



**THE SCHOOL OF PHARMACY AND
PHARMACEUTICAL SCIENCES
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**A JOURNEY FROM ETHNOMEDICINE IN RURAL
KENYA TO NETWORK PHARMACOLOGY USING A
CRITICAL RESEARCH APPROACH**

Ciara Mary Smyth

Being a thesis submitted towards the degree of
Doctor of Philosophy in Pharmacy and Pharmaceutical Sciences

Supervisor: Professor Helen Sheridan

Co-supervisor: Dr Junying Liu

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Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work with the exception of the following: Headspace-GC/MS analysis was performed by Professor Niko Radulovic visiting TCD from the Department of Chemistry, Faculty of Sciences and Mathematics, University of Nis, Serbia with Ms Olga Knutova and Professor Fabio Boylan of the School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin; the cell biology study was carried out with the assistance, guidance and training of Dr Ismael Obaidi, The NatPro Centre, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin; and molecular dynamics was performed by Dr Shouli Yuan, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China.

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Summary

Traditional medicine (TM) is widely practiced globally, with inter-country figures on usage ranging from 30 to 90%. Research on TM is conducted for a multiplicity of reasons, among them health care, sustainability, preserving of traditional knowledge and bioprospecting. Ethnodirected bioprospecting research has been conducted since the colonial era but has increased substantially over the last few decades. This thesis explored TM research beginning with historical field research in Kenya in the 1990s, through an analysis of *Carissa spinarum* L. (*C. spinarum*), as a case study in bioprospecting research. Finally, a network pharmacology (NP) approach was used to study one metabolite *C. spinarum* in relation to traditional therapeutics.

Chapter 2 examined qualitative ethnomedical research that I conducted in the early 1990s in a rural area in northwestern Kenya. There were 740 use reports (URs) recorded for 144 botanical species. The most common plant families used were Fabaceae, Vitaceae and Rutaceae, accounting for 28% of all URs; 61% of species were woody, 80% of URs used perennial plant parts; 81% of preparations were aqueous and 83% were taken orally. The biomedical (BM) interpretation of local illness terms was challenging, ultimately finding that several Pokot illness terms were probably malaria and that this was the most common BM illness treated (28% URs). Digestive disorders and neurological complaints accounted for 19% and 16% of URs respectively. Twenty of the identified species had a significant volume of literature with intersections in cross-cultural usage, including *Hoslundia opposita* Vahl., *Zanthoxylum chalybeum* Engl., *C. spinarum* and *Dichrostachys cinerea* (L.) Wight & Arn.

Chapters 3 and 4 were literature-based analyses of *C. spinarum*, searching online databases such as PubMed and Science Direct. Microsoft Excel was used to organise and analyse the data. Chapter 3 analysed the ethnobotanical research with a particular focus on ethnomedicine and categorised the studies using Hunn's phases of ethnobiological research. This species was used across three continents, mainly as a medicine and in diet. The 284 documents reviewed contained 513 URs, mostly from East Africa and South Asia. The majority (66%) of preparations used root and bark, were aqueous (37%) and were used orally (45%). The dominant traditional usage was in the treatment of digestive complaints (23% URs) and infections (18% URs), mainly malaria and STI. Most of the studies (75%) presented little socio-cultural context to TM usage. The majority of studies (65%) met criteria for Hunn Phase I, presenting decontextualised lists of plant species and their uses.

Chapter 4 analysed the phytopharmacological data of *C. spinarum* root and compared this to the ethnomedical usage. Key compounds were selected by yield for in-depth analysis: 2'-hydroxyacetophenone (2-HAP) (**2**), odoroside H

(4), carissone (5), (-) nortrachelogenin (6), carissanol (7), carinol (8), ursolic acid (9), rutin (10), quercetin (11), betulinic acid (12). Some have known activity in relation to traditional usage such as 6, which has anti-inflammatory, antibacterial and anti-plasmodial activity while 4, a cardiac glycoside, has potential toxicity. Some key activities of extracts that correspond to TM usage include anti-spasmodic, anti-convulsant, anxiolytic, analgesic, and anti-HSV activities. The investigations of root extracts (60) included some aqueous preparations (12) with the majority of studies being of organic solvent extracts (48), thus not reflecting TM practice overall. Metabolomic analysis of test extracts is limited to one study presenting a metabolic fingerprint of a MeOH extract. One study analysed the composition of the aqueous decoction and identified one phytochemical. The lack of adequate analysis of composition of extracts limits the usefulness and comparability of the studies, especially in relation to traditional preparations. Toxicity assessment of *C. spinarum* L. is not adequately assessed in most studies with only a few measures (6 studies) of selectivity provided, most showing moderate toxicity.

Chapter 5 applied NP methods to predict the bioactivity of 2 in relation to the traditional usage of *C. spinarum* root as an inhalant in chest conditions, stress and memory impairment. Potential targets of 2 were identified using online databases such as PharmMapper and SEA. The Metascape analysis of these targets predicted that pneumonitis, cancers and CNS disorders were potential targets of 2. A case-study of 2 in pneumonitis constructed a protein-protein interaction (PPI) network using STRING, and identified overlapping KEGG pathways and GO processes using Metascape. The key intersecting targets of 2 and pneumonitis were subjected to molecular docking and molecular dynamics simulations using AutoDock Vina software and Gromacs 2020.1. These predicted that COX2, NOS3, ALB and MAPK14 form the most stable docking complexes with 2. COX2, NOS3, and MAPK14 are each involved in inflammatory processes including pulmonary inflammation. Preliminary cell biology studies in iBMDM cells found anti-inflammatory activity of 2 against IFN β , IL6, both of which are implicated in pulmonary inflammation. The NP and cell biology studies support a role for 2 and potentially of volatilised *C. spinarum* root in inflammatory processes, in particular lung inflammation. Further NP analysis that examines the wider metabolome would be beneficial, followed up by well-designed *in vitro* and *in vivo* research based on the NP predictions.

The results of these analyses, TM, phytopharmacological and NP research illustrate the potential for improvements at all levels towards a TM-focused ethnopharmacological research.

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Research outputs

Book Chapter

Smyth, C., Sheridan, H. *Carissa spinarum* L.: A case study in ethnobotany and bioprospecting research. In: Kumar S, editor. Medicinal Plants. London: IntechOpen; 2022 DOI: 10.5772/intechopen.104665

Conference posters

Carissa spinarum L. (Apocynaceae): A case study in ethnomedical research

- Prize-winning poster at: GA 2021 in Bonn Gesellschaft für Arzneipflanzen- und Naturstoff- Forschung e. V. Society for Medicinal Plant and Natural Product Research

Carissa spinarum L.: a study using ethnomedicine-guided systems pharmacology in identifying a mechanism of action of a medicinal plant

- Poster at GA 2022 conference in Thessaloniki

Conference presentation

The impact of global and national governance structures on the practice of traditional medicine with particular reference to Kenya

- ISPA 2018 Postgraduate and Early Career Researcher Conference at the Carmelite Centre, Aungier Street, Dublin 2.

Upcoming paper to be submitted for publication

Carissa spinarum L.: a study using ethnomedicine-guided network pharmacology, molecular simulations and cell biology in identifying a mechanism of action of a medicinal plant.

Projected paper

Creating a blueprint for future ethnopharmacological research: Rebalancing of research models with ethnomedical source data.

Return of Pokot ethnomedical data to Kenya

- Report on some key Pokot medicinal species submitted to Ortum Mission Hospital in 1996 for hospital medical staff.

Pending ethical approval from Kenyan government agencies:

- Pokot ethnomedicine: accessible document in English, Swahili and Pokot for deposit in an agreed location within the community
- Pokot ethnomedicine: scientific report for deposit with the East African Herbarium

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Abbreviations

2-HAP: 2'-hydroxyacetophenone (2)

ADMET: absorption, distribution, metabolism, excretion and toxicity

BALF: broncho alveolar lavage fluid

BGCI: Botanic Gardens Conservation International

BM: biomedicine or western style medicine

BSA: bovine serum albumin

BSC: biological safety cabinet

C. spinarum: *Carissa spinarum* L.

CAT: catalase – CAT

CBHC: community-based health care

CC₅₀: the concentration causes a reduction of cell viability by 50% when compared to untreated controls

CCL5/RANTES: Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted

CHCl₃: chloroform

CHM: Chinese herbal medicine

CNS: central nervous system

COPD: chronic obstructive pulmonary disease

COVID 19: disease caused by SARS-CoV-2

CQ: chloroquine

DBT: *Danggui Buxue Tang*

DCM: dichloromethane

DEG: differentially expressed genes

DMR: direct matrix ranking

DMSO: dimethyl sulfoxide

DPPH: 1,1-diphenyl-2-picrylhydrazyl - DPPH

ELISA: enzyme-linked immunosorbent assay

EM: ethnomedicine

EMA: European Medicines Agency

EtOH: ethanol

EVM: ethnoveterinary medicine

FDR: false discovery rate

FGC: Female Genital Cutting

FL: fidelity level

FRAP: ferric reducing antioxidant power assay

GC-MS: gas chromatography-mass spectrometry

G-CSF: granulocyte colony-stimulating factor

GEO: Gene Expression Omnibus

GSH: glutathione

GUI: genito-urinary infection
 HCMV: human cytomegalovirus
 HIV: Human Immunodeficiency Virus
 HSV: Herpes Simplex Virus (HSV)
 iBMDM: immortalised bone marrow derived macrophages
 IC₅₀: how much of a drug is needed to inhibit a biological process by 50%
 ICF: informant consensus factor
 ICPC: international classification of primary care
 IFN β : beta interferon
 IL6: interleukin 6
 iNOS/NOS2: inducible nitric oxide synthase/nitric oxide synthase 2
 i.p.: intraperitoneal
 IUCN: International Union for Conservation of Nature
 KDH: Kapenguria District Hospital
 KEGG: Kyoto Encyclopaedia of Genes and Genomes
 KEMRI: Kenya Medical Research Institute
 LD₅₀: lethal dose in 50%
 LPS: lipopolysaccharide
 MAPK/ERK: mitogen-activated protein kinase/ extracellular signal-regulated kinases
 MAPK14: mitogen-activated protein kinase 14
 MeOH: methanol
 MIC: minimum inhibitory concentration
 MOA: mechanisms of action
 MRSA: methicillin resistant *Staphylococcus aureus*
 MU: mobile unit (Outreach service of Ortum Mission Hospital)
 NACOSTI: National Commission for Science Technology and Innovation
 NF κ B1: nuclear factor kappa B 1
 NOS3: nitric oxide synthase-3
 NP: network pharmacology
 NS: not stated
 OMH: Ortum Mission Hospital
 PBS: phosphate buffer saline
 PDB: Protein Data Bank
 PE: petroleum ether
 PPI: Protein-Protein Interaction
 PRR: Pattern Recognition Receptors
 PTGS2/COX2: prostaglandin-endoperoxide synthase 2/ cyclo-oxygenase-2
 RAF: residual aqueous fraction
 RFC: relative frequency of citation

RI: relative importance
 RMSD: root mean square deviation
 RNS: reactive nitrogen species
 ROS: reactive oxygen species
 RTB: rootbark
 RTI: respiratory tract infection
 SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2
 scRNA: Single cell RNA
 SI: selectivity index CC_{50}/IC_{50}
 SNNPR: Southern Nations, Nationalities and Peoples Region (Ethiopia)
 SOD: superoxide dismutase
 STI: sexually transmitted infection
 STRING: Search Tool for the Retrieval of Interacting Genes/Proteins
 TB: tuberculosis
 TCM: Traditional Chinese Medicine
 THP1: macrophages.
 TLR4: Toll-like Receptor 4
 TM: traditional medicine
 TMB: 3,3',5,5'-tetramethylbenzidine
 TNBS: trinitrobenzenesulfonic acid
 TNF α : Tumour Necrosis Factor α
 TRAIL: Tumour Related Apoptosis-Inducing Ligand
 UR: use report
 URTI: upper respiratory tract infection
 UTI: urinary tract infection
 UV: use value
 VHW: village health worker
 VL: visceral leishmaniasis/kala-azar
 WHO-ICPC: World Health Organisation- International Classification of Primary Care
 XO: xanthine oxidase

Chapter 1: Introduction

This story begins in West Pokot in northwestern Kenya in 1992. I arrived to work as a pharmacist at Ortum Mission Hospital with an interest in community work and herbal medicine. From pre-departure reading [1], I was aware of traditional medicine (TM) usage in the area. It became apparent after arrival that many patients had used medicinal plants prior to attending hospital. Some staff were disapproving and angry at its use as it was considered to delay biomedical treatment. In a few cases, I learned of deaths certified as poisoning by local “*dawa*” (medicine). A diagnosis of poisoning would have been based, then, as now, mainly, on clinical judgement [2]. It was my view, at the time, that such certifications could have been influenced by prejudice against the use of TM. I was interested in learning about what plants were in use and wrote to several people to learn about TM use in other places – the Zimbabwean healer’s association; Sue Bottomley, a pharmacist involved in a community herbal medicine project in the Philippines; and Amanda le Grand working with the WHO on health systems research, each providing further leads and valuable advice. At the same time, I was spending some time, in addition to pharmacy duties, with the community-based healthcare programme of the hospital. The programme trained traditional birth attendants and community health workers following WHO policy on using traditional practitioners to expand the health workforce. This group of women became the core of this research into traditional herbal practice in West Pokot. We collected voucher specimens of plants, sent them to Nairobi for identification, recorded information about their use, and made preliminary investigations of the cross-cultural use of these species in East Africa using Kokwaro’s book on the subject [3]. My first research assistant, Rhoda, then a curious and challenging early school leaver, wondered if I would make money from the knowledge and plants we were gathering. The women we were working with were also curious to know if there might be some exciting cure in their plants that could be brought to help people outside the area. I had recently completed a research degree in pharmacognosy and explained that their enthusiasm was unlikely to be rewarded in the near term, given the lengthy research processes involved. In addition, I was not collecting plants for phytochemical investigation but simply for identification. I planned to investigate the literature on the known phytochemistry and pharmacology of the Pokot species and feed that back both to the community and to the hospital.

On my return to Ireland, I found the workload to be extensive, with 144 full species identifications along with difficulties in the translatability of Pokot illnesses to biomedical terms. Without adequate library access in pre-online days and a busy schedule with work, study and family commitments, I initially settled for a summary document of the most commonly used 25 species as a

reference for the hospital. I postponed the return of a fuller and more accessible document for the community to a later date.

My recent attempts to return the information to the community brought me into confrontation with interrelated national and international governance issues through the Convention on Biodiversity (CBD), national policies concerning traditional knowledge (TK) and biodiversity, Intellectual Property rights (IPRs), WHO TM policies and with the pitfalls of ethnobiological research, each of which forms a backdrop to all ethnomedical research in the present day.

1.2 Traditional Medicine Background

The World Health Organisation (WHO) defined TM in their most recent strategy document: *WHO Traditional Medicine Strategy: 2014-2023*.

Traditional medicine has a long history. It is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness [4].

This is a very commonly used definition of TM in the literature of this field. An alternative view is taken by Kim Taylor [5] in her historical study of the development of Chinese medicine in the mid-20th century. She, along with Chiang [6], describes how Traditional Chinese Medicine (TCM) was developed in Communist China, not as a continuation but as a transformation of ancient traditions into a standardised, scientific and institutionalised new “Traditional Chinese Medicine”. A different approach again is taken by other writers. Thorsen [7] examining therapeutic landscapes in Nepal and Dutfield [8] discussing international law on TK choose not to define TM but rather challenge the relevance of the assumption of a dichotomy of modern and traditional in describing healthcare knowledge and practice. Gale [9], in her analysis of the sociology of traditional, complementary and alternative medicine (TCAM), describes TM as a term:

...commonly used to describe long-established, empirically-adapted healing practices originating in a geographically specific area and contrasted with ‘modern’ or ‘professional’ medicine [9].

She, like Dutfield and Thorsen above, also challenges the dichotomy of biomedicine and TCAM practices arguing that this binary reflects and reinforces the power imbalance relative to biomedicine and is, in fact, an inaccurate depiction of the reality of healthcare practices. So, the concept of TM is a contested one. Nonetheless, there are broadly agreed ideas about it in the

literature. TM is considered to be practised widely in less developed countries, providing an essential resource to populations in regions where biomedical healthcare is inadequate or unable to meet the needs of the communities served.

1.1.1 Global context of Traditional Medicine

Caring for health is assumed to have been an integral aspect of human life throughout our existence, adapting and increasing in response to the ever-changing conditions of our living environment. In many geographical areas, no early historical records of healthcare practices support this assumption. However, in some areas such as in present-day China and India, there are written accounts of various healthcare practices dating back several thousand years [10]. In modern times, allopathic medicine or biomedicine (BM) [11]¹, based in the scientific method, dominates the official healthcare systems of most countries. Globalisation brought the expansion of BM as the dominant form of national healthcare [10, 12, 13]. This expansion took place in Sub-Saharan Africa (SSA) with the gradual displacement of traditional forms of healthcare from the dominant position they held before the advent of BM with colonisation [14]. TM has nonetheless survived in SSA [10], occupying an essential role as part of a pluralistic healthcare landscape [14-17]. As such, there is nowhere that BM stands as the sole healing model as other forms of healthcare are available to those who, either through want or need, choose an alternative to BM. Some of these alternatives are based in ancient traditions, and others have more modern roots. In China and India, for example, Traditional Chinese Medicine (TCM) and AYUSH² (respectively) are officially recognised and integrated elements of the national healthcare systems. In many other countries, irrespective of socioeconomic status, they occupy a more marginal position [18].

1.1.1.1 Global prevalence of traditional medicine usage

TM is widely practiced, particularly in countries of the global south with UNESCO [19] citing an often recirculated WHO figure of 80% usage of TM in developing countries, a figure which dates back 36 years [20] and is still in use in some WHO documents [21]. This 80% figure has been challenged by TM researchers, including Willcox and Bodeker [22] in their research on malaria treatment and

¹ Several terms are used in the literature to represent this system of medicine. The US National Cancer Institute describes biomedicine as being alternatively called allopathic medicine, conventional medicine, mainstream medicine, orthodox medicine, and Western medicine.

² AYUSH = Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy. The Government of India promotes and develops these medical systems as a whole <http://ayush.gov.in/>

Oyebode *et al.* [23] in their research on TM in middle-income countries. Additionally, this figure was initially understood to mean total dependence on TM for healthcare needs. However James *et al.* [17] and Bodeker *et al.* [24] among others have found that TCAM is often used concurrently with biomedicine.

Any average estimated figure masks a wide range globally, between and within WHO regions, countries, and different health conditions [17]. At the regional level, the WHO Western Pacific Region usage of TM has been found to range from 30% in New Zealand to over 90% in China, [25] while in the WHO South East Asia Region, the figures are in the range 45-85% across the region [26]. In a review of the limited literature on prevalence in Australia among Aboriginal peoples, Oliver [27] found that 32.1% were using TM. In Latin America, there is an estimate by Caceres Guido *et al.* [28] that 66% of the population uses traditional/natural and/or complementary/alternative medicine³. In the WHO Africa region, the prevalence of TM usage is cited as 70-80%⁴, a figure which will be discussed further in the next section.

1.1.1.2 Traditional Medicine in Sub-Saharan Africa

Traditional practitioners in SSA include, among other categories of healers, herbalists, bonesetters, traditional birth attendants (TBAs), psychiatrists and spiritual healers [29]. The causes of ill-health in TM can be grouped under natural/physical, spiritual/ancestors and supernatural causation [30], in contrast to the theories of disease in BM which rely on physical causation in the main. While TM is widely practiced in Africa, the profile of present-day TM varies across SSA. TM policies and legislation are developing at different rates throughout the region. In West Africa, Ghana, Mali, Niger and Nigeria have the most extensively developed policy and legislative frameworks [30, 31]. Some West African countries such as Ghana [32], Mali [33], Cameroon and Niger [34] have some TMs on their Essential Medicines lists. In some areas of SSA, notably in Zimbabwe [35] traditional healers have been well organised as a group since the 1980s. However, throughout Africa, such organisations have been fraught with difficulties, as described by Wreford [36] and Scott [30]. In East Africa, Tanzania is the more advanced with regard to recognition and regulation of TM [30]. Throughout SSA, TM systems have been transmitted orally through the generations, often in an apprenticeship system similar to many other geographical areas. Although there are no ancient historical records, more

³ The figure cited was 400 million and the estimated population of Latin America is currently 626 million (<http://worldpopulationreview.com/continents/latin-america-population/> accessed 23.04.2019)

⁴ <https://www.afro.who.int/health-topics/traditional-medicine> accessed 11.04.2019

recent records dating to the colonial period are available. Early accounts of medicinal plants in South Africa dating back to the 17th century were described by Scott [30]. One extensive colonial era publication centred on medicinal plants of Southern and Eastern Africa, includes research dating to the 19th century[37]⁵ Estimates of the prevalence of TM usage vary somewhat from the average figure of 80% cited by the WHO. Busia and Kasilo [31], writing for the WHO Africa Region, cite a range of 70% in Benin to 90% in Burundi and Ethiopia. A recent systematic review of TCAM use in SSA [17] aimed to examine the evidence for the prevalence of TCAM usage and patterns of use. The reviewers found that there was a shortage of research in general, little research of high quality and that three-quarters of the research was from four countries: Nigeria, Ghana, South Africa and Uganda. Nigeria (40%) far outstripped the other countries in research on TM prevalence. The studies revealed a broad range of TCAM usage from 4.6-94%, with variation depending on socioeconomic status, education, age, gender, location and religion. Still, they found a high average usage of 58% in SSA across the examined studies [17]. They found that the use of TCAM was more likely among people in lower socioeconomic groupings and with lower levels of education. The reviewers noted that the studies focused heavily on TCAM use in HIV/AIDS and obstetric patients, with relatively little research on the growing area of non-communicable diseases [17].

1.1.1.3 Traditional Medicine and International Governance

TM intersects with multiple fields of international governance, including health care, biodiversity, TK and intellectual property (IP) law. The WHO has been a key player in guiding governance of TM in recent decades with other roles held by UNESCO, the secretariat of the CBD, the World Trade Organisation (WTO) and the World Intellectual Property Organisation (WIPO). An outline of international and Kenyan governance of TM is presented in Figure 1.

Since 1969, the WHO has been actively developing policies and strategies aimed at integrating traditional, and latterly complementary and alternative medicine, into national BM healthcare systems [38]. A WHO meeting of international experts on TM in 1977 aimed to develop a plan of action around TM promotion, development and integration[39]. At the Alma Ata Primary Health Care Conference of 1978, the WHO shifted its focus from secondary hospital-based care to primary care and the policy of “Health for all by the year 2000” [40] which has more recently developed into the “Universal Health Coverage” policy [41]. The policy was a rallying call to use all available healthcare resources to

⁵ John Mitchell Watt and Maria G Breyer-Brandwijk were physicians who extensively catalogued African medicinal plants. First edition 1932.

meet the challenge of providing universal health care. The term “Traditional Medicine” has become increasingly used since 1978, covering a wide range of practices in different geographical areas, overtaking other terms in public and official discourse, such as indigenous medicine and folk medicine in describing the field. The policy reinforced the outcomes of the 1977 expert meeting in promoting the inclusion of TM practitioners in the primary healthcare team [40] and has continued through many key stages including the WHO TM strategies of 2002 and 2014 [4, 42]. TBAs, more than any other group of practitioners, gained much attention in terms of training for integration with BM. Their training in fact, predated Alma Ata [43] with the WHO and UNICEF training TBAs in BM techniques from the 1950s onwards and the Rockefeller Foundation doing so even earlier from the 1930s, as described by Kadetz [44]. For many decades, Kadetz [44] argued, they were only group of healers that were targeted for integration into national health services. The path of TBAs as acceptable health workforce members has not been smooth with WHO and national governments shifting from acceptance of TBAs to prohibition to somewhere in between over the intervening decades [45, 46]. Since the Alma Ata general statement on the inclusion of traditional practitioners in national programmes of primary health care, the WHO strategy has become increasingly refined with a focus on safety, quality and efficacy of practitioners, practices and *materia medica* [47, 48] and aiming, with a better legislative, knowledge and practice base, towards the integration of TM into national healthcare systems. The WHO defines the *materia medica* of Traditional Medicine⁶ as medicinal plants in the main, as exemplified in the 2014 strategy [4] and various other policy and strategy documents. At international, WHO regional and national levels, the WHO provides support in the development of policy and legislation towards these ends. The WHO also supports and monitors the modernisation of TM in member states through practical measures such as promoting and supporting the existence of TM research institutes, TM practitioner registration, National Herbal Pharmacopoeia, evaluation and registration of TM products and TM training in higher education for traditional and BM practitioners [31].

UNESCO has a remit to promote cultural plurality and supports TM from this perspective [19]. UNESCO mirrors the WHO in its requirement for safety, quality and efficacy of TM supporting as it does the human right to benefit and

⁶ T&CM products include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants, other plant materials or combinations thereof as active ingredients. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g., animal and mineral materials). (WHO 2013 p 31)

the avoidance of harm while supporting the right of people to have access to culturally pluralistic health care.

The CBD [49] and the 2010 Nagoya Protocol, an instrument of the CBD [50], support the use of TM by promoting the maintenance of TK, the sustainable use of associated genetic resources (GR) (e.g., medicinal plants) and the equitable sharing of benefits from the use of GR and TK. Access and benefit sharing (ABS) agreements with Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) are each key components of any research relating to TK and biodiversity, such as ethnomedical research. The CBD changed the status of GRs from being a common good, as they were considered under colonialism, to being the property of nation-states with the right to their exploitation [51, 52]. The conduct of all research, commercial and non-commercial alike, has been affected and impeded by the implementation of the CBD [53], although the approach differs between signatory countries, and its effects in Kenya will be examined more thoroughly below.

The WTO also contributes to international policy and legislation relating to TM through the TRIPs agreement (Trade-Related aspects of Intellectual Property Rights)⁷. Both the use of GRs and the associated TK in TM indicate the need to protect IP relating to these elements of TM [54]. However, the responsibility for the debate over the right to protection of IP in TK was moved in the late 1990s from WTO to WIPO, a relatively weaker organisation. Harrington *et al.* [55] describe this move as preventing strong IP protections for developing countries around TK under the WTO system. While there is some possibility of protecting TK and associated GRs under WTO-based protections such as copyrights and trademarks [56], international protection of TK is still under discussion without any clear resolution in sight under WIPO [55].

The global promotion of TM by the WHO, and other elements of the UN, has been, at least in part, responsible for a gradual shift in policies and attitudes towards TM at global, WHO-regional and national levels. The required country-level policy and regulatory changes have been progressing globally with significant increases in regulation of TM products, practices and practitioners - more recently broadened to Traditional and Complementary Medicine (T&CM) - noted in WHO TM Strategy [4] relative to the earlier policy document [42].

⁷ This is an agreement between all member nations of the WTO.

Timeline of TM governance – Global and Kenya

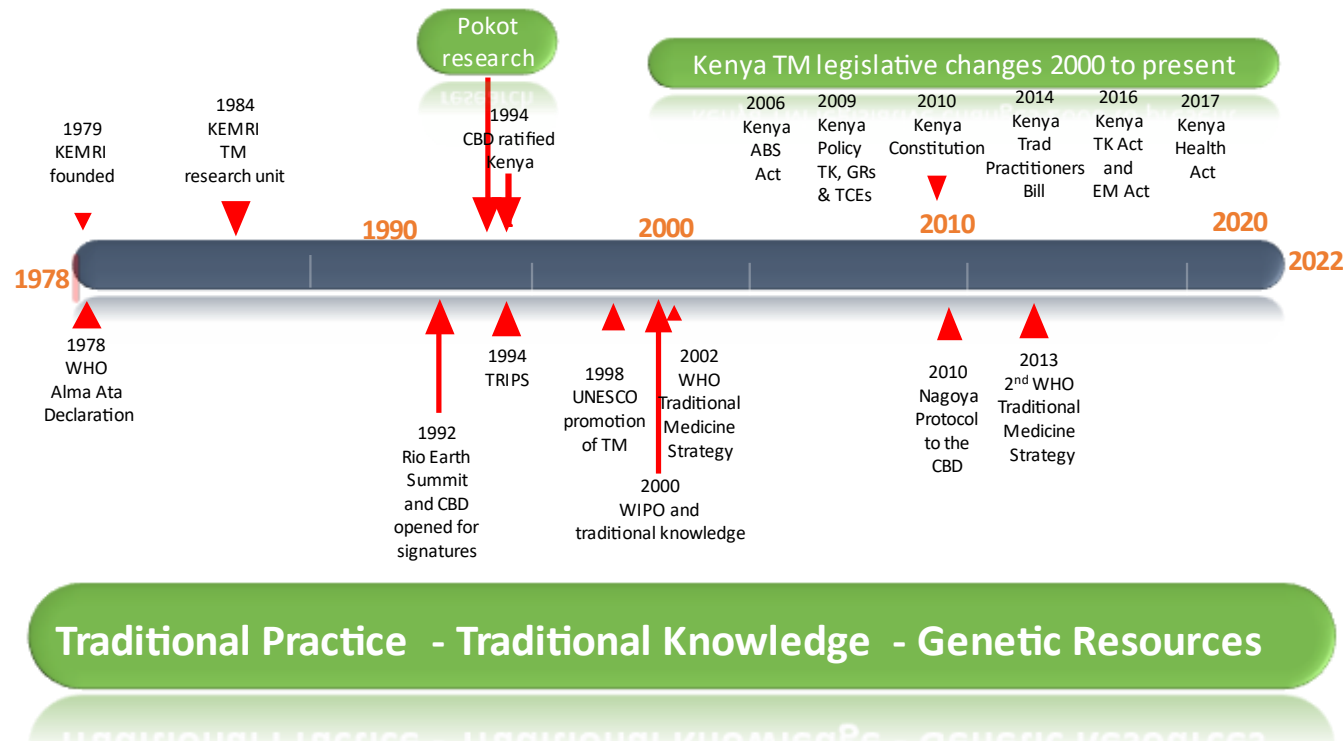


Figure 1: Outline of timeline of international and Kenyan governance of TM

1.1.2 The Kenyan Context for Traditional Medicine

1.1.2.1 Healthcare in Kenya

Kenya gained independence in 1962 and inherited a BM healthcare system of uneven and inequitable service distribution, primarily urban and hospital-based. From that point, there was a shift towards expanding healthcare to the whole population, resulting in huge improvements in life expectancy and mortality figures [14]. Unfortunately, many of these gains were lost throughout the 1990s from the impact of Structural Adjustment Programmes and the HIV/AIDS epidemic [16]. There have been some improvements since the 2000's in healthcare statistics for Kenya [57]. However, Kenya, like other low- and middle-income countries, has experienced a significant increase in non-communicable diseases (NCDs), most notably cardiovascular diseases, cancer and diabetes [57-59], creating new challenges for healthcare systems nationally. This is reflected in 2007 research cited by Nugent *et al* [59]. It is also seen in IHME [60] whereby NCDs as cause of death increased substantially between 2009 and 2019. This is thought to be attributable to smoking, obesity, more sedentary lifestyles and changing demographics towards an older population [59].

1.1.2.2 Traditional Medicine in Kenya under Colonial and Post-Independence Governments

Good [14] argued that during the colonial period, TM practices continued without much interference from the colonial authorities. However, the prohibition on witchcraft, embodied in law in Kenya since 1925, has been confused with TM, albeit unwittingly, a confusion said to continue in some quarters to this day [51]. Direct opposition to TM came from Christian missionaries, BM staff and Europeans who denigrated it as irrational [15, 61]. Post-Independence, hostility and dismissal of TM continued among Kenyan elites and authorities. The late President Jomo Kenyatta, the "father of the nation", was reported to have referred to traditional practitioners as "lazy cheats" [61]. The opposition by BM staff is still evident in the present day, as described by Harrington [51], and evidenced by proposals for punitive regulatory control of traditional practitioners.

1.1.2.3 Governance of Traditional Medicine in Kenya

As with other countries, official and population-level attitudes to TM have been changing in recent decades. Kenya has had several policy and legislative initiatives since the early 2000s aimed at regulating the sector, outlined in Figure 1 [15, 62]. The new Constitution of Kenya [63], promulgated in 2010, values culture, indigenous technologies and the natural resources of Kenya. Due to

these values, the new Constitution has birthed several pieces of legislation impacting TM. The Health Act 2017, signed into law in June 2017, sets out an approach to promoting and regulating TM practitioners, plants and practices with a view to their ultimate integration into the health care system. TM practitioners are brought in under the Ministry of Health rather than the Ministry of Culture as had been the case heretofore, a move which has been welcomed by advocates. The Traditional Health Practitioners Bill 2014 sought to regulate practitioners, requiring each person to register and reach a certain level of training in order to meet quality, safety and efficacy standards in practice. The bill also includes significant disciplinary procedures. This bill has not passed through parliament to date as it met with significant opposition. However, if it is passed, it leaves several questions unanswered. The question of conducting the registration of such a large body of people, estimated to be 40,000 is not addressed as it would require significant funding. Many current practitioners could be excluded from registration such as through lack of education or finance, with consequences for livelihoods and community membership [51]. The *materia medica* of TM in Kenya, in theory, must be registered. However, there is insufficient capacity to test and approve products and therefore this element of ensuring quality, safety and efficacy is not enforced [51]. The approach to practitioners, thus, is one of control with assumed safety issues with the practitioners and the products they use and the need to reduce the risk associated with traditional practitioners' practice.

On the other hand, the government is more supportive of the potential of *materia medica* of TM, seen as key to the development of a lucrative export industry in herbal medicine [55]. This process has been ongoing since the establishment of the Kenya Medical Research Institute (KEMRI) in 1979. As described in section 1.2, TM also intersects with laws to prevent the loss of biodiversity globally. Kenya, as a party to the CBD, is committed to the sustainable use of and benefit sharing concerning plant resources and TK. Kamau [64] described the current ABS legislation in Kenya dating from 2006, though under review, as very restrictive and of a mindset to prevent biopiracy rather than encourage research and access and benefit sharing processes. The Protection of Traditional Knowledge and Cultural Expressions Act 2016 gives IP including *sui generis* IP protection to the owners of TK. However, this is done without acknowledging the complex web of ownership that may be involved in traditional knowledge within and across communities and countries [65]. Harrington [51] described the system as not working well, with a lack of clarity on who gives consent to research and who shares the benefits, finding that there have been minimal benefits accruing to ordinary Kenyans. Kenya, as a member of WIPO and the WTO, is also keen to maximise the potential economic benefits

of medicinal plants through invoked IP issues. These elements are also exemplified in The Environmental Management and Coordination (Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing) Regulations, 2016.

The appeal in the constitution to exploit Kenya's natural resources with the drive to document traditional medicinal knowledge for bioprospecting and to reduce the range of species used by practitioners to those with proven benefit and safety [51] could unwittingly drastically alter the landscape of traditional medicine practice in Kenya in the future, in opposition to the aims of the CBD to maintain TK

1.3 Ethnobiology, Ethnopharmacology and Bioprospecting

Research in the traditional use of medicinal plants is inextricably linked with ethnobiology, a discipline with its academic beginnings during the colonial era in the late 1800s [66] although pre-academic work extends for millennia [67]. The discipline of ethnobiology is defined as “the scientific study of dynamic relationships among peoples, biota, and environments” [66, 68].

Ethnobiology has undergone many changes since its academic beginnings, from descriptive lists of species and their uses, through ethnographic and symbolic studies to more ecological concerns from the 1970s and, latterly, a focus on indigenous rights and IP from the 1990s onwards. This has resulted in the increasing visibility of research participants toward more collaborative and ethics-driven research. As a result, ethnobiology is now a wide and varied discipline with research agendas covering the natural and social sciences such that in research on medicinal species, the research focus could involve maintaining a cultural record, improving health care, environmental sustainability, indigenous rights and ethnopharmacology.

Ethnobotany, ethnomedicine and ethnopharmacology are major subdisciplines of ethnobiology [66]. Ethnobotany is understood to be the study of the inter-relationships between people and plants [69]. Ethnomedicine, as a discipline, examines the cultural understandings of health and illness, healing practices and health-seeking behaviours [70]. Ethnomedicine, as a term, is also an alternative word for the practice of TM [71]. Ethnopharmacology combines ethnomedical research with pharmaco-toxicological study in the context of traditional usage [72]. In this thesis, I will be using ethnobiology as an overarching term with ethnobotany to refer to all aspects of relationships of people and plants and the ways in which plants are used including medicinal use. I use the term ethnomedicine as an aspect of ethnobotany while

ethnopharmacology is used to refer to ethno-directed laboratory-based and clinical research, in this case on medicinal plants only.

Ethnomedical research has historically been conducted for ethnopharmacological and bioprospecting purposes [66]. Osseo-Asare [52] has described the search for new drugs as a vital element of the colonial project, extracting TK on medicinal and poisonous species and storing live and preserved specimens in European botanical gardens [73]. The search for arrow poisons with their potential for cardio-activity was a particular interest and is frequently listed in ethnomedical uses in a colonial-era treatise on African ethnomedicine and ethnopharmacology [37]. Ethnopharmacology conceptually combines in-depth cultural information with phytochemical and pharmacological investigation of activities based on traditional usage. However, this conceptual combination is infrequently seen in practice. There is a historical prevalence, in ethnomedical and ethnopharmacological research, of decontextualised lists of useful species lacking the necessary insights into cultural usage that would better guide pharmacological investigations [74-76]. Ethics has become a central element of all social and healthcare research. One aim of ethical ethnobiological and ethno-directed bioprospecting research is that it should benefit the provider community [77] although this is hard to achieve in practice in terms of monetary, social or health benefits [8, 18]. There is the added risk of overharvesting of a newly “discovered” species as happened with *Hoodia* in southern Africa [52]. Researching traditional knowledge and medicinal plants thus raises ethical issues around health, IPRs, benefit sharing and the protection of biodiversity.

1.4 Outline of the research

Chapter 2 is an investigation of medicinal plant-based TM in a rural community in West Pokot County in north-western Kenya. The field research used qualitative methods spanning fourteen months in the early 1990s to learn about illnesses treated and how they were treated. The research participants were female traditional practitioners and laywomen who treated friends and family. Key medicinal species were examined for cross-cultural similarities in medicinal plant use and the scientific support available for that usage.

Chapter 3 involves an in-depth analysis of the ethnobotanical literature on *Lagetetwo*, a Pokot medicinal species identified as *Carissa spinarum* L. (*C. spinarum*). This analysis is used as a model of ethnobotanical study of a varied and environmentally tolerant species.

Chapter 4 examines the research on *C. spinarum* from ethnomedical and bioprospecting perspectives, investigating the known phytochemical and pharmacological data of the root. This chapter critically examines laboratory methods and their relationship to ethnomedical usage.

Chapter 5 uses network pharmacology (NP) to predict the activity of 2'-hydroxyacetophenone (2-HAP) which forms up to 99.9% of the volatile root oil. Biological processes and diseases predicted to be affected by 2-HAP are compared with traditional usage. Potential modes of action are explored followed by preliminary exploration of activity in cell-based assays.

Chapter 2:

Pokot Ethnomedicine

2.1 Introduction and Background

This chapter will examine ethnomedicine in a community in the Ortum area, West Pokot County, Kenya (Figure 2 and Figure 3). It will present an analysis of

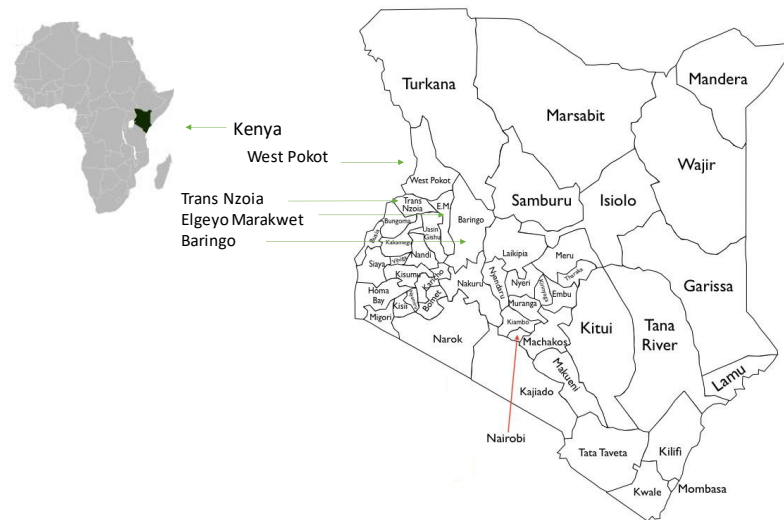


Figure 2: Maps illustrating Africa, Kenya and West Pokot with neighbouring counties



Figure 3: Some Euphorbia near Ortum with Cherangani hills in the background (photo by Ciara Smyth)

ethnomedical research conducted by the author in the early 1990's whilst working as a pharmacist at Ortum Mission Hospital (OMH). The chapter will give an overview of the Pokot people and will examine a variety of other research relating to Pokot traditional medicine at the time.

2.1.1 Pokot people

The Pokot are an east African people living mainly in West Pokot, Baringo and Elgeyo-Marakwet counties in Kenya's Rift Valley (Figure 2) [78]. They are part of the Southern Nilotic Kalenjin group with overlap in language, culture and economic activity across the constituent ethnic groups. It is thought that they came originally from Southern Ethiopia and moved down through Kenya, differentiating into the different language and cultural groups over time [79]. Davies and Moore [80] wrote that the earliest records of the Pokot close to the study area was as livestock keepers in Sigor in 1750, 24km northwest of Ortum (Figure 4). From that time there were ongoing displacements of people, westwards and eastwards towards the plains where they specialised in pastoralism and southwards and northwards into the Cherangani and Sekerr hills where they specialised in agricultural production. The division persisted historically with agricultural Pokot constituting about two thirds of the population. Pastoralists were the remaining one third, occupying the semi-arid areas in West Pokot and Baringo counties [78]. The majority of the population of the county lives in poverty [81], 35% living in absolute poverty, rising to 53% in rural areas [82]. At the time of the study, there were limited but increasing educational facilities in the region, at primary and secondary level with primary education being compulsory [1].

2.1.2 The Study area

This study was conducted in the West Pokot District of Kenya, situated 460km north-west of Nairobi on the border with Uganda. West Pokot District comprises an area of 9064 sq. km. It is located in the area that was then called Rift Valley Province. In 2013, the province system in Kenya was dissolved, and a county system replaced it. The area formerly known as West Pokot District is now called West Pokot County and is divided into sub-counties. The study area is located in what are now the South and Central Pokot sub-counties [83]. The County is bordered by the counties of Turkana, Baringo, Elgeyo-Marakwet and Trans-Nzoia, with Uganda to the west. The study was conducted in the Chepareria division (1400 sq km) (limited to Batei and Kipkomo locations) in villages between Ortum (1 26 N and 35 21 E) and Chepareria (1 19 N and 35 12 E), all within 25km radius of Ortum. The villages included in the study were Chepareria, Kapromtim, Kapchemogem, Sebit, Sobukwo, Kasongwor, Chepokeriung and Ortum (Figure 5). West Pokot was (and still is) a predominantly rural area [82]. The population of the Chepareria division was 35,094 in 1989, with a population density of 41 per sq. km and 89% ethnic Pokot [84]. The study area is in the vicinity of the tarred trunk road running northwards towards Sudan, though other road infrastructure in the area was poor.

The vegetation type is variable. The map in Figure 6 shows the study area to be semi-arid to transitional - transitional from semi-arid lowland to the highlands with wetter and cooler climate – and the majority of plants collected were from semi-arid areas along with more fertile riverbanks (Muruny river) and highland areas. The geographical variation in the district is reflected in the variety of plants used medicinally, as highlighted by Timberlake [87].

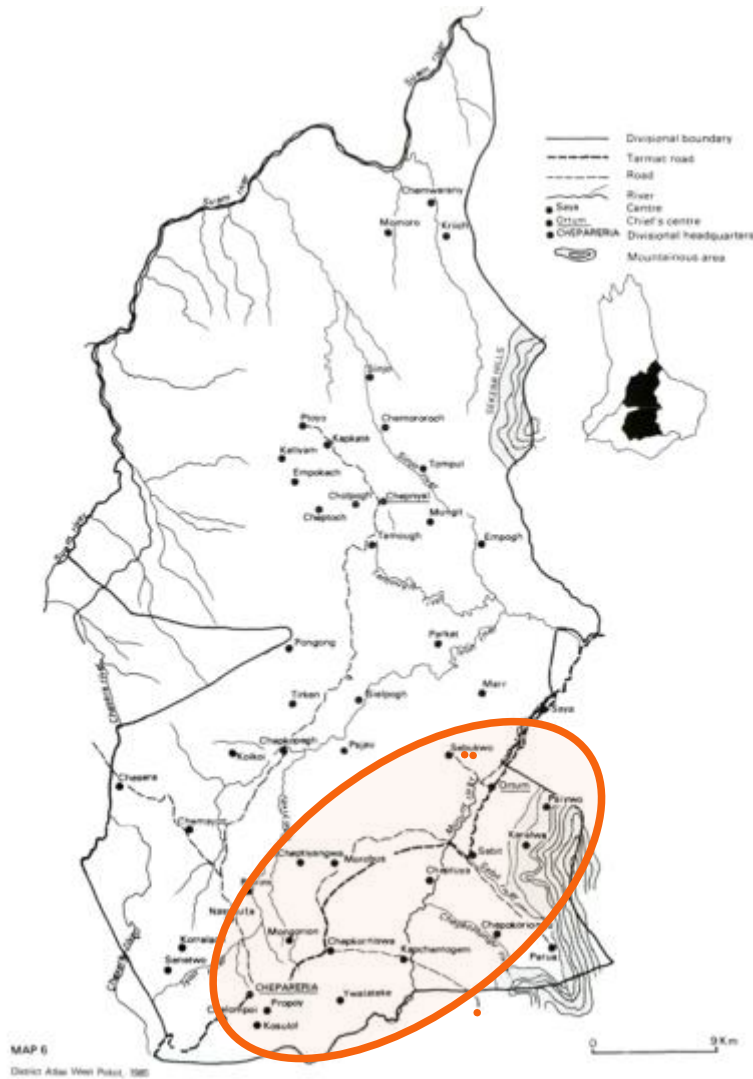


Figure 5: Map of Chepareria Division indicating several of the villages involved in the study (circled area) [85]

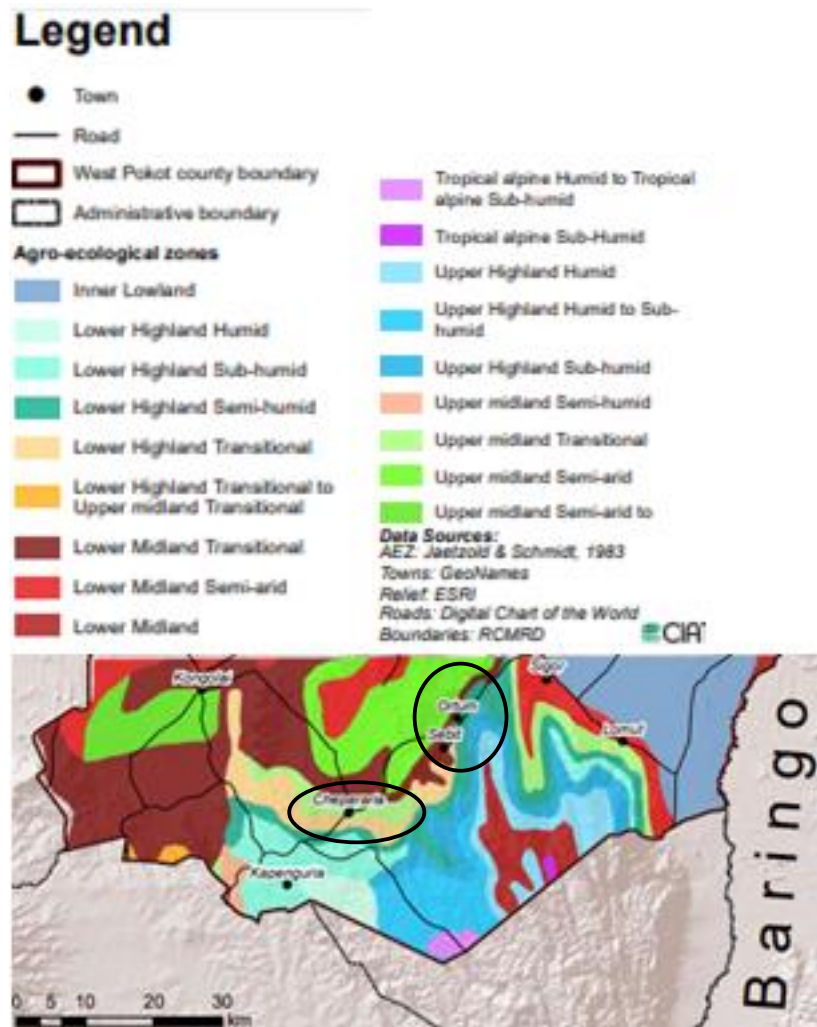


Figure 6 Agro-ecological zones of southern part of West Pokot County including study area [88]

2.1.3 Ethnomedical research

At the time of this study, Nyamwaya [89] had conducted detailed anthropological research relating to healthcare decision-making and ethnomedicine among the Pokot and illustrated the dynamic nature of this behaviour in the context of the availability of traditional and BM forms of health care. This research was conducted between 1980-82 around Chepareria village (Figure 5), and investigated Pokot beliefs and practices in relation to health and illness and the relationship of traditional and western medicine in the study area. Nyamwaya [89] described an active practice of Pokot TM and identified 13 different types of healers. These included: *Chepsaktian* (herbalist), *Kama-echo* (TBA), *Mutin* (circumcisor), *Kokomelkong* (clitoridectomy expert), *Mutindo ngaliamunget* (uvula remover and tonsils remover), *Ghotin* (tooth extractor), *Cheposogoyon*, *Pkwanyan*, *Pkweghion* (diviners), *Kapolokion* (mystical power), *Tsin*, *Parparin* (ritual experts), *Liokin* (can withstand *pan* – witchcraft or sorcery) and *Werkoyon*

(prophet). Nyamwaya [89] wrote that Pokot beliefs around illness causation worked on a few planes: primary causation relating to the “how” of an illness - a physical cause such as rain or sugar or a mosquito causing malaria and a secondary layer that could be interpersonal or spiritual causation, the “why” of an illness. Treatment could then relate to both the “how” and the “why” of illness where treatment of the biological cause with herbs, *sakt*⁸, and dietary recommendations being specific to physical symptoms. Accompanying treatments for interpersonal and spiritual causes could include a ritual element, *tum* (a short glossary of Pokot terms is included in Appendix III). Some ailments, such as infertility and mental illness, were considered to have an interpersonal or spiritual basis while others such as malaria were thought to have a more naturalistic causation.

It was thought that illnesses with a spiritual causation that manifested physically could not be fully healed by *mzungu*⁷ medicine/ BM. He found that *sakt*, whether traditional or biomedical, could be used to treat the physical symptoms of an illness at the primary level of causation. Treatment of illness would normally begin with the *Chepsaktian* who would then refer to another practitioner to treat a secondary cause of illness if necessary. The *Chepsaktian*⁹ (herbalist) (pl. *Chepsaktis*) equated to the BM doctor or nurse.

The treatment of *kakatan* (malaria), for example, could be physical with purging and emetic treatments in addition to chloroquine and combined with a prohibition on eating sugar and an instruction to eat plenty of goat's soup. *Tum* would normally also be required for complete healing of *kakatan*. Thus, TM and BM were syncretised in Pokot concepts of illness and health.

The use of purgatives and emetics by the Pokot to prevent and treat illness was understood in the context of illnesses residing in the gut. The process was thought to “clean up the system” [89]. The treatment of malaria, for instance, could involve the use of pharmaceuticals complemented by the subsequent use of purgatives and emetics to ensure the complete expulsion of the illness. The use of emetics and laxatives was likewise discussed by Timberlake [87] in the context of Pokot ethnobotany and among the Luo by Geissler *et al.* [91].

Nyamwaya [89] found that the conflict in health and illness was not between TM and BM but rather was between *sakt* and *tum* treatments. *Mzungu* medicine/BM expanded the range of *materia medica* available to the *Chepsaktian*. He described a range of illnesses that were mainly to be treated by one or other form of treatment e.g., epilepsy, sterility, impotence, mental

⁸ *Sakt*: Pokot word for medicine/medicinal plant; *Tum*: Pokot word for ritual part of healing; *Mzungu*: Swahili word for European/white person; *Chepsaktian* = Pokot herbalist

⁹ Also called Chesaktian in Timberlake 1987 (by the Pokot in Baringo) and Chesaktin in Meyerhoff (1981) (near Sigor in West Pokot).

illness, bone-setting and constipation to be treated with Pokot medicine; acute illness, illness requiring surgery, kala-azar/visceral leishmaniasis (VL), sexually transmitted infection (STI) and TB to be treated by BM. However, these categories were flexible with choices varying over time and with perceived efficacy of treatments such that the author found painkillers, available at the local shop, to be present in all homes in his study as they were found to be very effective.

In later research in the study area, O'Dempsey, who worked as a doctor in OMH, investigated traditional belief and practice in relation to maternal and child health [1, 92]. He reiterated some of the information on Pokot medicine described by Nyamwaya [89] and highlighted some traditional practices relating to pregnancy, contraception, lactation and neonatal health. Dietary taboos in pregnancy could have helped protect the mother from infection but some may also have contributed to low neonatal birthweight in an effort to ease delivery. Meyerhoff [93] reported on pregnancy-related and initiation practices in her research on the roles of women in Pokot society. Women's lives included many actions to maintain the health of their families and livestock. Such actions included ritual purifications and the observing of food and sexual taboos. While the study was not about healthcare as such, the *Kokeogh* (midwife) and *Kokomelkong* (clitoridotomy expert) featured significantly in Meyerhoff's thesis and some plant species were used symbolically and in washing ceremonies (*Lapow*), ritual cleansings and wound healing¹⁰.

Recent research in West Pokot indicates that TM is still in use [94, 95]. KNBS [96] reports that 74% of births are home deliveries, with 69% of these assisted by TBAs. The 2012 study by ACF-USA [97] found 15% using traditional healing to treat sick children while the later surveys in 2014 and 2018 found just 1.5% using TM [98]. These studies indicate that TM is currently in use in West Pokot County although diminishing radically, at least in relation to the treatment of children. This could be due to the expanded biomedical facilities in the county and the local promotion of the importance of attending qualified health workers as described in the reports [98, 99]. Other factors such as education levels, local morbidities, availability of pharmaceuticals or research design may be contributing factors to the major reduction in usage. On the other hand, in research conducted in 2020 on TM governance in western Kenya, the busy town of Makutano in West Pokot had a significant presence of medicinal plant sellers, indicating that a demand exists [100]. However, with no in-depth studies since that of Nyamwaya [89] in 1981, there is no clear indication of the extent to which

¹⁰ Meyerhoff described her use of the term clitorodotomy rather than the more commonly used terms, clitoridectomy, excision and infibulation as they do not precisely describe what she witnessed in *rotwo* ceremonies in West Pokot.

wider changes have impacted upon the practice. There is a recognised impact of western-style education on worldview and TK transmission systems [101]. In addition, the integration of societies into the cash economy could impact upon customary methods of payment-in-kind for traditional treatments [102].



Figure 7: Diagram of Ethnomedical Systems in Kenya (Adapted from Good, 1987)

Nyamwaya [89] described the Pokot concepts of treatment of illness using Pokot or western medicine as part of one system rather than in terms of two separate systems. This view was coherent with that expressed by Good [103] in his description of ethnomedical systems in Kenya in 1987. The diagram in Figure 7 illustrates the multi-component character of healthcare options available when seeking treatment of illness at the time of his research. These included home treatment, government, church and NGO services, traditional practitioners, shops, pharmacies, market herb sellers, spiritual healing and back-street doctors. All of these forms of healthcare were available in the study area in the 1990s in West Pokot. Taken more broadly, there are indications that, while very often in plural medical systems people may choose primarily BM where this is available and affordable, there are some illness categories for which TM is still the treatment of choice. In neighbouring Tanzania, for instance, convulsions were sometimes treated preferentially by TM before referral to a biomedical

facility although this is reported to have changed significantly since the 1990's [104, 105]. In a 2013 study among the Sabiny, an ethnic group related to the Pokot, across the border in Uganda, women chose overwhelmingly to give birth alone or attend TBAs – as they do in West Pokot [89]- rather than give birth in a health facility for a variety of cultural and social reasons [106] indicating a complexity in healthcare decision-making.

2.1.4 Ethnobotanical research with Pokot

From the literature, it appears that the largest ethnobotanical survey conducted with the Pokot is that of the botanist Timberlake [87], who reported on extensive ethnobotanical fieldwork conducted in 1976-78 in East Pokot, with data relating to 1,833 ethnobotanical samples. At around the same time, Tanaka [107] also conducted an ethnobotanical investigation in that area. In the study area itself, Nyamwaya [89] collected information on 38 medicinal species around Chepareria village (Figure 5) which were deposited in the East African Herbarium in Nairobi with 25 of these overlapping with the species in this study. A more recent study in 2015 by Kanyongi [95] investigating medicinal plant use around Chepareria village identified 40 species used medicinally by lay people and traditional healers, 21 of which are additional to the current study.

2.1.5 Health and healthcare choices

2.1.5.1 Biomedical health care options

At the time of the study there were very few BM facilities available in West Pokot. In 1980, there were seventeen BM health facilities including hospitals, dispensaries and health centres in West Pokot [89]. In 1993, West Pokot district was served by two hospitals; the government run Kapenguria District Hospital (KDH) in the south and OMH run by the Catholic Holy Rosary Sisters. The district hospital was free to access but was frequently out of stock of medicines. OMH charged for services but did have a good supply of medicines. In 1993, the study area also had two church-run dispensaries in Chepareria. There was a mobile unit (MU) operating from OMH making regular visits to surrounding villages offering Maternal and Child health and general care. A community-based health care (CBHC) or primary health care programme operated from OMH. Up to the health access report in the 2018 County Development Plan, it was recognised that BM health care was still inadequate in the county due to historical marginalisation [86] reflecting the general national imbalance whereby BM healthcare is disproportionately available in urban areas and to higher socio-economic groups [65].

2.1.5.2 Epidemiology

At the time of the study in the early 1990s, malaria was the primary cause of morbidity in the area, illustrated by statistics for OMH where malaria was responsible for about 50% of hospital admissions (Table 1). Other key morbidities at OMH, with incidence rates of 5 - 11%, were pneumonia, anaemia, upper respiratory tract infections (URTI), gastrointestinal (GI) infections and meningitis in 1991 as there was a devastating epidemic that year. Changes in some of these figures from 1991 to 1996 may be related to circumstances, e.g., reduction in TB could relate to the TB programme, which was growing as a result of outreach campaigns and availability of medicines through a TB control

Table 1: OMH in-patient morbidity statistics (n/l denotes category not listed; frequency given as a % of total of figures listed, not total annual morbidity figures)

Illnesses - OMH morbidity statistics	Frequency (1996) (%)	Frequency (1991) (%)	Frequency (1987) (%)
Malaria alone	1642 (49.1%)	2631 (48%)	2626 (52%)
Malaria with anaemia	579 (17.3%)	n/l	n/l
Anaemia	n/l	621 (11%)	n/l
Pneumonia	344 (10%)	558 (10%)	521 (10%)
URTI	n/l	470 (9%)	n/l
Gastro-enteritis	272 (8.1%)	288 (5%)	549 (11%)
Kala-azar (VL)	164 (4.9%)	158 (3%)	126 (3%)
Typhoid	107 (3.2%)	n/l	79 (2%)
T.B.	103 (3.1%)	122 (2%)	521 (10%)
Dysentery	53 (1.6%)	n/l	49 (1%)
Amoebiasis	n/l	110 (2%)	322 (6%)
Meningitis	40 (1.2%)	374 (7%)	33 (0.6%)
UTI	37 (1.1%)	n/l	n/l
Gonorrhoea	n/l	n/l	58 (1%)
Accidents	n/l	104 (2%)	n/l
Measles	n/l	n/l	129 (3%)
Bronchitis	n/l	n/l	84 (2%)

programme. Increase in VL figures in 1996 to 4.9% could relate to a clinical trial of a generic form of sodium stibogluconate in that year which made the treatment affordable for the hospital relative to the branded Pentostam™, thus recruiting

more patients [108]. The MU treated malnutrition and more minor concerns such as scabies, intestinal worms, conjunctivitis, otitis media and urinary tract infections (UTIs) (Table 2). Nyamwaya's research had reported similar data on the main causes of morbidity in KDH: acute respiratory tract infections (RTIs) and pneumonia, malaria, diarrhoeal diseases, skin infections, acute eye infections, intestinal worms, gonorrhoea, accidents (e.g., fractures, burns), rheumatism/joint pains. Malnutrition, reported in the MU data (marasmus and kwashiorkor) may be masked in hospital data by illnesses consequent to malnutrition such as malaria, RTIs and GI disorders. The infant mortality rate for West Pokot was estimated at 108 per 1,000 in 1989 [109] which fell to 87 per 1,000 in 1999 [82]. Child (under 5) mortality rate in West Pokot was 127 per 1,000 in 1999, with key causes of mortality cited as malaria, anaemia, pneumonia, gastroenteritis and dehydration [82]. At a national level, there was a significant rise in child mortality (Under 5) from the late 1980's to the mid-1990's and this is thought to be due in part to the sharp rise in HIV prevalence [110]. However, in 1992-1994, the incidence of HIV in the study area was low and not reported in hospital morbidity data. In more recent data from 2018, West Pokot was considered to be a low prevalence area nationally with a county prevalence of 1.5% relative to national prevalence of 5.9% [111].

Table 2: OMH morbidity statistics for Mobile clinic 1996 (frequency and order not recorded)

Illness treated by OMH Mobile Unit 1996	
Malaria (most common)	Kwashiorkor
Amoebiasis	Marasmus
Anaemia	Otitis media
Bronchopneumonia	Scabies
Conjunctivitis	Ulcers
Intestinal worms	UTI

2.1.5.3 Literature accounts of illnesses treated with Pokot traditional medicine

In the context of local morbidities, Nyamwaya [89] described choice of health care specialist to attend, Pokot or *mzungu* (BM), to be dependent on causation (section 2.1.3) although these categories were not always reflected in practice in health-seeking behaviour. Integrating the qualitative data from the accounts of Nyamwaya [89], Timberlake [87] and Tanaka [107] of plants used for treating illness by *Chepsaktis*, the main ailments treated include malaria, fever, anaemia, GI disorders and intestinal worms, birth and pregnancy, genito-urinary infections

(GUIs), sterility, male virility, headache, coughs and colds, infections of the eye, ear and mouth, and skin wounds.

2.1.6 Changes in the current context of traditional medicine in West Pokot

The legislative context for the practice of TM and the use of biodiversity in Kenya, as described in the introductory chapter of this thesis, has the potential to markedly alter TM in West Pokot and elsewhere. Other significant and documented changes have occurred in West Pokot over the past decades which are also likely to affect TM practice in the present. These include changes in population, education, BM facilities, agro-economic activity and the environment. West Pokot County has experienced a four-fold increase in population since 1982 [86], with the consequent pressure on land and resources. In education, primary schools have almost trebled since 1996 and secondary schools have increased ten-fold in the same period [83]. Availability of BM facilities in West Pokot has increased from 17 in 1980 [89] to 156 in 2022 (including dispensaries, clinics and hospitals) [112] which undoubtedly has an impact on healthcare decision-making at community level.

Environmental changes described by Davies and Moore [80] found the current challenges of climate change, deforestation and soil erosion to be much worse than historical challenges. In addition, in a study describing land tenure systems around Chepareria, Nyberg *et al.* [113] points to the progressive enclosure and privatisation of land in West Pokot such that, since the 1980s, the area around Chepareria is now dominated by enclosures. This has potentially major consequences for the collection areas and habitats for growth of medicinal plants and a potential loss of biodiversity [114]. In a separate study, Bergmann *et al.* [115] described a progressive shift from mobile and subsistence-oriented cattle management to sedentary and market-oriented small stock production. This has been noted by other researchers, such as Ziegler and Lauderdale [114] in their research with Nandi healers to the south of West Pokot. These studies illustrate the complex interrelationship of loss of pasture, land degradation, bush encroachment, changes in biodiversity, traditional ecological knowledge and livelihood strategies. The complex of legal, social, cultural, economic and environmental changes in the present is likely to have profound impacts on the practice of TM and the use of medicinal plants in the study area.

2.1.7 The researcher as a pharmacist in the local mission hospital

I am a pharmacist and in 1992-1994 I worked in OMH. While working there, I became aware that there was a perception among the hospital staff that there

was a lot of usage of TM by the people attending the hospital. In addition, there was a generally negative perception of this by staff members. The hospital was working with TBAs at the time as part of the CBHC programme. It was proposed by OMH that I investigate the usage of medicinal plants within the community. This was to be followed up subsequently by other hospital staff to develop a working relationship with traditional practitioners in the area.

2.1.8 Ethical issues in this research

The negotiations on the CBD culminated in its signing in Nairobi in 1992 and ratification in July 1994 [116]. This research study thus predates the ratification and is technically exempt from its requirements in relation to TK and biodiversity. The study was conducted under the auspices of the CBHC programme of OMH and thus had permission under agreements with local authorities for healthcare-related activities within the community. Verbal informed consent was obtained from the participants although no physical record of this consent was obtained. This form of consent does not meet current academic standards for prior informed consent in social research, nor with current ethical requirements around TK and biodiversity. The consent process requires consent, not just from participants but also from local, regional and national administrative levels.

Due to the issues as described above, it is not possible to publish the material associated with this study on ethical grounds. We attempted to get permission to publish through the Kenyan authorities but a few problems arose. I attended a conference on the Nagoya Protocol of the CBD in Goettingen, Germany in 2012 where I met with members of National Commission for Science Technology and Innovation (NACOSTI), the national body for approval of scientific research in Kenya. Initial contacts suggested that retrospective approval for the research may be possible. However, when the time came to put that in motion, I was told that I would need to obtain full research approval and perhaps conduct the research again. The process was prohibitively costly and difficult and was abandoned. Advice was then sought from Professor Michael Heinrich, one of the world's leading experts in ethnobotanical and ethnopharmacological research at University College London. He advised that the Pokot voucher specimens, having been lodged in the East African Herbarium prior to ratification of the CBD, could be used as a secondary source of data and this would be a legal and ethical route for publication of the data in this study. In 2016, on meeting staff at the herbarium in Nairobi, it emerged that just one third (60) of voucher specimens that had been submitted could be found. As so much of the interlinked Pokot data would then be without the voucher specimens for independent verification, it was decided that the route of secondary data analysis could not be used. For these reasons, this part of the thesis cannot be published

as there is currently no ethical or legal basis for publishing as primary or secondary data.

2.1.9 The aims of the research

The aims of the study were to:

- document the traditional herbal medicine used in the Pokot community served by OMH around Chepareria and Ortum villages.
- document culturally significant emic (local) illness categories.
- examine their relationship to biomedical (etic) disease categories.
- compare Pokot medicinal usage of the most important species with wider ethnomedical use.
- compare known pharmacological studies on the most important species with regard to Pokot medicinal usage.
- valorise the skill and knowledge of the study participants.

2.2 Materials and Methods

2.2.1 Data gathering

2.2.1.1 Participant recruitment

An initial meeting was held in September 1992 at OMH to introduce potential participants to the research. This was done as part of the CBHC programme, which was training *Kama-echo* (TBAs) and Village Health Workers (VHWs). Many of these women were already known as *Chepsaktis* in the community. Following this introductory meeting, potential participants were contacted directly through the CBHC programme, while others were contacted indirectly through the initial contacts using snowball sampling.

2.2.1.2 Use of translation

Interviews were conducted in the Pokot language with the aid of local research assistants, as none spoke English. The first set of interviews was conducted with Rhoda Lodio assisting and translating Pokot directly into English; the second set of interviews from September 1993 to January 1994 was conducted by translating Pokot into Kiswahili which I then translated into English. Veronica Ling'areng, one participant *Chepsaktian*, later became a research assistant.

2.2.1.3 Interview structure

Interviews involved groups of *Chepsaktis* in focus group discussions (FGDs) and more detailed interviews conducted with one or two participants. Semi-structured interviews and open-ended questioning were the primary methods of

data gathering. The interview guides are available in Appendix I. Open-ended questioning was used when interviewing on cultural understandings of health and illness. Some complex illness categories also led to further open-ended discussions in order to get a deeper understanding of the illness being described. From September 1993 to January 1994, fifteen participants kept a diary of treatments with the help of their families. In these diaries, they recorded the patients' details, illnesses, presenting symptoms, treatments given and outcomes. These were then discussed at subsequent meetings.

2.2.1.4 Plant collecting walks and plant identification

At the end of each interview, voucher specimens of the plants were collected where possible. Where none was available, a note was made to collect at the following meeting. Specimens and photographs of most of the plants discussed were collected with the research assistant and *Chepsaktis*. Two collection expeditions were made to Parua and Marich for commonly used plant species that grow in more fertile areas (Figure 5). Herbarium voucher specimens were deposited with the East African Herbarium in Nairobi for identification and storage. There were several batches of specimens submitted over the course of the year. The botanists identifying the specimens were Dr. Geoffrey Mwachala, Dr. G. M. Mungai and Dr. F. C. Magogo.

2.2.2 Data analysis

Data analysis was commenced in the field, resulting in modifications of the interview guide in an iterative process in response to issues arising in interviews (Appendix I). This could help, for instance, to better understand illness terms or the particulars of plant usage. Analysis of therapeutic use data included emic illnesses and symptoms. Etic or outsider interpretation of the illnesses was conducted at the study site with a colleague at OMH, Mrs Miriam Lopus SRN, a Pokot nurse who translated the illness terms into English. After the study, Dr Deirdre O'Gorman, MD, who had worked at OMH at the time of the study, examined the illness descriptions (167) and made tentative interpretations from a biomedical perspective. These etic illnesses are classified according to the ICPC classification with some modifications as recommended by Staub *et al.* [117] for ethnobotanical studies.

The primary plant data was entered in an Excel spreadsheet with data on Pokot plant name, voucher number, botanical species, plant family, plant part used, preparation, route of administration, ailment treated and other comments such as side effects or cautions. Where different plant parts or different methods of preparation were used for the same plant, each of these was treated as a separate use report (UR). The interviews or discussions in a particular village

often had the same participants with a risk of double counting. Where a particular UR was already recorded in an earlier interview with overlap in participants, this was only counted as one UR even though the information may have been repeated many times. Ethnobotanical indices such as Use Value, Cultural Importance and Informant Consensus were not calculated as the number of participants was too small to provide meaningful information. The variance in the data would be too large, as described by Weckerle *et al.* [77] and Leonti [118], and thus the primary data showing the number of participants citing usage is presented here instead of ethnobotanical indices. Some of the arithmetic analysis and graph production is done using the Pivot function in Excel.

2.2.3 Literature search and analysis

Literature search on the Pokot data while in Kenya was difficult. I commenced by reference to Kokwaro [3], a valuable and extensive record of medicinal plants used in East Africa. In addition, I contacted NAPRALERT at the University of Chicago to obtain literature data relating to the identified species. I could not access their online facility at the time as there was no internet access in the study area, so correspondence was by post. NAPRALERT is a database of literature available on natural products, ethnomedicine, phytochemistry, pharmacology and biochemistry, and, at the time, they provided a limited *pro bono* service to institutions in developing countries. They sent printouts for selected species with summaries of ethnobotanical, phytochemical and pharmacological information collated from the literature about each species (Figure 8). A sample printout is available in Appendix II. This service facilitated the early analysis of comparative ethnobotany and pharmacological literature of some of the species identified in the study. At time of writing, NAPRALERT is unavailable for online searches.

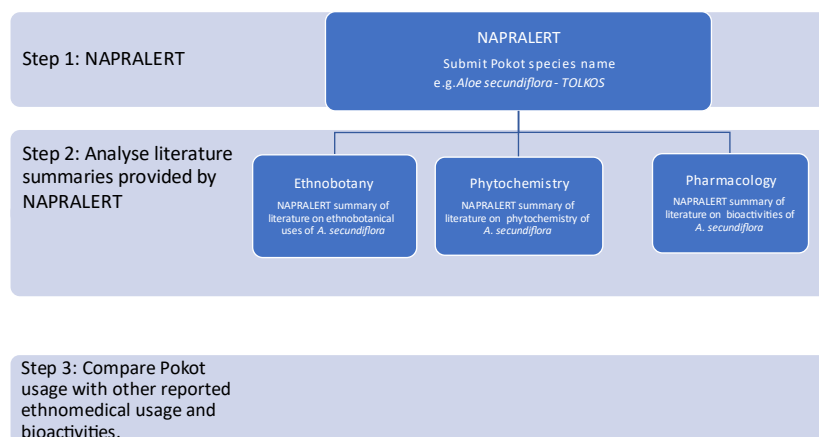


Figure 8: Process for utilising the NAPRALERT database for ethnobotanical, phytochemical and pharmacological information on Pokot medicinal species

A further literature search in Ireland relating to Pokot medicinal species accessed PubMed, Science Direct, Google Scholar, Scopus, library databases, medicinal plant databases, books, theses, grey literature including conference proceedings, workshop reports and manuals. The primary research included broad ethnobotanical studies, human ethnomedical studies, ethnoveterinary studies and ethno-dietary studies.

2.3 Results and Discussion

2.3.1 Participant profile

In this study, all of the participants were ethnically Pokot. The way in which the participants were recruited through the VHW and TBA training programmes of OMH (2.2.1.1) meant that all initial participants were women and further snowball sampling also recruited women. The participants had varying levels of experience and expertise. There were 29 participants in total, 16 of whom were *Chepsaktis* (traditional herbalists) and *kama-echo* (TBAs), 5 were *kama-echo* who had a limited number of illnesses they felt competent to treat and a further 8 whose role and experience is unknown. I will, in the main, use the term “participants” to cover the range of expertise found in the group as to use the term *Chepsaktis* or herbalist would be inaccurate. The main body of information was gathered from nine *Chepsaktis* who were met with on a regular basis while some of the 29 women were met with only once or twice. The ages of the participants were not recorded and was difficult to judge. All were mothers and most were grandmothers, although one of the grandmothers had a young baby of her own. The fact that most of the participants were neither literate nor did

they speak Swahili implies that they are unlikely to have attended school although this was not recorded.

2.3.2 Interviews and focus group discussions

This study was conducted over a 14-month period from November 1992 to January 1994, starting with preparatory meetings in November 1992. In total, 44 meetings were held in 8 villages in the study area from March 1993 to January 1994 (Figure 5). The meetings held with dates, participants and locations are listed in Appendix IV. The meetings included FGDs and more in-depth interviews with key informants listed in Table 3.

Table 3: Meetings held with participants in Pokot ethnomedical study

Meeting type	Number of meetings
Focus Group discussion (3 or more participants)	17
Two participants – in depth	17
One participant – in depth	10

Key informant interviews were held with Veronica Ling'areng, Lonah Chekee, Josephine Lousot, Kamaloripo, Rosemary Lokosoywan, Kochepekmoi, Elizabeth Pkukat, Priscillah Long'ura and Kophilip Siwanoi. The interviews took place at participant's homesteads. The initial focus of the interviews was a disease such as malaria, followed by its treatment and the plant species mentioned.

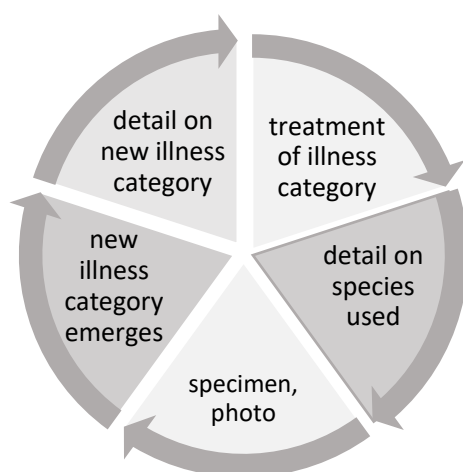


Figure 9: Interview cycle with emergence of new illness categories and new species to investigate over time

A subsequent plant-focused interview would generally mention a new or ill-defined illness term (that could be treated by a particular species) which was then explored separately. As time progressed, more illnesses and treatment options were explored in each interview (Figure 9).

2.3.3 Pokot Therapeutic Use Categories

To understand the Pokot treatment of illness, ideally a full exploration of the participants' understanding of the body and its functioning would be conducted to understand diagnosis and treatment decisions. However, this was beyond the scope of this research. The interviews used emic illness terms in the Pokot language and the most treated illnesses and Pokot terms are listed in Table 4. The full descriptions from which these are drawn are in Supplementary file 1 (Illness descriptions). A quantification of the data is presented in section 2.3.9 but the most treated illnesses are described here. As described above (section 2.2.2), in the field translations of Pokot illness terms into BM terms were made by a local nurse, Miriam Lopus although it became clear that it was not possible to make definitive or one-to-one correlations. Proposed correlations with BM illness terms are discussed below in section 2.3.7 following analysis of the descriptions post-fieldwork by Dr O'Gorman. While the illness terms were in the Pokot language, some English terms including "*malaria*" and "*typhoid*" were used interchangeably with *Kakatan* (febrile illness with pain, headache and vomiting) and *Chemwaror* (severe illness with fever, pain and GI symptoms) respectively, two of the more common illness categories. "*Gonorrhoea*", "*Gono*" and "*STD*" likewise were used interchangeably with *Pkata*. I use inverted commas as the illnesses might not have corresponded to BM terms and can, thus, also be considered emic terms. Sample descriptions of *Kakatan* and *Chemwaror* are given here:

Kakatan: Person has headache, fever, sweating, yellow vomit; malaria and typhoid together can have breathing problems and ribs pull together; diarrhoea is watery and mucussy, some swelling in the feet, anaemia, jaundice and is weak (Kakatan: Elizabeth Julias, Kapromtim)

Chemwaror: Person has black tongue, black diarrhoea. It can be fatal at this stage. Starts with abdominal pains, headache, weakness, painful joints, occasionally fever (not always), abdominal pulse is quick (in umbilical/sursum region). It can cause abortion. Some believe that it can come from cow's milk. Illness may last for up to one year (Chemwaror: Veronica Ling'areng, Sebit)

Table 4: Composite of symptoms of Pokot illness categories described by multiple participants

Pokot term (no. of descriptions recorded)	Composite Description of Symptoms by Multiple Chepsaktis (Figure in brackets = number of times this sign/symptom was recorded in descriptions of this illness)	Summary description (no. of URs)	Proposed most likely BM correlations
Kakatan, Kaghatan (6)	Fever (5), fast pulse (3), sweating (3), headache (1), cough/breathing (3), vomiting (3), diarrhoea (3), anaemia (2), swelling in feet (2), pains (1), jaundice (2), weak (2).	Fever, headache with vomiting (52)	Malaria (mostly)
“Malaria” (7)	Fever (6), headache (7), vomiting (2), diarrhoea (1), oral symptoms (3), fast pulse (3), pains (2), weak (1), cough/breathing problems (2), sweating (3), fainting/dizziness/convulsions (cited as “malaria may occur with”).		
Kuprirr (1) Type I: infant Type II: 6–24mo	Type I: Neonate – red spots all over body, swollen, cries, wastes, urine and skin yellow. Can’t die from it. Type II: 6–24-month-old: also called seme wa nyariluh (yellow illness). A serious illness affecting older babies. Symptoms and signs are of a highly contagious and potentially (though not always) rapidly developing illness with jaundice, fever, vomiting, hepato-splenomegaly, anaemia, body	Illness with red body and jaundice (4)	Type I – neonatal jaundice Type II – jaundice due to viral hepatitis

Pokot term (no. of descriptions recorded)	Composite Description of Symptoms by Multiple Chepsaktis (Figure in brackets = number of times this sign/symptom was recorded in descriptions of this illness)	Summary description (no. of URs)	Proposed most likely BM correlations
	pains and swellings. Also described as being the same as Cheptukon.		
Ptokuot (4)	Black tongue (3), weak (3), umbilical pain (3), fever (3), headache (3), breath smells bad (2), fast pulse (2), nausea (2), vomiting (2), diarrhoea (1), fast breathing (1).	GI with fever and black tongue (5)	Unclear – malaria, typhoid, gastroenteritis
Chemwaror/ “typhoid” (17)	Abdominal pain (9), diarrhoea (9; 6 black or with blood), vomiting (5), swollen abdomen (2), breath smells bad (2), weakness (7), raised pulse (12), fever (8), body pains (6), headache (7), may be prolonged (4), black tongue (7), cough/respiratory problems (3), chest pain (2), threatened abortion (3), may have anaemia in long term (1), may have Tionypomut (dizziness/fainting) (2), oedema (1), may be fatal (2).	Fever, headache, GI symptoms (88)	Malaria (mostly)
Chepkolomut (11)	Child (commonly but not always) (11), fever (6), body sores (5), mouth sores (8), mouth/tongue white (4), GI symptoms (7) (includes: diarrhoea (4), constipation (2), stomach sounds (3)), loss of appetite/grows thin/not thriving (5), cries a lot (3), chills (3), can be fatal (1, cited twice by same person).	Oral thrush with fever, gastro-intestinal (GI) and other symptoms (86)	Malaria with oral thrush (mostly)

Pokot term (no. of descriptions recorded)	Composite Description of Symptoms by Multiple Chepsaktis (Figure in brackets = number of times this sign/symptom was recorded in descriptions of this illness)	Summary description (no. of URs)	Proposed most likely BM correlations
Cheposkut (6)	Fever (4), white tongue (4), mouth sores (1- in addition to descriptions with white mouth/tongue), GI symptoms (5) includes: diarrhoea (2), abdominal pain (4), vomiting (1), loss of appetite (2), headache (1).		
Kapusan (3)	Fever (3), white tongue (3), GI symptoms (3) includes: diarrhoea (3), vomiting (2), weak (1), fast pulse (1), headache (1).		
Cheptukon (14)	One participant said there are two types of Cheptukon – one acute and makes you faint, the other not acute and gives headache. May occurs with Pkata, with “malaria”, can become Yomut. Faint (6), dizzy (8), weak (2), fever (5), oedema (5), twitch (2), convulsions/meningitis (2), shaking (1), anaemia (3), jaundice (3), psychiatric (5 – includes insane, talk a lot as if mad, becomes stupid), urinary symptoms (3) (includes: urine frothy (3 – 2 with oedema), urine yellow (2), urine strong tea colour (1), urine white (1)), long-term (months or more)(8), recurs (3) (included in long term ones where specified as recurring), fast pulse (4), headache (1), pains (2), eye symptoms	Convulsions with fever (22)	Convulsions – many potential causes in described cases – malaria, epilepsy, pre-eclampsia, kwashiorkor

Pokot term (no. of descriptions recorded)	Composite Description of Symptoms by Multiple Chepsaktis (Figure in brackets = number of times this sign/symptom was recorded in descriptions of this illness)	Summary description (no. of URs)	Proposed most likely BM correlations
	(3) - includes red eyes (1), blue under eyes (1), painful eyes with pus (1).		
Tionypomut (3)	Dizziness (3), eye pain (3), with anaemia (2), with Chemwaror (2), with malaria (1).	Dizziness/fainting/mild seizures/headache (72)	Perhaps faintness of anaemia
Ptangtang (2)	Dizziness with severe headache and sometimes fainting.		
Yomut (8)	One participant said two types of Yomut – one where you can walk, the other you can't walk Can't move (4), body pains (4), chronic/progressive (6), association with rain (3), psychiatric symptoms (3), weakness (4), loss of appetite/gets thin (4), comes and goes (2), one sided (1), one or both sides of the body (1), body sores (3), twitching (2), lose muscles quickly.	Serious illness with progressive paralysis (10)	Paralysis perhaps as a consequence of convulsions, polio, post-cerebral malaria
Termes (3)	Two types - acute with malaria or chronic (2), acute with malaria or fever (6), long-term where patient thin (2), spleen enlarges (2), stomach	Splenomegaly (13)	Splenomegaly caused by VL or malaria

Pokot term (no. of descriptions recorded)	Composite Description of Symptoms by Multiple Chepsaktis (Figure in brackets = number of times this sign/symptom was recorded in descriptions of this illness)	Summary description (no. of URs)	Proposed most likely BM correlations
	enlarges (2), spleen not as large as with acute form (2).		
Ng'asat	Swollen liver.	Hepatomegaly (7)	Hepatomegaly with anaemia caused by VL
Pkata (11)	Dysuria (7), abdominal pain (6), vaginal discharge (6), back pain (4), haematuria (3), walking difficult (1), infertility (1), abortion (1), urine yellow (1), low urine volume (1), urine frothy (2), body itch, fever (1).	Painful urination with or without discharge (33)	GUI
Korupoi 3 types Type (I) (6) Type (II) (2) Type (III) (1)	Type (I): neonate (5) red body (6), follows bleed in pregnancy (2), cries a lot (3), body swollen (1), GI symptoms (1) - includes: stomach pain (1), diarrhoea (1), not feeding (1). Type (II) swelling (3), fever (2), skin cracks (2), pimples (2), may be fatal (2), jaundice (3), loss of appetite (2), fast pulse (2), urinary symptoms (2), was mostly children (2). Type (III) swelling of lower limbs only and treated topically.	Some are swelling that can kill such as cancer (13)	Cancer such as Burkitt's lymphoma

Oral symptoms such as oral thrush in *Cheposkut/Chepkolomut/Kapusan* were also very commonly treated. Dizziness and fainting such as in *Tionypomut/Ptangtang* and convulsions in *Cheptukon* were also a significant feature of prevalent illnesses. Splenomegaly/*Termes* was very well understood as a symptom of illness requiring treatment. There were some illnesses that were said to have a few types as seen in Table 4 including: *Kuprirr* (illness with red body and jaundice), *Yomut* (illness with paralysis), *Cheptukon* (illness with fainting/convulsions) and *Korupoi* (illness with skin rash +/- oedema, swelling) and this was a potential source of confusion in interpreting the results and is a limitation in the study.

2.3.4 Diagnosis of illness

The recognition of signs and symptoms is the first step in treatment of illness. The data shows the range of signs and symptoms reported by participants and illustrates a deep familiarity with normal and pathological states of the body (Table 4). Some of the most common signs and symptoms recorded include: the appearance and smell of the mouth, the characteristics of stool, urine and vaginal discharge, fever, pain, pulse, blood flow, skin colour and feel, swelling, respiratory symptoms, appearance of the eyes, weakness, dizziness, fainting, convulsions and head pain. Pulse diagnosis was particularly prevalent in *Chemwaror/ "typhoid"* (severe illness with fever, pain and GI symptoms), especially the abdominal pulse, feeling the strength and direction of blood flow around the umbilicus. Severe thumping of this pulse was seen as a sign of serious illness. In diagnosing *Tionypomut* or *Ptangtang* (illnesses involving dizziness and/or fainting) in a baby, the feel of the pulse of the abdomen was used. Some plants aided diagnosis such as *Chepteya* (*Cycnium tubulosum* (L.f.) Engl. ssp. *montanum* (N.E.Br.) O.J. Hansen) which was used in the diagnosis of *Ptokuot* (GI illness with fever and black tongue). If the tongue turned black after taking *Chepteya* leaf (*Cycnium tubulosum* (L.f.) Engl. ssp. *montanum* (N.E.Br.) O.J. Hansen), then this was a positive diagnosis of *Ptokuot*.

2.3.5 Causation

Illness causation was not investigated in this research. However, some participants said that "*malaria*" was caused by the mosquito, by eating too much sugar or by drinking too much fresh, unfermented milk. *Chemwaror / "typhoid"* (severe GI illness with fever and black tongue) was thought by one *Chepsaktian* to be caused by drinking too much alcohol while another thought that it could be caused by bathing in the river. *Termes* (enlarged spleen) was thought by some to be caused by drinking fresh unfermented milk but also to be a symptom of other illness such as "*malaria*". *Yomut* (a progressive paralysis) was thought by a few *Chepsaktis* to be caused by rain. *Ptokuot* was thought to be caused by

eating sheep's meat. *Korupoi* I (red, swollen body in neonate) was thought to be caused by a vaginal bleed in pregnancy.

2.3.6 Treatment approaches

In the treatment of *Yomut*, a rare and serious illness with paralysis, a ritual element was reported by one *Chepsaktian* who used ash and "colours" from the soil and goat's hide bracelets to treat a patient. These were blown onto the skin and the patient subsequently recovered. In a separate treatment for *Chemoyo* (like *Yomut* – illness with paralysis), *Kophilip* applied ash for three days:

*.....after 3 days gave medicine to wash the whole body.
Likwon (Maerua crassifolia Forssk.) roots and leaves for
washing and given (orally). Also slaughtered a goat and
tied bracelets of goat's hide at joints. Recovered after 1
week completely (Kophilip, Sebit 24/11/1993).*

Vomiting or therapeutic emesis was used as a treatment for a wide range of illnesses, to "finish off an illness" after other treatments including treatments at the hospital. This was thought to get rid of the disease from the body. One *Chepsaktian* said that it was not the plant(s) that caused the vomiting but rather the disease leaving the body that caused the vomiting. Several commonly encountered illnesses were treated with emesis including *Dizziness/Ptangtang*, *Pkata (GUI)*, *Kakatan/ "Malaria"*, *Cheptukon* (illness with convulsions/fainting), *Chemwaror / "typhoid"*, *Tionypomut* (illness with dizziness) and *Cheposkut* (illness with oral thrush/oral infection). The treatment usually involved a decoction of several plants – up to 20 – boiled for an hour and then:

*.....slaughter a goat, boil and the soup mixed with the
medicine. The mixture is drunk and the patient vomits
black. The meat is roasted and given to the patient (Lonah
Chekee, Kasongwor, 13/11/93)*

Vomiting was more important as a treatment in this study than purgation but, in a few cases, purgatives were used to rid the body of the disease. *Ketaiwo (Cissus ruspolii Gilg.)* root was boiled and given orally "to diarrhoea the dirt of *Chepkolomut*" (Rosemary, Kapromtim 5/10/1993). Nyamwaya [89] discussed the use of emesis and purgation with the Pokot understanding that most diseases resided in the gut.

Emesis and purgation as a treatment is seen in other Kenyan studies, among the Pokot and Marakwet [87, 119] and among the Samburu and Maasai [120, 121]. Kokwaro [122] listed dozens of species that were used for purgation and emesis in East Africa. More broadly speaking, emetics and purgatives were

used in European medicine in the nineteenth century “to clear ‘impurities’ from the body” [123] and therapeutic emesis is used in Ayurvedic medicine as a treatment and prevention of disease [124].

2.3.7 Biomedical interpretations of Pokot therapeutic use categories

An analysis of the 167 combined illness descriptions from an etic perspective are summarised in Table 4. It was considered that most cases of *Kakatan* and “*malaria*” were probably BM malaria given the disease prevalence although some could have been hepatitis. *Chemwaror/* “typhoid” was most likely to be malaria with some being typhoid (typhoid was rare in OMH at the time), pneumonia or anaemia. *Chepkolomut*, *Cheposkut* and *Kapusan* frequently reported fever so most cases were likely to be malaria with oral thrush. Those with extensive mouth sores could have been *Herpes simplex*. *Termes*, (splenomegaly) can occur with malaria as described by the *Chepsaktis*. Severe splenomegaly was most likely to have been VL. VL also leaves the person more vulnerable to other infections such as malaria. Some other potential causes of splenomegaly in the study area included typhoid, HIV/AIDS, TB and brucellosis [125]. *Ng’asat*/hepatomegaly and anaemia frequently occur with splenomegaly in VL [125] with one description given corresponding with the clinical picture of VL with anaemia:

.....*Termes and Ng’asat with breathing difficult and noisy,
fast weak pulse, body weak, feet swollen, coughing a lot,
high fever, anaemia, no jaundice (Dorcas, Kapromtim,
16/11/1993)*

Some other potential causes of hepatomegaly in the study area included malaria, TB, hepatitis and amoebiasis [125]. The descriptions of *Cheptukon* were very varied and in the absence of more detail, could not be definitively diagnosed but was considered to involve convulsions. There were several potential causes of convulsions in the study area including fever, malaria (and sequelae), meningitis (and sequelae), VL, tetanus, pre-eclampsia, kwashiorkor and epilepsy. The paralysis of *Yomut* could indicate polio or be a consequence of convulsions. *Kuprirr* type I could be neonatal jaundice, mild cases clearing up in a few weeks, and type II was likely to be jaundice caused by viral hepatitis. It was noted that TB and pneumonia, which were relatively common, did not feature much in the illness descriptions. Some descriptions of *Kakatan/* “*malaria*”, *Lodومتim/* “tonsillitis” and of *Chemwaror/* “*typhoid*” with breathing difficulties could correspond with pneumonia. It is also possible that cases of TB and pneumonia were recognised and referred to hospital for treatment as described in one case

of pneumonia by Lonah Chekee and by Nyamwaya [89]. *Korupoi*, which was said by the *Chepsaktis* to be rare, could indicate cancer such as Burkitt's lymphoma which occurs in malaria-endemic areas in Africa [125]. The main risk factors for endemic Burkitt's Lymphoma are considered to be intense, repeated infection with *P. falciparum* and with Epstein-Barr virus with potential mechanisms through dysregulation of the immune response and alteration of the lymphoma phenotype [126].

The difficulties involved in the translation of local illness categories to BM diseases have been highlighted by several researchers and are illustrated in this study. Supplementary file 1 illustrates that some descriptions were contradictory such as in *Tionypomut* which may or may not be associated with fever depending on the participant. Some difficulties which arose in this study are recognised phenomena in ethnomedical research such as the lack of clinical expertise on the part of the researcher [127, 128], researcher limitations around language and cultural translation [129]. There is a recognised difficulty in BM diagnosis in the field in the absence of laboratory facilities such as of anaemia and in the recognition of key symptoms such as fever and respiratory symptoms in the absence of technology [125, 130, 131]. In addition, there may be a lack of correspondence between emic and etic categories [91, 127] such as in this study where one emic category may explicitly have several forms [132].

2.3.8 Treatment diaries

The treatment diaries resulted in 85 reports. The data from the treatment diaries are presented in Supplementary file 2 (Treatment Diaries). The data is qualitative as there are insufficient reports and participants for statistical analysis [118]. It includes information on signs and symptoms, diagnosis, choice of treatments, previous treatments, outcomes and follow-up if relevant. The patients were children and adults with two thirds of children being under 2 years. Ailments treated in the main were mouth-based infections – *Cheposkut*, *Chepkolomut*, *Kapusan* – along with “Malaria”/*Kakatan*, *Chemwarorl* “Typhoid” and *Pkata*. The signs and symptoms reported reflect the main body of the study (section 2.3.3).

In one report, a two-year-old child had “*bad Chepkolomut*” with the symptoms:

.....fever, coughing with sputum, raised pulse, bad
Chepkolomut. Treated with *Kamil* (*Combretum molle* G.
Don.) leaf and *Tolcos* (*Aloe secundiflora* Engl.) root (orally)
 and tisan (ash) for *Chepkolomut* (applied to the mouth).
 The child was improving after 5 hours and was sent home
 with medicine (Lonah, Kasongwor 26th September, 1993)

Here, the illness and the oral symptom are both called *Chepkolomut* and the treatment was both systemic and topical. In another instance, *Cheposkut* (illness with oral thrush/infection) was reported as a symptom of *malaria*. A one-year-old child was diagnosed with “*malaria*” having:

.....pains in body, headache, sweating, not much fever and
Cheposkut. *Sulan* (*Momordica boivinii* Baill.) root was
crushed in cold water for *Cheposkut* with *malaria*, *Songowo*
(*Zanthoxylum chalybeum* Engl.) bark in cold water, *Koloswo*
(*Terminalia brownii* Fresen.) bark was just boiled and all
were given orally. *Cheripar* (*Pavetta subcana* var. *longiflora*
(Vatke) Bridson) root ground for back pain, mixed with
water and put into nostrils and swallowed. This is very
strong, sharp, makes you sneeze. Child recovered
completely (Lonah, Kasongwor 26th September 1993)

In this instance, the ingredient used to treat *Cheposkut* as a symptom of “*malaria*” was *Sulan* (*Momordica boivinii* Baill.) root. The “*malaria*” itself was treated with *Sulan* (*Momordica boivinii* Baill.), *Songowo* (*Zanthoxylum chalybeum* Engl.) and *Koloswo* (*Terminalia brownii* Fresen.). *Cheripar* (*Pavetta subcana* var. *longiflora* (Vatke) Bridson) was used for pain specifically. This illustrates the breaking down of treatment into symptomatic treatment as well as treating the overall diagnosis of *malaria*. Pulse characteristics were important in diagnosis as described previously with the feeling of pulses at various points around the body – in the abdomen, chest, temples and the top of the leg – though with the abdominal pulse there was more detail given. When the umbilical pulse was very strong in *Chemwaror* (severe illness with fever, pain and GI symptoms), then lower doses of treatment were given because high doses could be dangerous (Kamaloripo 18th November 1993). The direction of flow at the umbilicus was significant in diagnosis: flow down the left-hand side or the right-hand side or circling the umbilicus. The pulse at the temple was used to diagnose *Ptangtang* (severe headache, may also have dizziness) in infants. In one instance, the pulse and *Tolcos* (*Aloe secundiflora* Engl.) leaves were used to aid diagnosis. The baby had symptoms of:

.....headache, high fever, no sweating, fast strong pulse,
breathing fast, ribs pulled in on breathing, no anaemia.

And the treatment process followed was:

.....started with *Tolcos* (*Aloe secundiflora* Engl.) leaf in cold
water, 2 teaspoons, recovered. Sweated when gave *dawa*
(medicine). Give *Tolcos* (*Aloe secundiflora* Engl.) to see if

pulse goes down. Then know that it's typhoid and malaria.

Then gave Kurion (Vepris nobilis (Delile) Mziray) (for typhoid and malaria) when pulse became normal. Root boiled. Child still had fever and pains in the body but mostly recovered. Korkidowo (Capparis tomentosa Lam.) given for fever. Then recovered after one week (Elizabeth Julias, Kapromtim, 28th August 1993).

Thus, a baby with a fast, strong pulse was given Tolcos (*Aloe secundiflora* Engl.). When the pulse became normal after being given Tolcos (*Aloe secundiflora* Engl.), the diagnosis of “*malaria*” and “*typhoid*” together in this child was confirmed and the choice of treatment followed on from this. Swelling of different parts of the body was also a sign frequently recorded. Swelling of the whole body or of a limb, the abdomen, of different sides of the body, swelling of the back, swelling of *Termes* (spleen) or *Ng'asat* (liver), swelling (or depression) of the fontanel in infants were all recognised as significant signs of illness and aided diagnosis. Psychological or psychiatric signs and symptoms were rarely mentioned, only arising in cases of the more serious illnesses of *Cheptukon* (illness with convulsions/fainting) and *Yomut* (illness with progressive paralysis). This could be because people with primarily psychological symptoms attend different types of healers.

Treatments were mostly administered orally. There were a few treatments involving external application of a wash or a paste to treat a body rash. Therapeutic vomiting was used to treat patients of all ages. Referral to the dispensary or to hospital was reported in several cases, especially in babies. Kamaloripo and her colleagues (18th November 1993) stated that the illnesses that would always be referred to hospital were meningitis, bad malaria, *Takat* (lung - respiratory illness) and where symptoms were very bad such as severe anaemia (seen in eyes, hands and mouth or having swollen feet). In several cases, patients came to the participants having already been to the hospital or dispensary. This happened for various reasons: not feeling better after hospital treatment; main complaint cured at hospital but still some symptoms remaining; medications making them feel ill and looking to the *Chepsaktian* for an alternative or a better cure. At the time of the last interview with Kophilip, a woman who had been sick for a long time in OMH came to her for treatment. The woman's “blood was going very fast and she was weak and had to lie down”. Kophilip diagnosed her with *Chemwaror* (severe illness with fever, pain and GI symptoms) and *Yomut* (illness with progressive paralysis). Her treatment plan was as follows:

.....first gave dawa (medicine) for Chemwaror and then for Yomut. Ketaitou (Cissus ruspolii Gilg.) root, Tolcos (Aloe

secundiflora Engl.) leaf and Skakaght (Aloe sp.) roots for Chemwaror. Along with medication, tied (goat) skins at joints for Yomut. Then will slaughter a chicken and drink with dawa (medicine) to finish treatment for Yomut. After that, will look again to see if Chemwaror still there.

(Kophilip January 1994)

This example illustrates some of the choices being made by patients and *Chepsaktis* in the search for healing. Hospital treatment had failed and Kophilip treated her patient with physical and ritual elements to try to rid the body of a difficult to treat illness. In a case of “*malaria*” being treated by Lonah, the patient presented with:

.....fast breathing, bad headache, very fast pulse, very bad smell in mouth, sweating, no vomiting, can't go to the toilet, yellow green stools, weak

Lonah diagnosed “*malaria*” with *Chepkolomut* changing to *Ptokuot* (abdominal illness with fever and black tongue). The treatment process she followed was:

.....Siriowo (Searsia natalensis (Bernh. ex C.Krauss) F.A. Barkley) leaves in the morning and improved a bit but breath smelled worse and still had headache and high temperature. Gave Chloroquine and got better. Sent home with Siriowo (Searsia natalensis (Bernh. ex C.Krauss) F.A. Barkley) leaves dried and ground for Ptokuot (Lonah, Kasongwor, 30th September 1993).

This illustrates the use of Chloroquine, available in local shops, as a treatment option for *Chepsaktis* and participants such that they were not limiting their *materia medica* to plants alone as described by Nyamwaya [89]. The treatment diaries proved very useful in that they presented a clearer picture of the treatment process and patient choices of different treatment options.

2.3.9 Quantification of Pokot Illnesses treated using ICPC classification

In order to analyse Pokot illnesses in an etic manner, the likely biomedical interpretations (Table 4) are categorised using the ICPC classification recommended by Staub *et al.* [117] and then quantified using URs. The total number of URs recorded overall is 740 (Supplementary file 3). The key emic Pokot illnesses treated are represented in Figure 10 showing *Chemwaror* / “typhoid” to be the most commonly treated illness (88 URs) followed by

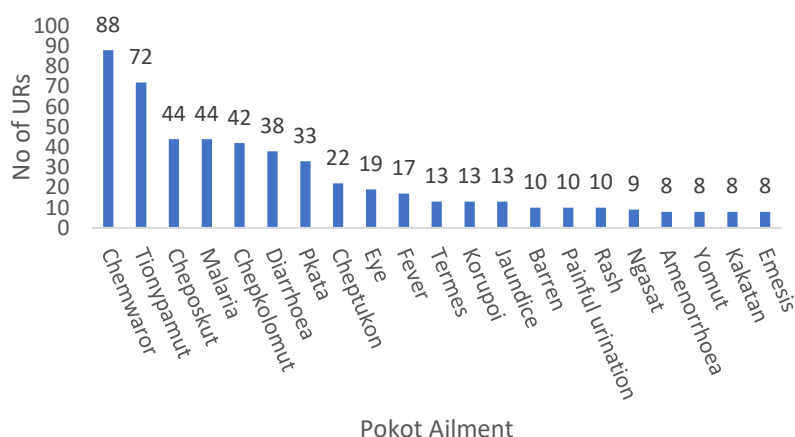


Figure 10: The most commonly treated Pokot illnesses

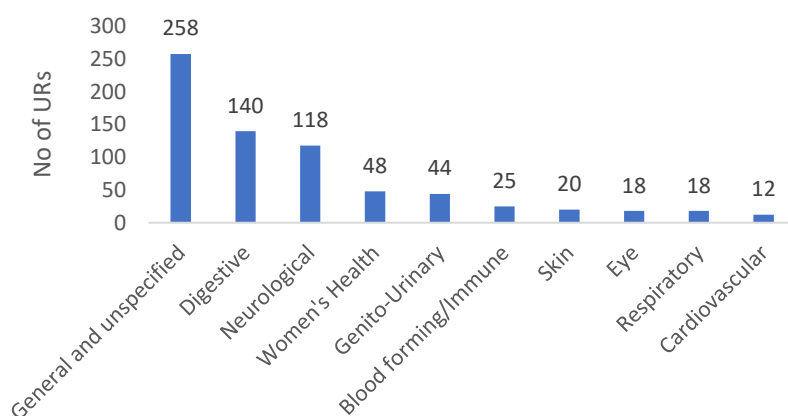


Figure 11: Top 10 ICPC Body Systems treated by Pokot Chepsaktis

Tionypomut (72 URs) while *Kakatan* / “Malaria” has 52 URs (combining the figures for *Kakatan* and “*malaria*”). Using ICPC classification, the top 10 body systems treated are presented in Figure 11 showing that the “General and Unspecified” system accounts for 258 URs (35% of total). The breakdown of this category (Table 5) indicates that 80% of these (205 URs) were presumed malaria (i.e., combination of “*malaria*”, *Kakatan*, *Chemwaror*, “*typhoid*” and the *Cheposkut*/*Chepkolomut*/oral thrush group selected for those with febrile and headache symptoms) and combined with fever, accounts for 31% of all URs. By comparison, OMH morbidity data shows almost half of all causes of morbidity as malaria (Table 1). Digestive disorders account for 130 URs (18% of total) with the most common illnesses being diarrhoea (39 URs, 5%) and oral

Table 5: Top 10 ICPC Body Systems treated by Pokot Chepsaktis with key Ailments treated (UR >2) (=89% of all URs)

Body Systems	Ailment Categories	URs
General and unspecified	Malaria	205
	Fever	24
	Malignancy	13
	Pain general	3
Digestive	Diarrhoea	39
	Mouth/tongue/lip disease	28
	Jaundice	17
	Abdominal pain/cramps general	11
	Gastroenteritis presumed infection	10
	Hepatomegaly	9
	Vomiting	8
	Constipation	4
	Teeth/gum symptom/complaint	4
Neurological	Dizziness/Fainting	74
	Convulsions/seizures	27
	Paralysis/weakness	10
	Headache	7
Genito-Urinary	GUI	44
Women's Health	Infertility/subfertility	10
	Menstruation absent/scanty	8
	Antepartum bleeding	7
	Abortion spontaneous	4
	Menstrual pain	3
	Sex of child in womb	3
	Antepartum pain	3
Blood forming/Immune	Splenomegaly	21
	Anaemia	4
Skin	Skin injury	9
	Rash	9
Respiratory	Cough	9
	Throat symptom/complaint	5
Eye	Eye symptom/complaint other	9
	Red eye	4
Cardiovascular	Oedema	7
	Pulse	4

disorders (mainly oral thrush without fever or headache) (28 URs, 4%). By comparison, OMH morbidity data shows gastroenteritis combined with dysentery

and amoebiasis accounted for 10% of morbidity in 1996. Neurological disorders accounted for 118 URs (16% of total URs), the majority being dizziness/ fainting (74 URs, 10%) and convulsions (27 URs, 4%). These could relate to anaemia, malaria and meningitis in OMH morbidity data (section 2.1.5.2). The category of Genito-urinary disorders is composed entirely of GUI and accounted for 44 URs (6% of total). Prevalence of GUI was low in OMH statistics although UTIs were seen in MU clinics and gonorrhoea reported in earlier regional hospital data (section 2.1.5.2). It is also possible that many attended the KDH rather than OMH for GUI. Women's health included pregnancy related issues and menstrual problems, accounting for 38 URs (5% of total), the majority representing fertility problems. Local morbidities associated with fertility issues were malaria and associated anaemia, prevalence of STIs and Female Genital Cutting (FGC). FGC is known to cause menstrual and urinary problems, vaginal discharge, habitual abortion and infertility [133], all of which are represented here. The Blood forming/immune system group (25 URs, 3% of total) is primarily splenomegaly (21 URs). Splenomegaly was not listed in OMH morbidity statistics but in mild form, is common in malaria [125] and more enlarged spleens are found in VL (section 2.1.5.2). Anaemia as an illness category is low in the Pokot data relative to the frequency in local morbidity statistics (11% in OMH 1991, section 2.1.5.2). It was often a symptom of malaria, poor nutrition, pregnancy and VL in the study area [125].

The relatively low figures for treatment of respiratory symptoms by participants contrasted with OMH and MU statistics. These indicated a high level of respiratory disorder when figures for URTI, pneumonia, TB and bronchitis are combined ranging from 13-22% of morbidity (section 2.1.5.2). The low level of spontaneous reporting of respiratory symptoms could be due in part to referral of more severely ill patients with respiratory symptoms to the hospital or clinic as described above (section 2.3.8). However, more recent research in Uganda and Nigeria has found low levels of recognition of respiratory symptoms by caregivers which could also have been a factor in this study [134, 135]. The Cardiovascular group accounts for 11 URs (1.5% of total) and comprises oedema and pulse rate. Oedema was included in descriptions of severe forms of Cheptukon, Korupoi, Kupirrr and Chemwaror. The oedema described was widespread oedema or oedema below the knees. There were many potential causes in the area including Kwashiorkor (severe malnutrition), TB, HIV/AIDS, cancer and pre-eclampsia in pregnancy.

2.3.10 Plant details

In total, 180 identifications were made accounting for 144 species, with most identified to species level (listed in Supplementary file 3). This section identifies the plant habit, parts used, methods of preparation and routes of administration.

2.3.10.1 Plant habit

The plant habits of species identified as being used in Pokot medicine are shown in Figure 12. Woody categories (shrubs plus trees) accounted for 61% of the total. Creepers and herbs accounted for a substantial proportion of 21 and 15% respectively (Supplementary file 3). This may relate to the area being semi-arid with more consistent availability of woody species and plant parts

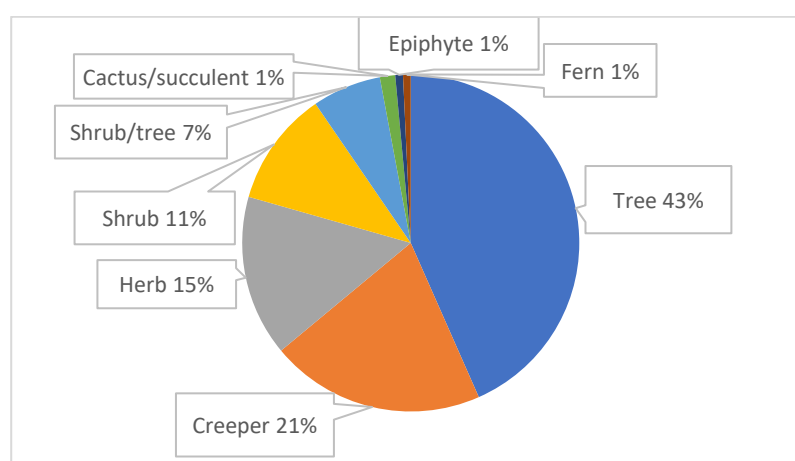


Figure 12: Plant habit of species used in Pokot medicine

2.3.10.2 Plant part

Root and bark were the most used plant parts accounting for 54% and 23%, respectively while the leaf was used in 12% of preparations (Figure 13). Roots were the predominant plant part used across all plant habits though with herbs, leaf and stem were often used (25% and 15% respectively) (Figure 14) while among trees, the perennial plant parts - root and bark – account for most URs (85%). Trees and shrubs are the predominant medicinal plants used in this study and the predominance of the use of root and bark is coherent with Kokwaro's study of East African medicinal plant use [122]. Studies of other arid and semi-arid regions in Kenya likewise reported on the predominance of bark and root in

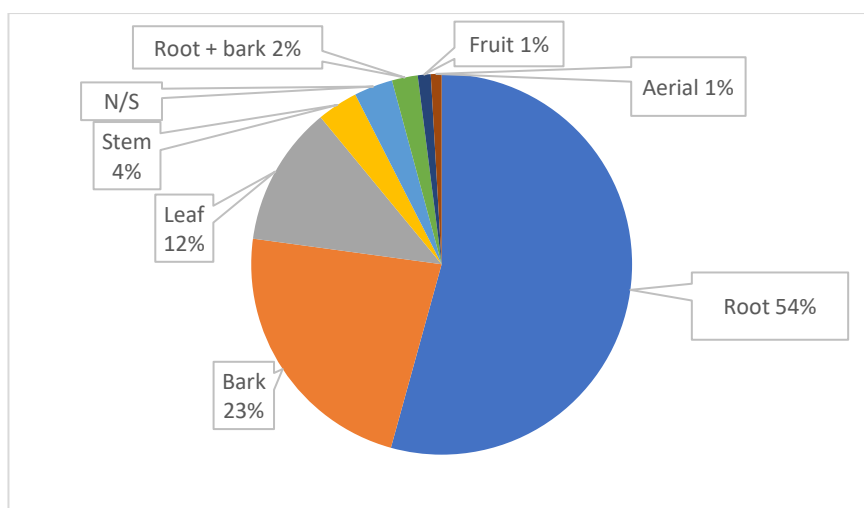


Figure 13: Key plant parts used in Pokot medicine

treatments [87, 119, 120, 136]. A further discussion of choice of plant part in relation to culture and efficacy is found in Chapter 3.

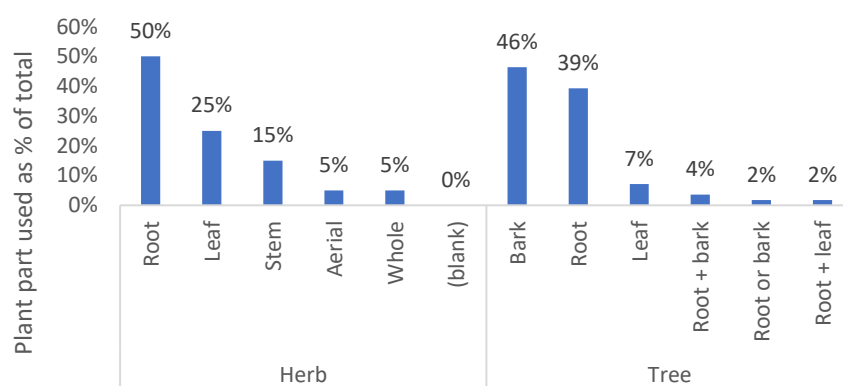


Figure 14: Comparison of plant part used as % of total for herbs and trees used in Pokot medicine

2.3.10.3 Plant preparation

Decoction was the most common method of preparation (69% URs) (Figure 15). This varied in that some plants were boiled for a long period (such as *Kembirwo-Acalypha volkensii* Pax.) as the root is very hard. In some cases, short boiling time was recommended in order to reduce the extent of bitterness in the extract. Some plants were extracted by infusion (2.2% UR's) such as *Kapkopf (Cyphostemma bambuseti)* (Gilg & M. Brandt) Desc. ex Wild & R.B. Drumm) with a strict instruction not to boil. *Chepomoi (Cyphostemma heterotrichum)* (Gilg & R.E.Fr.) Desc. ex Wild & R.B. Drumm.) was boiled twice in order to reduce

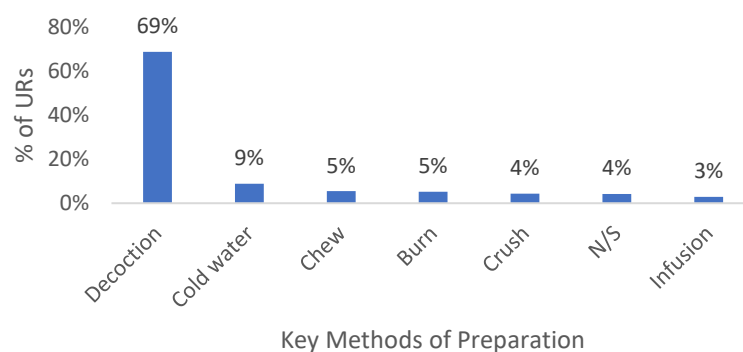


Figure 15: Key methods of preparation of Pokot plant medicines

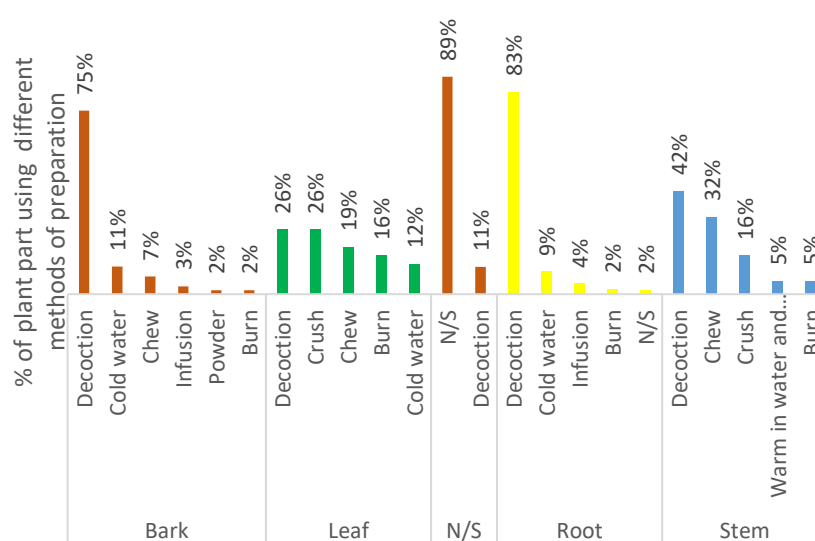


Figure 16: Key methods of preparation for each plant part used in Pokot medicine

bitterness and itching. Burning a plant or plant part to ash (5% UR's) was used in some treatments of oral infection. Squeezing or chewing plant parts such as leaf, fruit, stem or bark accounted for 9.5% URs. Treatments of eye complaints usually required the application of leaf juice directly into the eye (e.g., Solion - *Tetradenia riparia* (Hochst) Codd). The difference in preparation of different plant parts is illustrated in Figure 16. Bark and root were predominantly prepared by decoction (75% and 83% respectively) whereas leaf preparation was more evenly divided across the range of decoction, crushing, chewing, burning and mixing with cold water. For the stem, decoction was used most (42% URs) with chewing and crushing employed in 32% and 16% of URs respectively. Decoction as the preferred method of preparation was found in several regional studies such as Jeruto *et al.* [137] and Ssegawa, Kasenene [138] and was also reported in Kokwaro [122] for the wider East African region. There may have been a cultural preference for decoction over other methods given that it was a regional

norm. Decoction may also help in extracting more of the active ingredients as with hard roots. There is a further discussion of medicinal plant preparation in relation to culture and efficacy in Chapter 3.

2.3.10.4 Route of administration of plant remedies

The most common route of administration for plant treatments in this study was oral (83% URs) followed by application to the skin and mouth (9 and 5% URs) (Figure 17). The oral route of drug administration is common in TM and BM [139, 140] and may be so through cultural norms, acceptability, safety and efficacy (Chapter 3). In contrast, van Andel *et al.* [141] have found the rectal administration of medication to be common in parts of West Africa, reportedly due to tradition, rapid action and avoiding the unpleasant taste of herbal medicines.

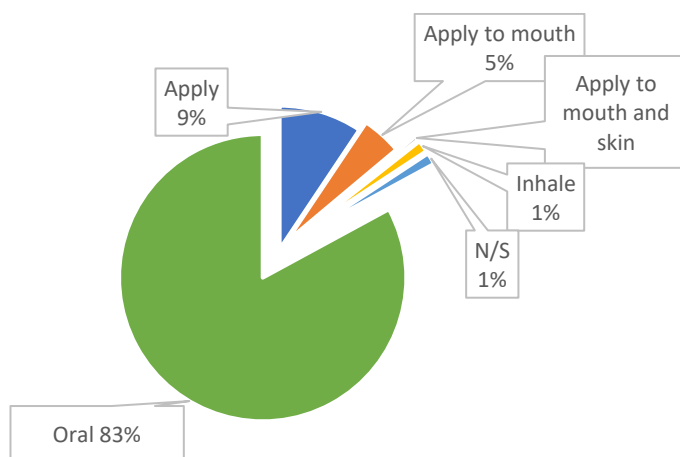


Figure 17: Key routes of administration of plant remedies in Pokot medicine

2.3.10.5 Correspondence of Botanical and Vernacular plant names

There is not a one-to-one correspondence between Pokot and Latin plant names, a common phenomenon in ethnobotany [142] which highlights the need for correct identification by a botanist so that data can be compared across cultures. In some cases, the same Pokot name applied to more than one species (Table 6) while there were nineteen species with more than one Pokot name (Table 7). This phenomenon was also seen in Timberlake's study [87], one example being that several *Aloe* species were referred to as Tolkos. Martin [142] recommended the collection of multiple samples of each local taxon to correct for under and over-differentiation of species. In this study, only a few species have multiple samples which is a study limitation.

2.3.10.6 Plant families

There were 55 Plant Families with 144 species having ethnomedical usage.

Table 6: Pokot taxa with >1 Botanical name

Pokot Name	Botanical names
Koyopko	<i>Bauhinia thonningii</i> Schum. (Fabaceae) (tree)
Koyopko	<i>Tylosema fassoglensis</i> (Schweinf.) Torre & Hillc. (Fabaceae) (creeper)
Cheptukamkam	<i>Psydrax schimperiana</i> (A.Rich.) Bridson (Rubiaceae)
Cheptukamkam	<i>Strychnos usambarensis</i> Gilg ex Engl. (Loganiaceae) (from riverside – <i>lalwa</i>)
Kawatian (three types, one only identified)	<i>Cyphostemma heterotrichum</i> (Gilg & R.E.Fr.) Desc. ex Wild & R.B.Drumm. (Vitaceae)
Kaparsamugh	<i>Phyllanthus nummulariifolius</i> Poir. (Phyllanthaceae) (from Kasongwor)
Kaparsamugh	<i>Flueggea virosa</i> (Roxb. ex Willd.) Royle (Phyllanthaceae) (from Kapchemogem)
Paraterit (creeper) – also a Paraterit tree (not identified)	<i>Plicosepalus curviflorus</i> (Benth. ex Oliv.) Tiegh. (Loranthaceae) (creeper)
Chepkoteyon	<i>Acacia mellifera</i> (M.Vahl) Benth. (Fabaceae)
Chepkoteyon	<i>Maerua angolensis</i> DC. (Capparaceae)
Kembirwo	<i>Acalypha volkensii</i> Pax (Phyllanthaceae)
Kembirwo	<i>Phyllanthus sepialis</i> Müll.Arg. (Phyllanthaceae) (from riverside – <i>lalwa</i>)
Kunjirwo	<i>Cussonia arborea</i> Hochst. ex A.Rich. (Araliaceae)
Kunjirwo	<i>Indigofera tinctoria</i> L. (Fabaceae)

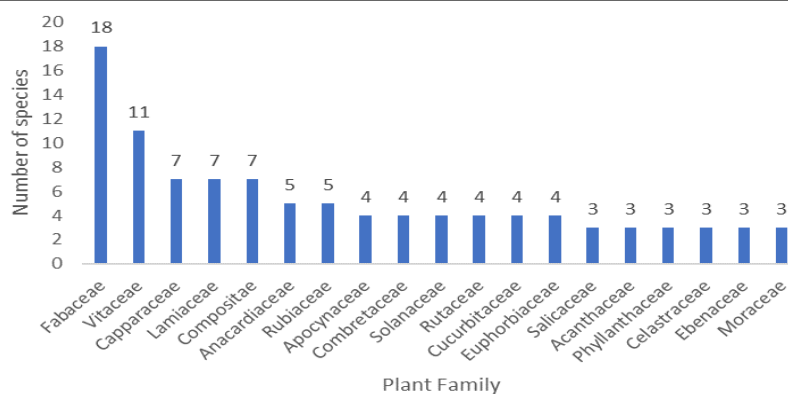


Figure 18: Plant families with three or more species used in Pokot medicine

The plant families with three or more species used are illustrated graphically in Figure 18. The family with the greatest number of species was Fabaceae with 18 species. However, Figure 19 illustrates the plant families with the highest number of URs. Fabaceae was also the family with the greatest usage (102 URs) followed by Rutaceae (64 URs, 4 species) and Vitaceae (42 URs, 11

species). Looking at the families in more detail, Table 8 illustrates that three of the top ten species in terms of total URs belong to the Rutaceae family.

Table 7: Botanical species with >1 Pokot name

Botanical name	Pokot names
<i>Acacia mellifera</i> (M. Vahl) Benth.	Chepkoteyon / Panyerut
<i>Acacia tortilis</i> (Forssk.) Hayne	Ses / Yomut
<i>Ampelocissus africana</i> (Lour.) Merr.	Choyowo/ Akutan
<i>Cassine aethiopica</i> Thunb.	Chepkresmeywon/ Seremonyon
<i>Combretum molle</i> R.Br. ex G. Don	Kamil (from Kasongwor)/ Parasinde
<i>Cordia ovalis</i> R. Br.	Adomeyon/Chirkitutwo
<i>Cucumis ficifolius</i> A. Rich.	Angaleru/ Silangwapkuombes
<i>Cyphostemma adenocaulis</i> subsp. <i>adenocaulis</i> (Steud. ex A.Rich.) Desc. ex Wild & R.B.Drumm.	Chepkatagh/ Ketpomorbus
<i>Cyphostemma heterotrichum</i> (Gilg & R.E.Fr.) Desc. ex Wild & R.B.Drumm.	Chepomoi (from Kapromtim) / Kawatian
<i>Diospyros abyssinica</i> (Hiern) F.White	Kamuke / Murkitwo
<i>Ficus cyathistipula</i> Warb.	Cheptukelat/Nyorpotwo
<i>Gardenia ternifolia</i> subsp. <i>jovis-tonantis</i> (Welw.) Verdc.	Kapilwo/Akorion/Kamokodo
<i>Gouania longispicata</i> Engl.	Chepchan/ Chepkalas
<i>Maerua crassifolia</i> Forssk.	Likwon / Asiokonion
<i>Maerua triphylla</i> A. Rich.	Saretech / Saretechpochebolomin
<i>Manilkara butugi</i> Chiov.	Posion/ Anuun
<i>Senna singueana</i> (Delile) Lock	Chepkopil / Ketparor
<i>Solanecio angulatus</i> (Vahl) C.Jeffrey	Sarrekatch/ Shelshel
<i>Terminalia brownii</i> Fresen.	Koloswo; Kurteswo
<i>Uvaria scheffleri</i> Diels	Kamketwo / Oichun
<i>Vangueria madagascariensis</i> J.F.Gmel.	Kamolwo/ Tapirpirwo
<i>Vepris nobilis</i> (Delile) Mziray	Kamugh/ Kurion

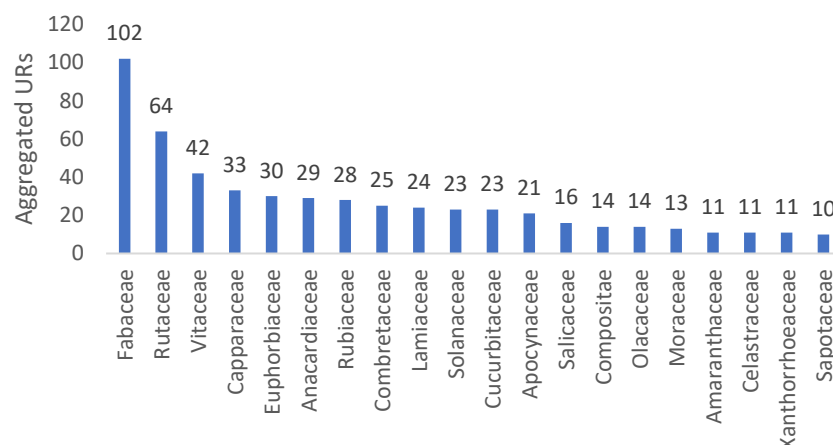


Figure 19: Top 20 plant families by number of aggregated URs in Pokot medicine

Table 8 shows that the species with the greatest usage was Songowo (*Zanthoxylum chalybeum* Engl.) (24 URs) followed by Siria/Siriowo (*Searsia natalensis* (Bernh. Ex C. Krauss) F.A. Barkley) (19 URs), Sulan (*Momordica boivinii* Baill.) (18 URs), Kinyotwo (*Ximenia americana* L.) (18 URs), Kamugh/Kurion (*Vepris nobilis* (Delile) Mziray) (17URs), Cheripar (*Pavetta subcana* var. *longiflora* (Vatke). Bridson) (17 URs), Tiyin (*Dichrostachys cinerea* (L.) Wight & Arn.) (16 URs), Kerelwo (*Croton dichogamus* Pax.) (16URs), Manempelion (*Vepris glomerata* (F.Hoffm.) Engl.var *glabra*) (16URs) and Lagetetwo (*C. spinarum* L.) (14 URs). There is no botanical survey of the study area available to compare species use to the total available flora. However, the predominance of Fabaceae in Pokot medicine was replicated in other studies in East Africa [87, 119, 136, 143-146]. This may, in part, be a reflection of its being one of the largest plant families globally [147]. Gras *et al.* [148] also reported it as one of the most important medicinal plant families across geographical areas. Chassagne *et al.* [149] found the Fabaceae to be among the most represented families in relation to reported antibacterial activity, validating its use in ethnomedicine. Species of the Fabaceae family are a rich source of isoflavones, phenolic compounds, alkaloids, lectins and saponins [150] and a recent review found it to be the most frequently studied family for anti-plasmodial and anti-malarial activity [151].

A study by Gottlieb and Borin [152] combined qualitative ethnobotanical data with quantitative evolutionary data on dicotyledon plant orders and families to test the predictive ability of ethnobotany in relation to bioactivity. They analysed the ethnobotany of three indigenous Amazonian groups and compared them to the large Brazilian database of Pio Corrêa. Plant families were analysed with respect to the ethnobotanical uses: medicinal, food, nutraceutical and poison using two methods. They used the Dahlgrenogram, qualitatively assigning plant orders to different ethnobotanical groupings. The quantitative

measure of the Sporne Index (SI) was also used as a measure of the evolutionary progression of plant families [153]. Families were pooled into their orders and the mean SI of the order with regard to families represented in the data was calculated. The South American data showed that most medicinal families had a higher SI ranging up to 80 while orders with a lower mean SI

Table 8: Twenty-three Pokot medicinal species with ten or more URs and number of uses (42 % of total URs)

Pokot name	Species name (Family)	URs	Uses
Songowo	<i>Zanthoxylum chalybeum</i> Engl. (Rutaceae)	24	8
Siriowo/Siria	<i>Searsia natalensis</i> (Bernh. ex C.Krauss) F.A.Barkley (Anacardiaceae)	19	8
Kinyotwo	<i>Ximenia americana</i> L. (Olacaceae)	18	7
Sulan	<i>Momordica boivinii</i> Baill. (Cucurbitaceae)	18	6
Kamugh/Kurion	<i>Vepris nobilis</i> (Delile) Mziray (Rutaceae)	17	6
Cheripar	<i>Pavetta subcana</i> var. <i>longiflora</i> (Vatke) Bridson (Rubiaceae)	17	7
Kerelwo	<i>Croton dichogamus</i> Pax. (Euphorbiaceae)	16	8
Tiyin	<i>Dichrostachys cinerea</i> (L.) Wight & Arn. (Fabaceae)	16	7
Manempelion	<i>Vepris glomerata</i> (F.Hoffm.) Engl.var <i>glabra</i> (Rutaceae)	16	7
Lagetetwo	<i>C.spinarum</i> (Apocynaceae)	14	6
Koloswo	<i>Terminalia brownii</i> Fresen. (Combretaceae)	12	8
Chepkopil / Ketparor	<i>Senna singueana</i> (Delile) Lock (Fabaceae)	12	6
Kamil/Parasinde	<i>Combretum molle</i> R.Br. ex G.Don (Combretaceae)	11	6
Mukurkong	<i>Harrisonia abyssinica</i> Oliv. (Rutaceae)	11	6
Putapuk	<i>Aerva lanata</i> (L.) Juss. (Amaranthaceae)	11	5
Posion/Anuun	<i>Manilkara butugi</i> Chiov. (Sapotaceae)	11	5
Tolcos	<i>Aloe secundiflora</i> Engl. (Xanthorrhoeaceae)	11	7
Cheptukelat/ Nyorpotwo	<i>Ficus cyathistipula</i> Warb. (Moraceae)	10	6
Chuchuwen	<i>Dovyalis macrocalyx</i> (Oliv.) Warb. (Salicaceae)	10	6
Lopotwokanyerput	<i>Solanum</i> sp. (Solanaceae)	10	7
Chepchilowo	<i>Capparis</i> sp. (Capparaceae)	10	7
Simoyowo	<i>Hoslundia opposita</i> Vahl (Lamiaceae)	10	6
Saretech/ Saretechpo- chepolomin	<i>Maerua triphylla</i> A. Rich. (Capparaceae)	10	6

(up to SI=50) were predominantly food species. This analysis was used to compare the Pokot medicinal data with Gottlieb and Borin [152], though the comparisons are partial as only Pokot medicinal and not food data was collected. The most prominent medicinal orders in the Gottlieb and Borin study were

replicated in the Pokot data as seen in Table 9. Gentianales, Lamiales, Solanales, Asterales and Caryophyllales have a mean SI range of 55-72 (total SI spread = 37-80 for individual families), corresponding with fully medicinal families in the South American study. Other orders in Gottlieb and Borin [152] that were characterised as being used for food and medicine with somewhat lower SI range were also relatively high ranking in the Pokot study. These orders in the Pokot data were Santalales, Apiales, Boraginales, Saxifragales and Zygophyllales with a mean SI range of 55-60. Proteales (mean SI=57) were not represented in the Pokot data at all. This could be due to the fact that they are principally found in other geographic areas: Australia, South Africa, Madagascar, tropical America and Southeast Asia [154]. Icaciniales (Pokot mean SI=55) was not found in the Gottlieb and Borin data. However, this order was only added to the APG system in 2016 [155] and could not have been listed in the Gottlieb and Borin study. Euphorbiales was an outlier in both sets of data with a low mean SI in South American (37) and in Pokot data (41) and yet was significantly represented as a medicinal species in both studies, with 13 Pokot species in this order.

2.3.10.7 Key Pokot medicinal species and their therapeutic uses

Of the total of 144 species with associated Pokot ethnomedical knowledge, 23 species (16%) account for 42% of all URs (Table 8). This table shows that Songowo (*Z. chalybeum* Engl.) was the species most commonly used by participants and also had the highest number of different uses (8) as did Siriowo (*S. natalensis* (Bernh. ex C.Krauss) F.A.Barkley), Kerelwo (*C. dichogamus* Pax.) and Koloswo (*T. brownii* Fresen.). There were 64 species with fewer than 4 URs (Figure 20) which may imply that they were not important culturally or alternatively, that they were known only by select individuals with specialised or family knowledge. To investigate Pokot medicinal plant usage relative to the literature on ethnomedical usage and pharmacological activities, the top 15 Pokot medicinal species were selected for more detailed analysis. This selection was based on a combination of URs and importance in treating the most commonly treated Pokot illness categories. A summary of this information is presented in Appendix V and photos of some of these species are presented in Figure 21, Figure 22 and Figure 23. For some key species, there is very little literature data including Cheripar (*P. subcana* var. *longiflora* (Vatke) Bridson), Kapkopf (*C. bambuseti* (Gilg & Brandt) Wild & Drum), Manempelion (*V. glomerata* (F.Hoffm.) Engl.var *glabra*) and Posion/Anuun (*M. butugi* Chiov.) while *Lopotwokanyerput* was only identified to genus level – *Solanum* sp. – and *Solanum* is too broad a genus for comparison.

Table 9: Dicotyledon Orders and Families of Pokot medicinal species with Sporne indices (SI) and number of species compared with Pio Corrêa data [152]

Order	Pokot Families (SI)	SI range Pokot families (avg for order)	# Pokot spp	Pio Corrêa mean SI (#spp)	Pio Corrêa Main uses
Fabales	Fabaceae (48) Polygalaceae (55)	48-55 (52)	20	48 (206)	Food and medicine
Lamiales	Acanthaceae (72) Linderniaceae (Scrophulariaceae – 67) Orobanchaceae (72) Lamiaceae (72) Bignoniaceae (80) (<i>K. africana</i> (Lam.) Benth.) Oleaceae (62)	62-80 (71)	14	72 (80) (Lamiales)	Medicine
Malpighiales	Phyllanthaceae (was in Euphorbiaceae) Euphorbiaceae (37) Salicaceae (44) Ochnaceae (43) Passifloraceae (45)	37-45 (41)	13	37 (71) (Euphorbiales)	Medicine
Gentianales	Apocynaceae (57) Rubiaceae (48) Loganiaceae (61)	48-61 (55)	11	58 (144)	Medicine
Sapindales	Sapindaceae (45) Rutaceae (45) Anacardiaceae (52)	45-52 (47)	11	51 (26)	Food and medicine
Rosales	Rosaceae (43) Ulmaceae (52) Moraceae (48) Rhamnaceae (45)	43-52 (47)	9	43 (41)	Food, medicine and nutraceutical
Vitales	Vitaceae (38)	38	9	45 (6) (Rhamnales)	Medicine
Asterales	Compositae (72)	72	7	72 (111)	Medicine

Order	Pokot Families (SI)	SI range Pokot families (avg for order)	# Pokot spp	Pio Corrêa mean SI (#spp)	Pio Corrêa Main uses
Brassicales	Capparaceae (52)	52	6	56 (23) (Capparales)	Food and medicine
Myrtales	Combretaceae (47) Myrtaceae (45)	45-47 (46)	5	52 (110)	Food
Cucurbitales	Cucurbitaceae (54)	54	4	45 (88) (Violales)	Food
Solanales	Solanaceae (68)	68	4	64 (79)	Medicine
Caryophyllales	Amaranthaceae (63) Plumbaginaceae (60)	60-63 (62)	3	57 (56)	Medicine
Ericales	Ebenaceae (45) Sapotaceae (50)	45-50 (47)	3	45 (7)	Toxic
Ranunculales	Menispermaceae (47) Ranunculaceae (53)	47-53 (50)	3	48 (20)	Medicine
Santalales	Santalaceae (64) Loranthaceae (65) Olacaceae (46)	46-65 (58)	3	56 (5)	Medicine
Apiales	Araliaceae (55) Pittosporaceae (63)	55-63 (59)	2	56 (25) (Araliales)	Food and medicine
Celastrales	Celastraceae (47)	47	2	47 (12)	Food and medicine
Boraginales	Boraginaceae (60)	60	1	60 (23)	Food and medicine
Ilacinales	Ilacinnaceae (55)	55	1	(New 2009)	-
Magnoliales	Annonaceae (40)	40	1	38 (4)	Medicine
Malvales	Malvaceae (48)	48	1	40 (31)	Food and medicine
Saxifragales	Crassulaceae (55)	55	1	52 (4)	Medicine
Zygophyllales	Zygophyllaceae (55)	55	1	51 (26) (Sapindales)	Food and medicine

Some species have a large volume of ethnomedical and pharmacological information. Of these, Lagetetwo – *C. spinarum* L.– was chosen for more in-depth analysis of ethnobotanical, phytochemical and bioactivity analysis in subsequent chapters of this thesis.

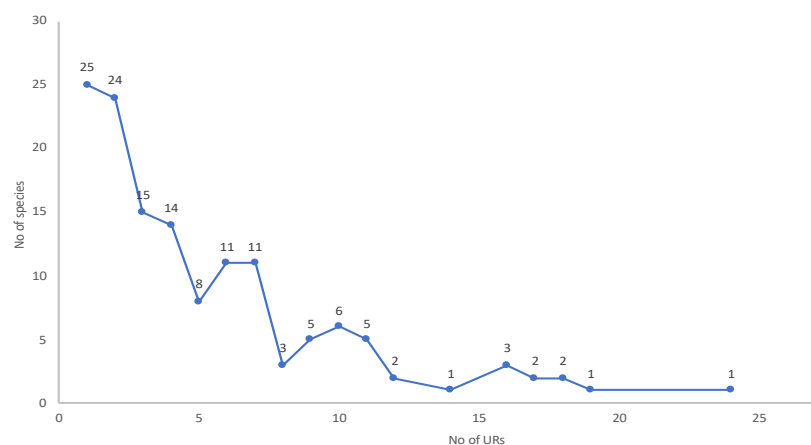


Figure 20: Pokot medicinal species with number of URs - 17 species have more than 10 URs, 64 species have 3 or fewer URs

Songowo – *Z. chalybeum* Engl. - (24URS) was used by the participants to treat probable BM diagnoses of malaria, fever, GI disorders, fainting and dizziness, pain, skin disorders and eye complaints. The preparations included mainly root and bark decoctions with a few preparations of leaf as a decoction. These uses are supported by the ethnomedical literature whereby *Z. chalybeum* Engl. is used throughout Eastern Africa to treat malaria, fever, convulsions, pain and gastrointestinal disorders (Appendix V). In pharmacological studies, it has demonstrated anti-plasmodial, antibacterial and anti-inflammatory activity *in vitro* and anti-malarial activity *in vivo* which support its ethnomedical usage (Appendix V). *Z. chalybeum* Engl. has had several alkaloids isolated, the most prominent being the benzo[c]phenanthridines alkaloids of which nitidine has strong anti-plasmodial activity in ng/mL concentrations as well as antimicrobial, anti-inflammatory and analgesic activity [156] and chelerythrine which has antibacterial and antifungal activity which contribute to the overall activity of the species in traditional usage [157]. In the 2015 study by Kanyongi in the study area, the fruit of *Z. chalybeum* Engl only was used to treat toothache and abdominal disorders so there is no clear correlation between his findings and this historical study [95].

Siriowo/Siria - (*S. natalensis* (Bernh. ex C. Krauss) F.A. Barkley) - (19URs) was used by participants to treat probable BM diagnoses of GI disorder, malaria, oral thrush, habitual abortion, vaginal bleed in pregnancy, bleeding gums, cough and measles. The Pokot preparations included the chewing of leaf or mixing with cold water, bark or root decoctions and fruit mixed with cold water.

The ethnomedical literature on *S. natalensis* (Bernh. ex C. Krauss) F.A. Barkley supports these uses in that it is used to treat GI disorders throughout eastern Africa and malaria in eastern Africa and Namibia. It is used to treat vaginal bleed in pregnancy in Tanzania and other women's gynaecological issues, skin infections and wounds, cough and oral infections in Eastern Africa. In pharmacological studies, it has demonstrated antibacterial, anti-plasmodial, antimalarial, anti-measles virus and anti-nociceptive activity which supports some of its ethnomedical usage (Appendix V). In its phytochemistry, *S. natalensis* (Bernh. ex C. Krauss) F.A. Barkley has had several flavanones and flavones isolated including 7-O-Methyl isokaemferide, 7-O-methyl naringenin and eriodictyonone and genkwanin which had antioxidant activity which may contribute to wound healing. The biflavonoid, rhuschromone, which is active against *S. aureus*, may contribute to the activity of this species in treating skin and respiratory tract infections [158]. [157]. Kanyongi's finding, whereby the leaf was chewed to treat toothache, compares with this 1990s study where the leaf was chewed to treat bleeding gums and indicates some overlap in usage over time [95].

Kinyotwo (*X. americana* L.) (18URs) was used by participants to treat probable BM diagnoses and symptoms of malaria, fever, oral thrush, diarrhoea, oliguria, dysmenorrhoea and amenorrhoea, splenomegaly and fast or irregular pulse. The Pokot preparations were mainly decoctions of bark and root. Where the leaf was used, it was chewed for Cheposkut (oral thrush) or as a paste applied for urinary retention. Correspondingly, *X. americana* L. is used to treat GI disorders throughout sub-Saharan Africa and has demonstrated antibacterial, anthelmintic, analgesic, anti-inflammatory and anti-ulcer activities in pharmacological studies which support some of its ethnomedical usage (Appendix V). In phytochemistry, there has been significant interest in riproximin isolated from fruit kernels which has anti-neoplastic activity in ng/mL concentrations. This does not contribute to known ethnomedical use but is interesting nonetheless. *X. americana* L. has also shown significant activity against *C. albicans* which has a clear correlation with traditional usage. Researchers in Brazil have experimented with tablet formulations containing this species for the treatment of oral candidiasis (thrush) [159, 160]. Procyanidins B and C and catechin/epicatechin isolated from *X. americana* are thought to contribute to ulcer protective activity [161]. In Kanyongi's Pokot research, the leaf was steeped in water to treat stomach ache and eye disorder and this does not correspond directly to historical usage in the 1990s study [95].

Kamugh/Kurion (*V. nobilis* (Delile) Mziray) (17URs) was used in this study to treat probable BM diagnoses of dizziness and fainting, convulsions, malaria, GI disorder, vomiting in pregnancy, infertility, conjunctivitis and pain. In

comparison, it has been used to treat convulsions in Tanzania, malaria in several areas in Eastern Africa and in Ethiopia to treat stomach ache (Appendix V). In pharmacological studies, it has been shown to have anti-plasmodial, antibacterial, anti-inflammatory, antipyretic, analgesic and weak antibacterial activity which support some of its ethnomedical usage (Appendix V). Phytochemically, *V. nobilis* (Delile) Mziray, has had several alkaloids isolated including furanoquinoline alkaloids: skimmianine, kokusaginine and maculine, monrifoline and acridone alkaloid: arborinine which have anti-plasmodial activity at μM concentrations [162] and skimmiamine and kokusaginine have antibacterial and anti-leishmanial activity at μM and sub μM levels, the antibacterial and anti-plasmodial activities of these alkaloids potentially being significant contributors to activity in relation to traditional usage in malaria and GI disorders[163].

Kerelwo (*C. dichogamus* Pax.) (16 URs) was used by participants to treat oral infections and GUI. Elsewhere in Kenya, it was used by the Marakwet to treat oral thrush [164] and by the Sekanani Maasai as a toothbrush [121]. It was used to treat malaria by the Samburu, Nandi, Kamba and Suiei Ndorobo [120, 137, 165, 166]. In pharmacological studies, it has been shown to have antibacterial, anti-plasmodial and anti-inflammatory activity which support some of its ethnomedical usage [167].

Tiyin (*D. cinerea* (L.) Wight & Arn) (16URs) was used by study participants to treat probable BM diagnoses and symptoms of malaria, GI disorder, oral thrush, dizziness and fainting, dysuria and pain. Correspondingly, it is used in east, west and southern Africa to treat malaria, pain and GI disorders. It is used in east and west Africa and in India to treat GUI and in Tanzania, Congo and Namibia for oral thrush. Pharmacological studies have demonstrated anti-plasmodial, antimalarial, antiviral, anti-inflammatory, analgesic, anticonvulsant, diuretic and antispasmodic activities which support some of its ethnomedical usage (Appendix V). *D. cinerea* (L.) Wight & Arn. has had meroterpenes isolated (flavono-labdanes, dichrostachines) from root and stem bark which exhibited potential anti-cancer activities [168] and the hydroxycinnamic acid amide, clovamide, exhibited activity against Influenza A and has activity against HIV at μM levels which may indicate other antiviral potential of this compound. Tannins extracted from the root exhibited antibacterial activity *in vitro* which may contribute to its activity in GI disorders [169, 170].

Lagetetwo (*C. spinarum* L.) (14 URs) was used in this study to treat probable BM diagnoses of dizziness and fainting, convulsions, malaria, GI disorder and amenorrhoea. The Pokot preparations were mainly decoctions of root including for the treatment of malaria and Chemwaror. It has been used elsewhere in Kenya for the treatment of dizziness, for convulsions in Kenya,

Zimbabwe and Malawi and for brain tension and stress in Ethiopia. It has been used to treat malaria throughout eastern Africa and in Benin and Mozambique. It has been used to treat GI disorders in sub-Saharan Africa and India and to treat menstrual disorders in Kenya, Ethiopia, Saudi Arabia and South Africa. Pharmacological studies have shown it to have anti-plasmodial, antibacterial, antispasmodic and anticonvulsant activity which support some of its Pokot medical usage (Appendix V). *C. spinarum* L. contains the lignan, nortrachelogenin, which has anti-inflammatory, antifungal and antibacterial activity in µg/mL concentrations and the eudesmane sesquiterpene, carissone with anti-inflammatory activity in µg/mL concentrations, both of which along with other phytoconstituents may contribute to overall activity in traditional treatment of GI disorder and convulsions [171-174]. In Kanyongi's 2015 research, the root was prepared as a decoction to treat malaria according with some of the usage of it in the historical Pokot study [95]. This species will be looked at in more detail in Chapters 3 and 4.

Kamil/Parasinde (*C. molle* R.Br. ex G. Don.) (11URs) has significant ethnomedical data from across SSA supporting its use in Pokot medicine in the treatment of cough and malaria. Substantial pharmacological data indicates antibacterial, anti-mycoplasmal, anti-plasmodial, antifungal and anti-inflammatory activity *in vitro* and anti-malarial and respiratory activity *in vivo* (Appendix V). In its phytochemistry, *C. molle* R.Br. ex G. Don., contains iridoid glycosides [175], a class which has shown hepatoprotective and anti-inflammatory activity [176]. Oleanane-type pentacyclic triterpenoids and oleanane-type triterpene saponin, β-D-glucopyranosyl 2α,3β,6β-trihydroxy-23-galloylolean-12-en-28-oate were isolated and demonstrated *in vivo* anti-inflammatory activity which may contribute to the treatment of pain and fever in malaria [177].

Mukurkong (*H. abyssinica* Oliv.) (11URs) has supporting ethnomedical and pharmacological data for its use in genito-urinary issues in East Africa, in particular for GUI. In its pharmacology, it has demonstrated anti-plasmodial, antibacterial, antifungal and antiviral activities. Phytochemical studies have identified tetranortriterpenes including obacunone, harrisonin and acetoxyharrisonin; tetranortriterpenoid [178]. Obacunone has exhibited antiproliferative and neuroprotective activity in nM concentrations. Thus it is not clear what metabolites may contribute to efficacy in traditional treatment of GUI [179].

Putapuk (*A. lanata* (L.) Juss.) (11URs) has supporting ethnomedical data for its use in the treatment of malaria, oral thrush, pain and tropical skin ulcers in East Africa and South Asia (Appendix V). In bioactivity studies, it has shown antibacterial, antifungal, anti-plasmodial, antiviral, immunomodulatory,

anti-inflammatory, analgesic and hepatoprotective activities. In its phytochemistry, it contains ecdysones, betulin, acylated flavone glucosides, ferulic acid, sulfonoquinovosyldiacylglyceride and alkaloids [180]. Indole alkaloids isolated include canthin-6-one which has antifungal and benzodiazepine receptor binding at μM levels, which may contribute to

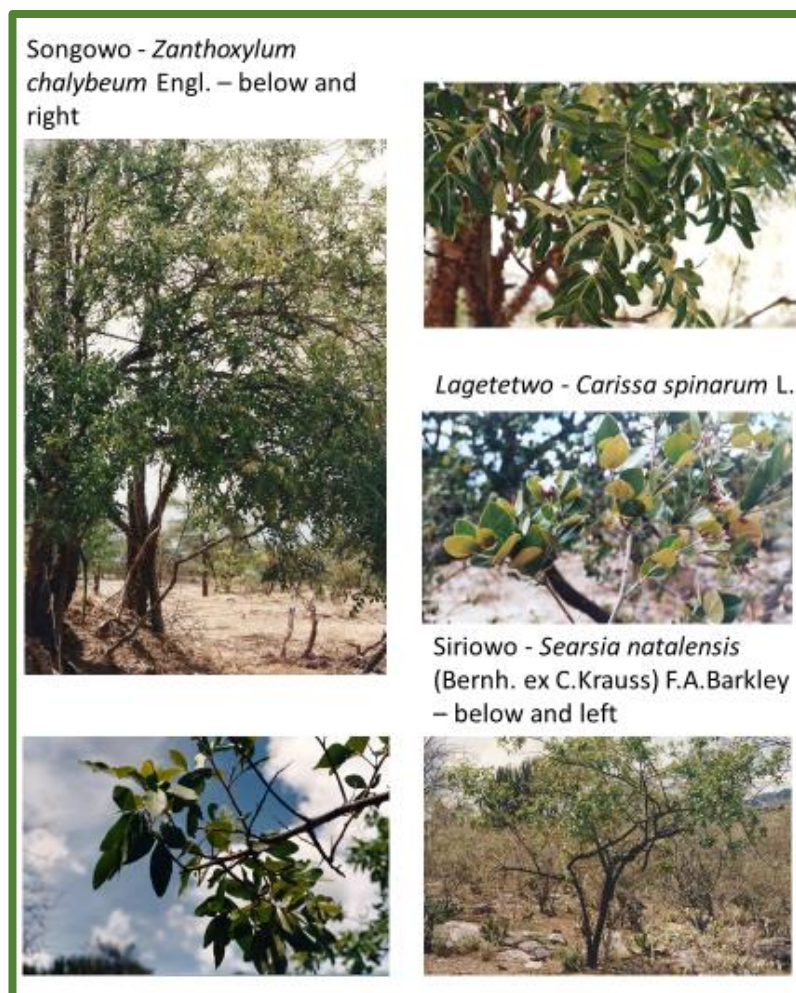


Figure 21: Photos of medicinal species used by the participants: Songowo (*Z. chalybeum* Engl.), Lagetetwo (*C. spinarum* L.), Siriowo (*S. natalensis* (Bernh. ex C.Krauss) F.A.Barkley) (photos taken by author)

pain relief and skin wound treatment in traditional use. Sulfonoquinovosyldiacylglyceride has antibacterial and anti-HSV activity in $\mu\text{g/mL}$ concentrations, possibly contributing to efficacy in oral thrush and wound treatment, and feruloyltyramine anti-inflammatory activity at μM levels which may contribute to treatment of pain and fever in malaria [179].



Figure 22: Photos of medicinal species used by the participants: Manempelion (*V. glomerata* (F.Hoffm.) Engl.var *glabra*), Posion (*M. butugi* Chiov.), Cheripar (*P. subcana* var. *longiflora* (Vatke) Bridson) and Kerelwo (*C. dichogamus* Pax.) (photos taken by author)

and two 6-furanoflavones and four methylpyranoflavonic analogues isolated showing anti-leishmanial activity [181].

Malaria had a high prevalence of treatment in the study if the BM interpretation of several Pokot illness categories is correct. It corresponds with locally high incidence of malaria as well as historic and current morbidity levels [182, 183]. Broad ethnomedical studies have identified malaria as the most treated illness [120, 143] while several malaria specific studies have been conducted in Kenya [136, 137, 184, 185]. In a review by Omara [186], the author identified 61 studies and 286 species in use in Kenya for the treatment of malaria. Of the most commonly used species identified in this study for the treatment of presumed malaria, Songowo (*Z. chalybeum* Engl.), Tiyin (*D. cinerea* (L.) Wight & Arn.), Kamugh/Kurion (*V. nobilis* (Delile) Mziray), Lagetetwo (*C. spinarum*),

Kamil/Parasinde (*C. molle* R.Br. ex G.Don), Putapuk (*A. lanata* (L.) Juss.) and Simoyowo (*H. opposita* Vahl) each has been used more widely in the ethnomedical treatment of malaria and have proven anti-plasmodial and in some cases *in vivo* anti-malarial activity with some active anti-plasmodial compounds isolated as described above.

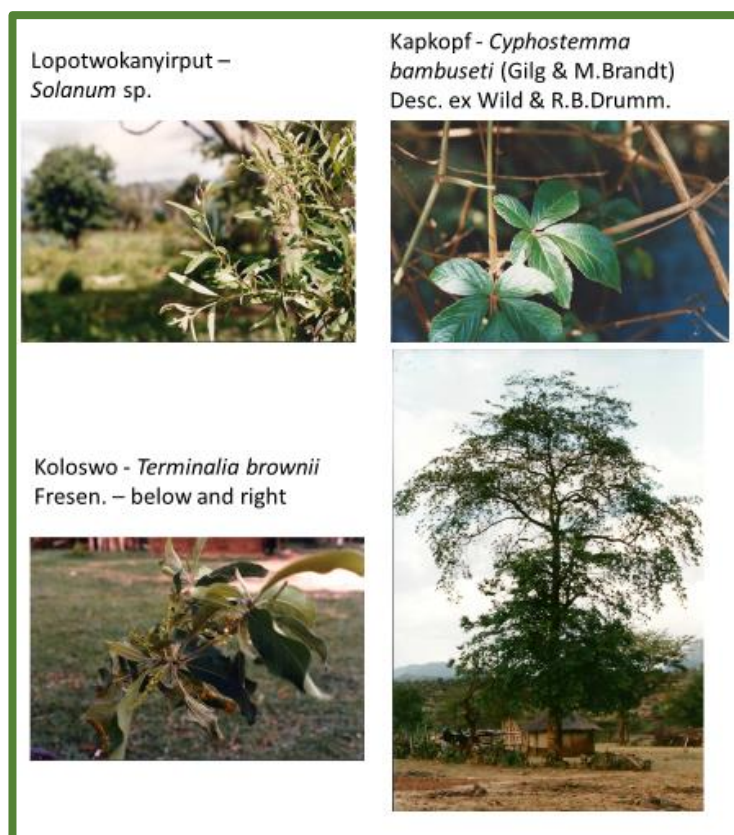


Figure 23: Photos of medicinal species used by the participants: Lopotwokanyirput (*Solanum* sp.), Kapkopf (*C. bambuseti* (Gilg & M.Brandt) Desc. ex Wild & R.B.Drumm) and Koloswo (*T. brownii* Fresen.) (photos taken by author)

2.3.10.8 Under-reported species for further research

There are many species used in this study which are not otherwise reported in the literature. Of the commonly used species, Cheripar (*P. subcana* var. *longiflora* (Vatke) Bridson), Kapkopf (*C. bambuseti* (Gilg & M.Brandt) Desc. ex Wild & R.B.Drumm), Manempelion (*V. glomerata* var. *glabra*) and Posion/Anuun (*M. butugi* Chiov.) are under-reported in the ethnomedical and pharmacological literature. Cheripar (*P. subcana* var. *longiflora* (Vatke) Bridson) (17 URs) is the species most commonly used by participants for the treatment of illnesses that correspond biomedically to fainting, dizziness and convulsions. There is very little information in the literature relating to this species and none supporting the Pokot usage in CNS symptoms. However, other species of *Pavetta* have shown good anti-plasmodial activity as well as antimicrobial, antitumor, anticonvulsant,

hepatoprotective and anthelmintic activity. These activities may also exist in *P. subcana* var. *longiflora* (Vatke) Bridson and may contribute to its ethnomedical efficacy in the treatment of convulsions and dizziness which can have numerous aetiologies [187, 188]. Manempelion (*V. glomerata* (F.Hoffm.) Engl.var *glabra*) (16 URs) was used by study participants to treat probable BM diagnoses of dizziness and fainting, convulsions, malaria, fever, eye infection, rash, oedema, GI disorder and cancer. There is not much information on this species. However, it has been used in Tanzania to treat convulsions, in Tanzania and Ethiopia to treat malaria, in East Pokot to treat headache, in Tanzania to treat eye disease and body swelling and in Ethiopia to treat GI disorder. In anti-plasmodial studies, 80% ethanolic extracts were shown to be inactive *in vivo* in mice infected with *Plasmodium berghei berghei* [189]. Thus, there is some ethnomedical data supporting its use in Pokot medicine but no supporting pharmacological data (Appendix V). In its phytochemistry, *V. glomerata* (F.Hoffm.) Engl.var *glabra* has much less available data but contains flavonoid and furoquinoline alkaloids isolated which have demonstrated antioxidant activity and antibacterial activity [190] and the flavonoid, hesperetin has antioxidant and anti-inflammatory activities, each of these activities with clear potential to contribute to traditional use in treating fever, infections, GI disorders and oedema [163, 191]. A recent review of the genus *Vepris* reported several species within the genus with anti-plasmodial activity which may indicate similar bioactivity in this species [163]. Posion (*Manilkara butugi* Chiov.) was used by the study participants to treat hepatomegaly, splenomegaly, vaginal bleed in pregnancy, diarrhoea, dizziness and pain. There is very little in the literature about this species. The only corresponding ethnomedical use reported elsewhere is in the treatment of diarrhoea in Ethiopia. However, other *Manilkara* species have demonstrated analgesic, anti-inflammatory, anti-diarrhoeal, anthelmintic, hepato-protective, antifungal and antibacterial activities which would support most of the Pokot usage [19]. *C. bambuseti* Gilg & M.Brandt) Desc. ex Wild & R.B.Drumm was used mostly in the treatment of malaria. Other species of *Cyphostemma* have had phenolic compounds, flavonoids and terpenoids identified in phytochemical screen but there is very little available bioactivity data on this genus [245]. These are important areas of research and merit further pharmacological investigation.

2.3.10.9 Toxicology and Pokot medicinal plants

Medicinal plant toxicity is multi-faceted. Toxicity may be intrinsic to the plant material itself or to extrinsic factors. Individual toxic metabolites or combinations thereof may contribute directly to toxicity. Some metabolites, such as cardiac glycosides, may have a narrow therapeutic index with associated toxicity [192].

Some medicinal species are highly interactive, such as St John's Wort, which is a cytochrome P450 inducer, interacting with many drugs including anti-coagulants with potentially life-threatening effects [193]. Among the Pokot species, *H. abyssinica* Oliv. is known to interact with this system [194]. The combination of hundreds of metabolites in a single- or multi-herb extract, may add to the interactive and toxic potential of herbal medicinal preparations relative to a single pharmaceutical drug and complicate a safety evaluation. Alternatively, multiple ingredients may reduce the toxic potential or side effects such as in the case of a standardised mixture of cinchona alkaloids relative to quinine alone in the treatment of malaria [195]. Variability in traditional preparations is a known factor in the unpredictability of herbal safety. At the primary level, environmental conditions during the growth of the plant, can have a significant impact on the presence and concentration of metabolites such as during drought stress, soil conditions, herbivory dangers or across the plant's life-cycle and diurnal and annual cycles [196, 197]. Phenotypic plasticity may be evident where there are variations in water, light and nutrients with resulting variation in concentrations of plant secondary metabolites [197]. Intraspecific plant secondary metabolite polymorphisms are known to occur and are proposed to be caused by intersecting geographic and temporal factors leading to variability and unpredictability in composition of a species [197].

Extrinsic factors may contribute to concerns regarding herbal medicine safety. Physical factors, such as the presence of intentional or unintentional adulterants and contaminants, are well-publicised [198]. Contaminants may include heavy metals and pesticides while adulterants may include the incorrect plant species or the addition of pharmaceutical drugs. Problems developing on storage or processing may include fungal, or bacterial contamination [193]. In a study of Nigerian medicinal preparations, *X. americana* L. (Kinyotwo) was found to have developed carcinogenic nitrosamines from bacterial contamination of water-soaked rootbark at room temperature [199]. Lack of precision in preparation of remedies, such as in the proportion of plant material to solvent, degree of comminution, duration and temperature of extraction, may result in variability in the range and concentration of metabolites, contributing to toxicity [198]. Social issues such as the perception that herbal medicines are natural and safe may lead to use of high doses or to their concomitant use with pharmaceuticals, creating the potential for unpredictable pharmacodynamic and pharmacokinetic interactions [193]. The possibility of people giving up a life-saving HIV treatment due to faith in a particular herbal medicine was illustrated in northern Tanzania in relation to treatment with *C. spinarum* L. [200]. Cultural issues in healing such as the deliberate use of emesis or purgation based on understandings of illness can be dangerous, with some recognition of this among

the Pokot study participants. Dehydration is a clear risk in these treatments. The vomiting of “black” as described in section 2.3.6 could indicate haematemesis which is considered a medical emergency but may not have been recognised as such by participants.

As described by Moyo *et al* [198], there are very few studies focusing on safety of African traditional medicine. Safety evaluation of medicinal plants used in herbal medicine is thus very difficult, even in a small study such as this one in West Pokot, where little is known of the majority of the species. Aydin *et al* [201] recommend a battery of, *in vitro* and *in vivo* studies for traditional herbal medicines, with a focus on aqueous extracts where this is the dominant traditional preparation method. However, Moyo *et al* [198] argued that there is little funding available for such studies. Willcox *et al.* [202] described the difficulty of safety monitoring for pharmaceuticals and traditional medicines alike. They recommended a pharmacovigilance programme for herbal treatments piloted in a study in East Africa promoting the use of cultivated *Artemisia annua* for the treatment of uncomplicated malaria. The pooling of data through pharmacovigilance may improve causality assessment, which is often difficult to prove as described by Neergheen-Bhujun [193] and could prove useful where full safety assessments are prohibitively costly.

In this historical study of Pokot TM, the study participants reported on some of the dangers of using medicinal species. The most common cautions in use were in relation to use in pregnancy and in small children. Of the key species selected for analysis, Kinyotwo (*X. americana* L.), Mukurkong (*H. abyssinica* Oliv.) and Simoyowo (*H. opposita* Vahl) were contraindicated in pregnancy as they could cause abortion. Species to be used in reduced dosage in pregnancy were Songowo (*Z. chalybeum* Engl.) and Cheripar (*P. subcana* var. *longiflora* (Vatke) Bridson).

There is some limited toxicity data on the Pokot medicinal species in this study. Several laboratory studies have identified potential toxicity of *X. americana* L. [203-205] and the leaf, fruit and root are known to contain the toxin HCN with the root also containing oxalates and saponins which contribute to toxicity [206, 207]. Active and toxic doses are considered to be too close with a lack of selectivity [203-205]. The contraindication in pregnancy may additionally relate to the activity of saponins which can increase uterine contractility [208]. The alkaloid, candicine is thought to be responsible for the known toxicity of *Z. chalybeum* Engl. extracts [157]. *Z. chalybeum* Engl. has, in general shown conflicting *in vitro* results from non-toxic through to toxicity [204, 209-212]. The stem bark and root bark of *H. abyssinica* Oliv. have demonstrated brine shrimp toxicity [213]. The root extract of *H. opposita* Vahl has not been toxic to brine shrimp in one study but in another study, has shown poor selectivity in

antiplasmodial activity [214, 215]. Other key species with toxicity data include *V. glomerata* (F.Hoffm.) Engl.var *glabra* with low toxicity *in vitro* [216], *A. lanata* (L.) Juss. with non-acute toxicity but potential long-term tissue damage [217] and *D. cinerea* (L.) Wight & Arn. where most extracts were non-toxic or mildly toxic [218-220]. *C. spinarum* L. contains some toxic metabolites including cardiac glycosides, which will be examined further in Chapter 4. There is no pharmacological data relating to *P. subcana* var. *longiflora* (Vatke) Bridson.to the best of my knowledge.

Thus, several of the key Pokot medicinal species have the potential for toxicity at certain dosages or with long-term usage. It would be useful to conduct pharmacovigilance and safety studies on the currently used key species in all regions where TM is in use, with a focus on the most commonly used form of extract in order to guide traditional medicine practice.

2.3.11 Correspondence of historical Pokot data with more recent research

A relatively recent study by Kanyongi [95] in the study area investigating medicinal plant use around identified 40 medicinal species of which 21 overlapped with this historical study. Four of these overlapping species are among the selected most commonly used Pokot species from the 1990s. These include Songowo (*Z. chalybeum* Engl.), Siriowo (*S. natalensis* (Bernh. ex C.Krauss) F.A.Barkley, *X. americana* L. and Lagetetwo (*C. spinarum* L.). however, only the usage of Lagetetwo (*C. spinarum* L.) overlaps in both studies. It is unclear whether or not this indicates a change in usage over time. Each study was conducted with a small number of participants in statistical terms and were not comprehensive in their remit and thus represent a snapshot of usage rather than a full analysis of TM in the study area.

2.3.12 Conservation status of selected Pokot medicinal species

A search on the IUCN red list of endangered species indicates that not all species have been evaluated including Cheripar (*P. subcana* var. *longiflora* (Vatke) Bridson), Kapkopf (*C. bambuseti* (Gilg & M.Brandt) Desc. ex Wild & R.B.Drumm), Manempelion (*V. glomerata* var. *glabra*), Posion/Anuun (*M. butugi* Chiov.), *A. lanata* (L.) Juss, and *H. opposita* Vahl. All other species are listed as being of “Least Concern” with the exception of *C. spinarum* L. in the Seychelles where it was listed as endangered in 1998 with no more recently updated status [221]. However, the absence of data does not guarantee that the species is not endangered as evidenced by local areas of concern for *C. spinarum* L. in the Seycehlles [222]. In addition, Mahunnah *et al.* [223] described this species as

being locally endangered due to overharvesting following significant media attention. This fact indicates that the IUCN red list does not provide the full picture in relation to conservation.

2.3.13 Shared Knowledge

The graph in Figure 24 illustrates that there are a small number of species that are well known within the community of *Chepsaktis*, in this case there is one plant known by 9 Chepsaktis, 2 plants known by 8 Chepsaktis while the bulk of the knowledge is idiosyncratic, with 94 plants known by one or two participants. Thomas *et al.* [224] describes this phenomenon as being common in the ethnobotanical literature. There were 29 participants in total though most

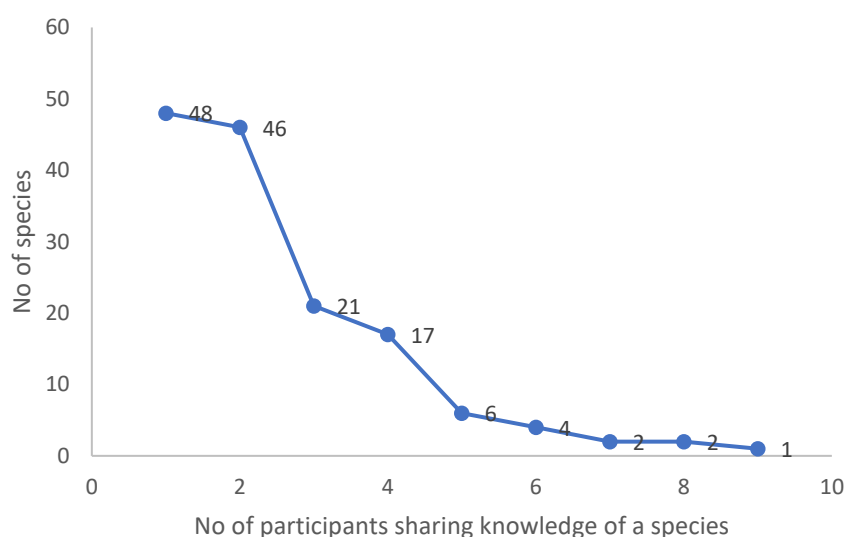


Figure 24: Degree of Shared Knowledge among Participants

interviews were conducted with a smaller number of key participants. The shared knowledge on an individual use is relatively small with three species having 8 or 9 URs for the same treatment indications: Songowo (*Z. chalybeum* Engl), Siriowo/Siria (*S. natalensis* (Bernh. ex C.Krauss) F.A.Barkley) and Kinyotwo (*X. americana* L.).

2.3.14 Evidence of therapeutic use of non-plant-based materials

The therapeutic use of non-plant-based materials was not explored in this research. In a few instances, particularly in the treatment diaries, it was apparent that Chloroquine was used following failure of plant treatment. Conversely, plant medicines were used following discharge from hospital if people still felt unwell. A small animal like a hedgehog known as *porpor* was used in a mixture with several plants, burned to ash and given to treat *Chepkolmut* (probably oral thrush or malaria). In one instance, oil from rock (*mnagh po kaogh*) (tar?) was used to

treat the skin. Coloured soils and ash applied to the skin were used to treat difficult to treat illness as described in section 2.3.6.

At the time of the study in the 1990s, with a few exceptions, there were not many people in the community on long term medication so that the risk of interactions with medicinal plants was not very high. Examples of long-term medications in use were peri-natal iron, TB and VL medications, each of which could interact significantly with other medications including plants. At the time, the area had a low incidence of HIV infection and ARVs were not yet in use there. However, interactions with ARVs would be an issue in the present day, as would the use of medications for NCDs, which have an increasing prevalence in Kenya [57-59]. Thus, in considering the use of traditional and complimentary medicines in the present day, the issue of concomitant use of pharmaceuticals needs to be assessed.

2.3.15 Translation in the research

The need for interpreters was an important and limiting element of the research. There are ethical and practical issues which arise in this. Temple and Young [225] outline how the act of translation is not a neutral process simply about translating words but involves translating meaning, across language, culture and power differentials and can raise questions around the validity of the research. Many of the issues cannot be overcome. I was a white, female, pharmacist, from Ireland, English speaking and in a position of relative power. I placed myself in a position as interpreter of meaning and experiences of people who don't speak English for an English-speaking audience. If this primary document was to be published, it would not be accessible to the participants or many older members of the community. I am also, not currently in a position to review the material with the participants which would be a normal part of a research process such as this. At a more basic level, simple misunderstandings of language and meaning were bound to happen due to language and cultural barriers and a lack of preparation on my own part. At the time, I had not studied the basics of ethnobotany, nor was I aware of the pitfalls such as those of folk classification or of vernacular vs scientific nomenclature or indeed the basic ethics of conducting such research in the first place.

2.4 Limitations of the study

Translation as described above is the key limitation of this research, including the non-translatable nature of some ethnomedical knowledge into a biomedical framework. In addition, the limits of time, financial resources and skills – particularly language and cultural skills - of the researcher at the time of the research were significant. Skill was needed to appreciate the anthropological nature of the research which would then have required more time to explore emic

understandings of illness, the body and meanings around treatment. Botanical expertise would have been useful in identifying, for instance, different Pokot names for the same species. Clinical biomedical input into the interviews, at least through the design of the interview schedule and at best to work together in the field, observing the *Chepsaktis* and patients during treatment, would have resulted in more clarity in the research, though raising ethical issues. The treatment diaries were a valuable addition and would have been better if introduced at an earlier stage of the study as they gave a clearer insight into treatment processes. Including specific questioning on the use of pharmaceuticals or non-plant-based treatments would have given a fuller representation of treatments used. The low number of participants is another limitation of the study as the research is necessarily qualitative as a result and does not meet the current standards of ethnobotanical research. As with any study, it is a snapshot in time and place. While there is overlap in species reported by studies in the same community by Nyamwaya [89] and Kanyongi [95], the differences also point to the incomplete nature of this and other ethnomedical research. Having met with women only as participants in this study is both a limitation and adds value to the research. *Chepsaktis* can be both men and women among the Pokot so I was missing a male perspective, particularly on men's health as this did not emerge in the study. The majority of ailments treated cross gender boundaries but genitourinary health would differ by gender. Thus, a male perspective of *Chepsaktis* would be likely to expand the repertoire of medical species in use. On the other hand, women are often more difficult to access in research. Being female and able to meet with the women over several months allowed a relationship of trust to develop. The CBD and Nagoya Protocol rightly protect traditional knowledge and biodiversity but that I do not meet the ethical standards of this or of academic publication of this material is a clear limitation of this research.

If it were possible to do further similar ethnomedical research in the study area, I would build in a number of changes as described above into the research protocol following best practice guidelines [77]. In the first instance, the study community would be involved throughout the research design process, including the conduct of research that would be relevant to the community. The prior informed consent process would be documented, including changes during the conduct of the research. If the aim was to conduct a representative survey, there would be a large number of participants to lend statistical power to the results. The participants would include a representative sample of community members including practitioners (female and male), community leaders and people from different socioeconomic and ethnic backgrounds. The team would be multidisciplinary including local collaborators, botanical, ethnobotanical,

anthropological, clinical and statistical expertise in the design and conduct of the research. Several local research assistants with good knowledge of the Pokot language and English would be trained to perform the research. This would be capacity building for research in the area and contribute to benefit sharing from the research. Multiple samples of each Pokot taxon would be collected to correct for lack of one-to-one correlation between Pokot and botanical taxa. In-depth qualitative methods would form a core element of the research to understand the cultural, healthcare, environmental and economic contexts of the healthcare landscape in the study area. Such a research project would be costly and funding would be very likely to be a major limiting factor in addition to CBD-related difficulties in TM research.

The limitations identified in the conduct of this research are acknowledged more broadly in critiques of ethnomedical research [77]. Having first-hand experience has informed my analysis in Chapters 3 and 4 of the ethnobotanical and ethnomedical research. Knowing at first hand the pitfalls of such research has resulted in the drilling into the data on *C. spinarum* L. from the perspective of these limitations.

2.5 Outputs of the research

In 1996, a preliminary document was sent to OMH with the 25 most commonly used species reported by the participants. A Kenyan doctor who worked at the hospital at that time was coordinating with KEMRI to gain a better understanding of TM usage in the community. The content of this chapter will be disseminated back to East Africa Herbarium in Nairobi where some of the voucher specimens remain. I hope to be able to return the data to the community in some form if permission can be granted. I know from friends who still live in the study community that this is something the original participants are interested in although, as it stands, this would be in breach of the CBD and national regulations. There are changes to national legislation proposed [64] which may improve the prospects for this happening.

2.6 Conclusions

This research, documenting traditional medicinal knowledge in a rural community in Kenya from the early 1990s, is a valuable source of knowledge from this time for the community and the wider Kenyan population. It demonstrates the knowledge and expertise of the participants in the plant-based treatment of illness in their community in the context of significant health challenges and the availability of a variety of healthcare options. The research shows some awareness of plant toxicities among the participants. Literature research adds to this base of knowledge on, especially chronic, toxicities in the medicinal flora

which may be more difficult to recognise in daily usage. The research reveals continuities of medicinal plant usage across East Africa and at times throughout Sub-Saharan Africa and in India with significant pharmacological data in support of some of the most commonly used species. In addition, a number of species emerge as locally important in the treatment of malaria and CNS disorders but without associated ethnomedical or pharmacological data indicating potential for further research on these species.

As described in the introductory chapter, the current context of traditional medical knowledge in Kenya is one of both diminishing traditional medicinal knowledge and increasing interest in the subject from cultural heritage, healthcare and economic perspectives [51]. From recent visits to West Pokot, it is clear that habitats have substantially changed with a likely loss of biodiversity and associated traditional knowledge. Future research on traditional medical knowledge in the study area would be in the context of significant social, cultural, economic, environmental, health and healthcare change since the 1990s. Added to this is the altered legislative environment for the practice of Kenyan traditional medicine and bioprospecting related to the new constitution and the CBD as described in the introductory chapter to this thesis. This historical research would make for an interesting comparison with contemporary ethnomedical study in the research area.

Beyond the value of the research in the study area, the difficulties and limitations of the research are replicated to the present day in ethnomedical research which focuses on medicinal plant use, as will be explored in Chapter 3. As such, it highlights potential for more ethical and relevant research in the future.

Chapter 3: *C. spinarum* a case study in ethnobotany and bioprospecting research

3.1 Introduction

In August 2010, Reverend Ambilikile Mwasupile, a retired pastor in the Loliondo area of northern Tanzania began to treat people for a range of chronic ailments including diabetes, cancer, epilepsy, herpes, HIV/AIDS, liver disorders and asthma. A report on the phenomenon by Malebo and Mwambo [226] described how he was instructed in dreams to use a decoction of the root of the *Mugariga* tree to heal people with chronic illnesses. He would pray over the root before preparing the decoction, and pray again before personally administering it to the patient using Babu's own "miraculous cup" known as *kikombe cha Babu* (grandfather's cup) [200]. He was, for a brief period, a media sensation throughout East Africa and was known as *Babu wa Loliondo* (the grandfather of Loliondo) providing what was known as the "miracle cure". People travelled long distances by road and helicopter in order to get the once-off treatment. The flocks of people making their way to the remote village caused great disruption locally (Figure 25). The Loliondo cure was reported in local, national and international media including by the BBC [227] and in the New York Times [228].



Figure 25: Queues of traffic of 26km as people make their way to Loliondo (photo with permission from Jonathan Kalan)

After initial scepticism, the Tanzanian government, local health officials and the national research hospital endorsed *Babu wa Loliondo* [200]. Popularity waned after ten months, at least in part because the pastor called for a break due to the chaos caused locally. It was also reported that some elderly people died as they waited in queues for days [228] and that some HIV positive patients died, having stopped their ARV treatment subsequent to treatment with *Mugariga* [200]. Vähäkangas [200] described how *Babu wa Loliondo* was, during that brief time, able to bridge the gap for people in the region between the scientific, traditional and Christian worldviews and between physical and spiritual healing.

Mugariga was identified as *C. spinarum* L. (*C. spinarum*), a plant known among several local ethnic groups as having healing properties including among the Pokot of Kenya (Chapter 2) [226]. In fact, prior to this, KEMRI (Kenya Medical Research Institute) was investigating *C. spinarum* for its antiviral properties and in 2014 produced Zedupex™ containing *C. spinarum* [229]. Interest in the Loliondo “miracle cure” has re-emerged recently in relation to COVID-19 and was reported in the media that KEMRI is investigating Zedupex™ for efficacy against the virus [230, 231]. This is a species with a large body of associated ethnobotanical data, not just in East Africa, but throughout SSA, Australia, and parts of Asia, dating from the late 1800s. Recent review articles have focused on phytochemistry and pharmacology with limited ethnobotanical context [232-236]. While *C. spinarum* is most widely reported for use in healing, it also has many uses including practical and ritual. Despite its widespread usage, it is not well-known outside the oral and folk traditions of Africa, South Asia and Australia. Written records began during the colonial era as well as the recent media interest. There is limited recorded usage in the ancient texts of Traditional Chinese Medicine (TCM) and Ayurvedic and Siddha medicines although a similar species, *C. carandas* L., is listed in Ayurvedic databases [237]. The focus on healing properties may be a function of the studies that have been conducted where the emphasis is often on ethnomedicine (EM) and the ultimate potential for bioprospecting. Broad ethnobotanical studies and anthropological research would help to situate this healing focus in the context of the wider value of biodiversity as integral to existence in communities of the Global South [238].

Ethnobiology is the discipline from which most *C. spinarum* research emerges. As described in section 1.3, ethnobiology is concerned with the material and symbolic aspects of interactions between humans and their environments, thus having multidisciplinary foundations and outlooks. It has undergone many changes since its academic beginnings in the late 1800s. Hunn [239] described four phases in the development of ethnobiology (Table 10). Hunn’s Phase I from the later 19th century to the 1950s was primarily descriptive presenting lists of plant species and their medicinal and other uses [66]. Phase II from the 1950’s to the 1970’s, examined the role of cognition in human relationships with their environments researching folk classification, linguistics and symbolic aspects, representing the perspective of the person whose culture was being studied. It is described as the *emic phase* [240]. Phase III developed from the 1970’s through to the 1990’s, examining indigenous knowledge and the broader ecological context which resulted in the formation of the ISE Code of Ethics [68]. Phase IV from 1990’s onwards is characterised by the rise to prominence of concerns around the rights of local communities, specifically the exploitation of indigenous knowledge and IPRs of indigenous knowledge

holders. The indigenous voice is heard in this phase as a research collaborator rather than as a participant. A developing Phase V involves deeper networking and collaborative work between researchers across geographies and disciplines to address pressing issues of ecological and cultural crises [66]. D'Ambrosio [240], identified the same phasing as Hunn though he found ethnopharmacology to be a significant element temporally in each of Phases I, II and III as illustrated in Table 10. In Phase IV he identified both biocultural and ethical components to the focus and conduct of research.

Table 10: Elements of History of Ethnobiology (adapted from D'Ambrosio [240])

Time period	Hunn Phases	Character of era D'Ambrosio	Some Academic Fields
Late 19 th C – 1940s	I	Ethnography Cultural Anthropology Uses of biota	Ethnography Economic Botany
1950s-1970s	II	Cognitive and emic classification Secondary metabolites	Ethnoscience Linguistics Ethnopharmacology Phytochemistry
1970s-1991	III	Ethnoecology Ethnopharmacology	Ecology and conservation Traditional Ecological Knowledge Bioprospecting
1992- present	IV	Indigenous Ethnobiology Bioculture Ethics Reflexivity	Indigenous rights Biocultural Diversity Research Ethics Global Change

Ethnobiology has, thus, historically had a significant focus on ethnomedicine (EM). The main emphasis within EM research from its inception has been on finding new drug leads in what Reyes-García [76] called the “romance of ethnopharmacology”. The interest in mining medicinal plants for economic purposes dates back to the colonial period [52]. This search for new drugs has resulted in the prominence of Phase I-type research in Hunn's classification where medicinal species and their therapeutic uses are documented [66, 129].

3.1.1 Aims and Objectives

The aim of this chapter is to analyse ethnobotanical and bioprospecting research through the lens of the medicinal species, *C. spinarum*, in the context of broader critiques of such research.

The specific objectives within this aim are to:

- examine the botanical and ethnobotanical aspects of *C. spinarum* relating to distribution and nomenclature
- analyse the presentation of emic or insider's perspectives in relation to *C. spinarum*
- conduct a fine-grained analysis of the available indigenous knowledge regarding the cultural role of *C. spinarum* across the reported domains with a particular focus on the ethnomedical domain
- apply a cross-cultural focus to compare all reported cultural domains with a particular focus of the ethnomedical domain
- analyse the data from the late 19th Century to the present using the framework of Hunn's phases of ethnobiology

Finally, this research questions whether this analytical approach to the literature can inform future research on *C. spinarum* and other medicinal species with substantial associated biocultural knowledge?

3.2 Methods

A literature search in Ireland relating to *C. spinarum* accessed PubMed, Science Direct, Google Scholar, Scopus, library databases, medicinal plant databases, books, theses, grey literature including conference proceedings, workshop reports, manuals and non-academic books. Hand searches of bibliographies of journal articles were conducted, authors were contacted for further detail on their research, and I visited the library in Kew (London) for material that was otherwise difficult to access. Primary research was used for the most part. In some cases, secondary sources were used when the primary data could not be traced back through the bibliography. The primary research included broad ethnobotanical, human ethnomedical, ethnoveterinary and ethno-dietary studies. Some broad studies had a particular focus such as conservation, taxonomy and economic botany. The secondary research included reviews, books and reference books. Microsoft Excel was used to organise the ethnobotanical data. The pivot chart function in Excel was used to analyse some data elements.

3.3 Botany of *C. spinarum*

3.3.1 Description and distribution

The species *C. spinarum* (Apocynaceae) grows as a thorny highly branched evergreen shrub or small tree of up to 6m tall with evergreen leaves and masses of fragrant white flowers tinged with pink/purple. Fruits are green, then red ripening to purplish black and are edible when ripe [241] (Figure 26).



Figure 26: *C. spinarum*, by SAplants, CC BY-SA 4.0 via Wikimedia Commons

C. spinarum is among the most widely distributed of all Apocynaceae species [242]. It occupies a range of habitats from wet or dry forest, to thickets and savannahs, at altitudes of 0-2450m though it is not found in very wet climates or in equatorial rainforests [242]. Its broad ecological tolerance is reflected in the geographic distribution of botanical and ethnobotanical samples. *C. spinarum* is found in 64 countries and territories across 3 continents (Figure 27): Australia, throughout SSA and the Indian Ocean islands, the Arabian Peninsula; and in countries of South, East and Southeast Asia.

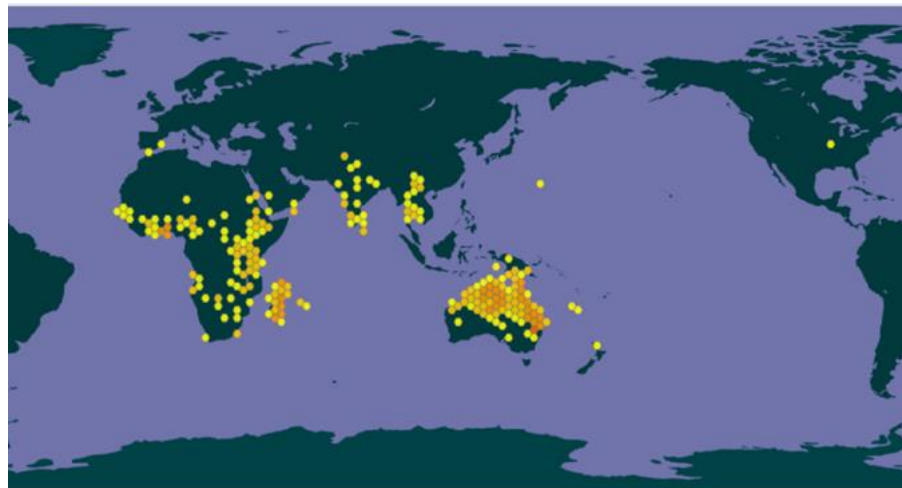


Figure 27: *C. spinarum* in GBIF Secretariat [243]

3.3.2 Botanical nomenclature and synonymy

C. spinarum is the currently accepted name of the species. It is listed in World Flora Online [244] (formerly The Plant List) as having 98 synonyms. The genus *Carissa* is described as having significant inter- and intra-species variation [245] and this, along with its wide geographic distribution, may contribute to its numerous botanical synonyms. While all listed synonyms were included in the literature search, the key synonyms that appear in the ethnobotanical literature

are *C. spinarum* (accepted name), *C. edulis* (Forssk. Vahl.) (SSA), *C. opaca* Stapf ex Haines (India and Pakistan), *C. congesta* Wight (India), *C. xylopicron* Thouars (Mauritius) and *C. lanceolata* R.Br. (Australia). As a heterogeneous species, there are disagreements around plant identification, naming and synonymy with the added problem of misspellings. The complexities in the naming of *C. spinarum* can carry through in all subsequent study findings as described in best-practice documents [77, 246, 247].

3.4 Ethnobiology of *C. spinarum*

This section will analyse the available indigenous knowledge regarding the cultural role of *C. spinarum* across the multiple reported domains. It begins with the ethnobiological distribution and nomenclature of the species. It then examines how the relationship of people and plants has been explored through qualitative and quantitative means. Specific cultural domains of use are examined, with a particular focus on ethnomedical use. The data on which this analysis is based is available in Supplementary file 4.

3.4.1 Ethnobiological distribution and nomenclature of *C. spinarum*

C. spinarum has a wide ethnobiological distribution. Overall, a total of 284 documents were reviewed for this study of the ethnobiology of *C. spinarum*. There are ethnobotanical reports from 35 countries across 3 continents (Figure 28). This is less than the botanical distributions and could indicate that it is not considered a useful species in the remaining 29 countries, that such research has not been conducted or that the knowledge has been lost. For the purposes of our analysis, the geographical regions have been divided as follows: Eastern Africa (Kenya, Tanzania, Rwanda, Uganda, Ethiopia); Southern Africa (Angola, Botswana, Mauritius, Madagascar, Mayotte, Malawi, South Africa, Zimbabwe); Central Africa (Chad, "Congo Belge" and "Ruanda-Urundi", DRC, Burundi); West Africa (Benin, Côte d'Ivoire, French West Africa, Ghana, Guinea, Mali, Nigeria, Senegal, Togo); Northern Africa (Sudan); Arabian Peninsula (Saudi Arabia); Eastern Asia (China); Southeast Asia (Indo-China – Vietnam) and South Asia (India, Pakistan). The proportional geographical distribution of these studies is illustrated in the chart in Figure 29 showing that most of the research has occurred in Kenya, Ethiopia and India with Uganda, Pakistan, South Africa, Tanzania and Australia being proportionally in a second tier of the data. The Kew Medicinal Plant Names Service [248] lists 162 common names. In this study, the most common vernacular names found were *Agam* (and variations - Ethiopia), *Muyonza* (and variations - Kenya), *Legetetwo* (and variations – Kenya), *Lamuriei* (and variations – East Africa), *Olamuriaki* (and variations – East



Figure 28: Map of ethnobotanical distribution of *C. spinarum* (courtesy of Robbie Kelleher)

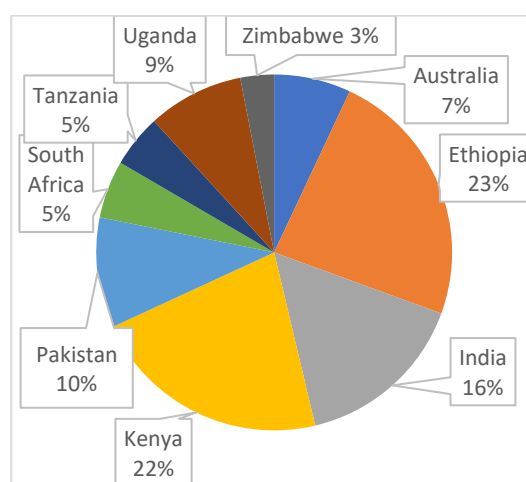


Figure 29: Proportion of Documents per country (>6= 80% of total) citing ethnobotanical use of *C. spinarum*

Africa); *Bois drone* (Mauritius), *Konkerberry* (and variations - Australia), *Currant bush* (and variations - Australia), *Gara-asur* (India), *Karamarda* (and variations India) and *Karaunda* (and variations – India and Pakistan).

3.4.2 Some emic perspectives on *C. spinarum* ethnobiology

Anthropology has historically been an integral part of ethnobotanical investigations dating to the colonial era. The emic approach in anthropology came to the fore in the 1950s whereby the researcher attempted to experience and interpret the culture from within, from the insider's perspective. In ethnobiological research, techniques to gain the insider perspective are used in order to understand and interpret the relationship of people and plants. In ethnomedical research, for instance, this can take the form of understanding local illness aetiologies, concepts of health and healing, the meaning of different

elements of the healing process and the meaning attached to different plants and habitats. In Hunn's four phases, the emic aspect becomes significant in Phase II though Frazão-Moreira illustrates clearly the ongoing centrality of ethnographic research in ethnobiology [249]. An anthropological perspective in ethnobotanical and ethnomedical studies has been considered vital for decades [74, 77, 142, 246]. This view can give valuable context for the medicinal use of plants and can help direct further study and practice in many fields such as health care provision, sustainable plant use, environmental protection and bioprospecting. There is very little of the insider's understanding available in this analysis on *C. spinarum*. Of all primary ethnomedical studies involving *C. spinarum*, 75% have no contextual information at all. Some 25% of studies, provide, usually minimal, emic information with short descriptions of selected aspects of local traditional medicine such as causation and diagnosis, specific illness understandings and descriptions, knowledge distribution and transmission and information on the place of plants in healing [121, 250-264]. However, a limited view on how this species has a role in health and healing in some communities is presented in research from Australia and Eastern Africa.

3.4.2.1 Australia – “All trees are good for something”

The phrase “*Boonyja Bardag Gorna* - All trees are good for something” is the title of the book authored by Esther and Sandy Paddy and Moya Smith [265] and is an illustration of the centrality of biocultural knowledge to life for the authors. Attempts to record that knowledge are ongoing in parts of Australia.

There are elements of Aboriginal biocultural knowledge that makes Australian ethnobiological research different to that conducted in other cultural contexts. These elements relate to the historical context, cultural norms relating to biocultural knowledge, knowledge transmission and norms around plant usage. *C. spinarum* is a widely distributed species in northern Australia [266]. However, there is relatively little ethnobotanical data in comparison to the high level of botanical sampling. This might be explained by the fact that significant ethnobiological research in the area is relatively recent, being concentrated in the past 35 years with the greater part of that local ecological knowledge already lost (Glenn Wightmann, Ethnobiologist, Northern Territory Government pers. comm 2020). Most of the research analysed in relation to Australian Aboriginal ethnobiology is not published in online academic journals but is mostly in the form of Botanical Bulletins. The research is collaborative with community members as primary authors and is published for the purposes of transmission of biocultural knowledge, a cultural requirement for Aboriginal Elders [267]. In research with Wik peoples in Queensland, Edwards *et al.* [268] have described how ethnobotanical research methods need to be culturally appropriate. They

stated that for Aboriginal Communities with whom they have conducted research, widely used ethnobotanical methods such as those described by Cotton [269] and Martin [142] are not appropriate in this environment. Factors contributing to this include: categories of public and secret biocultural knowledge, inappropriate questions and inappropriate questioning. Researchers need to be aware that only selected people may speak on behalf of “country” by law. In this analysis of the ethnobotany of *C. spinarum*, the reality of public and secret knowledge is described by several authors [267, 270-273]. These studies highlight the fact there is a vast amount of knowledge of Aboriginal medicines including gendered knowledge, which is secret, culturally sensitive and cannot be shared in publications.

In a large study of Australian Aboriginal medicinal plant usage, Latz [274] found that, in contrast to western medicines, fewer than 10% were taken internally and that about 70% were used as a wash or an ointment. The use of fumes from heated plant parts was also common in Australia especially for children in a process known as “smoking” [274-276]. These general findings are reflected in the research on *C. spinarum* in Australia. The most common routes of administration were fumigation and external application. Latz [274] stated that most important species have several uses. Connelly and Wallis [275] added that there is knowledge of all of the different elements of a plant and how they may each be used in different ways. In these Australian studies, *C. spinarum* was used medicinally and ritually but was also included in diet, as an insect repellent, for carving and for firewood. This multifunctionality of plant species is seen across the global range of *C. spinarum*. The possibility of multiple uses of species recorded in ethnobotanical research may be important to bear in mind when asking why a particular species may be chosen by local people. It’s very multifunctionality may be a central reason for its choice as a medicinal species. While emic description of Australian aboriginal EM is provided in some documents, as elsewhere, detailed anthropological information in relation to *C. spinarum* in particular is limited. McDonald [277], in an anthropological study in the East Kimberley region on concepts of health and illness, described the “smoking” process: Aboriginal peoples “smoked” the bodies of children and adults with medicinal plants in order to clear the body of “rubbish” – debris that can clog the flow of *birlirr*, the body’s life-force. In the study community, *C. spinarum* was an excellent smoking medicine [277]. Aromatic leaves were believed to be strong medicinally as the smoke could be felt entering the body through the senses and the skin:

*You can feel the jiluwa working, feel the blood flowing
[during a smoking treatment]. I was heavy before, just like
sin. I’m fresh and light now. We can feel the medicine*

*going into us. Feel cold coming through the body. When we
take gardiya tablets, we don't feel anything.*

*Gija woman, [277] p. 90. [Note: Jiluwa are body channels,
gardiya tablets are whitefella medicine or pharmaceuticals.
Coldness is a healthy state].*

For Aboriginal peoples, bush medicine is considered to have been in the ground from the Dreaming time, placed there by the ancestors. This elicits trust in users of bush medicine. *C. spinarum* is included in some Dreaming stories and thus is part of Aboriginal cosmology, which links plants, animals, humans, and places, past and present [278]. The inclusion of this and other aspects of the social and natural environment in these stories indicate a cultural importance that extends beyond the utilitarian realm.

The loss of biocultural knowledge is a well-established phenomenon in the ethnobotanical literature in general [279, 280]. Latz [274] and Wightmann (2020 pers. commun.) described the loss of many aspects of traditional Aboriginal knowledge due to changes in the way of life and the influx of new diseases. Other authors described how many of the plant species and the practices involving them were still in use by many adults as “domestic” medicine and need to be documented [272]. The value of the publication of the Dalabon biocultural knowledge from North Australia was asserted by Bordulk *et al* [267]. Given that traditional modes of transmission of knowledge relating to native species, from elders to the next generation, are being interrupted through the lack of access to sacred sites and ceremonial practices, some elders felt that the transmission of knowledge through publication was a way of fulfilling their duty.

3.4.2.2 Evil Eye in Ethiopia

Spirit-related illness, usually *Buda* (Evil Eye) was commonly reported to be treated with *C. spinarum* in Ethiopian studies and was often used in combination with other species in the form of fumigation. Literature shows that there is a wide geographical and historical spread of Evil Eye. It was referenced in Classical Greek literature, the Bible, Islamic literature and was reported among some Asian peoples and in most European, African and American countries [281]. However, there was no description of the presentation of *Buda* or other spirit-related illness in any of the Ethiopian documents on *C. spinarum*. In other Ethiopian research with a sociological rather than ethnobiological focus, *Buda* has been studied. Amenu *et al*. [282] wrote that the ability to inflict Evil Eye /*Buda* is innate and that the identity of people who can inflict *Buda* is secretly known within a community. Abbink [283] wrote that the Amhara people of Ethiopia believed *Buda* to be held by people outside of the Amhara group, who were

perhaps landless or with no permanent home or people of certain professions such as blacksmiths or potters. They had a hereditary, malevolent power which was used, perhaps involuntarily, to cause harm. He wrote that the victims of *Buda* became weakened with a sense of being drawn to their death. *Buda* was described as causing many animal and human health problems as well as damage to property and was commonly treated by ritual [282-285]. Jacobsson [286] wrote that it may cause any kind of illness including neuroses and psychosomatic illness, though it often manifested as GI disorders. Jacobsson argued that there is no clear distinction between physical and mental disorders, and consequently there is none between associated healing methods. Thus, physical and spiritual means may be used as part of the healing process. The treatment with medicinal plants can have ritual and physical components, and the burning of *C. spinarum* in the treatment of *Buda* disorders could be interpreted in this light. The most common method of preparation of *C. spinarum* for treatment of *Buda* was the administration of root smoke by fumigation e.g., Teklehaymanot *et al.* [262]. In a few cases, the root was mixed in water alone and inhaled or taken orally or the root was tied around the neck as protection [287-289]. Ritual and physical effects may combine in the use of *C. spinarum* to treat *Buda*. In an examination of ritual plant use in Benin and Gabon from a BM perspective, Quiroz *et al.* [290] illustrated that ritual use does not imply a lack of pharmacological activity such that pharmacological activity of the smoke of, say, *C. spinarum* may augment the socio-cultural healing benefits of the ritual components of healing.

The example of spirit-related illnesses illustrates that an understanding of the context of healing, can help to situate the use of plants in the healing process and to interpret it from a cross cultural perspective.

3.4.2.3 Kenya –Luo health-seeking behaviour

There are two anthropological studies from Kenya, whereby the context of *C. spinarum* use was provided through in-depth emic information in relation health and illness in the study areas. The Pokot study conducted in 1980/81 [89] has already been described in section 2.1.3. In 2002, Geissler, an anthropologist, conducted an ethnomedical study with the Luo of Western Kenya [91]. There are other Kenyan ethnomedical studies referencing *C. spinarum* with limited emic information [87, 119-121, 164, 165, 291-294].

The ethnobotanical research of Geissler *et al.* [91] with Luo mothers was conducted with the backdrop of the authors' earlier extensive anthropological research with the Luo of western Kenya and historical Luo anthropological data. It is an instance of rich emic understandings presented in this analysis of *C. spinarum*. As with the Australian example above, there is an amount of secret

knowledge among the Luo mothers, especially in the treatment of more complex ailments resulting in lacunae in the recorded knowledge. Their research found that GI illnesses were the most commonly treated with medicinal plants while fever and headache were rarely treated using plant medicines despite the high prevalence of malaria in the study area. The authors attributed this to the availability of pharmaceuticals in local shops to treat these symptoms. The extent of secret knowledge and the availability of effective pharmaceuticals could result in an overall skewing of the ethnobotanical findings towards GI use. Illness itself was considered to be a constant element of life and plant remedies were used, not to eliminate illness but rather to find a balance in a constant process. In the case of helminth infections, worms were considered to live permanently in the gut and it was important to maintain worms in a healthy equilibrium in the gut for the health of the individual. Treatment of other illnesses, such as diarrhoea, required the illness to be removed from the body with purgatives. This concept of healing also finds the BM healing by suppression of illness to be unhealthy such that sometimes the use of BM was prohibited in the pursuit of health:

Luo medicine makes the illness come out; injections push it back inside. If you inject, it moves through the body and you swell and may die (Luo mother, Geissler et al. [91] p.44)

Many illnesses including diarrhoea were thought to be caused by *Yamo*, an overarching illness concept that could manifest in many ways but was treated through eliminating it from the body. The constant presence of illness in the body, even when healthy, required that a child be constantly treated to make illness emerge from the body and as such keep the forces of *Yamo* and other forces of illness at bay.

A (young) child ought to be ill regularly (nyathi onego otuore) lest it would die, once the illness (i.e., Yamo) finally comes out (Luo saying, Geissler et al. [91] p.44)

In their research, Geissler *et al.* [91] reported the use of *C. spinarum* (Ochuoga) in the treatment of diarrhoea and *Ang'iew* (a childhood febrile illness with rash). While no individual species was singled out as culturally important, the presentation of in-depth emic understandings of health and illness described in brief here gives valuable context to the listing of 91 medicinal species. From a BM perspective, such as researching health-seeking behaviour or bioprospecting, it is important to understand the socio-cultural understandings of health and illness as a backdrop to the choice of treatments. The use of *C. spinarum* as a purgative to treat malaria, for instance, does not imply that it is ineffective as an anti-malarial but the knowledge could modify the perspective of

the bioprospecting ethnopharmacologists. For BM healthcare workers, the knowledge of local purgative and emetic practices is important in providing care to the communities served.

3.4.3 Ethnobotanical uses - *C. spinarum* in human and animal medicine

EM analysis of *C. spinarum* took into consideration the following basic elements: plant parts used, method of preparation, route of administration and ailments treated, followed by analysis of the use of different plant parts for different ailments. Plant harvesting details were searched but were only reported on in three studies out of a total of 284 documents and 513 URs analysed. URs are the number of studies reporting any use of a species. Each of these aspects of ethnomedical plant use bears a relationship to the phytochemistry, bioactivity and toxicity of plant extracts which then have a bearing on the outcomes of ethno-directed bioprospecting studies. The global geographical variation in usage of *C. spinarum* and the ethnoveterinary use of *C. spinarum* were also examined.

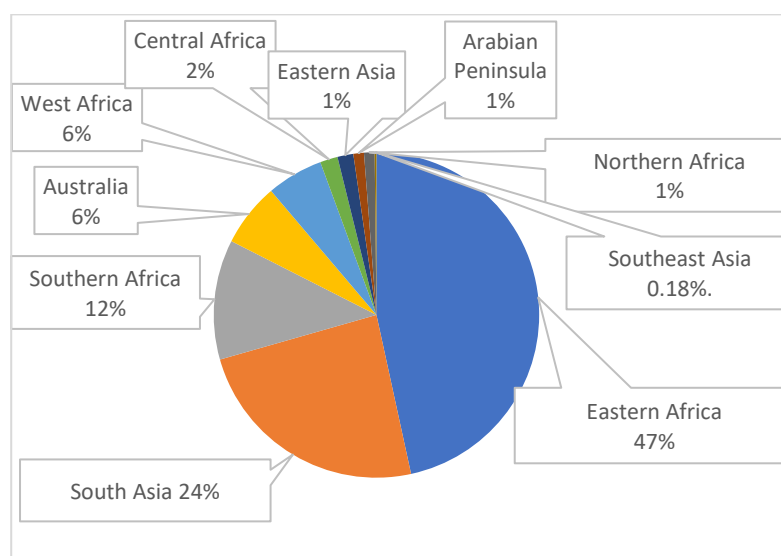


Figure 30: Geographical distribution of human ethnomedical URs for *C. spinarum*

The geographical distribution of the ethnobotanical data is illustrated in Figure 30. The key areas reporting the ethnomedical use of *C. spinarum* are Eastern Africa (mainly Kenya and Ethiopia) and South Asia (mainly India and Pakistan). *C. spinarum* is also used across the rest of SSA, Northern Africa and the Arabian Peninsula, East and South East Asia and Australia. There is very little available data on *C. spinarum* use in TCM.¹¹

¹¹ The only listing was in the National Herbal Compendium, Wan 1977 ISBN 14048-3571. Personal communication with David Middleton, Singapore National Botanic

3.4.3.1 *C. spinarum* in human ethnomedicine: plant parts used

A summary of *C. spinarum* plant part used in medicinal preparations for human use across all regions is presented in Figure 31. It illustrates that, for *C. spinarum*, the primary plant part used was the root followed by leaf, fruit, rootbark, stem and bark while 9% of reports did not state the plant part. In general, where the root was used, it was not specified which part. Where rootbark was stated, this was analysed as a separate category in the data. In a few instances in Australian data, more detail was given. The inner orange root core was used, or the root was scraped (Supplementary file 6) [272, 275]. The data was analysed across 5 key geographical regions: Eastern Africa, Western Africa, Southern Africa, South Asia and Australia.

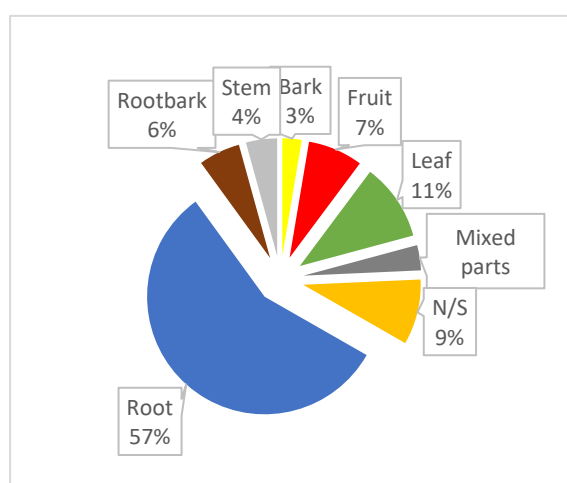


Figure 31: *C. spinarum* for human use: plant parts used in the preparation of EM

The summary of key plant parts used is illustrated in Figure 32 and shows that, at regional level, the root was still the most commonly used plant part. However, in Eastern Africa, the predominance was more marked where the root and rootbark together accounted for three quarters of all URs. Fruit and leaf use were minimal by comparison. In Western Africa, the root and rootbark together accounted for most usage with leaf, fruit and stem used infrequently. In Southern Africa the root accounted for over half of usage with lesser usage of stem and other parts. In South Asia, root use was less dominant and followed by leaf and fruit. In Australia, the different plant parts used were more evenly divided

Gardens and Apocynaceae author stated that he could find no reference to usage of this species in the Asian taxonomic literature (7/2016). Personal communication with Christine Leon, National Botanic Gardens, Kew stated that she could find no reference to use in TCM literature. She said that it could be used in Chinese folk medicine rather than in official TCM and thus not show in her records (7/2016).

between root, stem and leaf. In most areas a proportion did not state what plant part was used, particularly in Southern Africa.

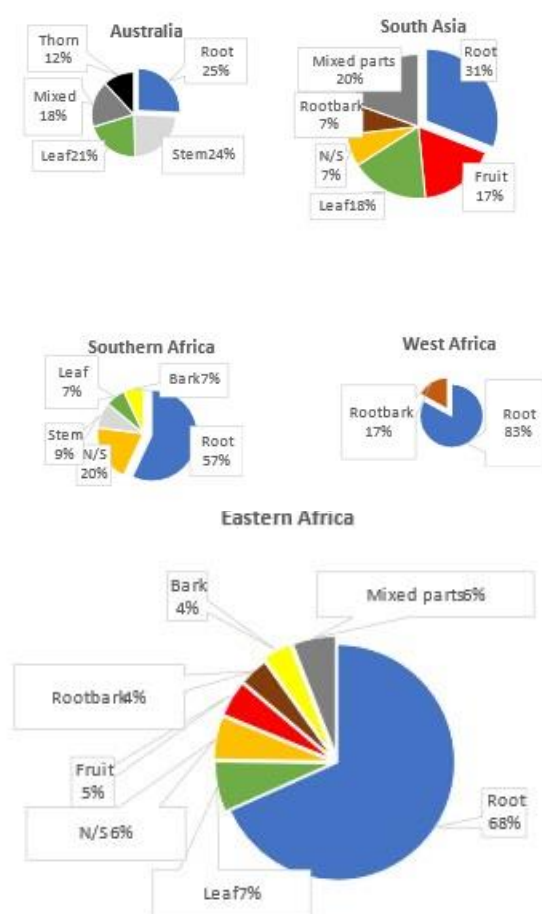


Figure 32: *C. spinarum* and variation in plant part usage across global regions (UR>3, 85% of all URs; N/S = Not Stated; chart areas approximately reflect relative nos. of URs)

More broadly in ethnomedical research, there are a number of reasons hypothesised for differences in the choice of plant and plant part such as availability and related cultural norms, the presence of bioactive metabolites, organoleptic qualities, appearance, time of year, apparency and location of a species. In describing the most commonly used plant parts in East Africa, Kokwaro [122] stated that with trees and shrubs, the bark and root were more commonly used. Timberlake [87], in a study with the Pokot people in a semi-arid region of Kenya, found that most medicinal plants are trees and shrubs, and their bark and roots were the most commonly used parts though no reason was given. Monteiro *et al.* [295] found that in the *Caatinga* (semi-arid) region in North-eastern Brazil, the species and plant parts that are available throughout the year are the most important species medicinally with the stem-bark being the most commonly used plant part. They reported that herbaceous plants and leaves,

which are only available for a few months of the year, were less commonly used in the same study. The authors examined tannin concentration in two medicinal species, assuming tannins to be responsible for much of the medicinal activity though they acknowledge that this may not be wholly correct. They found that both leaf and stem-bark contained significant quantities of tannins although the stem-bark is the part used medicinally. They postulated that the community develops a stronger relationship with the perennial woody plant parts regardless of the fact that the leaf may be as effective as the stem.

Organoleptic qualities of taste, smell, colour and texture were reported by Etkin [74] to be important to decision making around medicinal plants and were linked to explanatory models of illness. In a study in Peru, Shepard [296] illustrated how sensation was at the centre of medicinal plant selection connecting culture and the environment. Medeiros *et al.* [297] found that taste and smell were associated with therapeutic indications in a community in Brazil. Studies in Mexico indicated a similar connection between taste, smell and therapeutic indication where, for instance, bitter and aromatic plants were valued in the treatment of GI disorders [298]. Nyamwaya [89] found that among the Pokot, bitter medicines were considered to be more effective at treating all illness. Sensory attributes of plants were, likewise, seen as an important indicator of healing power among the Suiei Dorobo of Northern Kenya [165]. The “doctrine of signatures” may be a contributor to the choice of plant or plant part such that, for instance, a root with a red extract may be considered to be useful in blood disorders [74, 299].

The presence of bioactive components may also direct the choice of plant part in ethnomedicine, an assumption underlying ethnodirected bioprospecting studies [300]. Secondary metabolites may benefit plants through action as pollinator attractants, as simple feeding repellents or as toxicants to prevent herbivory, indicating bioactivity [301]. Voeks [301] stated that this phenomenon, referencing a study on the presence of alkaloids, was more pronounced in low-latitude countries and was more likely in herbs and shrubs. Albuquerque *et al.* [300] reported that plants invest more heavily in phenolic secondary metabolites based on a several Brazilian studies including in the semi-arid Caatinga and the dry forest Cerrado. They referenced Gottlieb and Borin [302] who examined ecogeographic variation between humid forests and Cerrado. They found higher concentrations of shikimate-derived metabolites including lignans, neolignans and benzylisoquinoline alkaloids in humid forest species whereas there were more acetate-derived metabolites such as steroids, polyacetylenes, iridoids and pyrrolizidine alkaloids in Cerrado species. The authors found higher oxidative levels in humid forests due to availability of light and water leading to an enhanced shikimate pathway and a higher production of lignified biomass. In

contrast, in the Cerrado, alternative metabolites such as polyphenols predominated. Separately, Marchica showed phenolic compounds to be part of the anti-oxidant protection of the plant, protecting from environmental and internal stressors and reducing and neutralising reactive oxygen species (ROS) [303]. Albuquerque *et al.* [300], based on the pooled research findings from the Caatinga and Cerrado, found high levels of phenolic compounds in the medicinal plants of the Caatinga. The plants used are reportedly potent as anti-inflammatory and antimicrobial species. Another consideration in bioactivity and choice of medicinal plants is that intraspecies phenotypic plasticity is known to occur across different habitats in response to different environmental stressors [300]. Thus, woody species growing in semi-arid areas could have lower concentrations of bioactive ingredients in the leaf than in perennial plant parts due to the short growth-period of the leaf, thus favouring the perennial parts as medicinal ingredients.

How do these general hypotheses and findings in relation to the choice of plant parts in ethnomedicine relate to *C. spinarum*? While *C. spinarum* has a wide ecological tolerance, it can be seen from the map of ethnobotanical usage (Figure 28) that many of the areas in which *C. spinarum* were used are semi-arid. This lends credence to the use of the perennial parts of this shrub, as these are available throughout the year. Organoleptic qualities may be a factor with choice of *C. spinarum* root. It is somewhat bitter in taste which, as described above, may lead to its being considered medicinal [226, 304]. The root has an oily, volatile sap with a pleasant smell which lends itself to use as a rub for chest and muscle conditions and as an inhalant. *C. spinarum* is a member of the Apocynaceae family, a family which is known to produce toxic metabolites. The choice of plant part may be related to the avoidance of toxic components in other plant parts. Omino, Kokwaro [305], in a review of the ethnobotany of Apocynaceae species (25 in all) in Kenya, found that the root is the most commonly used plant part for medicinal use (63%). In contrast, Bhadane *et al.* [306], found that there is no particular preponderance of root use in a review of some medicinally important Apocynaceae species. Rather, ethnomedical use in different species was found to be generally distributed across plant parts. This correlates with the finding in India and Pakistan where the plant part usage of *C. spinarum* is more evenly distributed across plant parts than in Eastern Africa. This difference could point to factors such as availability of plant material, cultural norms around medicinal preparations or ecological differences across its range leading to different secondary metabolite patterns.

3.4.3.2 *C. spinarum* in human ethnomedicine: methods of preparation

The predominant methods of preparation of *C. spinarum* globally are summarised in Figure 33. In the data as a whole, where a method was given, it could be simply written as a single word such as decoction, infusion, paste, juice or raw. There was relatively little detailed description of the preparation of *C. spinarum* in the studies analysed. In the reviewed studies, over one third did not to include a method of preparation. The preparation of medicinal products by

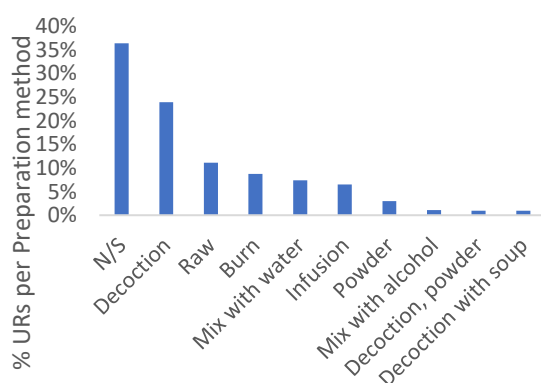


Figure 33: Methods of preparation of human EM containing *C. spinarum* (UR>4)
 ("Decoction, powder" where unclear which method used per ailment)

decoction i.e., boiling of the plant part in water for a length of time, was by far the most common method reported followed by use of the untreated plant, burning for smoke, cold water mixture, infusion, powder, juice, decoction with soup and mixing with alcohol (4 citations).

Methods of preparation varied across the geographical range represented in Figure 34. A significant proportion in each geographical area, except Australia, did not state the method of preparation. In South Asia, Eastern Africa and West Africa, decoction was the most common method used. In Southern Africa, infusion was the most popular method of preparation. In Australian studies the most common method was burning followed by decoction and untreated. Burning as a method of preparation globally was relatively high. When we examined the data in relation to burning, it was apparent that this was greatest in Ethiopia and in Australia with the emic context described above in section 3.4.2. In Ethiopia the primary illness category where burning was used was *Buda* (Evil Eye) while in Australia burning was used in the main as a treatment to protect children by "smoking"[277].

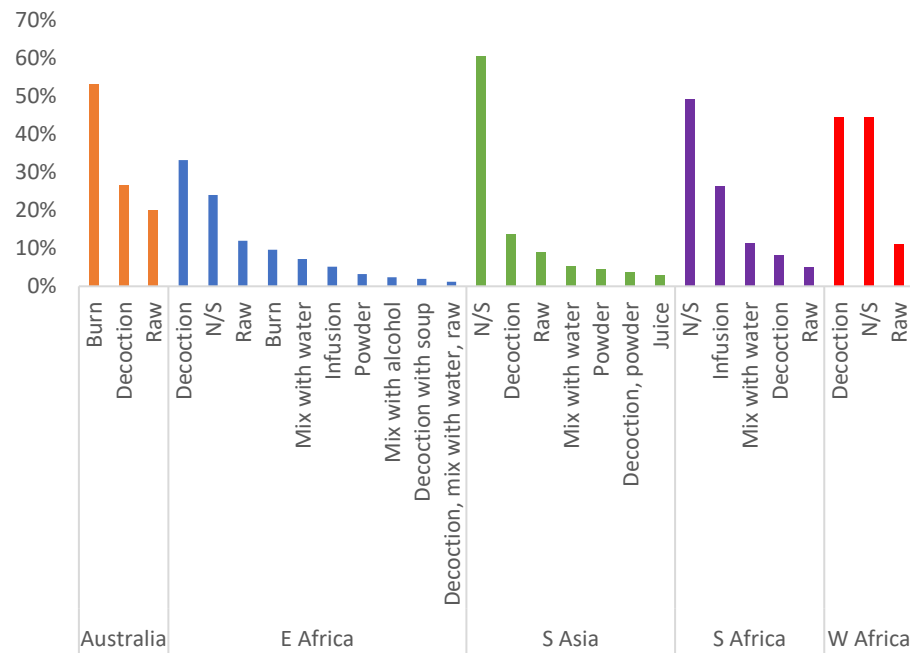


Figure 34: Regional variation in method of preparation of *C. spinarum* expressed as % URs per region (UR > 2). Methods with low occurrence are excluded.

3.4.3.3 *C. spinarum* in human ethnomedicine: key methods of preparation x key plant parts used

It is useful to take a closer view of the methods of preparation that are used for

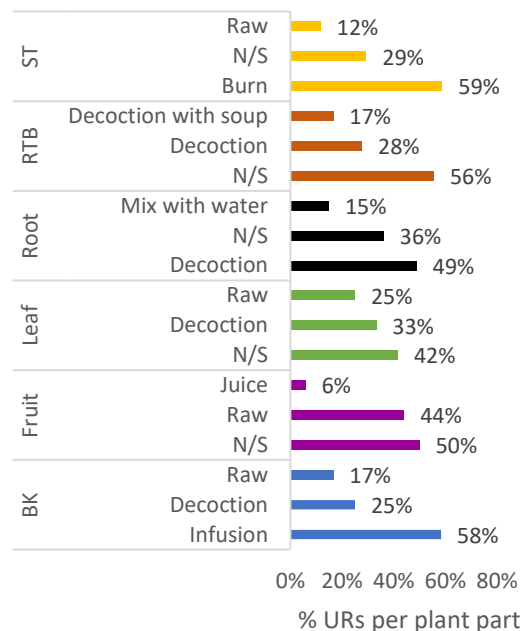


Figure 35: Salient methods of preparation for each plant part (Top 3, excludes combinations; 54% of total URs) (BK= Bark; ST = Stem; RTB = Rootbark)

each plant part. The chart in Figure 35 summarises the most common methods of preparation for each plant part, while the correlation with route of

administration is examined in the following section (3.4.3.4). The chart shows that the fruit was most often used relatively untreated; the bark was most often prepared by infusion; the leaf, root and rootbark were each most commonly prepared by decoction and the stem by burning.

Where fruit was treated, the minimal treatment is illustrated as follows:

Fruits are crushed, dried, pounded into powder and sprayed on wound ([307] Ethiopia).

and of leaf:

Leaves are crushed, squeezed and liquid taken (orally) with coffee (to treat febrile illness) ([307] Ethiopia).

Simple decoctions were prepared as follows:

Among the Mitakoodi, it [root] is scraped and soaked in water and then boiled. The resultant liquid is drunk as a tea to treat aches and pains and used as an eyewash" ([275] Australia)

Leaves are boiled in water and liquor is used to treat jaundice [308] Pakistan

Sometimes, additional ingredients were added as part of the treatment prescribed:

Rootbark is crushed and mixed with black pepper and given (for typhoid fever) [309] India

Fresh root of C. spinarum pounded mixed with cold water. One cup of tella [beer] is used as a drink for three days [to treat gonorrhoea] [260] Ethiopia

Sometimes, other medicinal species were added to make a concoction as in the following example:

Root barks [of C. spinarum] are mixed with dry stem bark of Mtanga (Spirostachys africana Sond.) boiled and a tea cup of the concoction is drunk three times daily (to treat cancers) [213] Tanzania

A bark infusion was described as follows:

Grate the bark and prepare an infusion, drink 1 cup 1–2 times per week” (to treat prostate disease) [304] Rodrigues Island.

The infusion rather than decoction of the bark may relate to the relative ease of extraction of metabolites from the bark rather than the harder root. While the leaf was most commonly prepared by decoction, it was also used fresh in a relatively untreated form such as by squeezing, illustrating that the metabolites are relatively more accessible than from roots and bark.

The method of preparation of medicinal plants can have a significant influence on the extraction of active constituents. This can be seen in Chinese Herbal Medicine (CHM) whereby a variety of processing methods may be used to improve efficacy and/or to reduce toxic effects [310]. Extraction methods were described by Sheridan *et al* in the preparation of CHM with special preparation instructions that relate to different physical, chemical and pharmacological activities of active compounds [311]. The authors described the preparation of the two-herb formula, *Danggui Buxue Tang* (DBT), illustrating the variability in yields of marker compounds with duration of boiling, the drug/solvent ratio, the number of extractions and co-extraction vs individual extraction of each plant. In relation to *C. spinarum* preparation in the studies analysed, the level of detail seen in CHM research is not provided. However, the example of DBT illustrates the importance of accurate data recording in ethnomedical studies. The use of decoction or maceration for root preparation, as seen with *C. spinarum*, can be seen to aid extraction of active compounds in harder plant materials such as roots, bark and stem [312]. It may also have a role in reducing the toxicity of phytochemicals or plant extracts given that *C. spinarum* belongs to the Apocynaceae family, known to produce toxic metabolites. In bioprospecting, detail on ethnomedical preparation can be a crucial guide for ethnopharmacologists in their investigations [311]. Detailed ethnographic descriptions on preparation give a clearer view of the elements of the healing process as a whole which has relevance across other research foci such as cultural record, health care provision and sustainable plant use [77].

3.4.3.4 *C. spinarum* in human ethnomedicine: route of administration

The oral route was the most commonly used route of administration globally for *C. spinarum* (Figure 36) while external application, fumigation and inhalation were the most commonly used routes otherwise. This was true across all geographical areas except Australia (Figure 37). However, the route was not specified at all in 40% of URs.

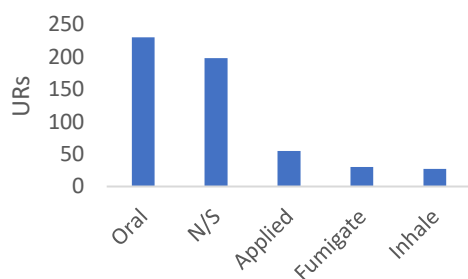


Figure 36: Most common routes of administration of *C. spinarum* globally (Top 5)

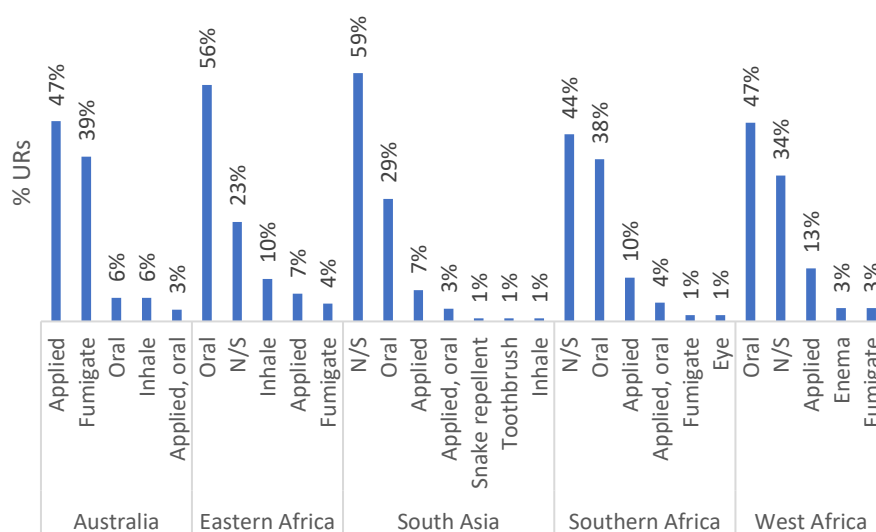


Figure 37: Key routes of administration of *C. spinarum* (by %) in different geographical regions (URs > 9)

Table 11 lists the primary methods of preparation and route of administration for each plant part. The table illustrates that the root and rootbark were most commonly prepared by decoction and administered orally. Where the root or rootbark were used untreated or mixed in cold water, it was most commonly applied externally, e.g., as a poultice or a paste. The fruit usually underwent no preparation and was administered orally. The stem was burned and used as a fumigant or inhalant. The leaf was most commonly burned as an inhalant although overall it was most commonly taken orally, whether decocted or untreated. The prevalence of burning and inhalation for all plant parts relates to the importance of Evil Eye in this analysis of *C. spinarum* as described above (sections 3.4.2.2 and 3.4.3.2).

The oral route of administration is common in EM, as in BM [139, 313]. This may be explained by factors such as safety, convenience, cost and acceptability [139, 140]. Route of administration may also be related to the ailments treated. *C. spinarum*, as we shall see below, was commonly used to

Table 11: Most common route x preparation x plant part in *C. spinarum* used in ethnomedicine.

Plant part	Preparation	Route	URs
Root	Decoction	Oral	70
Root	Burn	Fumigate/ Inhale	21
Root	Mix with water	Oral	18
Root	Infusion	Oral	17
Fruit	Raw	Oral	15
Root	Decoction	Applied	9
Stem	Burn	Fumigate/ Inhale	9
Root	Mix with water	Applied	8
Root	Raw	Applied	8
Leaf	Burn	Inhale	8
Bark	Infusion	Oral	7
Leaf	Decoction	Oral	7
Root	Mix with alcohol	Oral	6
Root	Powder	Oral	6
Leaf	Raw	Oral	5
Root	Raw	Inhale	4
Leaf	Raw	Applied	3
Root	Powder	Applied	3
Root	Raw	Oral	3
Rootbark	Decoction	Oral	3
Rootbark	Decoction with soup	Oral	3
Leaf	Decoction	Applied	2
Leaf	Raw	Inhale	2

treat GI ailments and oral administration would thus be logical. In addition, some theories of health and illness in the study areas necessitated the oral route of administration where emesis or purgation was required [91]. The choice of route could be related to the bioactive metabolites present. It was asserted by Le [140] that some phytochemicals such as alkaloids may be more active orally whereas terpenoids may be well absorbed through external or nasal administration. In some cases, a mixture of routes was used for one treatment. In the following example oral and topical routes were combined for the treatment of syphilis:

Infusion (of root) drunk in malwa, sitz bath (Tabuti et al. [314] Uganda; malwa is local beer made from Eleusine coracana).

In Tanzania, for the treatment of chest conditions, oral and topical use were combined whereby the crushed root was scraped and the pulp used as a poultice

while a root extract was drunk at the same time [315]. In Uganda, epilepsy was treated using a combination of internal and external by drinking the powder and bathing and smearing the body at the same time [316]. The case of treatment of epilepsy may be related to the condition itself and ease of administration. In a study by Tabuti *et al* [314] in Uganda, of eight different species used to treat epilepsy, most were applied in some manner rather than taken orally. Methods used included smearing, bathing, instilling into eye or nostril, fumigating, inhaling or licking. Use of non-oral routes could be a reflection of the practical difficulty of administering orally if a person is having a seizure, or may be a reflection of a ritual component of treatment of a complex illness, or both [290].

The multiplicity of plant parts, methods of preparation and associated routes of administration may have their origins in cultural as well as practical, efficacy and acceptability reasons. However, from a phytopharmacological perspective, each has different profiles, an issue which will be addressed in Chapter 4.

3.4.3.5 Human ailments treated using *C. spinarum*

This section will first outline the form of classification used for this analysis on human ailments treated. The classification is then applied to the medicinal usage of *C. spinarum*.

The WHO-ICPC system (International Classification of Primary Care) of illness classification was developed for the biomedical primary care setting as a patient centred model of classification [317]. The ICPC template is promoted by Staub *et al*. [117] and Weckerle *et al*. [77] to classify certain ethnomedical studies which often lacks precise biomedical diagnoses required of the ICD system. This analysis of *C. spinarum* utilises the ICPC classification system for cross-cultural comparison of ethnomedical usage. This is a bi-axial system of Body Systems and Ailment Categories. Modifications of the ICPC system are made to both axes to reflect salience in the *C. spinarum* data of particular body systems and ailments. A summary of the overall classification system is presented in Table 12. Some Body Systems listed in Table 12 are self-explanatory. The General and Unspecified grouping is often used where the ailment relates to the whole body such as malaria or other infectious diseases/symptoms. The *C. spinarum*-data-specific classifications of Spirit-Related and Ill-Defined are created to reflect their prevalence in the data, with the former including Evil Eye, bewitchment, sorcery and evil spirits. The classification of Ill-Defined is created to reflect ambiguity or lack of descriptions of illness e.g., “Cold-sick” where it is not clear what this means from the text [318]. Genito-urinary ailments are frequently cited in many global studies [319] and in African *C. spinarum* related studies [122, 145, 314, 320-323]. The ICPC Body System classifications of women’s health,

andrology and urology are modified in this analysis of *C. spinarum* such that the Genito-Urinary ailments are grouped under four Body System classifications: *Women's Health*, *Genital (not gender specific)*, *Urology (not gender specific)* and *Andrology*. *Women's Health* includes the ICPC "Female Genital" and "Pregnancy, Childbearing, Family Planning and Female Reproductive System"; *Genital* includes ailments which are not gender specific and this mainly refers to STIs; *Urology* refers mainly to UTIs and *Andrology* refers mainly to virility. The ICPC modification is made so that the true prevalence of women's' health issues can be evaluated rather than splitting the data into smaller categories. The original ICPC Ailment Categories of Infection, Injury, Symptom/Complaint, Neoplasm and Other are retained while some symptoms, diagnoses and injuries are added as Ailment Categories to aid the analysis. These occur frequently in

Table 12: Modified ICPC classification for *C. spinarum*

BodySystem (Plus)	Ailment Category
Blood, Blood Forming Organs and Immune Mechanism	Infection
Cardiovascular	Injury
Digestive	Neoplasm
Endocrine/Metabolic and Nutritional	Symptom/Complaint
Eye	Other
General and Unspecified	Added Ailment
Ill-defined	Categories:
Musculoskeletal	Abdominal pain
Neurological	Asthma
Psychological	Cough
Respiratory	Constipation
Skin	Diarrhoea
Spirit-related	Evil Eye
Genito -Urinary sub -classification:	Fever
Andrology	Pain
Genital (gender not specified)	Seizure
Women's Health	Snakebite
Urology (gender not specified)	

the data and would otherwise be subsumed under a broad generic Category such as Symptom/Complaint (Table 12). The added Ailment Categories are Abdominal Pain, Asthma, Cough, Diarrhoea, Evil Eye, Fever, Pain, Seizure and Snakebite. Examples of the bi-axial classification include: amoebiasis classified as Digestion (Body System) and Infection (Ailment Category); gonorrhoea classified as Genital (Body System) and Infection (Ailment Category); post-

partum haemorrhage classified as Women's Health and Symptom/Complaint. This is a means of analytical cross-referencing of the data. The key Ailment Categories treated with *C. spinarum* are Infection, Pain, Evil Eye, Symptom/Complaint, Diarrhoea and Abdominal Pain with a more complete list of 15 Categories presented in Table 13. These Key Ailment Categories are subdivided into the key ailments within those Categories. It illustrates that while Infection is the most common Category treated, Evil Eye is the most common specific Ailment treated followed by abdominal pain, malaria and fever.

Table 13: Key Ailment Categories with Key Ailments treated using *C. spinarum* (Ailment Category UR> 5; Ailment UR>4) (61% of URs)

Ailment Category and Ailment	URs
Infection	94
Malaria	24
Genital Infection	22
Worms/other parasites	14
Infectious disease other	11
Gastrointestinal infection	10
Upper respiratory infection	7
Pain	47
Teeth/gum symptom/complaint	15
Headache	14
Joint symptom/complaint	12
Pain general/multiple sites	6
Evil eye	27
Abdominal pain	26
Fever	22
Diarrhoea	21
Symptom/complaint	20
Impotence / Sexual function - male	7
Digestive symptom/complaint other	7
Lymph gland(s) enlarged/painful	6
Constipation	17
Ill-defined	14
Jaundice	10
Cough	13
Snakebite	13
Injuries	11
Asthma	8
Spirit-related (other)	6
Total	349

C. spinarum is used across multiple Body Systems with the Digestive system predominant (Table 14). The other key Categories treated are General/Unspecified, Spirit-Related and Genital. The breakdown of key ailments treated within the Body Systems is illustrated in Table 14. In the Digestive System, the key ailments treated are abdominal pain, diarrhoea and constipation. The main ailments of the General/Unspecified Body System are fever and malaria. Spirit-related illnesses are Evil Eye for the most part. STIs are the most common ailment treated in the Genital System, joint symptoms in the Musculoskeletal System, cough in the Respiratory System, headache in the Neurological System and snakebite is the most common ailment treated affecting the Skin.

Table 14: Main Body System (Plus) with key Ailments treated with *C. spinarum* (UR>9; 54% of total URs)

Body System (Plus) and Ailment	Sum of Citations
Digestive	116
Abdominal pain epigastric	26
Diarrhoea	24
Constipation	17
Teeth/gum symptom/complaint	15
Worms/other parasites	14
Gastrointestinal infection	10
Jaundice	10
General and Unspecified	58
Malaria	24
Fever	22
Infectious disease other	11
Spirit-related	32
Evil Eye	32
Genital (gender not specified)	22
Genital Infection	22
Musculoskeletal	14
Joint symptom/complaint	14
Respiratory	13
Cough	13
Neurological	14
Headache	14
Skin	10
Snake bite	13
Laceration/cut	11
Total	307

The four dominant geographical zones with ethnomedical data for the use of *C. spinarum* in humans are Eastern Africa and South Asia followed by Southern Africa and Australia. The top 4 ailments in each of these four regions are listed in Table 15, which illustrates a diversity in ailments treated across the regions. Spirit-related illness is the most reported ailment, mainly from Ethiopia. Eastern and Southern Africa both list abdominal pain and genital infection among the most commonly treated ailments. Southern Africa and South Asia both register fever, constipation and diarrhoea among the top 4 ailments treated. Southern Africa and Australia alone list respiratory symptoms among the top

Table 15: Key Ailments in each of the Top 4 Global Regions (31% of URs)

Top 4 Ailments in Highest Use Global URs Regions	
Australia	17
Upper respiratory infection	3
Teeth/gum symptom	3
Ill-defined	3
Breast/lactation symptom	2
Health maintenance/prevention	2
Eye symptom	2
Respiratory symptom	2
Eastern Africa	85
Spirit-related	30
Malaria	20
Genital infection	19
Abdominal pain epigastric	16
South Asia	43
Fever	16
Constipation	10
Jaundice	9
Diarrhoea	8
Southern Africa	19
Diarrhoea	5
Abdominal pain epigastric	4
Worms/other parasites	3
Cough	3
Genital infection	3
Fever	3
Constipation	3
Total	164

individual ailments treated. The treatment of malaria is high in Eastern Africa relative to South Asia corresponding to current and historical relative incidence of malaria [182, 183]. The relatively low level of treatment of fever with *C. spinarum* in Eastern Africa could reflect the choice of alternative treatments such as other plant species or pharmaceuticals as suggested by Geissler *et al.* [91] or the overdiagnosis of fever as malaria, a recognised phenomenon [324]. Jaundice and hepatic complaints are mainly reported in South Asia. Given the prevalence of malaria in SSA and other contributors to liver ailments such as hepatitis, typhoid fever, dengue and leptospirosis, this is unusual [125]. Possible explanations may be that jaundice may not be common in study areas other than South Asia, it may not be treated as a symptom *per se* in those regions or *C. spinarum* may not be a chosen treatment outside South Asia such as is the case in the West Pokot data where other species are chosen for its treatment (17 URs Table 5).

Examining the Body Systems classifications illustrates more inter-regional similarity in the top 5 Systems treated (Table 16). The Digestive and Respiratory Systems are commonly treated in each region. The Digestive System is the most commonly treated System in all regions except Australia though to varying degrees. Likewise, the General and Unspecified Body System is commonly treated in each region, mainly accounted for by malaria in Eastern Africa and fever in South Asia. The Skin has a high relative importance in all zones except Eastern Africa. The Musculoskeletal System is a key System treated with *C. spinarum* in Eastern Africa whereas the Cardiovascular System is commonly treated in South Asia. In Australia, the Body Systems treated are comparable to the other regions. Study aims and objectives contribute to variability in reports of ailments treated across different regions.

The Body Systems and Ailments treated by *C. spinarum* may be a true reflection of disease prevalence in the study areas. However, in the main there is no reporting of morbidity and mortality data in the ethnobotanical research analysed which makes it difficult to interpret the research results. Some studies have a particular disease focus such as malaria, respiratory disorders, HIV/AIDS, parasitic disease and others. However, the majority of ethnomedical information included is from general studies without focusing on a particular ailment. The most common specific research focus is malaria-related from Eastern Africa [136, 184, 291, 292, 325-330]. This could account for the relatively high ranking of malaria in this analysis. Geissler *et al.* [91] find that abdominal complaints are most commonly treated with traditional medicine in the study area and relates this to the availability of pharmaceuticals for the treatment of other common ailments. An alternative explanation is presented in early research by Good [103] who found that the Kamba people in Kenya had an organ hierarchy whereby the

abdominal and reproductive symptoms were the most important. This hierarchy may be relevant more broadly throughout the Eastern Africa region and could contribute to the prevalence of digestive disorders and treatment of genitourinary ailments with *C. spinarum*. The prevalence of treatment Genito-urinary conditions is a common finding in ethnomedical studies and may be related as much to cultural treatment norms as to disease prevalence [319]. Its absence from Australian studies may relate to “secret business” not suitable for discussion with a researcher [268].

Table 16: Top 5 ICPC Body Systems (Plus) treated by *C. spinarum* across 4 Geographical Zones (68% of total URs)

Region and Body System	URs	% of Total regional URs
Australia	18	50% (total 36)
Respiratory	6	17%
Skin	4	11%
General and Unspecified	4	11%
Digestive	4	11%
Eastern Africa	154	56% (total UR 274)
Digestive	52	19%
General and unspecified	50	19%
Spirit related	30	12%
Neurological	22	8%
Respiratory	21	8%
South Asia	97	72% (total UR 135)
Digestive	48	36%
General and unspecified	24	17%
Skin	14	10%
Respiratory	11	8%
Cardiovascular	9	6%
Southern Africa	41	60% (total UR 68)
Digestive	19	28%
General and unspecified	9	13%
Skin	6	9%
Respiratory	6	9%
Neurological	4	6%
Genital	4	6%
Total	347	513

3.4.3.6 *C. spinarum* in ethnoveterinary medicine (EVM)

Plant species used for human EM are commonly used for animal healthcare as well, a subject explored by Miara *et al.* [331] in their study of traditional veterinary knowledge in the Algerian steppe. The authors suggested that human use may stem from the observation of animal self-treatment, a behaviour known as zoopharmacognosy. In the case of *C. spinarum*, this may be an element in therapeutic choice. Animals are known to browse on the leaves of *C. spinarum* and it is used in EVM for the treatment of helminthoses and other digestive disorders in animals as in humans. In this analysis of *C. spinarum*, there are 39 documents citing its use in EVM. The geographical spread of documents includes SSA - mainly Ethiopia and Kenya and South Asia - mainly India and Pakistan.

3.4.3.6.1 *Ethnoveterinary use of C. spinarum: Plant part, preparation and route*

The plant part used followed that for human use and was predominantly the root, followed by the bark, fruit and leaf in Figure 38a. Comparing the plant parts used in South Asia to Eastern Africa, Figure 38b indicates that while the root predominated in both regions, there was greater variation in Eastern Africa. The root accounted for 48% of usage but the bark was also used a good deal (15%). This contrasts with human use in Eastern Africa where use of the root/rootbark far outstripped other plant parts used. This may imply that, in Eastern Africa, the root was considered to be less toxic to humans than other plant parts given the likelihood that greater care was taken with human than animal treatments. In EVM in South Asia, the root accounted for the vast majority of use and the leaf was used in a minority of cases. This was at variance with the plant parts used for human treatment in South Asia where there is a more even spread between root, leaf and fruit. This could be explained by a number of factors such as the narrower range of diseases treated in EVM, cultural norms, perceived toxicity or acceptability. Similar to human EM of *C. spinarum*, globally the route of administration of EVM is predominantly oral (Figure 38c). However, the external route is more common than oral in South (Figure 38d). This may be due to the predominance of skin diseases in animal ailments treated in South Asian studies (see below). In Ethiopia, Tanzania and India, it was applied in a similar way to treat worm-infested wounds in cattle whereby the root was ground and applied directly to the wounds [332-334]. The general methods of preparation were much more varied for ethnoveterinary use. The method was divided more evenly across decoction, cold water extract and raw preparation (Figure 38e) though in South Asia the plant was more likely to be used in a raw state relative to the variety of preparations seen in Eastern Africa (Figure 38f).

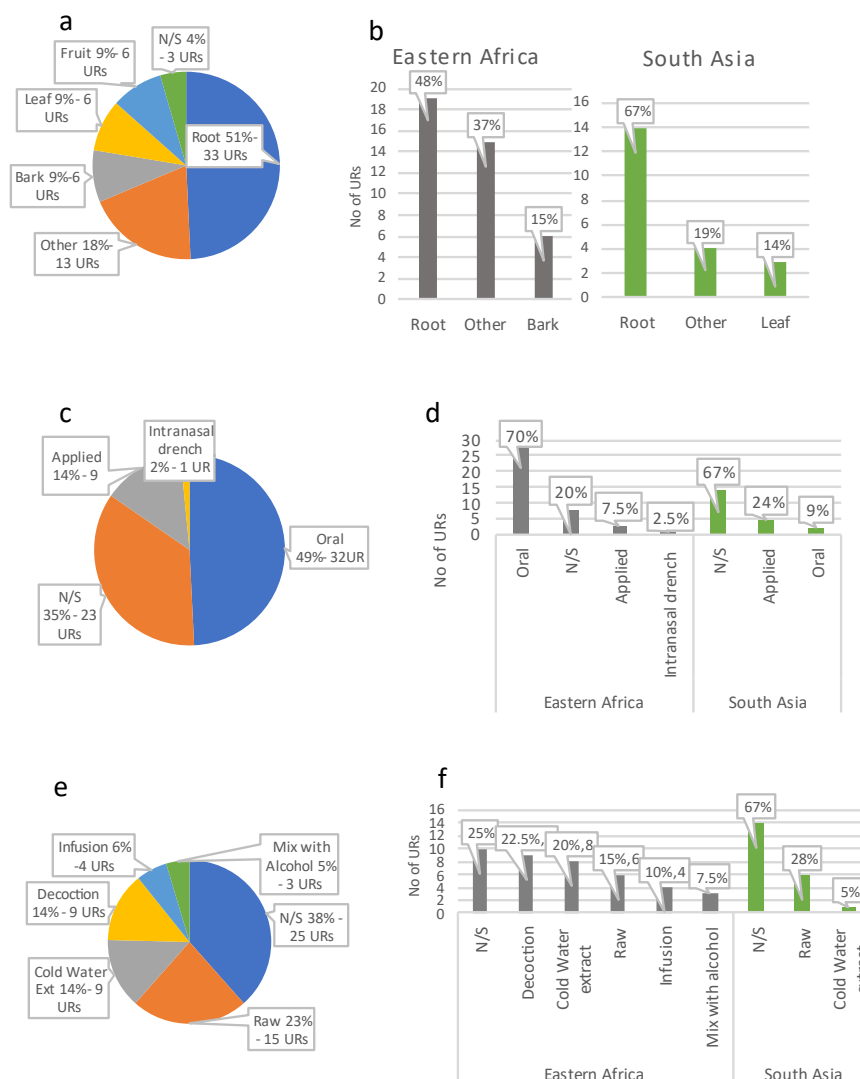


Figure 38: EVM of *C. spinarum*: a) Key plant parts used globally; b) Key plant parts used in Eastern Africa and South Asia; c) Key routes of administration; d) Key routes of administration in Eastern Africa and South Asia; e) Key methods of preparation; f) Key methods of preparation in Eastern Africa and South Asia

3.4.3.6.2 Ethnoveterinary use of *C. spinarum*: Ailments Treated

In examining the presenting ailments, they can be seen to be primarily related to infection (Figure 39a) with some infections affecting the skin such as ringworm (fungal), lice, ticks, scabies and maggot infested wounds (ectoparasites - all insect related), digestive system (endoparasites - helminthosis), respiratory system in pleuropneumonia (bacterial) or more systemic infections such as theileriosis (endoparasites - protozoa), heartwater, anaplasmosis and salmonellosis (all bacterial), foot and mouth disease, chicken pox, rinderpest and lumpy skin disease (all viral). Several studies from Eastern Africa had BM veterinary expertise on the research teams, providing BM diagnoses, which set

them apart from the human ethnomedical studies where it was rare to have BM diagnoses available [260, 335-342].

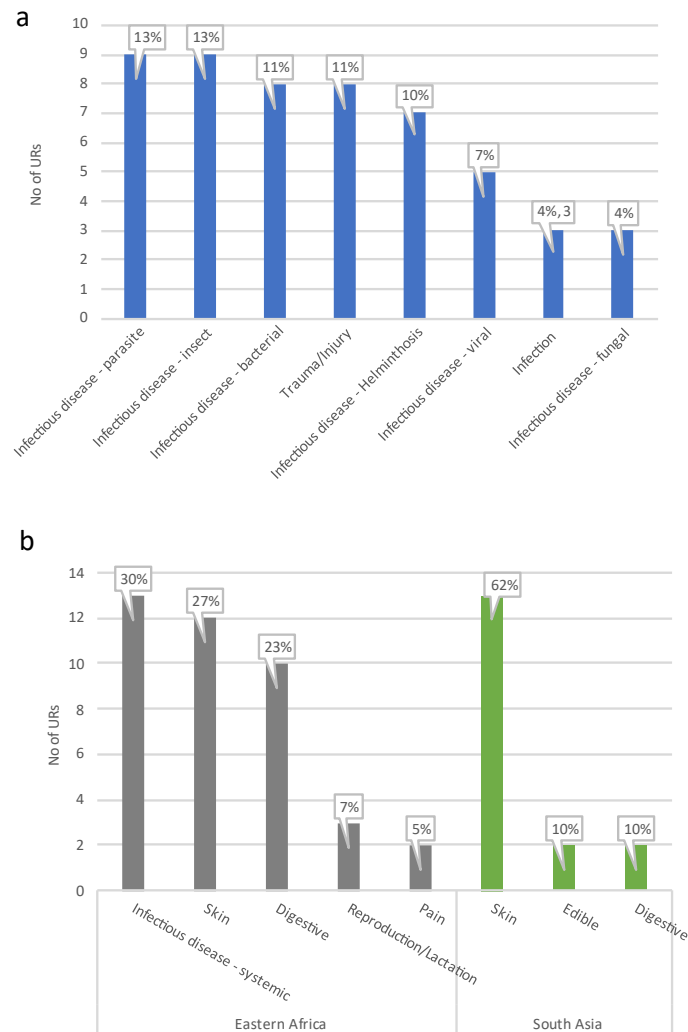


Figure 39: EVM of *C. spinarum*: a) Key ailments treated globally; b) Key body systems treated in Eastern Africa and South Asia

The majority of EVM ailments treated using *C. spinarum* were classified as Infections (Figure 39a). There was a marked difference in the classifications treated in Eastern Africa relative to South Asia. In the former, Skin infections, Digestive system and Systemic Infectious diseases were the most commonly treated Body System whereas in South Asia, Skin far outweighs any other System treated (Figure 39b). This was quite different to the BM systems treated with *C. spinarum* in human EM in South Asia (Table 16) where the digestive system predominated. Other EVM research in South Asia shows that many body

systems are commonly treated [343], which may indicate that *C. spinarum* is particularly effective in treating skin infections.

3.4.4 Ethnobotanical uses - *C. spinarum* non-medical uses

3.4.4.1 *C. spinarum* as a dietary additive – human and animal

The species *C. spinarum* was used throughout its range as a dietary additive with the majority of recorded instances being the eating of ripe fruit (Table 17). The black, ripe fruits were said to be very tasty and highly nutritious. Across its range, they were eaten raw, made into chutneys or fermented into wine and

Table 17: *C. spinarum* plant parts eaten (UR >1, N/S = Not stated)

Plant Part Eaten	No. of URs
Fruit	73
N/S	3
Root	8
Stem	4

vinegar whereas green unripe fruits were mashed and cooked in porridge [344-347]. The Me'en of Ethiopia had several emic categories of plant use, with fruits of *C. spinarum* considered a “famine plant” [348]. These were:

plants gathered by Me'en in times of food shortage (often in the dry season) or impending famine.... people move there (to the lowlands) when they feel they are going to be short of food or when rainfall appears to be insufficient (Abbink 1993).

In Ethiopia, the young stripped stem was eaten [349]. In East Kimberley, Australia, an infusion of the bark, leaf and twig was drunk as a tonic [277]. The stem and leaf were eaten in Madagascar [350]. In a study on medicinal plants included in food among the Maasai in Kenya, the bark, fruit and root were added to food [351]. In an earlier study, the root was said to be added to milk and meat-based soups by the Maasai of Kenya and Tanzania and the Pokot of North Western Kenya [87, 352]. In Uganda and Ghana, the root was used as a spice tea [353, 354]. Early colonists in Botswana soaked the root in rum or gin to produce bitters – a traditional bitter alcoholic drink flavoured with botanicals [345]. In South Africa, the root was soaked with other vegetables and eaten with porridge while in Vietnam, Burundi and Kenya, the root was used as a stomachic [299, 355-358]. *C. spinarum* can thus be seen to be valued in Asia, Africa and

Australia as a component of the human diet for nutrition, flavour and health benefits.

C. spinarum was also valued as animal fodder in studies in Asia and Africa. In districts of Jammu and Kashmir and the Lesser Himalayas region of Pakistan, *C. spinarum* leaf was used as fodder for livestock, where it was said to be good at increasing weight [253, 344, 359]. In semi-arid regions of Ethiopia, Kenya, Sudan and Botswana, it was used as forage and fodder for herds [87, 345, 360-363]. People in the study areas were often dependent on animals for their livelihoods and had a detailed knowledge of the browsing patterns of their animals [121, 260, 364]. Evergreen *C. spinarum* with its wide ecological tolerance may have been an important element in feeding livestock when other species were unavailable such as during times of drought.

Research on diet in ethnobiology was supported by Etkin [74] who proposed examining total exposure to “ingested botanicals” to include foods, teas, tonics and medicines. She considered the creation of these separate categories to be arbitrary and unhelpful. Most of the primary research on ethnodietary use of *C. spinarum* dates from 2000 onwards and reflects a growing international interest in traditionally used plant species with benefits for health, food security and market value as nutraceuticals [365-369].

3.4.4.2 *C. spinarum* – Miscellaneous uses

Both wood and leaf of *C. spinarum* were reported to be used as insect repellents in Africa, Asia and Australia. In India and Pakistan, *C. spinarum* was used as an insect repellent [370-372]. In different areas in Australia, the wood and leaf were burned with the smoke acting as an insect repellent [270, 275, 373-375]. In North-eastern Ethiopia, *C. spinarum* and other species are used as *Tush*, a traditional incense, which was lit in the home to create clean air with a good fragrance and to repel insects [376].

The use of *C. spinarum* as snake repellent can be seen to vary from its direct effect in snake holes to its use as a protective charm. In northeastern India and northwestern Ethiopia, the ground root of *C. spinarum* was mixed with water and poured into snake holes [332, 377]. In southern Ethiopia, the root smoke was used as a fumigant to protect against snakes while in Kenya, the Nandi fixed a stick to the roof as a charm to repel snakes [37, 378].

The usefulness of *C. spinarum* as a fuelwood was highlighted in several studies from Kenya, Ethiopia, South Africa, India, Pakistan and Northern Australia and it was thought to burn well when wet. In Ethiopia, it was valued for charcoal making. *C. spinarum* was useful throughout as a wood for construction, implements, carpentry and carving. In Asia and Africa, *C. spinarum* was found to be useful as living hedge and fencing attributable both to the abundance of

long thorns and the strength of the wood. Other uses in different regions included soil conservation, skin lightening, fabric dying and for the beauty and fragrance of flowers [120, 121, 253, 260, 344, 355, 359-361, 363, 372, 379-382].

3.4.5 *C. spinarum* - Structured Ethnobotanical methods and Cultural Importance

The relationship between people and plants can be examined through quantitative means in addition to qualitative means discussed in section 3.4.2. Structured quantitative methods provide an additional means of examining plant use in study communities [77]. The use of statistical methods in ethnopharmacological research dates back to the 1970's [383, 384]. A number of quantitative measures are employed that have been developed and modified over the years [385-387]. The most basic unit to measure is the UR. Other measures used are Informant Consensus Factor (ICF), Fidelity level (FL) Relative Importance (RI) and Use Value (UV). The value ranges for these measures vary by study and method of calculation, making it difficult to compare studies [77].

3.4.5.1 Relative importance of *C. spinarum* – Primary URs

Given the number of primary studies that are reviewed here, there is relatively little statistical detail on the ethnobotanical use of *C. spinarum*. While there is commonly accurate detail on the number of participants in the studies, few studies provide accurate detail on URs for individual use categories as recommended in current best practice documents [77, 246]. Of the primary studies reviewed: three quarters stated the overall number of participants; one quarter reported the number of URs (number of participants reporting any use of a species) with one third of these being aggregated URs rather than UR per single use category or ailment; one fifth of studies presented the primary data as the number of URs per use category. Thus, in 80% of primary studies, including recent ones, there was no indication of relative usage of any species. In overviewing the importance of *C. spinarum* where possible from UR data, it was of low to medium importance in most studies when compared to the use of other more frequently used species. The studies that indicated high importance of *C. spinarum* using the measure of URs included studies from Kenya, Sudan, Ethiopia, Angola, South Africa and Pakistan [254, 260, 388-394]. There were very few Asian studies presenting information on URs and most Australian studies involved participant-authors rather than large-scale surveys. Thus, the better assessments of value measured by UR were from African studies.

3.4.5.2 Relative importance of *C. spinarum*: statistical calculations

Primary research that included statistical calculations on the relative importance of *C. spinarum* came from Africa and Asia. The majority of studies that included *C. spinarum* had no measure of the cultural importance, contrary to current best practice recommendations [77, 246]. Overall, where such research included *C. spinarum* in its evaluations of importance, it was found to have a low-moderate relative importance. In Ethiopian studies, it was found in the main to have a relatively high importance over a broad range of ethnobotanical uses. In other areas where importance was reported, *C. spinarum* was of moderate or low cultural importance. Some early documents and the Australian studies included in this analysis contain no statistical information. The ethnobotanical information was often detailed but mainly involved very few participants. This would not be considered adequate in the present day but these studies are a valuable addition to ethnobotanical literature [87, 89, 119, 318, 395].

3.4.5.3 Relative importance of *C. spinarum* in Ethiopia: participatory ranking

Participatory ranking methods were developed as central tools in ethnobotanical research [269, 396]. They are a feature of several Ethiopian studies examined for usage of *C. spinarum* and are a semi-quantitative method of measuring the relative frequency of use of species. Ranking exercises create a more in-depth examination of species use in the study area and follow from more general earlier explorations with participants, which can select out key species to include in comparison exercises. Inclusion in a ranking exercise for specific or general utility of itself indicates a high relative importance among participants. Participatory exercises involve the selection of a small number of key participants, 5 to 10 people, and a selected number of key plant species, usually 5-7 species, identified during the research as having importance for broad or narrow purposes. The exercises examine a range of ethnobotanical uses through one of a number of methods: direct matrix ranking (DMR), pairwise ranking, preference ranking or simply by number of uses.

A total of 25 Ethiopian ethnobotanical participatory ranking studies were identified that included *C. spinarum* among the ethnobotanically known species of the area and 16 of these included *C. spinarum* in ranking exercises. It was evident that *C. spinarum* had a high relative importance in the study areas given its selection from a large pool of species.

3.4.5.4 Participatory general ethnobotanical rankings of *C. spinarum* in Ethiopia

Several Ethiopian studies examined the non-medical ethnobotanical importance of species using participatory ranking exercises. The range of ethnobotanical categories of importance in ranking exercises included medicine, firewood, furniture, construction, fencing, charcoal, forage, fodder and food. Sixteen studies are listed in Table 18 that included *C. spinarum* in their ethnobotanical species list, with 7 exercises from these studies listing *C. spinarum* as having significant general ethnobotanical importance. The study areas exhibiting above the median ethnobotanical relative importance for *C. spinarum* are distributed throughout the country. This analysis indicated a high relative importance for *C. spinarum*, which was also evident in the statistical ranking analyses such as UV and RFC in the previous section.

Table 18: Ethiopian rankings of *C. spinarum* use through participatory ranking exercises for general ethnobotanical use (N/R = Not Ranked)

Ranking method	Rank <i>C. spinarum</i> (total #) (1 is best)	Study authors
DMR	4 (8)	Amenu [260]
DMR	2 (6)	Amsalu <i>et al.</i> [360]
DMR	N/R	Araya <i>et al.</i> [307]
DMR	N/R	Ashagre <i>et al.</i> [346]
DMR	5 (6)	Jima, Megersa [380]
DMR	5 (11)	Kebebew, Leta [397]
DMR	1 (7)	Kebebew [398]
DMR	N/R	Kefalew <i>et al.</i> [399]
Uses	N/R	Kidane <i>et al.</i> [376]
DMR	11(46)	Kidane <i>et al.</i> [376]
DMR	N/R	Lulekal <i>et al.</i> [254]
DMR	N/R	Lulekal <i>et al.</i> [400]
DMR	N/R	Megersa <i>et al.</i> [401]
DMR	N/R	Mekonen <i>et al.</i> [402]
DMR	1 (6)	Meragiaw <i>et al.</i> [403]
DMR	N/R	Tefera, Kim [404]
DMR	N/R	Teklay <i>et al.</i> [405]

3.4.5.5 Ethiopian EM participatory rankings of *C. spinarum*

Several ranking studies specifically examined ethnomedical importance both for

Table 19: Ethiopian EM participatory ranking exercises in locations listing *C. spinarum*

Study	Ranking exercise	Ranking Method	<i>C. spinarum</i> Rank (total #) 1 is best
Abera [406]	General EM	Preference	N/R
	Gonorrhoea	Pairwise	1 (6)
	Malaria	Preference	N/R
Amenu [260]	Gonorrhoea	Preference	1 (5)
	Evil Eye	Pairwise	2 (6)
	General EM	DMR	2 (6)
Araya <i>et al.</i> [307]	Snakebite	Preference	N/R
Asnake <i>et al.</i> [407]	Malaria	Preference	N/R
Bekalo <i>et al.</i> [408]	Cough	Pairwise	N/R
Chekole <i>et al.</i> [409]	Stomach ache	Pairwise	N/R
	Wound	Preference	N/R
Chekole [410]	Stop bleeding	Preference	N/R
Jima, Megersa [380]	Stomach ache	Preference	4 (5)
	General EM	DMR	1 (5)
Kebebew, Leta [397]	General EM	DMR	3 (11)
Kebebew [398]	Diarrhoea	Preference	N/R
	General EM	DMR	2 (7)
	Gonorrhoea	Pairwise	1 (5)
Kefalew <i>et al.</i> [399]	Blackleg	Preference	N/R
Kidane <i>et al.</i> [378]	Ascariasis	Preference	N/R
Lulekal <i>et al.</i> [254]	Gonorrhoea	Preference	N/R
	General EM	URs	2 (10)
Lulekal <i>et al.</i> [400]	Atopic eczema	Preference	N/R
Lulekal <i>et al.</i> [411]	Diarrhoea	Preference	N/R
Megersa <i>et al.</i> [401]	Blackleg in cattle	Pairwise	N/R
	General EM	DMR	N/R
Mekonen <i>et al.</i> [402]	General EM	DMR	N/R
Meragiaw <i>et al.</i> [403]	Febrile illness	Preference	N/R
	Stomach ache	Preference	N/R
	General EM	DMR	1 (6)
Osman <i>et al.</i> [412]	<i>Mich</i> ¹²	Preference	N/R
Tefera, Kim [404]	Cancer	Preference	N/R
	Stomach Ache	Preference	N/R
	Wounds	Preference	N/R
Teklay <i>et al.</i> [405]	Abdominal Pain	Preference	N/R
	General EM	DMR	N/R
Teklehaymanot [413]	General EM	Preference	N/R

¹² Osman *et al.* describe *Mich* as a febrile illness with headache and sore lips and as not being well understood biomedically.

broad utility and for particular medical purposes. An overview of these rankings is shown in Table 19 and consists of 22 studies with a total of 37 ranking exercises. *C. spinarum* appears in 11 exercises using a variety of ranking methods: Preference Ranking, Pair-wise ranking and Direct Matrix Ranking (DMR).

In most exercises that included *C. spinarum*, it was ranked above the median value. The study areas where it exhibited importance in the general EM category were distributed throughout the central regions of Ethiopia, north to south along the Rift Valley. The most common medical ranking exercises other than the general ranking was for GI complaints and gonorrhoea. *C. spinarum* was ranked as the best for treating gonorrhoea in three studies, it was ranked in one GI ranking exercise and in rankings for malaria and febrile illness, *C. spinarum* was not ranked [260, 380, 398, 403, 406, 407].

From these participatory exercises, *C. spinarum* appears to have been highly ranked for the treatment of gonorrhoea and not for other specific ailments. It ranked high in several areas for general ethnomedical use. However, the very inclusion of a species in the choices presented to participants is based on preliminary research and implies a baseline value for the included species in the category presented to research participants.

3.4.5.6 *C. spinarum* and a comparison of cultural value based on statistical measures vs participatory ranking exercises in Ethiopia

Participatory ranking exercises and interpretation of cultural importance by UR yield sometimes conflicting information. Ethnomedical participatory ranking exercises found that *C. spinarum* did not rank high in the treatment of GI complaints. This is in contrast to the finding in section 3.4.3.5 whereby, in the ICPC classification, the most widely treated Body System with *C. spinarum* in Eastern Africa was the Digestive system while in Ethiopia, it was second most commonly treated Body System by UR (Table 20). This indicates a potential discrepancy between the two forms of analysis. However, in ranking for treatment of gonorrhoea, *C. spinarum* was ranked best in three out of four exercises. This corresponds with that found above in Table 13 and Table 15 which indicated its importance in treating genital infection globally and in Eastern Africa. That *C. spinarum* was not ranked in exercises for the treatment of malaria and for febrile illness is at odds with the findings in Table 13 and Table 15, where malaria and fever combined were among ailments most commonly treated with *C. spinarum*. There are other sources of variability between statistical and

Table 20: Ethiopia - key body systems treated using *C. spinarum* (UR>2)

Body System Treated	No of URs
Spirit related	30
Digestive	21
Skin	13
General and unspecified	9
Neurological	8
Respiratory	7
Psychological	5
Musculoskeletal	5
Genital	5
Women's Health	4
Andrology	3

participatory research. In participatory rankings, as in all research, there is a randomness to the choice of participant and all participants have necessarily a different knowledge base. There is also potential variability in the choice of species to include in the ranking exercises and it was not clarified how particular species were selected for analysis. The choice of a particular ranking subject is a function of the study design and researcher priorities and may not reflect the key ailments treated with TM in the study areas. This could add another layer of bias to the results.

The potential variability of outcomes, depending on research methods and study design should be borne in mind in any interpretation of the results or implementation of the research outcomes.

3.4.6 Phases in *C. spinarum* research

The framework of Hunn's phases of ethnobiology is applied to the ethnobiological documents analysed relating to *C. spinarum* following a method employed by Hidayati *et al* in a study of Southeast Asian ethnobiology [66]. There were 219 primary ethnobiological research documents analysed. Of these, 85% were ethnomedical only, while the remainder related mainly to diet and general ethnobotany. Within these studies, in whatever phase they were classified, there were shortcomings in detail provided on medicinal plant use (Table 21). There was almost total silence on plant harvesting. In 9% of cases, there was no information on the plant part used. In over one third of cases, there was no method of preparation given and where a method was given, rarely was close detail provided. The route of administration was not given in 40% of cases.

Table 21: Plant and cultural detail provided in *C. spinarum* papers

Detail provided	% with detail
Plant harvest	2%
Plant part used	91%
Method of preparation	64%
Route of administration	65%
Basic socio-cultural info	25%
In-depth socio-cultural info	2%

Added to these immediate aspects of medicinal plant use were the omissions on the fabric of local life. Culture is central to healing and plant use. However, cultural elements of ethnomedicine were omitted in the majority of studies such as the meanings associated with plants, disease concepts, therapeutic indications, or what “healing” actually means in the local context as distinct from biomedical understandings of being healed [414]. Other aspects that are rarely addressed include, for instance, local healthcare provision, morbidity and mortality, environment and economy, which, among other things, contribute to healthcare choices and to the relationship of people with plants.

The majority of the studies that included *C. spinarum* fall into the Phase I classification, representing two thirds of the total. As Phase I research usually represents a bioprospecting agenda, the findings show significant lacunae in the information available on *C. spinarum* for guiding further scientific study. One quarter of studies had Phase II character with some emic detail, providing cultural context to ethnomedical practices (Figure 40). There were some studies in the current analysis that had characteristics of each of Phases II to V even where they still contain catalogues of species and their uses. Those with some

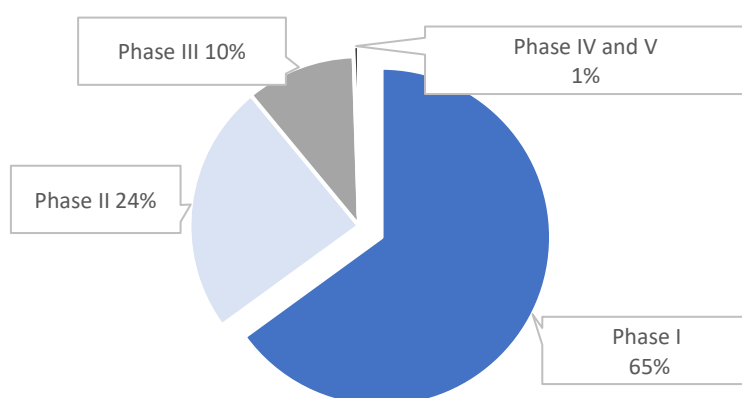


Figure 40: Character of *C. spinarum* research using Hunn's phases

descriptions of local understandings of illness and healing were ascribed Phase II character. Descriptions such as of disease and causation of TB in a Ugandan community, liver dysfunction in a community in Togo or the meanings associated with plant names in a South African community add to the reader's understanding

of ethnomedicine in those communities [257, 258, 264]. Five studies had more detail, such as Geissler's research with Luo mothers, already discussed in section 3.4.2.3, which situated the treatments of digestive disorders, worms and the use of pharmaceuticals in the study community. Two studies with the Marakwet of Kenya, from 1978 and 2014, gave good descriptions of disease perceptions in the study communities. Studies with the Maasai and Samburu in Kenya, gave good background descriptions of health in the communities and relationship to environment which allowed a deeper understanding of plant usage. Each of these more detailed studies offered an emic perspective which better situates how people use plants for life [91, 119-121, 164]. The studies ascribed to Phase III involved participatory research and accounted for 10% of studies (section 3.4.5). They gave a sense of how plants form an integral part of life. The participatory studies allow researchers to understand how people think about plants in their immediate environment and how particular species may have cultural value across multiple practical and symbolic domains. Studies conducted in collaboration and co-authorship with community members have elements of Phase IV or V character such as several Australian publications as described in section 3.4.2.1 and two Africa-based studies which mentioned benefit-sharing arrangements [121, 261, 265, 267, 270, 273, 373]. These represent 1% of the total.

The CBD and related issues such as conservation, sustainable use of resources, prior informed consent and equitable benefit sharing relate to later phases in ethnobiology from the 1970s and Phase III onwards. They were rarely discussed in any of the documents. Consent in any form was reported in 40% of studies though there is no description of the process in any instance. Ethics was referred to where ethical approval of the research has been granted in 16% of studies though the process was not discussed nor the ethical issues examined. The ISE code of ethics was referred to in six studies (3%) which may imply a greater attention to ethical standards in the research process. Research permits were cited in 15% of studies though the process was not elaborated upon. Intellectual property rights (IPRs) or benefit sharing arrangements were mentioned in eight studies (4%) [136, 270, 273, 373, 388, 415-417]. Half of all studies had no mention of consent, ethics, permits, IPR or other research ethics related issues.

3.5 Discussion on Ethnobiology of *C. spinarum*

Ethnobiology is a wide and varied discipline with research agendas covering the natural and social sciences including such diverse subjects as indigenous rights, migration, biocultural diversity, health, research ethics and reflexivity [235]. When conducting research on medicinal species, the research focus could

involve, for instance, cultural record, health care provision, sustainable plant use, environmental protection or bioprospecting. The ongoing preponderance of Phase I research, lacking cultural contextualisation and theoretical focus, is continually critiqued within ethnobiology and ethnopharmacology [74, 75, 77]. This holds true whatever the research focus. The phenomenon is reflected here in the studies containing *C. spinarum* with the majority falling into the Phase I category. There is an implication built into this rudimentary data collection, that the “how” and “why” of ethnomedicine has little to do with science. In the bioscience laboratory, studies based on ethnomedical catalogues of species generally use organic solvents to extract single or multiple metabolites and test these extracts or compounds in *in-vitro* systems, none of which bears any resemblance to the use of traditional medicines in the field. This disjuncture between TM practice and pre-clinical ethnopharmacological studies is an issue which is examined in detail in Chapter 4.

Phase I may be dominant in this analysis due to the nature of this analysis itself. Searching for an individual species may be biased towards cataloguing studies. *C. spinarum* is a medicinal and dietary species and this fact may self-select for Phase I studies. Another reason for the lack of later phases in this analysis of the ethnobiology of *C. spinarum* could be the nature of ethnobiological research itself. In ethnobiology in general, there has been a shift recognised in Hunn’s Phases towards an ecology and action-focused research agenda and away from its roots in anthropology [418]. These research agendas do not necessarily require the naming of particular species, which may explain the relative absence of later ethnobiological phases in this species analysis.

However, in ethnopharmacological research, the emic perspective is vital to an improved understanding of plant use. Its absence is a common phenomenon as described by Yeung *et al* whose analysis showed that the socio-

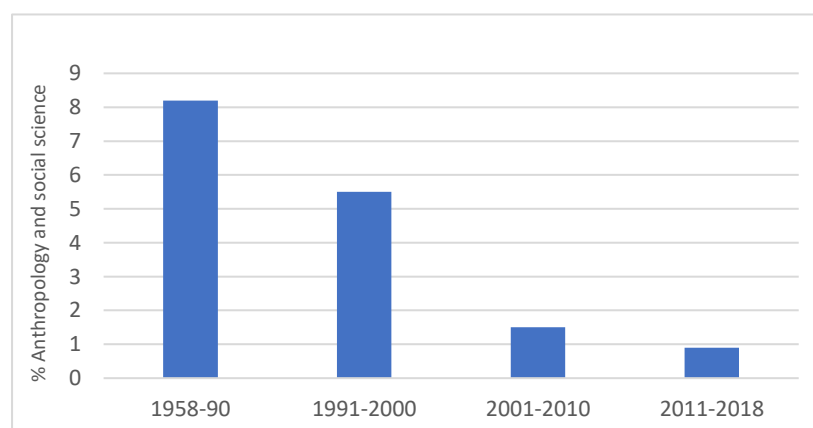


Figure 41: % Anthropology and social science in ethnopharmacological studies 1985-2018 (adapted from Yeung *et al* [419])

cultural focus has been in decline over the past three decades (Figure 41), at the same time as there has been a substantial increase in ethnopharmacological

research from 3545 articles in 1991-2000 to 38604 in the period 2010-2018 [419]. The relative lack of ethnographic content in ethno-directed bioprospecting has been criticised by numerous authors. Reyes-García [76] reiterated Etkin's early critique of ethnopharmacology as generating lists of species that extract them from their cultural contexts [74]. In the bioprospecting process, the subsequent extraction of active metabolites can then diminish indigenous knowledge, even where it is validated by laboratory science [76]. In either case, indigenous use of medicinal species is reduced to its chemical components in what Osseo-Asare called its "molecularisation", disregarding the wider cultural contexts of use and perceptions of efficacy [52, 129]. With regard to medicinal uses, there has been criticism by Albuquerque *et al* of the poor description and development of therapeutic indications leading to biased data and poor outcomes [129]. Weiskopf illustrated that there is an assumption that traditional ecological knowledge can be "plugged" in to "academic ecological knowledge" unchanged and that there is an assumed category and explanatory overlap between both as is the case, he argued, in the validation process in medical bioprospecting [420]. Taking the current analysis of *C. spinarum*, for example, a local diagnosis of malaria was assumed in some studies to equate to the biomedical category malaria or "typhoid" as typhoid [421]. The use of an emetic or a laxative might be assumed to be used for the same purposes as an emetic or laxative in biomedicine which may not be the case [87]. Category overlap thus cannot be assumed but must be underpinned by qualitative research exploring cognition among local participants. Ludwig [418] described a hierarchy of convergence in early ethnobiology research on folk classification between local and scientific categories whereby ethnotaxa that fit into the scientific categories were given priority and ethnotaxa that did not converge were marginalised. Such ethnotaxa could be names e.g., for plants, birds or for therapeutic indications in EM. One can interpret the analysis of *Buda* (Evil Eye) in Ethiopian studies in section 3.4.2.2 in light of hierarchies of convergence. While it was included as a therapeutic indication, there was no examination of this ethnotaxon in any of the Ethiopian studies analysed here. The local importance of this category was clear, but why was it not explored? Was it thought to be self-explanatory as other therapeutic indications are? Was it being marginalised as described above, a desire to ignore that which did not fit? Was there a lack of anthropological expertise in a natural science focused research team? Was it beyond the scope of the research agenda? Was it due to secrecy among research participants as described in Australian research in section 3.4.2.1? Whatever the reason, the lack of exploration of *Buda* in these ethnomedical studies is a missed opportunity to document local understandings of health and healing and perhaps illuminate the wider use of *C. spinarum* and other species.

The detailed ethnography needed for a substantial emic perspective in ethnopharmacological research requires collaboration between the natural and social sciences, but this is not common in practice [18, 76, 240, 422]. In the current analysis, five explicitly ethnobotanical studies have multidisciplinary teams. However, with two exceptions [87, 91], the team members belong to a natural science discipline [328, 423, 424]. D'Ambrosio [240] attributed this to the normal association of ethnobiology research with either anthropology or biology departments with few dedicated ethnobiology schools. He argued that the ethnobiology researcher, rather, takes the disciplinary approach of their primary discipline with either a natural science or a social science/humanities perspective, carrying through their disciplinary origins in their research questions. The natural sciences perspective is largely objective, quantitative and etic in its approach, while a social scientific outlook is more often subjective, qualitative and emic. The natural sciences perspective predominates in the Phase I character of *C. spinarum* studies analysed [240]. Significant roadblocks exist to collaborative research across disciplines including in ethnobiology. These barriers revolve around the interrelated aspects of status, funding, time and complexity. In an analysis of research collaborations between social and natural sciences, Barthel and Seidl [425] found that collaborative research has a lower academic status than natural sciences research. Funding across disciplines is difficult as funding streams generally follow single disciplines [240, 426]. Added to this, it is argued that social science research is chronically underfunded [427]. Within ethnopharmacology, there is a demonstrable lack of funding for ethnographically directed research compared with that for laboratory-based screening for bioactive metabolites [428]. Where social science and humanities research is conducted in collaboration with natural sciences, the latter tends to be the dominant partner in such research. The social science element may be tagged on with no true commitment of time and funding necessary for the incorporation of the social science discipline into the project [427, 429]. Similarly, the time required for communication across disciplines, for creating relationships and mutual understandings among research team members and for integrating research findings is rarely considered in project timelines [427]. Frequently, the social science elements of collaborative research are more difficult to translate into action relative to the faster, more reductive natural sciences research [427]. These limits on time and funding can hamper ethno-directed studies and result in poor outcomes in ethnopharmacological research [129].

Collaboration with study communities is a central feature of progressive ethnobiological research, with community voices heard rather than interpreted by academics [75]. However, in this analysis, there is near-total anonymity of

the research participants themselves. This is the norm in earlier phases of ethnobiological research and has been highlighted by researchers such as Osseo-Asare [52] and Langwick [430]. The collaborative approach suffers from some of the same barriers as cross-disciplinary collaborations including skills, funding, time constraints and academic norms. The marginalisation of social sciences in interdisciplinary collaborative research means that the social science skills needed for in-depth community collaboration may not be available. Likewise, the time and associated funding requirements may be lacking making it more difficult to establish the necessary relationships of trust. Academically, research design varies whereby the social sciences often have a more flexible approach than natural sciences [431]. Collaboration and co-authorship are integral to Phase IV and V research and are considered to be essential to the future of ethnobiology [75]. If ethno-directed bioprospecting is to be conducted within an ethical and collaborative framework, then these barriers need to be addressed. All of these elements - disciplinary silos, funding, status and time - militate against the collaboration with communities and across disciplines necessary for high quality ethnobiological research that can capture the interpenetrative nature of the relationships of humans and their environments in an ethical manner.

Research design and methods used in ethnobiology may produce results which do not properly represent the relationships of people and plants. Shortcomings in quantitative studies, in choice of participants and questionnaire design, in use of common ethnobotanical methods and the interview itself, may each contribute to an ethnobiology that is not representative of the local voice. Quantitative analysis in ethnobiology has become a common component of ethnobiological research especially since the 1990's [432]. It can be a component of a progressive ethnobiology when used judiciously. However, its inclusion has also been critiqued by several authors. The indiscriminate use of statistical methods without a basis in theory or hypothesis is challenged by Ferreira Júnior who proposes a better fit of methods with research questions [433]. The variability in the use and interpretation of statistics produces research that cannot be compared or synthesised across studies and geographies [77, 432]. In this analysis, the wide variation in sample sizes, the difference in formulae and terms used, different interpretations of use categories make comparisons and syntheses of data difficult or inaccurate. The varying application of statistical measures has led to the recommendation that primary data (URs) are reported to allow for comparison between studies and meta-analysis [77]. In health and medicine directed ethnobiology, there are pitfalls with statistical methods. The equation of the most popular species with the "best" could be misapplied since popularity may have more to do with ease of access

or symbolic value than efficacy in biomedical terms [129]. The format of many ethnobiological studies which results in catalogues of plant knowledge does not necessarily translate into real-world plant use such that what is known is not necessarily what is used [18].

It has been argued at a more fundamental level, that study design including choice of participants and questionnaire design lead to more variability in results than the choices around statistical indices or the grouping of use categories [434]. Sampling bias may emerge when indigenous knowledge is not homogeneously distributed across a community or region [435]. The recording and classification of ethnobotanical knowledge may focus mainly on practical utility of species and can ignore other elements of importance to local communities. Elements that may not be categories of interest in study protocols, such as the sacred or aesthetic, may be relevant to community members [436]. It was illustrated by Edwards *et al.* [268] how common ethnobotanical methods are not culturally appropriate in Australian aboriginal research and this necessitates the development of alternative culturally sensitive study designs [269, 396]. The use of tools such as free-listing were found to produce variable outcomes, even within a single participant (in separate interviews), or relating to age or the presence of a third party at the interview [437]. Similarly, a Bolivian study found that even subtle changes in the use of methods of free-listing and semi-structured interviews could produce markedly different results [438]. The core method of the interview itself is a challenged process when the recorded portion, the verbal, is only a fraction of the whole communication. The interviewer changes the outcome of the interaction through their very presence and the questions they ask, bringing their own etic category biases and resulting in an inaccurate representation of indigenous knowledge [439].

As the discipline of Ethnobiology has progressed, the ethics of research has become increasingly central to its conduct. Vandebroek [18] has argued that the normal method of documenting traditional medicinal knowledge and subsequent laboratory research on pharmacological activities has limited if any health benefits for study communities. There are clear ethical implications to such research. This has led to increasing calls to develop action-focused research protocols, which ask questions that directly address public health problems and make ethnobiological research more relevant to study communities [18, 76]. Vandebroek [18] refers to the work of Graz *et al.* [440] in Mali whereby ethnobiological research results were directly returned to the study community through a refinement of local knowledge on an anti-malarial traditional medicine and only secondarily directed towards the development of a phytopharmaceutical. The area of researching traditional knowledge and genetic resources raises ethical issues including of IPRs, benefit sharing and protection

of biodiversity. However, in the *C. spinarum* documents, there is little stated attention to ethics. Where ethics is mentioned, it is usually perfunctory. It is true that the lack of discussion of ethics does not mean that the research was conducted unethically. Some journals may require evidence of ethical research conduct as a condition of publication such as the *Journal of Ethnopharmacology*. This may not be the case for all journals. Where ethical issues are discussed as in Australian research such as in [272], there is a greater sense of the ethical conduct of the research. Likewise, benefit sharing and IPRs may be a component of some research permits. However, the mention or discussion of them within the research paper raises the reader's awareness of these essential aspects of ethical research which has a value in itself.

3.6 Limitations of the analysis

A key limitation of this analysis of *C. spinarum* is that, of itself, it is viewing a species out of its cultural context and may be criticised for doing so given the problems highlighted throughout with decontextualization of ethnomedical data. However, the choice of a single species has allowed a novel perspective on the nature of ethnomedical research, particularly that with a bioprospecting focus. A further limitation on this analysis of the ethnobotany and ethnomedicine of *C. spinarum* is in the access to primary data. I emailed the authors of the studies conducted since 2000 for access to the primary data on *C. spinarum* in cases where there was insufficient already provided. Most of the emails could not be delivered or recipients did not respond to the request, and three refused in the absence of a collaborative agreement with their universities. The Australian data is in print form in the main and was originally accessed on a visit to Kew library. However, further exploratory visits were not possible during the COVID emergency. Much of the South Asian research was also accessed on that visit to Kew but there is probably a lot of data in Indian libraries and available in Hindi which was not accessible for this study.

3.7 Concluding Remarks

This analysis of *C. spinarum* illustrates that it is a medicinal and dietary species in ethnodirected bioprospecting studies and other forms of ethnobiological research, across three continents. Most of the research is categorised as Phase I in Hunn's classification of ethnobiology with no ethnographic content. Where a local view on the use of this species is reported, it is seen to profoundly alter the perspective of the reader on the use of this and other species within the study community. However, even a minimal "thin description" is lacking in the research, where lists of ethnomedical species may be recorded without information on the plant part used, or on the preparation or administration of the

plant remedy. The omission of such essential data can invalidate the documentation of ethnobotanical data on this and other medicinal species, whatever the purpose, whether for cultural record, health care provision, sustainable plant use, environmental protection or bioprospecting.

There is more to be learned about this species and the many other species of broad biocultural value through the design and funding of research which allows local meanings and values to emerge. A more nuanced understanding of its meaning for indigenous people and local communities may be illuminated through study designs that incorporate a blending of qualitative and quantitative research methodologies as proposed in best practice documents in ethnobiology and in ethnopharmacology. A partnership approach with local communities can make for more ethical as well as more fruitful research. Allied to this, is the aim of trans-disciplinarity in ethnobiological research. Such a research process could reveal local ways of knowing biodiversity and environment beyond the categories of utility commonly captured in natural science-directed ethnobotanical research. If I was in a position to conduct ethnomedical research in the Pokot study community again, I would take the learnings from this *C. spinarum* analysis into the research process, first and foremost, using a partnership approach with the community as described in section 2.6.

The evidence of the current analysis of the ethnobotany of *C. spinarum* illustrates that such an approach is essential to ethical, sustainable, and effective ethnobiological research of this and other culturally valued species. A move beyond the current obstacles to collaborative research is needed to examine in meaningful and effective ways how people interact with plants and the wider environment in response to health challenges with dynamism and creativity.

Chapter 4: Phytochemistry and Pharmacology of *C. spinarum* root from an ethnomedical perspective

Reviews of the genus *Carissa* have been conducted in recent years [441, 442]. These reviews, which cover the whole genus, of necessity have a limited focus on traditional methods of preparation, primarily referring to traditional therapeutic

Chapter outline

Carissa spinarum root phytopharmacology

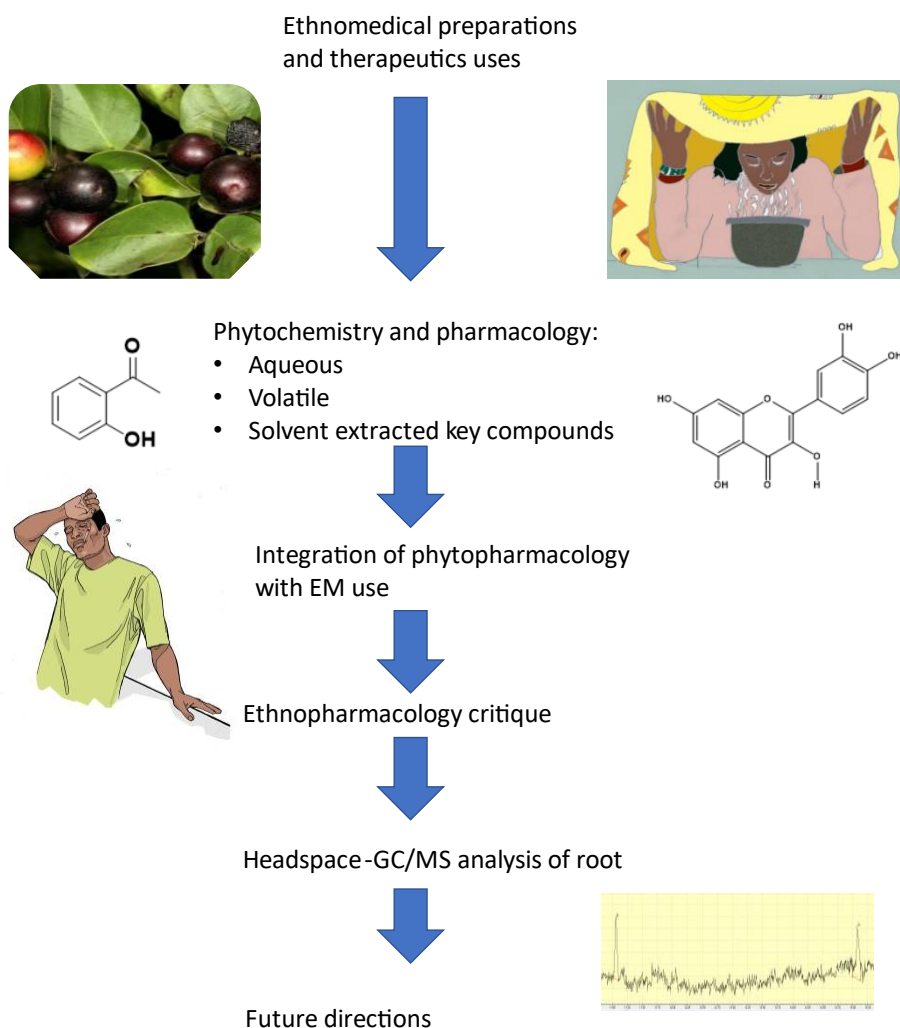


Figure 42: *C. spinarum* phytochemistry and pharmacology chapter outline

indications. The current study differs from these reviews in that the analysis herein focuses on *C. spinarum* root as the most commonly used plant part (Chapter 3). This analysis explores EM preparations at a granular level using data from Supplementary files 5 and 6. These results are then compared with phytopharmacological studies, analysing the laboratory methods used in the preparation of root extracts and the subsequent determinations of biological activity. These results lead us to question whether the phytochemical and pharmacological findings are relevant as an investigation of EM therapeutics. The outline of the chapter is presented in Figure 42.

4.1 Materials and Methods

4.1.1 Literature search

A literature search accessed PubMed, Science Direct, Google Scholar, Scopus, library databases, medicinal plant databases, books, theses and grey literature. The output of the literature search is outlined in Figure 43 and resulted in 167 documents after duplicates were removed and other articles excluded through ineligibility.

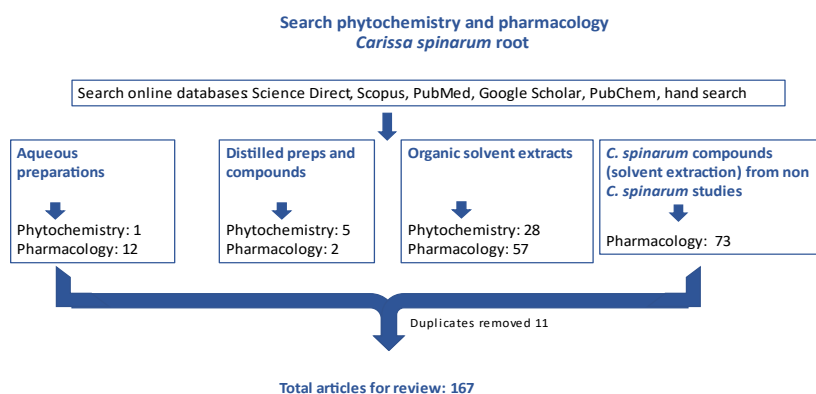


Figure 43: Search outputs for *C. spinarum* phytochemistry and pharmacology research

4.1.2 Experimental details

4.1.2.1 Plant material

A small sample (75mg) of botanically verified *C. spinarum* root was obtained from Château Pérouse Botanic Gardens in southern France by reaching out to the Botanic Gardens Conservation International (BGCI) network.

4.1.2.2 Headspace-GC/MS analysis of *C. spinarum* root fragment

The sample was cleaned, broken up and directly infused into GC/MS and analysed using the headspace-GC/MS technique for the determination of volatile constituents. Headspace analysis was performed with an auto-sampler AOC-5000 - Shimadzu (Tokyo, Japan) and volatiles were extracted from freshly ground plant material (75mg) prepared in a 20 mL headspace vial and incubated at 95°C for 15 min.

GC Settings

Instrument:	GCMS QP2010 SE - Shimadzu (Tokyo, Japan)
Column:	Zebron ZB-5plus 30 m × 0.25 mm ID × 0.25 µm column (5% diphenyl/95% dimethylpolysiloxane)] (Phenomenex, California, USA)
GC Oven Temp.:	from 40°C (1 min) to 290°C (5 min) at 5°C/min
Carrier Gas:	Helium
Linear Velocity:	36.1 cm/s (constant mode)
Pressure:	49.7kPa
Total Flow:	24.0 mL/min
Column Flow:	1.00 mL/min
Purge Flow:	3.0 mL/min
Injection Split	1:20
Injection Temp:	250°C

MS Settings

Ion Source Temp:	200°C
Interface Temp:	300°C
Scan Speed:	2500
Mass Range (m/z):	40-750

The volatile compounds were identified based on their retention times in gas chromatogram and peaks were matched with EI mass spectra in the National Institute of Standards and Technology (NIST) 17 mass spectral library using NIST mass spectral search program version 2.0 (United States of America, 2002). Data was acquired and processed using GCMSolutions V4.50 Shimadzu (Tokyo, Japan).

4.2 C. spinarum root and rootbark: EM preparation and usage

The main types of EM preparation associated with *C. spinarum* root included:

- Aqueous-based – oral, bathed
- Volatilising – inhaled or absorbed through skin
- Whole root/rootbark prepared with soup, fats, alcohol, milk, honey or by chewing taken internally
- Whole root or rootbark with little/no processing by application
- Burning - inhaled
- Multi-species preparations

Aqueous-based preparations included decoction, infusion, maceration and cold-water mixtures. Volatilising preparations included the use of steam, warmed plant parts and the use of body heat when the plant part is used as a rub or a poultice. The whole root/root part was taken internally when prepared with soup, fats, alcohol, milk, honey or by chewing or was used externally by application. Burning of the whole plant or plant part has been shown to have a different composition to solvent extracts in addition to the possibility of generating toxic compounds such as benzopyrenes [443]. Preparations which involved a mixture of species will not be explored in depth in this analysis due to the phytochemical complexity and the lack of such research in relation to *C. spinarum*.

Details of EM preparations are described here and their therapeutic uses are categorised using the ICPC system (Chapter 3). These are then linked to phytochemistry and bioactivities of extracts (section 4.3).

4.2.1 Aqueous traditional preparations of *C. spinarum* root

There were 319 URs of traditional usage of *C. spinarum* root or rootbark identified in this analysis. Of these, 82 (25%) did not state a method of preparation and 161 (50%) were prepared with water including by decoction, infusion, maceration or mixing (excluding mixing with other liquids such as soup, milk, honey, alcohol). Even where a method was stated, there was often very little detail provided. Methods of preparation are defined by the European Medicines Agency (EMA [444] as follows for the purposes of this analysis: decoction – boil in water (duration variable); infusion – pour boiled water over the plant material (like a tea); and maceration - plant is soaked or mixed in water at room temperature for a variable time. However, it is not usually clear that these were the definitions used in the manuscript.

4.2.1.1 *C. spinarum* root decoction – preparations and therapeutic uses

Decoctions accounted for 106 preparations, mainly from Eastern Africa. Most simply stated “decoction”, with some giving more practical detail on the process (Supplementary file 6 Table S1). The largest category of ailments treated with the root/rootbark decoction was Infection (41% of key ailments) (Figure 44). The Miscellaneous symptom category (22% of key ailments) included sexual issues, infertility, lymphadenitis, respiratory, psychological complaints and memory disturbance. Digestive disorders accounted for 16% of key ailments, pain for 10% and neoplasms for 6% of key ailments (breast, cervical and colon cancers where specified). Other ailments with 1 or 2 URs for root/rootbark decoction are snakebite, seizure, fever, cough, insect repellent and Evil Eye. The main

infections treated were STI and malaria with intestinal parasites, measles and URTI the next most common infections treated (Figure 45).

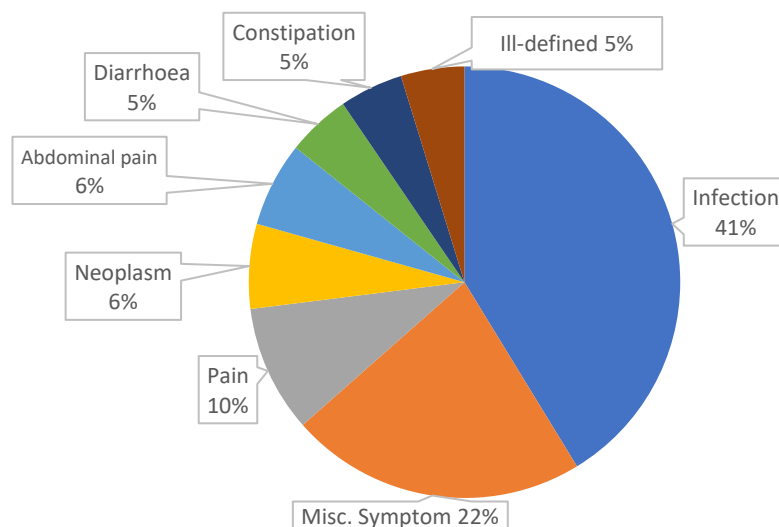


Figure 44: Key ailments (ICPC) treated with decoction of *C. spinarum* root/rootbark (UR>2) (86% of all decoctions) (data includes decoction only and excludes decoction plus other method)

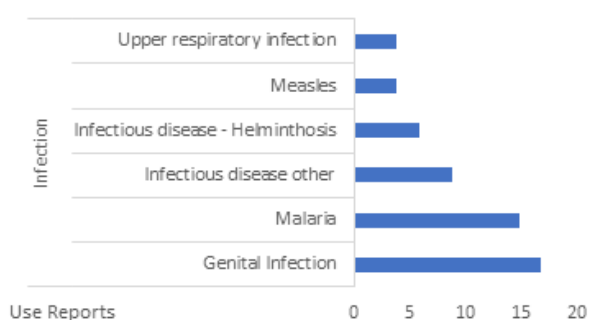


Figure 45: Key infections treated with *C. spinarum* root/rootbark decoction (UR>3)

4.2.1.2 *C. spinarum* root infusion – preparations and therapeutic uses

There were a small number of infusion preparations (22 URs) (Supplementary file 6 Table S2) and therapeutic use data is presented in Figure 46.

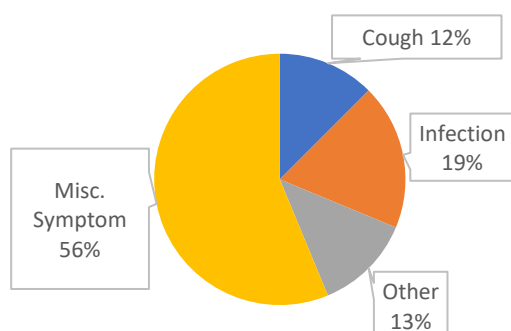


Figure 46: Key ailments treated with infusion of *C. spinarum* root/rootbark (UR>1)

Infections treated included malaria, TB and STI. Genital/sexual issues were the most commonly treated body system including impotence, reduced sexual desire and genital pain.

4.2.1.3 *C. spinarum* root extraction/mixture in cold water

Cold water root mixture/extraction/dispersion (28 URs) included making pastes, macerations, dispersals and homogenisations in water and were used across all

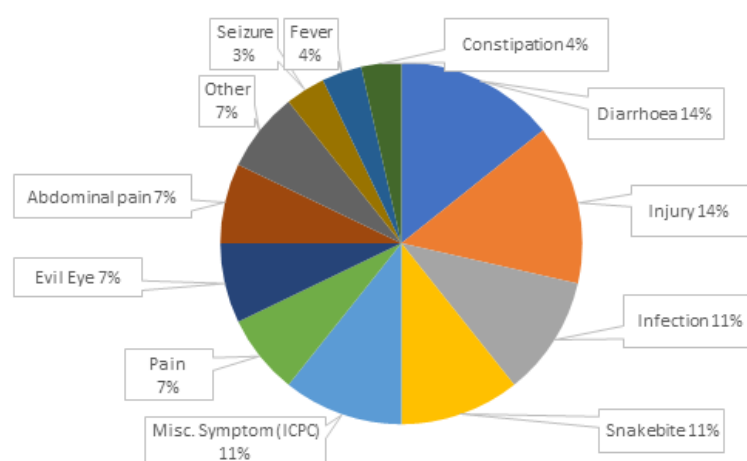


Figure 47: Key ailments treated with cold water mixtures of *C. spinarum* root/rootbark (UR>1)

regions (Supplementary file 6 Table S3). The key therapeutic uses (Figure 47) indicated that diarrhoea, infection, snakebite and injury were the most common ailments treated. The Digestive system (including diarrhoea, helminthiasis, abdominal pain and constipation) and Skin (including snakebite and skin injuries) were the key body systems treated.

4.2.1.4 *C. spinarum*: water extracts of root with additional ingredients

Root preparations of *C. spinarum* may involve the preparation with food ingredients (29 URs), mainly reported in Eastern Africa, e.g., boiling as a decoction in soup (normally meat soup) or animal fat or milk; mixing with alcohol or milk or food (Supplementary file 6 Table S4). The mixing of fresh root with alcohol was reported only in Ethiopia and was used to treat STI, impotence, stomach-ache and headache. Rootbark paste, mixed with black pepper and sugar cane juice, was given orally in India to treat fever of long duration. Rootbark decoction mixed with chili pepper was used as an anthelmintic in Senegal. All other preparations were boiled in soup or animal fat or milk with the key therapeutic indication being STI. Other ailments treated included pain,

digestive complaints, respiratory conditions, weakness and impotence. The preparation with meat soup was common among the Maasai and was also found among some other ethnic groups in Eastern Africa [445] with Orech and Schwarz [351] them as “medicinal soups” and Timberlake [87] as a “health drink”.

4.2.2 Volatilising traditional preparations of *C. spinarum* root

Methods such as inhalation (whole root or decoction), warming/grating/scraping chewing, and application of root involved the volatilising of root ingredients (13 URs) and were reported in Eastern and Western Africa and in Australia (Supplementary file 6 Table S5). The therapeutic uses included toothache, Evil Eye, chest infection, nasal infection, mental disorder, lymphadenitis and measles.

4.2.3 Non-aqueous-based whole root traditional preparation of *C. spinarum*

The use of whole root, pounded, powdered, crushed, chewed and roasted (11 URs), was reported in Africa, the Middle East and South Asia, where it was applied to the skin or taken orally (Supplementary file 6 Table S6). Wounds and pain were treated by direct application of the treated root. Respiratory conditions were treated both orally and by application to the chest.

4.2.4 Overlap in ailments treated with different preparations of *C. spinarum* root and rootbark

There was overlap in ailments treated with different root preparations of *C. spinarum* (Figure 48). GI disorders and infections were treated using all forms of preparation while respiratory disorders were treated using decoction, infusion and crushed root. In the treatment of infections (Figure 49), respiratory tract infection (RTI), GI and skin infections were treated with all forms of preparation while GUI and malaria were only treated with heated water preparations. Helminth infections were

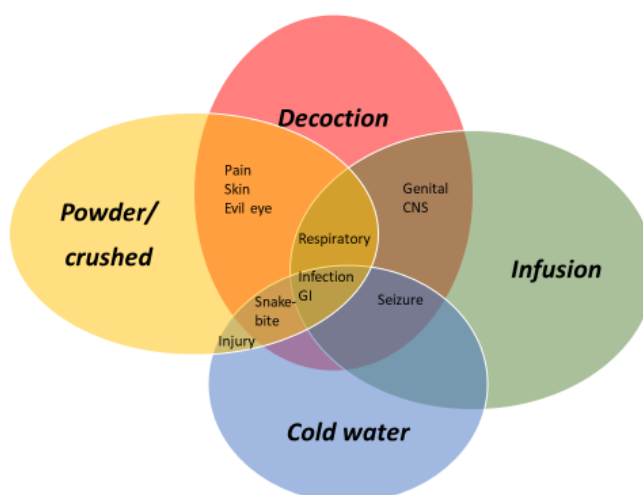


Figure 48: Diagram of overlap (only) in ailments treated with different formulations of *C. spinarum* root/rootbark

grouped under GI infection but only specifically mentioned as treated by decoction and cold-water preparations.

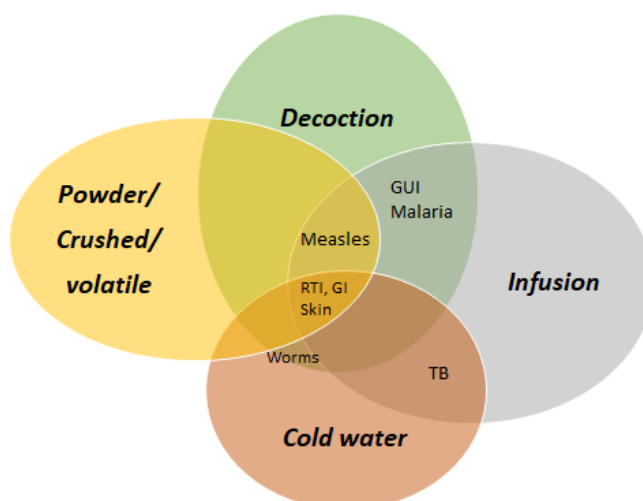


Figure 49: Diagram of overlap (only) in infections treated with different aqueous formulations of *C. spinarum* root/rootbark

4.3 Investigations of phytochemistry and pharmacology of *C. spinarum* root

The phytochemistry and pharmacology of *C. spinarum* root is examined separately according to method of extraction – aqueous extraction, distillation and organic solvent extraction.

4.3.1 Phytochemistry and pharmacology of aqueous extracts of *C. spinarum* root

4.3.1.1 Studies of cold-water extracts of *C. spinarum* root

Cold water extracts of *C. spinarum* have been studied for anti-plasmodial, anti-trypanosomal, anthelmintic, antibacterial and antifungal activity. The treatment of malaria and related symptoms with *C. spinarum* root was commonly reported in the literature (Chapter 3), though normally by decoction or infusion in water and in meat bone soup. Anti-plasmodial activity of the cold-water root extract was tested *in vitro* against Chloroquine (CQ) sensitive (NF54) and CQ (ENT30) resistant strains of *Plasmodium falciparum*. Kirira *et al.* [330] found the extract to have mild activity against NF54 (IC₅₀ 148.53 µg/mL) and to be inactive against ENT30 (IC₅₀ > 250 µg/mL).

Wurochekke *et al.* [446] tested *in vitro* anti-trypanosomal activity of the cold-water extracts based on EM treatment of trypanosomal infections in Nigeria with *C. spinarum*. Motility inhibition was achieved at 45 minutes incubation with root extract (aqueous bark extract more active). The aqueous extract was less active than MeOH extract and was therefore not included in *in vivo* testing [446]. However, there are inconsistencies in the representation of results in this study and the results may be included with this proviso.

The use of *C. spinarum* root in the treatment of intestinal worms in humans and animals is found in the EM literature [399, 400]. Harwansh *et al.* [447] report that *C. spinarum* was used as a purgative and in treating worm infested wounds in animals and used this as a basis for testing the anthelmintic activity of the root *in vitro*. The root extract was found to be inactive against *Pheretima posthuma* (Indian earthworms) at 25, 50 and 100 mg/mL giving a time to paralysis of worms at 98, 91 and 66 minutes (resp.) and time to death of worms at 466, 434 and 396 minutes (resp.) relative to piperazine control at 10 mg/mL giving 22/64 minutes to paralysis/death [447].

Pallant and Steenkamp [448] reported the use of macerated root of *C. spinarum* in the treatment of respiratory infections among the Venda in South Africa. They tested cold-water and MeOH extracts of *C. spinarum* and other Venda species for antimicrobial activity using disc diffusion assays against: *C. albicans* (CA), *Haemophilis influenzae*, *Klebsiella pneumoniae* (KP), *S. aureus* (SA), *Streptococcus pneumoniae* and *Mycobacterium smegmatis*. The results for *C. spinarum* were not listed indicating that the extract was inactive against all test species [448].

While there is some correspondence between the traditional indications of cold-water extract of *C. spinarum* root in EM and the chosen pharmacological studies e.g., treatment of fever [309, 449], wounds [450], intestinal worms [399];

diarrhoea [451]; stomach ache [410], the extracts were inactive or, at best, had poor activity.

4.3.1.2 Studies of aqueous decoction of *C. spinarum* root

Decoction in water was the most common EM preparation of the root of *C. spinarum*. *In vitro* and *in vivo* studies of aqueous decoction have been conducted to test for antiviral, antimicrobial, analgesic, tyrosinase inhibitory and anti-spasmodic activity.

Muthaura *et al.* [291] found the warm-water extract (60°C/1 hour) for anti-plasmodial activity to be inactive against D6 (CQ sensitive) ($IC_{50} > 100 \mu\text{g/mL}$) and W2 (CQ resistant) ($IC_{50} > 100 \mu\text{g/mL}$) strains of *P. falciparum* which the authors classified as inactive, a lower endpoint than Kirira *et al.* [330] (section 4.3.1.1).

Tolo *et al.* [452] *C. spinarum* root decoction for antiviral activity. Several studies report the use of *C. spinarum* root decoction in the treatment of viral and potentially viral infections including herpes zoster [423]; measles [91, 453, 454]; rabies [254]; polio [120]; unspecified skin infections [254, 277] and unspecified STIs [293, 455]. Tolo *et al.* [452, 456] conducted *in vitro* studies for activity against human cytomegalovirus (HCMV) and Herpes Simplex Virus (HSV). The aqueous decoction of rootbark showed a moderate activity against HCMV infected cells and a dose-dependent response in a dose range 10 to 100 $\mu\text{g/mL}$ ($n = 3$) [456]. The greatest activity was reported for 100 $\mu\text{g/mL}$ with 68% inhibition (IC_{50} 74 $\mu\text{g/mL}$). The CC_{50} was reported to be $>100 \mu\text{g/mL}$ with no exact figure given so it is not possible to calculate the Selectivity Index (CC_{50}/IC_{50}). The Selectivity Index (SI) is a measure of the toxicity of the extract or compound. It is the ratio between the cell cytotoxicity and the effective dose (CC_{50}/IC_{50}); the larger the ratio, the less likelihood of the extract/compound being toxic to the cell/host organism at the effective therapeutic dose i.e., it is selective for the pathogen relative to the host. Tolo *et al.* [452] also studied the aqueous decoction of rootbark for activity against four HSV strains including the aciclovir resistant strains (AP^r) 7401H HSV-1 and (TK⁻) B2006 HSV-1 measuring plaque inhibition and reduced virus yields ($n = 10$). The IC_{50} in plaque inhibition for all four strains ranged from 5.66 to 15.1 $\mu\text{g/mL}$ ((AP^r) 7401H HSV-1 $8.1 \pm 1.56 \mu\text{g/mL}$; (TK⁻) B2006 HSV-1 $11.1 \pm 5.66 \mu\text{g/mL}$) with CC_{50} of 480 $\mu\text{g/mL}$ indicating a good selectivity index. The virus reduction assay indicated 96.3 to 100% reduction at extract concentration of 200 $\mu\text{g/mL}$ compared to $\leq 3\%$ reduction for Aciclovir (5 $\mu\text{g/mL}$) for the two resistant strains ($p < 0.005$). An *in vivo* assay was conducted with mice whose skin was infected with (AP^r) 7401H HSV-1 and wild-type HSV-2 and treated with aqueous *C. spinarum* root extract at 250 mg/kg three times a day. The aqueous decoction was active, delaying onset of infection

for 2 days and reducing mortality significantly for each strain (70-100% reduction) ($p < 0.05$).

Antibacterial assays of EM species from Venda (South Africa) were conducted by Steenkamp *et al.* [457]. From the EM studies (4.2.1.1), the root decoction was used in the treatment of a wide range of infections which may be bacterial in origin including GUI, respiratory, GI, skin and lymphadenitis. Antibacterial assays using plate-hole diffusion and broth microdilution methods were conducted against *E. coli* (EC), *P. aeruginosa* (PA), *S. epidermidis* and *S. aureus* (SA). *C. spinarum* root extracts - aqueous decoction and MeOH extract – showed no zone of inhibition (ZOI) though the endpoint for inactivity was not stated.

Malik *et al.* [458] examined the *in vitro* tyrosinase inhibitory activity of *C. spinarum* aqueous and organic solvent extracts. Tyrosinase is involved in melanin production and inhibiting this enzyme could have a skin lightening effect, useful in hyperpigmentation disorders, in the cosmetics industry and potentially in melanoma formation [459, 460]. The *stem* rather than the root was reported to be used as a paste for skin lightening in Mayotte Island [382] although it can be seen in Table S10 (Supplementary file 6) that there is an overlap in the phytochemistry of root and stems. Malik *et al.* [458] reported an IC_{50} 24.33 $\mu\text{g/mL}$ for the aqueous root decoction and similar to +ve control Arbutin: IC_{50} 15.80 $\mu\text{g/mL}$ (CC_{50} not reported) ($n = 3$).

Oluoch *et al.* [461] cited EM literature from Kenya as the basis for their laboratory investigations of the anti-spasmodic activity of *C. spinarum*. Among the Luo and Keiyo communities, the aqueous root decoction of *C. spinarum* was used in the treatment of abdominal colic and diarrhoea. The broader EM data cited in section 4.2.1.1 indicate that *C. spinarum* root decoction was used widely in the treatment of gastrointestinal ailments accounting for 14% of all root decoction preparations, validating this laboratory investigation [461]. This *in vitro* study used rabbit jejunum to examine inhibition of contractility resulting from Acetylcholine (ACh), KCl and CaCl_2 -induced contractions ($n = 5$). At 1 and 3 mg/mL, the extract reduced ACh induced contractions by 39 and 56% (resp.) ($p < 0.001$); it reduced KCl induced contractions by 18 and 47% (resp.) ($p < 0.01$) and reduced CaCl_2 induced contractility by 31% ($p < 0.05$) and 61% ($p < 0.001$) (and 38 and 65% relative to basal tone). These results indicate significant anti-spasmodic activity of the extract at high test doses and correlate with EM use for digestive disorders.

Ibrahim *et al.* [462] listed toothache and other EM uses of *C. spinarum* as the basis for examining the analgesic activity of aqueous extracts of different plant parts, reflecting EM practice. The treatment of pain by root decoction accounts for 8% of all root decoctions (section 4.2.1.1). The *in vivo* analgesic

activity of the rootbark decoction was examined in mice through mechanical (tail-clip method) and chemical (acetic acid induced writhing) induction of pain (n = 6). In the mechanical method, 50 mg/kg intraperitoneal (i.p.) of extract was used with metamizole (10/20/30 mg/kg) as +ve control and distilled water as -ve control. Rootbark extract analgesic activity peaked at 45 minutes post extract dose and produced a similar response to metamizole control at 10 and 20 mg/kg but less than the 30 mg/kg dose ($p < 0.001$). The rootbark produced a poorer response than fruit, leaf and seed extracts but was better than stembark extracts. In the chemical method, the extract dose ranges were 50/100/150 mg/kg i.p. with Aspirin (150 mg/kg) as +ve control and normal saline as -ve control. The results indicated a dose dependent response measured by the mean number of writhings after administration of acetic acid. The rootbark extract at 150 mg/kg was the most active (9 ± 0.27 writhings) vs -ve control (23 ± 0.15) and +ve control (10 ± 0.36). The analgesic response was better for all other extracts (fruit, leaf, seed) excluding stembark ($p < 0.037$). One limitation of this study relates to the high dose of i.p. aspirin control which is beyond the normal human dose range given that 100mg/kg/day p.o. may cause salicylate poisoning [463].

The phytochemistry of the aqueous extract of root has not been examined in detail. Malik *et al.* [458] conducted organic solvent and aqueous decoction (2-hour) extractions of *C. spinarum* root and identified a single compound by Gas Chromatography-Mass spectrometry (GC/MS) in the aqueous decoction and MeOH extract. The sesquiterpene, 2,3,3-trimethyl 2-(3-methylbuta-1,3-dienyl)-6- methylenecyclohexanone (**1**) (Figure 50), was identified and was also identified by GC/MS in the MeOH extract by Saeed *et al* [464]. While GC/MS is useful for separating and identifying volatile and semi-volatile compounds that are sufficiently non-polar and of low molecular weight, as a method it cannot identify all constituents of an aqueous decoction [465]. More suitable techniques are HPLC, HPTLC and other high-resolution techniques. HPLC/HPTLC can be linked to a high-resolution detector such as UV/VIS HPLC detectors to identify the components of the sample. This compound has been isolated from several other species: *Pterocarpus macrocarpus* Kurz, *Alquilaria sinensis* Merr., *Atractylodes macrocephala* Koidz., *Artemisia capillaris* Thunb., *Flos chrysanthemi*, *Acilepis spirei* (Gand.) H. Rob. and *Curcuma zedoaria* (Christm.) Roscoe.

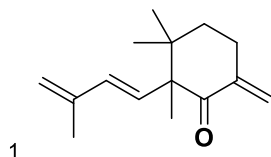


Figure 50: 2,3,3-trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecyclohexanone (**1**)

Malik *et al.* [458] found the aqueous decoction of *C. spinarum* (syn. *C. opaca*) roots to be active in a tyrosinase inhibition assay. To the best of our knowledge there is no identified bioactivity for this compound.

4.3.2 Phytochemistry and pharmacology of volatilised preparations of *C. spinarum* root

There have been no laboratory investigations to date examining the bioactivity of preparations of *C. spinarum* root which volatilise the root constituents. However, investigations of the bioactivity of individual volatile components at the phytochemical level may give a proxy measure of testing the bioactivity of the volatilised whole root.

4.3.2.1 Phytochemistry of steam and hydro- distillate of the root of *C. spinarum*

Laboratory studies [466-470] have examined the constituents of the distilled root

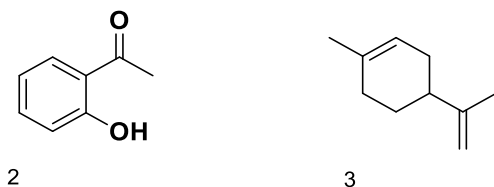


Figure 51: Compounds **2** and **3** from steam distillate of *C. spinarum* root

oil. The key component isolated across studies is the simple phenolic, 2'-hydroxyacetophenone (**2**) (2-HAP) (Figure 51), ranging between 89.5% to 99.9% of the root oil from samples from Australia, Kenya, India and Benin while it has also been isolated from organic solvent fractions (Table 22) [464, 471]. Some of the minor constituents identified include the terpenes, 10-epi- γ -eudesmol, 1,2-dehydrosesquiceneole and Juniper camphor (enantio-7(11)-eudesmen-4-ol) [469] and γ -eudesmol, Myrcene and Limonene (**3**) (Figure 51) [470]. For other minor components of the root oil, see Tables S8 and S9 (Supplementary file 6).

Table 22: Key compounds isolated from root and rootbark oil of *C. spinarum*, extracted by distillation (>0.3% of oil)

Synonym cited	Country/ Plant part	% Yield oil (method)	Compound ¹³	% Yield from oil	Ref
<i>C. edulis</i> (Forskal) Vahl	Kenya/ fresh rootbark	0.093 (steam)	2-HAP (2)	99.9	[466]
<i>C. lanceolata</i> R.Br.	Australia/ fresh rootbark	0.0551 (steam)	2-HAP (2)	N/S	[467]
<i>C. opaca</i> Stapf. ex Haines	India/ fresh root	0.20 (hydro)	2-HAP (2)	89.5	[469]
			10-epi- γ -eudesmol	2.4	
			1,2-dehydro sesquicineole	1.5	
			Juniper camphor	1.1	
			β -eudesmol + α -eudesmol	0.5	
<i>C. edulis</i> Vahl	Benin/fresh root	0.20 (steam)	2-HAP (2)	92.7	[470]
			γ -Eudesmol	1.2	
			Limonene (3)	1.2	
			Myrcene	0.8	

4.3.2.2 Bioactivity studies of phytochemicals isolated from *C. spinarum* root distillate

A literature search has yielded a few reports on the bioactivity of 2-HAP (2). Hettiarachchi [468] and Hettiarachchi *et al.* [467] conducted antibacterial assays on 2 (Supplementary file 6 Table S17). The authors reported strong activity against all four bacterial strains tested: *Escherichia coli* (EC), *Bacillus subtilis* (BS), *Pseudomonas aeruginosa* (PA) and SA. It displayed a minimum inhibitory concentration (MIC) of 1.25 mg/mL and a minimum bactericidal concentration of 2.5 mg/mL against all strains. Best practice suggests that strong activity would require MIC in the 30-50 μ M range [472] and 1.25 mg/mL represents a much higher concentration. This study has limitations in that the controls are not listed for comparison. Kim and Kang [473] conducted a study on the acaricidal activity of 2 and related compounds. 2-HAP (2) was found to have good activity against *Dermatophagoides* spp. and *Tyrophagus putrescentiae* with LD₅₀ of 1.12 to 2.1

¹³ All compounds identified by GC-MS. Hettiarachchi *et al* [467] also used ¹H-NMR and FTIR, Mallavarapu *et al* [469] also used RI, MS, ¹H-NMR and ¹³C-NMR for the identification of 2-HAP (2).

$\mu\text{g}/\text{cm}^2$ and was more potent than the control compounds, ranging from 8.5 to 21.2 times more potent than DEET and 4.02 to 10.04 times more potent than benzyl benzoate. In a xanthine oxidase (XO) inhibition assay, Saeed and Ahmed [464] reported that **2** had an IC_{50} of $11.66 \pm 0.27 \mu\text{g}/\text{mL}$ comparing well to allopurinol (control) (IC_{50} of $2.74 \mu\text{g}/\text{mL}$). In Chapter 5, *in silico* analysis of **2** is conducted to augment these known activities with network pharmacology (NP) predicted activities and compare them to some traditional uses of the root.

The antibacterial effect of **2** (at a high concentration) could correlate with the traditional use of *C. spinarum* root in chest congestion, nasal infection, toothache and adenitis. The acaricidal activity of **2** could correspond with other EM uses of *C. spinarum* root such as in scabies, other skin conditions and the burning of root as a fumigant. The use in skin conditions is in aqueous-based preparations and **2**, with moderate water solubility, could be effective therapeutically [179]. The XO inhibitory activity (relevant in gout) could correspond with the EM use of *C. spinarum* root decoction in the treatment of pain and arthritis.

Several monoterpenes were also isolated in minor yields from the steam and hydro- distillates (Supplementary file 6 Tables S8 and S9) with limonene (**3**) having the highest concentration (1.2% of oil) [470]. In a review of limonene (**3**), Erasto and Viljoen [474] described a wide range of biological activities including antimicrobial, antioxidant, anti-inflammatory, antinociceptive, anticancer and insecticidal, in addition to enhancing the transdermal penetration of drugs. It was reported to be active against *Salmonella* sp, EC, SA and *Pseudomonas* species although other studies found no activity against G-ve bacteria. The authors reported antioxidant and anti-inflammatory activity *in vivo* in rat lung, a reduction in bronchial obstruction and analgesic activity in mice. The anticancer activity of limonene (**3**) has been well documented [475]. It has been shown in cell-based and animal studies to reduce tumour growth, size, number of tumours and weight and increase survival and latency. It is thought to have a preventative role in cancer and is considered safe with no toxicity observed below 1,650 mg/kg in studied animals [475, 476].

Other minor volatile ingredients listed in Table 22, S8 and S9 are also relevant to the traditional use of inhaled root from steam and smoke, though concentrations may be too low to have an effect. 10-epi- γ -eudesmol (2.4% of oil) has shown activity as a tick repellent and has antimicrobial activity against SA and EC [477, 478]. *In silico* docking analysis predicts good topical anti-inflammatory activity for 10-epi- γ -eudesmol [479]. Myrcene (0.8% of oil) has been shown to have a wide range of biological activities with antioxidant, anti-inflammatory, analgesic, anti-ageing and anxiolytic activities, some of which support traditional indications of the applied and inhaled crushed root [480]. In

addition to these molecules, smoke of burned *C. spinarum* root is likely to be different in composition to aqueous or volatilised preparations although this has not been investigated for *C. spinarum* [443].

4.3.3 Phytochemistry and pharmacology of selected *C. spinarum* root secondary metabolites extracted using organic solvents

The whole root preparation of *C. spinarum* as it is used traditionally has not been investigated phytopharmacologically. It has not been possible to obtain an adequate sample of *C. spinarum* root for our own laboratory investigation. Therefore, the only available approximation of the whole root metabolome and traditional usage of whole root, ingested or applied, is a literature study of organic solvent root extracts and isolated phytochemicals with the proviso that these may not mimic extracts used ethnomedically qualitatively or quantitatively.

C. spinarum has been analysed phytochemically under various synonyms since 1954 with the isolation of the cardiac glycoside, odoroside H (**4**), and the sesquiterpene, carissone (**5**), from the roots of plant material harvested in Northern Queensland, Australia [481]. Since then, many studies have been carried out identifying different classes of molecules [441, 442]. An overview of the phytochemical classes of *C. spinarum* root is represented in Table 23 and Table S10 (Supplementary file 6). Key high yielding metabolites are illustrated below in Figure 52 while selected lower yielding compounds including, but not limited to, lignans, sesquiterpenes, terpenes, sterols, coumarins and phenolics are illustrated below in section 4.3.3.10 (and Table S10-S13 Supplementary file 6).

Lignans, such as the more *Carissa* specific carissanol (**7**) and carinol (**8**), represent one of the molecular classes that occurs in highest yield in *C. spinarum* root (Table 24 and S12) [468, 471, 482]. Other more common lignans are found such as (-) Nortrachelogenin (**6**), which is abundant in root extracts of *C. spinarum* [471]. Terpenes and steroids have also been identified in *C. spinarum* root. To date, a number of eudesmane sesquiterpenoids, have been isolated including carissone (**5**) and its hydroxylated derivatives: 6 α -carissanol, (+)-6 β -carissanol, 2 α -carissanol [468, 482]. Other ubiquitous terpenes have been isolated, mainly from MeOH extracts of *C. spinarum* roots and through distillation (4.3.2.1) [469, 470]. The highest yields of all terpenes isolated from *C. spinarum* root are betulinic acid (**12**), ursolic acid (**9**) and carissone (**5**) (Table 24). Phenolics and coumarins have been identified in *C. spinarum* root, mainly in low yield. 2-HAP (**2**) was isolated by distillation (0.19%) (4.3.2 and Table S8 Supplementary file 6) and from MeOH extract (0.048%) [464, 471]. The flavonoid, quercetin (**11**), was isolated from EtOH and MeOH root extracts

(0.49%) (Table 24) [464, 483]. Some of the most important cardiac glycosides to be isolated from *C. spinarum* root are of the odoroside scaffold, including odoroside H (**4**) with the remainder illustrated below in section 4.3.3.10 and in Table S13 (Supplementary file 6). The wide concentration range of odoroside H (**4**) (Table 24) isolated using different solvents from two countries (Australia and India) and three species which have since been taxonomically “lumped” as *C. spinarum* exemplifies the potential for metabolomic variety in ethnopharmacology [481, 484, 485], an issue which will be further discussed below (section 4.5.1). Recent publications detail other classes of glycoside including lignan and phenolic glycosides which are illustrated in section 4.3.3.10 [486, 487] and they occur in very low yields (Supplementary file 6 Table S13). Rutin (**10**), a glycoside of quercetin (**11**), is a significant metabolite isolated from *C. spinarum* root (section 4.3.3.7) [464, 483]. Other compound classes isolated from *C. spinarum* root in low or unknown yields include alkaloids, quinic acid derivatives and a benzodiazepine analogue (section 4.3.3.10 and Supplementary file 6 Table S10, S14, S15, S22) [488-490].

In this work we will examine ten high yielding secondary metabolites in detail (Table 24, Figure 51 and Figure 52), six that are more specific to *C. spinarum* (**2**, **4-8**), and four ubiquitous compounds (**9-12**). Selected bioactivities of the key secondary metabolites of *C. spinarum* root (**4-12**), which bear a relationship to EM usage, are discussed below with **2** discussed above (section 4.3.2.2). This pharmacological data will be integrated with the most potent reported activities of *C. spinarum* root extracts and analysed in relation to the traditional usage in section 4.4. The more *Carissa* specific compounds have limited bioactivity data available whereas there has been extensive research on the ubiquitous compounds

Table 23: Summary of phytochemistry of *C. spinarum* root from selected texts

Cpd Class	Ref [484]	Ref [471]	Ref [468]	Ref [488]	Ref [464]	Ref [491]	Ref [486]	Ref [492]	Ref [493]	Ref [490]
Lignan		✓ ¹⁴					✓			
Sesquiterpene		✓		✓						
Terpene				✓	✓	✓S			✓S	
Sterol			✓S		✓	✓S			✓S	
Phenolic		✓	✓S		✓					
Coumarin		✓					✓			
Flavonoid					✓	✓S		✓S	✓S	
FAs & esters					✓					
Benzodiazepine analogue										✓
Saponin						✓S		✓S		
Anthraquinone								✓S		
Glycoside						✓S	✓			
CG	✓		✓S					✓S	✓S	
Alkaloids			✓S	✓						✓S
Tannin						✓S		✓	✓S	

¹⁴ ✓represents compound specifically identified; ✓S represents compound class identified in phytochemical screening; CG = Cardiac Glycoside; FA = Fatty Acid

Table 24: Top ten secondary metabolites by yield isolated from *C. spinarum* roots¹⁵.

Compound	Class/ plant part	Top yield extract	ADMET*	Top yield % w/w	Yield range %w/w	Ref top yielding sample
2-HAP (2)	Phenolic Root	Distillation	Good	0.19	0.048 - 0.19	[470]
(-) Nortrachelo- genin (6)	Lignan Root	MeOH	Good	0.011	0.011	[471]
Carissanol (7)	Lignan Root	MeOH	Good	0.01	0.0024 - 0.01	[471]
Carinol (8)	Lignan Root wood	MeOH	Poor	0.01	0.007 – 0.01	[468]
Carissone (5)	Terpene Root	MeOH	Good	0.12	0.005 – 0.12	[482]
Odoroside H (4)	CG Root	EtOH	Good	0.02	0.0007 – 0.02	[484]
Ursolic acid (9)	Triterpene Rootbark	EtOH	Poor OB	0.09	0.09	[494]
Rutin (10)	Glycoside Root	EtOH	Poor OB	0.65	0.65	[495]
Quercetin (11)	Triterpene Root	EtOH	Poor OB	0.49	0.49	[495]
Betulinic acid (12)	Triterpene Rootbark	MeOH	Poor OB	0.05	0.05	[494]

¹⁵ All ADMET predictions used FAFDrugs-4 except Carinol with prediction from <https://admetmesh.scbdd.com/>.

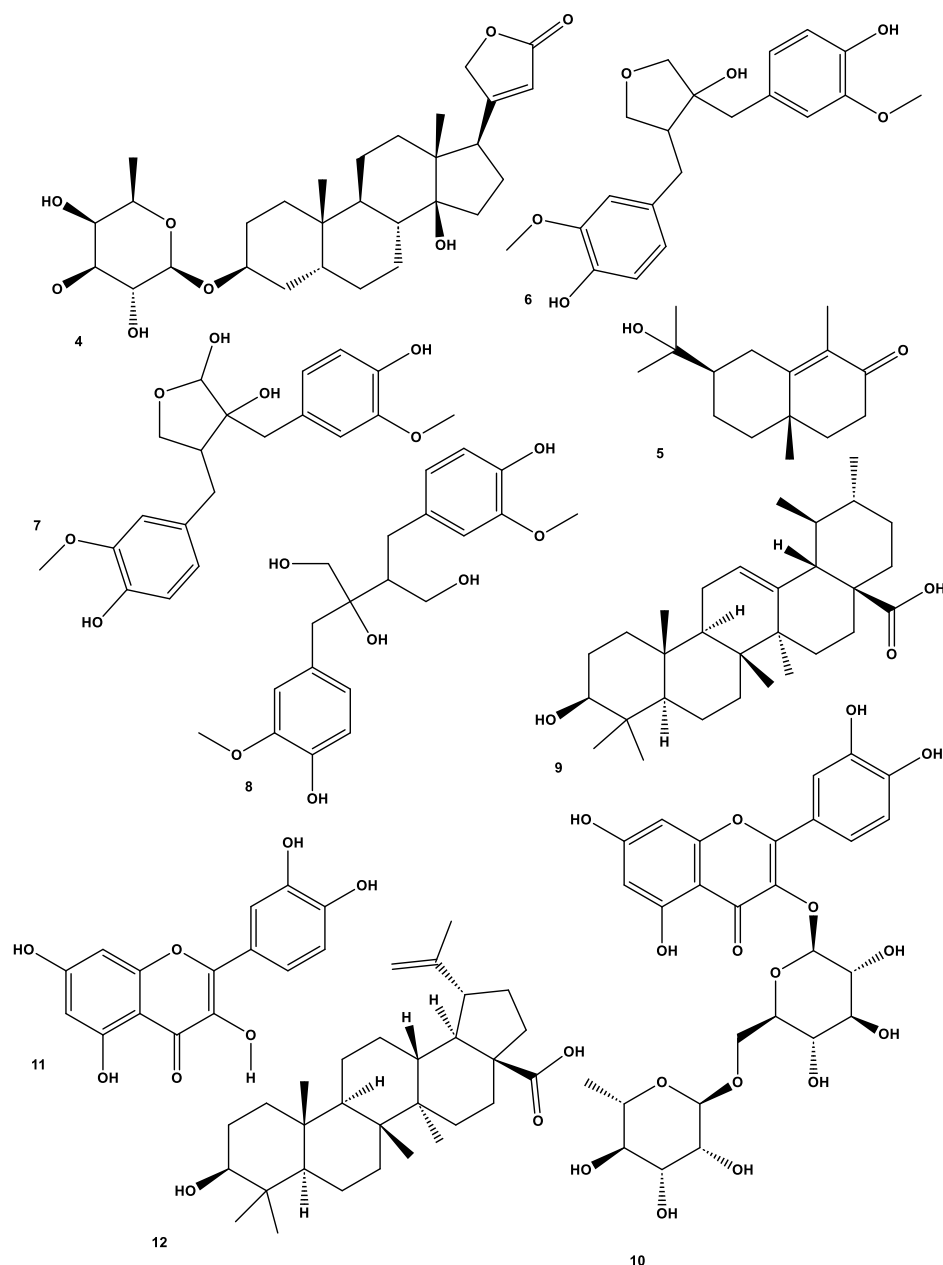


Figure 52: Compounds **4-12** - some key compounds by yield isolated from organic solvent extracts of *C. spinarum* root

4.3.3.1 (-) Nortrachelogenin (**6**)

Studies show that the lignan, (-) notrachelogenin (**6**), demonstrated strong antioxidant activity in a Nitro Blue Tetrazolium superoxide scavenging assay with IC_{50} values of 13.1 $\mu\text{g/mL}$ [496] and moderate antioxidant activity in DPPH assay (IC_{50} 35.8 μM) [173]. It reduced inflammatory mediators in murine J774 macrophages in a dose-dependent manner at 7 and 25 μM and *in vivo* at 100 mg/kg in carrageenan-induced paw oedema in mice ($p < 0.01$) [171]. It reduced IL-4 and IL-13 induced macrophage activation at 30 μM *in vitro* and reduced fibrosis *in vivo* in male C57BL/6N mice (250 μg intradermal) ($p < 0.05$ at least)

[497]. However, it was almost inactive in decreasing IL-6 production in TNF- α stimulated MG-63 cell line [496]. Kebenei *et al.* [498] conducted *in vitro* anti-plasmodial activity assays against D6 strain and they reported that **6** had moderate activity (IC_{50} 14.53 μ g/mL). Lee *et al.* [499] tested **6** for activity against ATCC strains of *Enterococcus faecium*, SA, methicillin resistant *S. aureus* (MRSA), EC O157, *Salmonella typhimurium* (ST) and antibiotic-resistant PA using the well-dilution method. MIC of 2.5-5 μ g/mL was obtained for all bacteria compared to melittin control (MIC 0.4–0.7 μ g/mL) ($n = 3$). However, it was inactive against 6 strains of SA up to a concentration of 128 μ g/mL [173]. (-) Nortrachelogenin (**6**) was found to be moderately active against CA, *Trichosporon beigellii* and *Malassezia furfur* with an MIC between 12.5 and 25 μ g/mL [500] and was inactive *in vitro* against HSV in Vero cells [173]. In anti-proliferative effects, **6** had low activity against cancer cell lines: lung (A549), breast (MCF7) and a normal human cell line (WI-38, lung fibroblast) with IC_{50} 29.0, 88.3, >100 mg/mL respectively [173]. Yatkin *et al.* [501] found low antiproliferative efficacy for **6** against PC-3M-luc2 human prostate cancer cells *in vitro* at 100 μ M ($p < 0.001$, $n = 3$) and moderate proapoptotic activity at 40 μ M ($p < 0.05$, $n = 3$). Peuhu *et al.* [502] found **6** to be effective at sensitising prostate cancer cells *in vitro* to tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis at 40 μ M ($n = 3$). At a cellular level, **6** is an inhibitor of Akt signalling and of tyrosine kinase activation, determined by Western Blot analysis, amplified TRAIL-induced caspase-3 activation (IC_{50} 32 μ M) and prevented colony formation in prostate cancer cells at 40 μ M ($n \geq 3$) (no normal cell comparison). Compound **6** did not prevent mammary and uterine tumour progression in a rat model; it had endocrine modulating activity in male and female rats; and it was found not to be converted to enterolactone by gut microbiota which is proposed to contribute to anti-tumorigenic properties of some lignans [503].

4.3.3.2 Carissanol (**7**)

The *Carissa*-specific lignan, carissanol (**7**), isolated in two studies, has shown moderate antioxidant activity in DPPH assay (IC_{50} 37.12 μ M and IC_{50} 33.4 μ M) [173, 504]. Carissanol (**7**) has shown moderate antiproliferative effects against cancer cell lines: lung (A549), breast (MCF7) with IC_{50} 11.0, 17.4 μ g/mL respectively. However, the strong toxicity of carissanol (**7**) to a normal human cell line (WI-38, lung fibroblast) (IC_{50} 6.2 μ g/mL) indicates a lack of selectivity for cancer cell lines [173].

4.3.3.3 Carinol (8)

The *Carissa*-specific lignan carinol (**8**) isolated in two studies, has shown moderate antioxidant activity in DPPH free-radical scavenging assay (IC_{50} 47.87 μ M and IC_{50} 20.2 μ M) [173, 504]. In antimicrobial studies, Carinol (**8**) was active against EC, BS and SA: MIC 1.25 mg/mL [468]. Mahmud *et al.* [505] tested carinol (**8**) *in silico* to determine efficacy against SARS-COV2 protease and it was predicted to have good characteristics for *in vitro* and *in vivo* testing. Carinol (**8**) had strong antiproliferative effects against cancer cell lines: lung (A549), breast (MCF7) with $IC_{50} < 1$ μ g/mL but with strong toxicity to a normal human cell line (WI-38, lung fibroblast) indicating a lack of selectivity for cancer cell lines [173].

4.3.3.4 Carissone (5)

The *Carissa*-specific eudesmane sesquiterpene, carissone (**5**), isolated from *C. spinarum* root in two studies, was investigated by Galipalli *et al.* [174] for anti-inflammatory activity. They found that it inhibited NO production in J774A.1 tumour cells with IC_{50} 20.1 ± 2.69 μ g/mL and inhibited inflammatory mediators at 30 μ M. Cell viability was shown to be good at $94.18 \pm 2.79\%$ (50 μ g/mL) ($p < 0.01$). Hettiarachchi [468] found **5** to have weak antibacterial activity against EC, BS and SA (MIC 5-10 mg/mL). Lindsay *et al.* [506] found a lower MIC for **5** against standard strains of SA (ACM844), EC (ACM845) and PA (ACM846) using Fluorescein diacetate assay (MIC 0.1-0.5mg/mL vs EC and SA and MIC 1-2 mg/mL vs PA) ($n = 3$). Carissone (**5**) was tested for activity against *Aspergillus niger* and *A. fumigatus* using the agar-streak assay (replicates not reported) and displayed low activity relative to other tested terpenes (maximum 60% inhibition at 1.0 mg/mL) [507]. Carissone (**5**) was predicted to have activity against HIV when tested *in silico* in a molecular docking study on HIV reverse transcriptase enzyme and HIV protease [508].

4.3.3.5 Odoroside H (4)

Oodoroside H (**4**) was isolated from *C. spinarum* root in two studies and from three other genera. It is thought to be responsible for some of the cardiogenic activity of the *C. spinarum* extract [234]. Apocynaceae-derived cardenolides are known to have anti-tumour activity [509] and **4** was found to have potent activity against renal and prostate cancer cell lines with LD_{50} ranging from 9.26 nM (786-0 cell line) – 86.4 nM (TK-10 cell line) [510]. Rashan *et al.* [511] tested the cytotoxicity of **4** (0.0003–3 μ g/mL) in a monolayer assay against ten human cancer cells lines ($n = 3$) and it was found to have potent activity (IC_{50} 0.010 μ g/mL [0.019 μ M] and IC_{70} 0.025 μ g/mL [0.047 μ M]) with no toxicity to normal cells. Odoroside H (**4**)

also demonstrated dose dependant CNS depressant effects in NMRI mice at 25mg/kg i.p. [512].

4.3.3.6 Ursolic acid (9)

The pentacyclic triterpene, ursolic acid (9), isolated in high concentrations from *C. spinarum* root in two studies, has wide-ranging activities, some of which are summarised here and are relevant to reported traditional use of *C. spinarum*. It has displayed antioxidant activity in a range of human and cell assays, reducing lipid peroxidation and oxidative damage [513]. In an *in vivo* model of Parkinson's Disease in Sprague Dawley rats (n = 6), 9 was found to have antioxidant activity through measurements of superoxide dismutase (SOD), glutathione (GSH) ($p < 0.01$) and catalase (CAT) and lipid peroxidation at 10mg/kg intranigral relative to +ve control ($p < 0.001$) [514]. Anti-inflammatory activity has been demonstrated in cell assays including of macrophages and phagocytes, inhibiting Cyclo-oxygenase 2 (PTGS2/COX2) and reducing inflammatory cytokines such as TNF α and Interleukin (IL) 6. Anti-inflammatory activity has been demonstrated *in vivo* across a range of body systems in rodents such as in models of acute lung injury, asthma and pleurisy, in oedema studies, induced arthritis, ulcerative colitis and Parkinson's Disease (dose ranges 20-25 mg/kg) [513, 515]. Ursolic acid (9) has displayed antibacterial activity against numerous organisms *in vitro* including, but not limited to, KP, *Bacillus cereus*, *Mycobacterium tuberculosis* (MIC 2.5-25 $\mu\text{g/mL}$), *Streptococcus mutans*, *Enterococcus faecalis*, PA (MIC 256 $\mu\text{g/mL}$), EC (MIC 256 $\mu\text{g/mL}$) and SA (MIC 64 $\mu\text{g/mL}$) [513, 515]. Shai *et al.* [516] tested 9 for antifungal activity against *Sporothrix schenckii*, *Microsporum canis*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, CA, *Candida guilliermondii* and *C. spicata* and had an MIC range of 12-63 $\mu\text{g/mL}$. Compound 9 was reported to have *in vitro* antiviral activity against HIV-protease (1 μM), Hepatitis C virus (MIC 0–125 $\mu\text{g/mL}$), rotavirus (MIC 10 μM) and influenza A (MIC 6–9.25 mM) [515]. It has shown antiproliferative activity across a wide range of human cell lines involved in gastrointestinal, genitourinary, blood, skin and CNS cancers, inhibiting proliferation and inducing apoptosis, inhibiting a number of processes through inhibition of e.g., PTGS2/COX-2 and nuclear factor kappa B (NF κ B), and modifying signalling pathways such as Mitogen-activated protein kinase/ extracellular signal-regulated kinases (MAPK/ERK) involved in cancer. It has shown *in vivo* anti-cancer activities in rodent models including of breast cancer, glioma, melanoma and prostate cancer [513]. In other activities, 9 has demonstrated neuroprotective effects *in vivo* through anti-depressant (0.1 mg/kg in mice), anxiolytic (10 mg/kg in Swiss mice) and hypnotic activity (0.3 mg/kg in male ICR mice), and is shown to be protective in early brain injury (25-50 mg/kg in SD rats)

[515]. Compound **9** was found to have weak anticonvulsant activity (50 mg/kg i.p.) in Swiss mice [517]. Ursolic acid (**9**) demonstrated hepatoprotective activity *in vitro* in cell-based assays (10-30 µg/mL) and *in vivo* such as restoring hepatic marker enzymes (e.g., in Wistar rats at 10-20 mg/kg) [515]. Compound **9** had cardioprotective activity *in vivo* (e.g., in C57BL/6 mice at 80 mg/kg/day); anti-hyperlipidaemic activity (e.g., in SD rats at 50 mg/kg) [515]; and spasmolytic activity *in vitro* [518].

4.3.3.7 Rutin (**10**)

A quercetin glycoside, rutin (**10**), isolated from *C. spinarum* root in two studies, has activities which are relevant to the traditional use of *C. spinarum* root, some of which are summarised here. Rutin (**10**) was shown to have antioxidant activity *in vitro* (60% free radical scavenging in DPPH assay at 8 µg/mL) [519] and *in vivo* in Wistar rats (100 mg/kg) [520, 521]. Compound **10** reduced granulocyte colony-stimulating factor (G-CSF)-induced hyperalgesia in Swiss mice (10-30 mg/kg) [522], was anti-inflammatory in carrageenan-induced inflammation in Wistar rats (80 mg/kg) [523] and was reported to inhibit XO *in vitro* (10.66 µg/mL) indicating potential benefits in gout [464]. Inhibition of GI inflammation is demonstrated by **10** (10 mg/kg) in trinitrobenzenesulfonic acid (TNBS) induced colitis in Sprague–Dawley rats [524]. It was immunomodulatory in macrophages infected with dengue virus ($p < 0.001$) [525] and had a range of anti-infective activities. It demonstrated antibacterial activity including but not limited to activity against KP (8 µg/mL) [526] and increased the effect of methicillin against MRSA [526]. It also inhibited biofilm production *in vitro* in *Streptococcus suis* at one quarter of the MIC concentration (0.0781 mg/mL) [527] and was active against *B. cereus*, PA and KP (MIC 30 µg/mL) using broth dilution method [528]. Rutin (**10**) demonstrated anti-plasmodial activity *in vitro* (IC₅₀ 7.10 µM) [529]. It has been shown to have antiviral activity *in vitro* against a SARS-COV-2 viral protease (11 µM) [530], against norovirus (1 µM) in infected murine macrophages (RAW 267.4) [531] and against Dengue virus ($p < 0.001$) in infected macrophages [525]. In addition, **10** is reported to have anti-leishmanial activity (IC₅₀ 10.4 µM) through inhibition of *Leishmania amazonensis* arginase [532]. In other activities, **10** has demonstrated antitumor activity *in vivo* against leukaemia cells in BALB/C mice (6-12 mg/kg) [533]; hepatoprotective activity (20 mg/kg) *in vivo* in Swiss mice with CCL₄ and paracetamol-induced liver damage [534]; anti-snake venom activity (14.4 mg/kg) in Swiss mice [535]; prevention of diabetic complications in the liver, kidneys, cardiovascular system and nervous system [521, 536]; and neuroprotective activities [520].

4.3.3.8 Quercetin (**11**)

The flavonol, quercetin (**11**), the aglycone of **10**, was isolated from *C. spinarum* root in two studies. It is the most abundant dietary flavonoid [537] with wide-ranging bioactivities, some of which are relevant to reported traditional use of *C. spinarum* and are summarised here. Quercetin (**11**) demonstrated antioxidant activity *in vitro* (5.7 μ M) and *in vivo* in phenylhydrazine-mediated oxidative stress in male SD rats (25 and 50 mg/kg) [538]. It had analgesic activity *in vivo* in a range of studies, e.g., in reducing mechanical allodynia in rats (100 mg/kg) [539]. Compound **11** had an anti-inflammatory effect in collagen-induced arthritis in C57BL/6 mice (30 mg/kg) and had XO inhibitory activity *in vitro* (8.40 μ g/mL) [464, 540]. In relation to other inflammatory conditions, **11** was active *in vitro* in cell-based assays of allergic asthma (40 μ M), allergic rhinitis (30 μ M) and atopic dermatitis (30 μ M), *in vivo* in allergic airways disease in Balb-c mice (8-16 mg/kg) [541] and was immunomodulatory in macrophages ($p < 0.001$) [525]. In anti-infective activity, **11** demonstrated anti-plasmodial activity *in vitro* ($< 14 \mu$ M) [529] and *in vivo* in *P. berghei* infected mice (50 mg/kg) [542] and had anti-giardial activity *in vitro* (26.5 μ g/mL) [543]. Antibacterial activity was demonstrated by **11** *in vitro* in diffusion-based assays against EC, BS and PA [544] and **11** reduced *Streptococcus suis* virulence *in vitro* (16-32 μ g/mL) and *in vivo* in C57BL/6J mice (100 mg/kg) [545]. In antiviral activity, **11** was active *in vitro* against a SARS-COV-2 viral protease (7 μ M), dengue virus in infected macrophages (24.5 μ M), human cytomegalovirus (HCMV) (5.931 μ g/mL), varicella zoster (3.835 μ g/mL) and Influenza A (1 ng/mL) [525, 537, 546, 547]. In other activities, **11** was shown to improve cardiovascular health *in vivo* e.g., reduction in blood pressure and heart rate in Wistar Kyoto mice (5-10 mg/kg p.o.); indicated potential anti-Alzheimer's effect in multiple *in vivo* studies e.g., in SAMP8 mice (25 mg/kg p.o.); had hepatoprotective activity in C57BL/6J mice (100 mg/kg p.o.); was cytotoxic *in vitro* against a wide range of human cancer cell lines, e.g., PA-1 (ovarian cancer) at 50-75 μ M and *in vivo* in lymphoma in mice (25-75 mg/kg i.p.); demonstrated wound healing in mice at 0.1% w/v; had spasmolytic activity *in vitro* in guinea pig ileum at IC_{50} 7.8 μ M [538, 548, 549]; and had tyrosinase inhibitory activity IC_{50} 10.73 μ M [550].

4.3.3.9 Betulinic acid (**12**)

The pentacyclic triterpene, betulinic acid (**12**), isolated from *C. spinarum* root in one study, has many pharmacological activities of relevance to the traditional use of *C. spinarum*, some of which are summarised here. In antioxidant activity, **12** reduced lipid peroxidation *in vivo* in rats with induced lung inflammation [551], improved haematological markers and lipid peroxidation in a dose-dependent manner (at 2-10 mg/kg) in carcinogen treated rats, [552] and prevented the loss

of hepatic antioxidants in CCl₄-treated Wistar rats (50-100 mg/kg) [551]. Betulinic acid (**12**) displayed anti-inflammatory activity across a range of models: it inhibited PTGS2/COX2 expression *in vitro* in lipopolysaccharide (LPS) - activated human peripheral blood mononuclear cells (1-2 µg/mL) [553]; it reduced inflammatory pain in mice with formalin-induced paw pain (10 mg/kg) and reduced inflammation in carrageenan-induced paw oedema in chickens (10 mg/kg) [551, 554]. In addition, **12** had anti-inflammatory activity in an acute lung inflammation model in mice (30 mg/kg) [551], it reduced levels of inflammatory cytokines *in vivo* at 67mg/kg in an acute lung injury model in Balb-C mice [555] and had immune-modulatory activity in mice immunised with sheep red blood cells (5 mg/kg) [551]. Betulinic acid (**12**) was active *in vitro* against CQ sensitive (T9-96) and CQ resistant (K1) strains of *P. falciparum* (IC₅₀ 25.9 and 19.6 µg/mL resp.) and showed a dose-dependent reduction in parasitaemia in *P. berghei* infected mice [551]. In antibacterial activity, **12** was found to be active against MRSA (MIC 4-64 µg/mL) [556] but in other studies was found to be inactive against SA, BS and EC at test concentrations up to 128 µg/mL [557]. It was tested for activity against a range of fungi including *Candida* species and had an MIC range of 12-47 µg/mL [516]. Compound **12** was also reported to reduce HIV replication *in vitro* (1.4 µM) [557]. In other studies, **12** demonstrated hepatoprotective activity *in vitro* and *in vivo*, reducing several markers of damage in ethanol-activated hepatic stellate cells (1 µM) [551] and in Kunming mice with alcohol-induced hepatotoxicity, **12** was protective at 0.25-1 mg/kg p.o. [558]. It had anti-proliferative activity *in vitro* against a wide range of cancer cell lines, e.g., SW1463 (colorectal cancer) (IC₅₀ 3.8 µg/mL) [551]. In addition, **12** reduced cardiovascular and renal complications of diabetes [551], displayed antidepressant activity *in vivo* in mice (10 mg/kg p.o.) [551] and had spasmolytic activity *in vitro* [518].

4.3.3.10 Phytochemistry of other *C. spinarum* metabolites

Compounds identified in lower concentrations in organic solvent extracts of *C. spinarum* root are presented below. The lignan, 3-(4-Methoxyphenyl)-2, 6-dimethylbenzofuran (**13**) [559]; sesquiterpenes: dehydrocarissone (**14**), carindone (**15**), carissol (**16**) [234, 488, 560], aristolone (**17**) and zierone (**18**) [559]; the triterpene, lupeol (**19**) [464, 561]; the sterol, β-sitosterol (**20**) [464, 562]; the phenolic compounds, coniferaldehyde (**21**) [471], caffeic acid (**22**) [563], vanillin (**23**) [464], *erythro*-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol (**24**), *threo*-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol (**25**), protocatechuic acid (**26**) and vanillic acid (**27**) [487]; and the coumarins, isofraxidin (**28**) and scopoletin (**29**) [471, 486, 559] are illustrated in Figure 53.

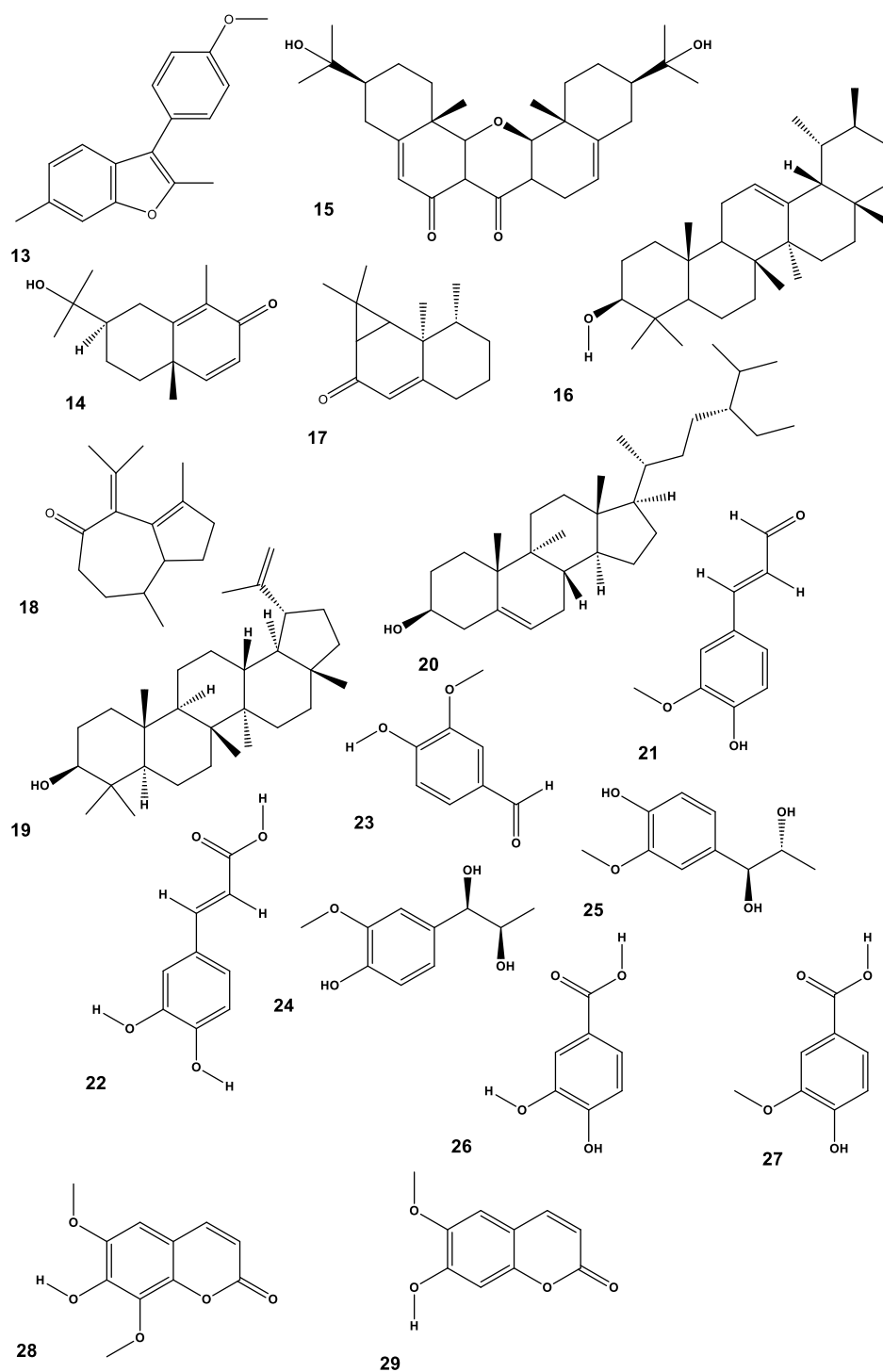


Figure 53: Selected lignans, isoprenoid and phenolic compounds (**13-29**) isolated from organic solvent extracts of *C. spinarum* root

The cardiac glycosides odoroside G (**30**) [484], evomonoside (**31**) and sarhamnoloside (**32**) [486, 564] and four lignan glycosides: (6*R*,7*S*,8*S*)-7*a*-[(β -D-glucopyranosyl)-oxy]- 1-methoxyisolariciresinol (**33**), (+)-isolariciresinol 3*a*-O- β -D-glucopyranoside (**34**), (-)- lyoni-resinol 3 *α* -O- β -D-glucopyranoside (**35**), (+)-lyoni-resinol 3 *α* -O- β -D-glucopyranoside (**36**) are presented in Figure 54.

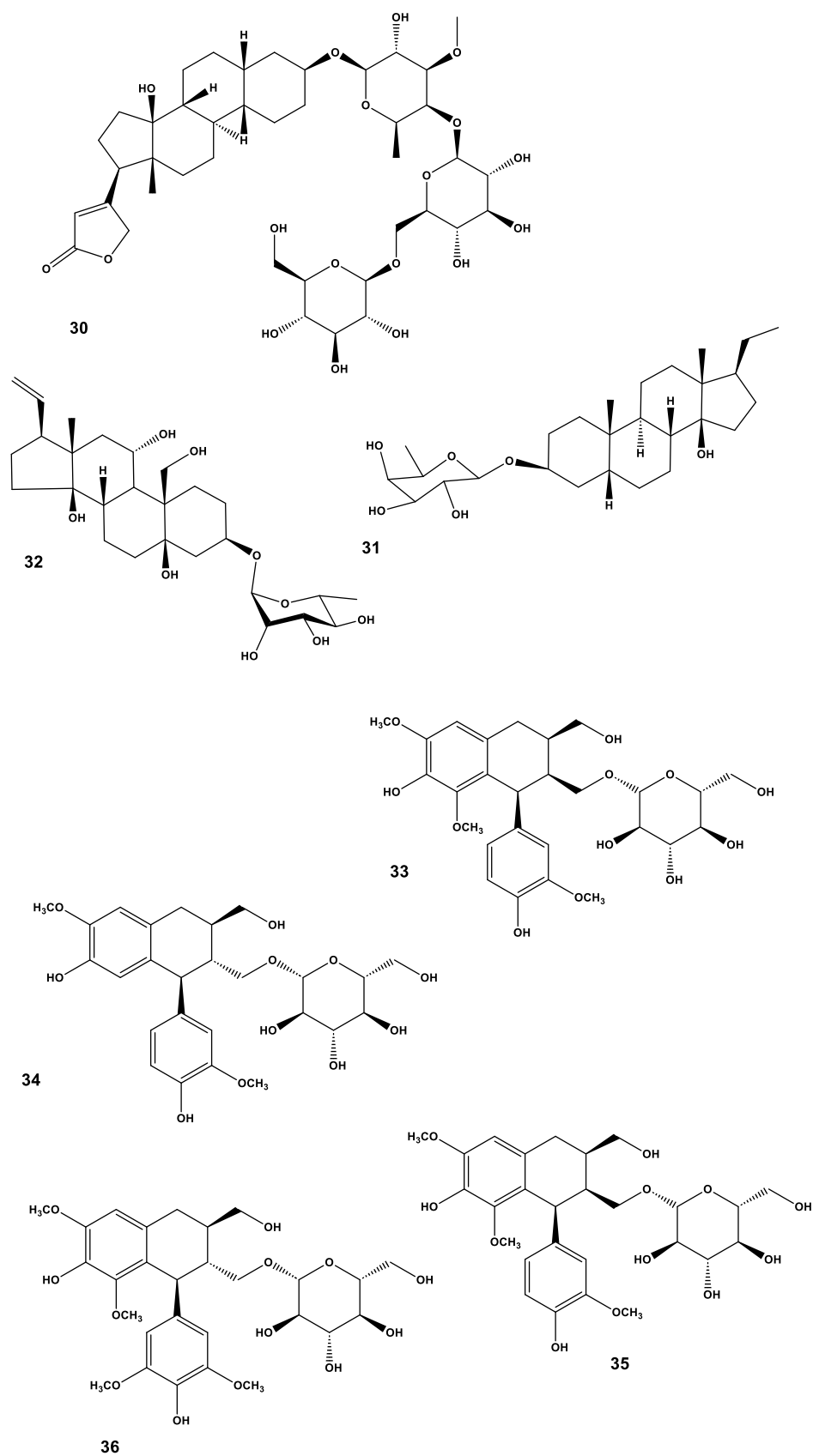


Figure 54: Compounds **30-36** – selected glycosides isolated from organic solvent extracts of *C. spinarum* root

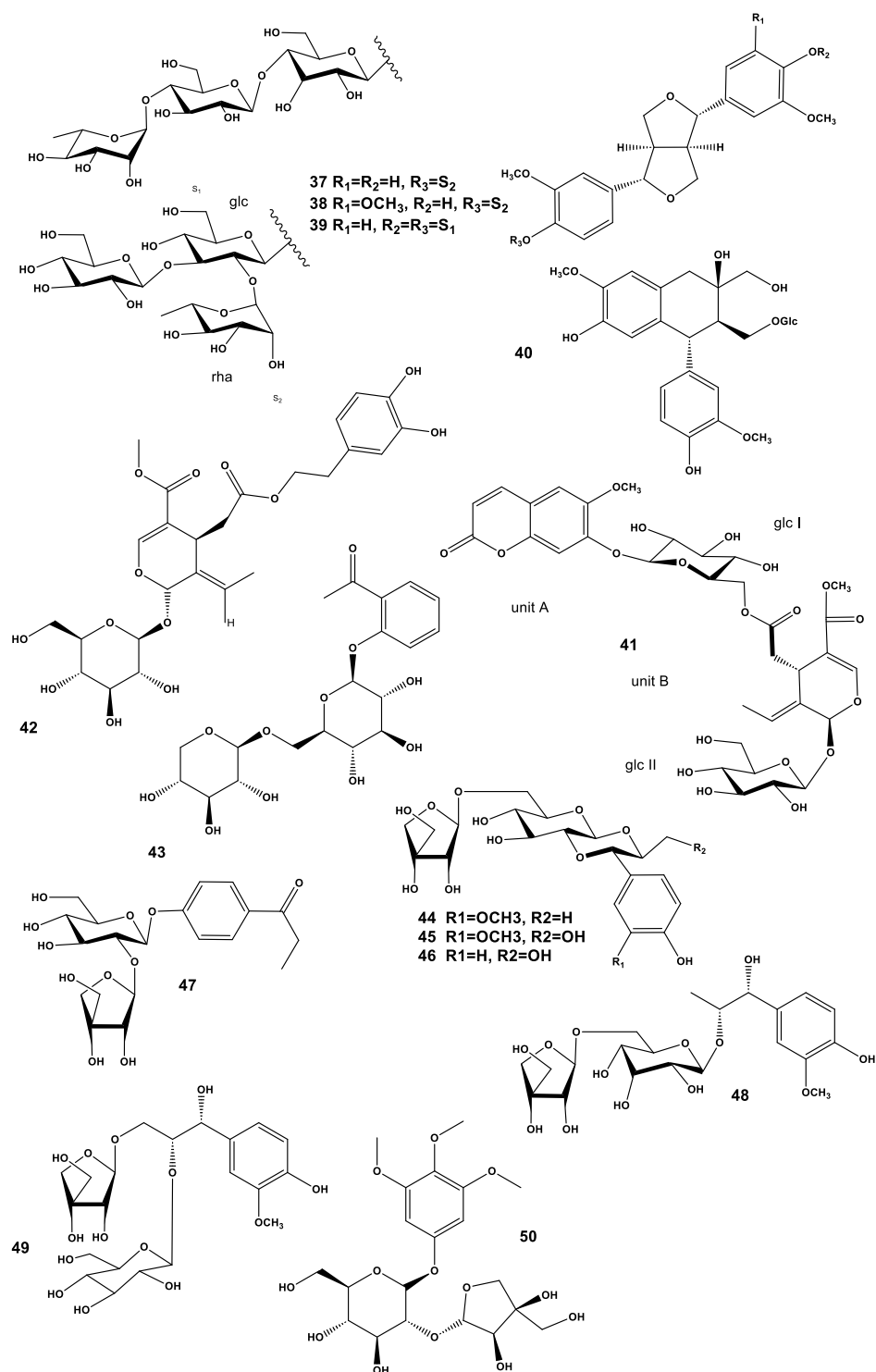


Figure 55: Compounds **37-50** - selected glycosides isolated from organic solvent extract of *C. spinarum* root

Figure 55 illustrates the lignan glycosides: carissaeduloside H (4-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- [β -D-glucopyranosyl-(1 $\rightarrow$ 3)]- β -D-glucopyranoside) (**37**), carissaeduloside I ((7*S*,8*R*,7'*S*,8'*R*)-medioresinol 4-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[β -D-glucopyranosyl-(1 $\rightarrow$ 3)]- β -D-glucopyranoside) (**38**), carissaeduloside J ((7*S*,8*R*,7'*S*,8'*R*) (+)-pinoresinol 4,4'-di-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside)

(**39**); the lignan monodesmoside [(1*S*,2*S*,3*S*)-1,2,3,4-tetrahydro-3,7-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-6-methoxy-2-naphthalen-yl] methyl β -D-glucopyranoside (**40**); the coumarin-secoiridoid hybrid glycoside carissaeduloside G (scopoletin-7-*O*-[11''-methyl-oleosidyl-(7'' $\rightarrow$ 6'')]- β -D-glucopyranoside) (**41**); the coumarin-secoiridoid hybrid glycoside, oleuropein (**42**) [486]; and the phenolic glycosides: acetophenone-2-*O*-xylopyranosyl - (1 $\rightarrow$ 6)-*O*- β -D-glucopyranoside (**43**), carissaeduloside A: (7*R*,8*S*)-3-methoxy-4-hydroxyphenyl-propane-7,8-[2',1'-*O*-(6'-*O*- β -D-apiofuranosyl)]- β -D-glucopyranosyl]-7,8-Diol (**44**), carissaeduloside B: (7*S*,8*S*)-3-methoxy-4-hydroxyphenylpropane-7,8-[2',1'-*O*-(6'-*O*- β -D-apiofuranosyl)]- β -D-glucopyranosyl]-7,8,9-triol (**45**), carissaeduloside C: (7*S*,8*S*)-4-hydroxyphenylpropane-7,8-[2',1'-*O*-(6'-*O*- β -D-apiofuranosyl)]- β -D-glucopyranosyl]-7,8,9-triol (**46**), carissaeduloside D: 4-hydroxyphenyl ethyl ketone 4-*O*- β -D-apiofuranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside (**47**), carissaeduloside E: *threo*-(3-methoxy-4-hydroxyphenyl)propan-2-yl)oxy)-7-hydroxy-8-*O*-(6'-*O*- β -D-apiofuranosyl)]- β -D-glucopyranoside (**48**), carissaeduloside F: *threo*-(3-methoxy-4-hydroxyphenyl)propan-2-yl)oxy)-7-hydroxy-8-*O*- β -D-glucopyranosyl-9-*O*- β -D-apiofuranoside (**49**) [487] and khaeuhuoside A (**50**) [486].

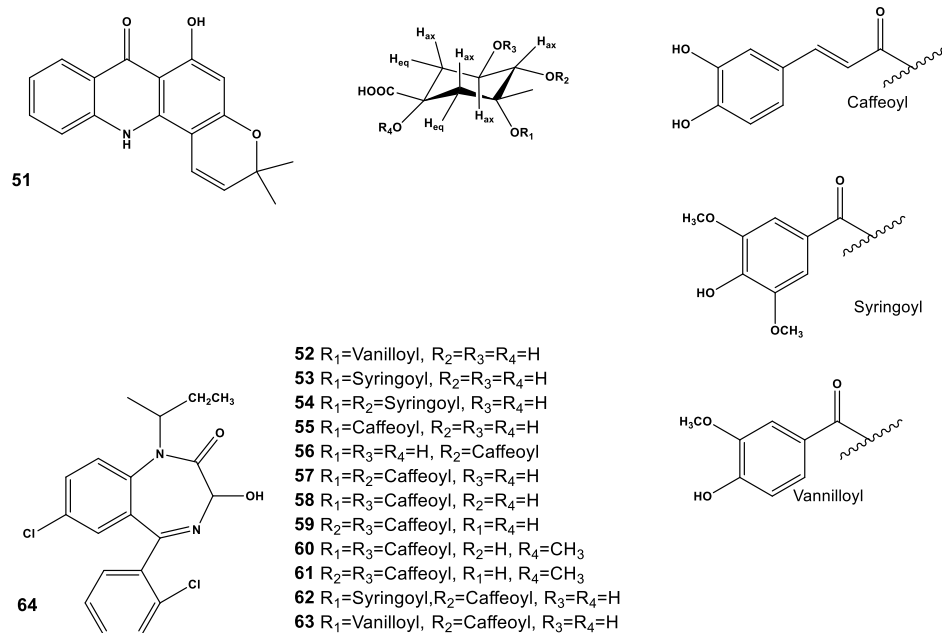


Figure 56: Compounds **51-64** isolated from organic solvent extract of *C. spinarum* root: des-*N*-methylnoracronycine, acyl quinic acid derivatives and benzodiazepine analogue

Figure 56 illustrates the acridone alkaloid, des-*N*-methylnoracronycine (**51**) [488]; the acyl quinic acid derivatives: 3-*O*-vanilloylquinic acid (**52**), 3-*O*-syringoylquinic acid (**53**), 3,4-di-*O*-syringoylquinic acid (**54**), neochlorogenic acid (**55**), cryptochlorogenic acid (**56**), 3,4-dicaffeoylquinic acid (**57**), 3,5-dicaffeoylquinic acid (**58**), 4,5-dicaffeoylquinic acid (**59**), methyl 3,5-

dicafeoylquinic acid (**60**), methyl 4,5-dicafeoylquinic acid (**61**), 4-*O*-cafeoyl-3-*O*-syringoylquinic acid (**62**), 4-*O*-cafeoyl-3-*O*-vanilloylquinic acid (**63**) [489]; and the benzodiazepine analogue 7-chloro-5-(2-chlorophenyl) 1-butyl, 3-hydroxy, 1,4-benzodiazepine-2-one (**64**) [490].

4.4 Integration of phytochemistry and pharmacology of *C. spinarum* root extracts

As outlined above, phytopharmacological studies on *C. spinarum* root do not, in the main, reflect traditional usage. A sole focus on those that do, i.e., aqueous extracts, would result in the omission of most of the research on *C. spinarum*. However, I have argued that examination of organic solvent extracts and their extracted phytochemicals may provide some limited approximation to ingestion or application of the whole root. In this section, we present a selection of the most potent activities of laboratory extracts of *C. spinarum* root and suggest potential correlations between laboratory studies, key secondary metabolites (**2**, **4-12**) and traditional therapeutic indications. This is followed by a review of toxicity in relation to *C. spinarum*.

4.4.1 Bioactivity of *C. spinarum* root and relationship to traditional therapeutic usage

A summary of the key bioactivity data is presented in Table 25 (summarised from Supplementary file 6 Tables S15-S24). In selecting studies for inclusion in this table, the best practice guidelines proposed by Heinrich *et al* [246] were used as cut-off limits such that only activity at pharmacologically relevant concentrations of extracts are listed (100-200 µg/mL *in vitro*; 100-200 mg/kg *in vivo*) [472]. The extraction solvents used in these studies were mainly MeOH and EtOH. Some cold and hot water extracts were studied but no aqueous infusion studies have been reported to date. Table 25 show extracts to have antioxidant, antiplasmodial, antibacterial (limited), antiproliferative, hepatoprotective, analgesic, antiarthritic, antipyretic, anti-inflammatory, immunomodulatory and CNS activity with potential correlation with many traditional therapeutic uses including malaria, pain, inflammation, fever, genitourinary, respiratory, gastro-intestinal, skin and CNS disorders. The ubiquitous and highly researched compounds (**9-12**) may be major contributors to the therapeutic efficacy of *C. spinarum* root in traditional usage with Quercetin (**11**) having the broadest range of activities. The more *Carissa*-specific carissone (**5**) and nortrachelogenin (**6**) are also active across several domains of use and may contribute to the specific therapeutic efficacy of *C. spinarum* relative to other medicinal species.

The demonstrated antipyretic activity *in vivo* could be related to the treatment of many infections including malaria and typhoid. In relation to

metabolites that may be contributing to this effect, the biological mechanisms involve many of the same enzymes and cytokines as the process of inflammation and hence an expected overlap in therapeutic efficacy of selected compounds such as **5**, **6**, **9**, **10**, **11** and **12** [565] although, to date, no specific antipyretic effect of these compounds has been identified, with the exception of betulinic acid (**12**) [566]. The widespread treatment of malaria with *C. spinarum* root in EM is not well supported by *in vitro* and *in vivo* studies where only CQ sensitive strains were sensitive to tested extracts in pharmacologically relevant doses [472]. However, the study by Kebenei *et al.* [172] which investigated *C. spinarum* root extracts alone and in combination with *Artemisia annua*, demonstrated synergism, albeit in a CQ sensitive strain. CQ resistance of *P. falciparum* has been shown to be reversed by co-administration of CQ with some medicinal species [195] and this is an avenue worth investigating further with species which have tested negative against CQ resistant strains of *P. falciparum*.

Plasmodium falciparum resistance to CQ has been a growing problem globally since the 1950s [567]. In 2001, the WHO has recommended ACT as first line treatment for malaria since 2001 [568] and Kenya replaced CQ with sulfadoxine-pyrimethamine (SP) as the first line treatment for malaria in 1998 [569]. In analysing the anti-plasmodial studies examined in relation to *C. spinarum*, they date from 2006 onwards, post-dating the growing CQ resistance in the relevant countries (Kenya and Ethiopia). Three *in vitro* studies used only the CQ sensitive strain, D6 [172, 325, 498]; three *in vitro* studies used a combination of CQ sensitive/CQ resistant strains (D6/W2 and NF54/ ENT30) [291, 329, 330] and the *in vivo* study used the CQ sensitive murine strain, *P. berghei* [570]. The D6 strain originated in Sierra Leone in 1988, W2 originated in IndoChina in 1988, NF54 originated in the Netherlands (airport) in 1981 [568]. ENT30 was a clinical isolate from western Kenya with the earliest identified *in vitro* use in 2001 [571]. Thus, only one of these strains is from East Africa (2001) and most are known to be from the 1980s and may no longer be appropriate for use in *in vitro* testing in the present day. *P. falciparum* is a genetically diverse species, making choice of *in vitro* strains more complicated and strengthens the argument for using locally relevant strains in laboratory studies, as is argued for vaccine trials [572]. *P. falciparum* also demonstrates significant geographic diversity [572] when compared across South America, Africa, Southeast Asia, and Oceania, while in Africa, there were also differences in strains between East and West Africa. However, the NF54 strain, likely to be of West African origin, was also found to overlap with recent clinical isolates from East and West Africa [572], supporting the use of NF54 in *in vitro* studies. Additionally, in a 2013 study of returning travellers by van Schalkwyk *et al* [568], the *P. falciparum* isolate from a single traveller to Kenya was considered highly sensitive to CQ (IC₅₀ 13nM),

also supporting the inclusion of CQ sensitive strains in laboratory studies. Overall, therefore, the use of D6 and W2 strains may not be appropriate in East African *in vitro* studies due to the age and geographical origins of the strains, but NF54 and ENT30 (used in [330]) may be more relevant. However, ideally, up to date genomic sequencing and drug sensitivity testing of geographically relevant clinical isolates and laboratory parasite lines would be useful to determine the best strains and controls for anti-plasmodial testing.

As with anti-plasmodial testing, antibacterial testing indicated some limited activity against PA, EC and SA using agar diffusion and agar dilution methods (Supplementary file 6 Table S17). None of the studies have taken account of the traditional use in treatment of STI with specific mention of gonorrhoea, the most commonly treated infection and this merits investigation. However, testing for direct antibacterial activity is only one element of *in vivo* efficacy [172]. Other elements include anti-virulence activity and the ability to modify bacterial resistance methods. Anti-virulence agents work through a number of mechanisms: preventing adhesion of the microbe to the host cell; inhibiting the bacterial density-dependant quorum-sensing mechanism; inhibiting bacterial biofilm formation; blocking the secretion of toxins into host cells and reducing bacterial defences. The potential to modify bacterial resistance methods include increasing bacterial membrane permeability to antibiotics; inhibiting bacterial enzymes that inactivate antibiotics and inhibiting bacterial pumps that expel antibiotics from the bacteria resulting in bacterial resistance [172]. These mechanisms of *in vivo* antibacterial activity broaden the investigative approach in future studies of plants used traditionally in the treatment of infections. Among the *C. spinarum* isolated compounds, it is known that rutin (**10**) moderately inhibits biofilm production in *Streptococcus suis in vitro* at one quarter of the MIC concentration, indicating much greater potential potency than previously thought [132]. In antifungal testing, there was low level activity against CA and AN using agar well diffusion methods. Some of this activity may be supported by compounds **5**, **6**, **9** and **12** (Table 25). The *in vitro* antiviral activity of *C. spinarum* aqueous decoction against HSV and HCMV was described in section 4.3.1.2 and may be associated with the known antiviral effects of compounds **9**, **11** and **12**. Antiviral activity may not be limited to these two pathogens but *C. spinarum* root may be active against other viruses treated ethnomedically, a possibility worth further investigation [573].

There are a few reports of treatments specific to the liver the treatment of jaundice, hepatitis and Yellow Fever in Tanzania, India and Chad with preparation methods not provided in these studies. The use in treatment of liver problems is supported by one study which examines the hepatoprotective effect of EtOH extract *in vivo* [574] as well as the demonstrated activity of **9**, **10**, **11**, **12**

described in section 4.3.3. Other isolated compounds besides these key *C. spinarum* compounds may also contribute to the hepatoprotective activity of *C. spinarum* root such as those isolated recently in low concentrations by Liu *et al.* [487]. In addition, liver problems can lead to non-specific GI symptoms, pain, fever and fatigue and the treatment of these symptoms may correspond more broadly to identified pharmacological activities of *C. spinarum* such as the EM use of root mixed with alcohol to treat stomach ache in Ethiopia [406] and the boiling of root in mutton soup to treat stomach ache in Uganda [575]. The specific treatment of cancer with *C. spinarum* root is rare but is supported by anti-proliferative effects *in vitro*. However, antioxidant activity may be related to the prevention of chronic diseases such as cancers and cardiovascular diseases [576]. As such, the long-term consumption of *C. spinarum* - based “health drinks” [351] as described in section 4.2.1 may be beneficial through an antioxidant effect. Symptoms of cancer such as wounds that don’t heal or fatigue are represented in the ethnomedical data and may be related to laboratory findings of anti-proliferative activity. Cardiogenic (ex-vivo), emetic (pigeons), hypertensive (cats) and musculotropic (guinea-pig ileum) activity of ethanolic *C. spinarum* root extract was reported by Vohra and De [577]. In contrast, Al-Youssef and Hassan [234] reported a significant and dose-dependent fall in blood pressure in rats with PE, EtOAc and aqueous extracts. Some of this activity is likely to be attributable to the cardiac glycosides **4**, **30**, **31** and **32**. Cardioprotective and anti-hyperlipidaemic activity is also attributed to **9**, **10**, **11** and **12** [515, 520, 538, 578].

It would be interesting to analyse the proprietary product, Zedupex™, containing *C. spinarum* and marketed for the treatment of *Herpes simplex* and to compare its preparation with traditional preparation. However, the preparation is under patent so that it is not possible to compare the formulations without having access to the product itself. The traditional use is as a decoction in the treatment of *H. zoster* and some other viral infections rather than *H. simplex* for which it is marketed.

4.4.2 Toxicity

*“It (C. spinarum roots) enters into the composition of
purgatives but if taken internally in too large a dose it
proves fatal causing purging which cannot be stopped”
[377]*

The potential toxicity of *C. spinarum* roots was recognised in Indian folk usage as reported by Rastogi *et al* [484] from 1933 research. In phytopharmacological research of *C. spinarum* roots, there are three studies which include an examination of the toxicity of aqueous extracts of *C. spinarum* root and eleven of

the toxicity of organic solvent extracts (Supplementary file 6 Table S24). The studies of aqueous extracts (decoction, boiled in distilled water for 45-60 mins) include *in vitro* brine shrimp and mammalian cell assays (Vero-6 and HEL cells) and one *in vivo* assay in a rodent model [330, 452, 456]. Each of the *in vitro* studies had an LD₅₀ of 100 µg/mL or greater but were not much higher than the active concentrations. This indicates a potential lack of selectivity of the extract with potential toxicity at therapeutic doses. In the *in vivo* study with the aqueous extract of *C. spinarum* root, the given dose of 250 mg/kg was not toxic. However, the maximal dose used in the toxicity study was the same as the active antiviral dose and LD₅₀ was not determined, limiting the usefulness of the study.

Three studies were identified analysing the *in vitro* cytotoxicity of organic solvent extracts of *C. spinarum* root. Tauchen *et al.* [579] found the 80% EtOH extract to be toxic to normal foetal lung cells relative to the cancer cell line tested at three times the active dose indicating a lack of selectivity towards cancer cells. Matata *et al.* [213] found the MeOH: dichloromethane (DCM) rootbark extract to be non-toxic with an LD₅₀ 342.5 µg/mL. Using the brine shrimp toxicity assay, the MeOH root extract had mild toxicity (LD₅₀ 186.71 ± 6.9 µg/mL) [330] and indicated a lack of selectivity relative to the tested anti-plasmodial activity (IC₅₀ 148.53 and > 250 µg/mL for different strains). Nine studies examine *in vivo* acute and subacute toxicity of *C. spinarum* root extracts. In Ya'u *et al.* [580, 581], the EtOH rootbark extract had a moderately good ratio of active to acute toxicity dose in anticonvulsant and sedative effects with active anticonvulsant activity at 20 mg/kg i.p. and behavioural and sedative effects at 5-20 mg/kg i.p. relative to LD₅₀ (oral) > 5000mg/kg and LD₅₀ (i.p.) 282.8 mg/kg. However, in Ya'u *et al.* [582], the active anticonvulsant dose of the residual aqueous fraction (RAF) of the EtOH extract was high at 600 mg/kg i.p. and was closer to the toxic dose for the fraction (LD₅₀ RAF = 2222.61 mg/kg i.p.), limiting the therapeutic usefulness of this fraction. Hegde *et al.* [583] did not determine the LD₅₀ for the 95% EtOH extract but found the single 2000 mg/kg p.o. dose to be non-toxic. However, the active anti-arthritic dose was 400 mg/kg, close to the tested toxicity dose indicating a possible lack of selectivity. Dossou-Yovo *et al.* [584] conducted a sub-acute toxicity study of *C. spinarum* root extract (50% EtOH) over 90 days and found the 1000 mg/kg p.o. dose to be non-toxic with limited adverse effects. Une and Doshi [585] in an acute toxicity test with petroleum ether (PE) root extract, found the 5000 mg/kg p.o. single dose to be non-toxic. Hassan *et al.* [586] examined the toxicity of the saponin fraction of the *C. spinarum* root EtOH extract relative to the anti-arthritic activity and calculated LD₅₀ to be 565.69 mg/kg i.p., under four times the anti-arthritic dose of 150 mg/kg indicating a lack of selectivity.

Table 25: Summary of key phytopharmacological data as it could relate to TM usage of *C. spinarum* root.

Pharmacological activities of extracts	<i>In vitro/vivo</i> (Solvent)	Lowest Effective dose	Key cpds. (known activity) ¹⁶	Related EM Use (Supplementary file 5)	EM preparation where stated (Supplementary file 5)	Possible clinical indications	Refs for lab studies
Antioxidant activity	<i>In vitro</i> EtOH; MeOH	FRAP 14.9 mmol Fe2+/g	6, 7, 8, 9, 10, 11, 12	Cancers, cardiovascular issues, memory	Decoction with other species (oral)	Protective vs cardiovascular disease and cancer	[487]
Anti-plasmodial	<i>In vitro</i> and <i>in vivo</i> MeOH (mainly); CHCl ₃	CQ sensitive only 1.95 µg/mL; 200 mg/kg	6, 10, 11, 12	Malaria, fever	Decoction (+/- food) (oral)	Malaria	[172, 291, 325, 329, 570]
Antifungal	<i>In vitro</i> EtOAc; Acetone	MIC 50- 100 µg/mL (AN, CA) (diff'n) MIC 7.8 µg/mL (CA) (diff'n)	5, 6, 9, 12	Skin infection, GUI, respiratory infections	Decoction (+/- other species, meat soup), maceration (oral), burned and inhaled/ wash, powder with porridge (oral), root inhaled	Skin, oral, respiratory and genital infections	[544, 587]

¹⁶ : 2-HAP (**2**), odoroside H (**4**), carissone (**5**), (-) nortrachelogenin (**6**), carissanol (**7**), carinol (**8**), ursolic acid (**9**), rutin (**10**), quercetin (**11**), betulinic acid (**12**)

Pharmacological activities of extracts	<i>In vitro/vivo</i> (Solvent)	Lowest Effective dose	Key cpds. (known activity) ¹⁶	Related EM Use (Supplementary file 5)	EM preparation where stated (Supplementary file 5)	Possible clinical indications	Refs for lab studies
Antibacterial	<i>In vitro</i> EtOH; MeOH; DCM (one study); EtOAc and acetone (one study)	MIC (Diff'n ¹⁷) 6.25 µg/mL vs PA, EC MIC (Dil'n) 110 µg/mL vs SA	5, 6, 9, 10, 11	Infection (respiratory, genito-urinary, skin, GI, typhoid), inflammation, fever	Decoction (+/- species, meat soup) maceration in water +/- other species, powder with porridge, root inhaled, mix with alcohol/milk (oral), burned and inhaled, burned and wash	Infections: respiratory, GI, GUI, skin	[467, 544, 587, 588]
Antiviral	<i>In vitro</i> Aq.	HCMV: IC ₅₀ 74 µg/mL HSV: IC ₅₀ 5.66 µg/mL	9, 11, 12	Skin and genital infections (other viral infections e.g., URTI, measles, shingles, rabies, polio)	Decoction, infusion, crushed (applied and oral)	Herpes Simplex virus Human cytomegalovirus Potentially other viral infections	[452, 456]
Tyrosinase inhibition	<i>In vitro</i> Aq.	IC ₅₀ 24.33 µg/mL	11	Skin bleaching (cosmetic)	Stem ground and made into a paste with water - applied	Hyperpigmentation, potential in melanoma	[458]
XO inhibition	<i>In vitro</i> MeOH	IC ₅₀ 156.0 µg/mL	2	Arthritic disorders, pain	Decoction (+/- soup, species, spices) (oral); Infusion; Cold water mixture; Burned	Gouty arthritis	[464]
Antiproliferative	<i>In vitro</i> EtOH; MeOH	HEP-G2 = 32.8 µg/mL	4, 5, 6, 7, 8, 9, 10, 11, 12	Cancers Others: Slow healing wounds, Weakness	Decoction with other species (oral); crushed or powdered and applied to wounds	Cancer	[579, 589, 590]

¹⁷ Diff'n = diffusion; Dil'n = dilution

Pharmacological activities of extracts	<i>In vitro/vivo</i> (Solvent)	Lowest Effective dose	Key cpds. (known activity) ¹⁶	Related EM Use (Supplementary file 5)	EM preparation where stated (Supplementary file 5)	Possible clinical indications	Refs for lab studies
		U1242 = 194.53 µg/mL U87 = 90 µg/mL U251 = 8 µg/mL					
Lipid peroxidation hepatoprotective	<i>Ex vivo</i> EtOH	200 mg/kg	9, 10, 11, 12	Jaundice, Hepatitis, Yellow fever Others: non-specific GI symptoms, pain, fever, fatigue	Jaundice: Not stated; weakness: mix with water and other species Other symptoms, see other entries	Protective vs cardiovascular disease, cancer, liver disorders	[574]
Analgesic, Antiarthritic	<i>In vivo</i> Aq.; EtOH; DCM:MeOH; PE	37.5-250 mg/kg	9, 10, 11	Toothache, headache, stomach ache	Decoction (+/- meat soup, food, other species); Infusion; Root mixed with cold water/ spices/ alcohol (oral, enema). Burned and applied or inhaled	Musculoskeletal disorders, pain, dysmenorrhoea	[583, 585, 586, 591]
Antipyretic	<i>In vivo</i> DCM:MeOH; EtOH	100-400 mg/kg		Fever, malaria, RTI	Decoction (+/- other species, meat soup), maceration in water +/- other species (oral), powder with porridge, alcohol, milk (oral), root inhaled, burned and inhaled/ washed	Fever, malaria, respiratory tract infection, GI infections	[491, 592]
Anti-inflammatory Immunomodulatory	<i>In vivo</i> EtOH; MeOH; PE	37.5-135 mg/kg	5, 6, 9, 10, 11, 12	Infections, pain, musculoskeletal, cancers, wounds	See other entries for infections, analgesic, cancers, wounds	Infections, immune problems, pain, skin and tissue inflammation, musculoskeletal	[585, 586, 593]

Pharmacological activities of extracts	<i>In vitro/vivo</i> (Solvent)	Lowest Effective dose	Key cpds. (known activity) ¹⁶	Related EM Use (Supplementary file 5)	EM preparation where stated (Supplementary file 5)	Possible clinical indications	Refs for lab studies
Sedative, Anxiolytic	<i>In vivo</i> EtOH	5 mg/kg	9, 10, 11, 12	mental disorder (brain tension, stress)	Infusion (oral); crush, burn and inhale	Anxiety, depression, insomnia	[581]
Anticonvulsant	<i>In vivo</i> EtOH	2.5-150 mg/kg	9 (weak)	seizure	Decoction/infusion with other species, applied as wash, smeared to body and oral; powder (oral)	Seizure control, epilepsy	[580, 582, 594]
Wound healing	<i>In vivo</i> MeOH	2.5% ointment	5, 6, 9, 10, 11, 12	Wounds, lacerations, snakebite	crushed or powdered or mixed with water and apply	Wounds, skin disorders, snakebite	[588]
Antispasmodic	<i>Ex vivo</i> Aq	3 mg/mL	9, 11, 12	GI disorders, cramps	Decoction (+/- other species, soup), root pounded and mixed with cold water/alcohol (oral)	Abdominal pain and cramps, irritable bowel syndrome	[461, 595]

Ngulde *et al.* [596] examined the *in vivo* toxicity of 95% EtOH extract of *C. spinarum* root and calculated oral LD₅₀ 3,807.9 mg/kg and i.p. LD₅₀ 288.5 mg/kg. The results were presented relative to *in vitro* antibacterial activity making it difficult to assess the selectivity of the activity relative to the toxicity. Overall, many studies where an LD₅₀ was calculated indicated a lack of selectivity of aqueous and organic solvent extracts *in vitro* and *in vivo* with a few exceptions. Where LD₅₀ was not examined, relative toxicity of the extracts cannot be assessed.

Some of the compounds isolated from *C. spinarum* have known toxicity, including the cardiac glycosides, of which *C. spinarum* has had four isolated, with **4**, at least, predicted to have good oral bioavailability (Table 24). In cytotoxicity studies, **4** was non-toxic to normal human cells at tested cytotoxic activities [510]. However, cardiac glycosides are known human toxins with a narrow therapeutic index. *In vivo* studies showed that odoroside H (**4**) had 40% mortality in mice at 50 mg/kg i.p. (n = 5) [564] and sarhamnoloside (**32**) had an LD₅₀ of 90 µg/kg (i.v.) in cats [173] while cardiac glycosides, odoroside G (**30**) and evomonoside (**31**) [476] may also contribute to toxicity. Odoroside H (**4**), at lower concentration of 25mg/kg i.p., caused potential signs of toxicity in CNS depressive effects including reduced activity, passivity, staggering, reduced responsiveness to touch and drooping eyes [597]. Cardiac glycosides act primarily via the inhibition of Na⁺/K⁺-ATPase – the sodium-potassium pump, disrupting ion homeostasis in cardiac and other tissues [598]. The main effects of poisoning include cardiac toxicity with bradycardia progressing to heart block and death occurring in severe poisoning, mainly due to ventricular fibrillation. Other effects of poisoning include GI symptoms (nausea, vomiting, abdominal pain, diarrhoea) and drowsiness [192], reflecting the finding of fatal purgation in high dosage cited in Indian reports above. Cardiac glycosides are also thought to cause an increase in blood pressure [598] although a reduction in blood pressure of the *C. spinarum* whole extract was reported previously [234]. Some of the other key compounds isolated from *C. spinarum* and tested for toxicity include carissanol (**7**) and carinol (**8**) which were found to be toxic to normal lung fibroblasts (IC₅₀ 6.2 µg/mL and IC₅₀ < 1 µg/mL resp.) [173].

Burning of plants as occurs with *C. spinarum*, may lead to unpredictable results. Braithwaite *et al* [443] compared the composition of acetone, methanol and inhalation extracts of *Pellaea calomelanos* by HPLC–UV chromatograms and found that the combustion process produced compounds which were not present in the solvent extracts. The inhalation had much greater antimicrobial efficacy than solvent extracts although it was also pointed out that smoke may contain toxic compounds and parallel toxicity evaluations to determine selectivity were not carried out in this study. In *C. spinarum*, volatile oils which are known

to be present in root samples may become oxygenated and result in different activities to the original compound [443]. Simoneit [599], in a review of biomass burning, wrote that high levels of polycyclic aromatic hydrocarbons including benzopyrene from woodsmoke are a health concern including being known carcinogens. Combustion of plant material may cause alteration in composition through oxygenation, aromatisation, dehydration and decarboxylation and vary also depending on burn time, temperature and degree of aeration producing unpredictable results and the potential for toxicity. As described by Braithwaite *et al* [443] and Mohagheghzadeh *et al.* [600], smoke as an element of healing is well-established but not well explored scientifically. While it may produce enhanced therapeutic effects, it is also associated with potential toxicity and these aspects deserve further study in the safety assessment of medicinal plants.

4.5 Phytopharmacological research on *C. spinarum* root and its relationship to ethnomedical use

The phytopharmacological research on *C. spinarum* root highlights some issues that are general in such research and other elements that are particular to ethno-directed phytopharmacological research.

4.5.1 General issues arising in phytopharmacological studies of *C. spinarum*

Multiple factors contribute to the composition of plant preparations which can lead to significant variation in biological activity *in vitro*, *in vivo* and clinically. Variability in plant material through genetics, environment, harvesting and storage can result in different bioactivities of plant extracts. During the growth of the plant, environmental conditions are a source of qualitative and quantitative variability in plant secondary metabolites as described in section 2.3.10.9. This is seen in this analysis in relation to the concentrations of limonene (**3**) in the root oil (Tables S8 and S9 Supplementary file 6) whereby limonene (**3**) is absent from the Mallavarupu *et al.* [469] sample and is present (1.2% of oil) in Moudachirou *et al.* [470]. It is also demonstrated in the varying concentration of Odoroside H (**4**) in different samples and in other key compounds in Table 24 (section 4.3.3). After harvesting, decomposition, light effects, oxidative changes, reactions between phytochemicals and enzymatic activity may occur altering the end composition of the tested extract [601]. In the laboratory, extraction technique is another variable: temperature used (and rate of temperature rise), solvent polarity, duration of extraction, ratio of solvent to plant material and degree of comminution of plant material are all sources of variation in the analysed studies (e.g., Supplementary file 6 Table S7) [601]. The use of moderate heat during

extraction such as slower rise in temperature towards boiling has been shown to alter the composition of extracts [602]. The duration of extraction used in the *C. spinarum* studies of heated aqueous preparations varied from 10 minutes to 3 hours for decoctions while one preparation [603] used reflux extraction for 2 hours and Muthaura *et al.* [291] used a temperature of 60 °C for 1 hour. Decoction (boiling) increases the dissolution of materials – including “impurities” – relative to maceration or percolation but is not useful for heat labile and volatile constituents. Decoction can cause more degradation of plant metabolites than cold extraction through reactions such as hydrolysis, dehydration, decarboxylation. Heat can inactivate β -glucuronidase in plants which can result in a higher concentration of glycosides in a decoction than in macerates and a comparably higher concentration of aglycone in macerates [602]. In the sole study that included phytochemistry of the aqueous decoction [458], the extraction method used (boiling for two hours) and identification by GC/MS would only allow the identification of semi-volatile, stable metabolites. The proportion of solvent to plant material varied such as in hot and cold aqueous extractions from 5% w/v [603] to 50% [446] (Supplementary file 6 Table S7). The lower % plant material results in a higher extract yield with a concomitant difference in composition but a longer drying process [601].

The antimicrobial assays used plate hole diffusion and broth microdilution method. There are variables in diffusion-based assays including different rates of diffusion for different compounds relating to solubility of metabolites, the solvent used, the concentration of extract and the thickness of agar medium. All of these affect the reliability of the results and some authors do not recommend the use of diffusion-based methods [196].

Other issues arising in *in vitro* testing in this analysis include the lack of endpoint criteria in some instances and significant differences in criteria were used making it difficult to interpret results. The choice of test organism, as discussed in relation to *P. falciparum* (section 4.4.1), should be relevant to circulating pathogens given the genetic variability of the organism and prevalence of resistance. The choice and use of controls may impact on the validity of the research such as where an antimicrobial with a high incidence of resistance among test organisms is used e.g., ampicillin [457], aciclovir [452], chloroquine [330]; the lack of +ve control [446]; and very high dose of control used, e.g., aspirin [462]. In order to test for selectivity of extracts and compounds, it is recommended that *in vitro* cytotoxicity assays in cell-lines are conducted in parallel to exclude the possibility of non-specific toxicity [604] and this is not done in most of the analysed studies. In addition, the representation of research methods and test results is inadequate in some of the analysed studies such as methods in a separate paper [457] and inconsistencies in data

representation [446]. Statistical validity measures are recommended and many studies have no evidence of replication of results nor of *p* value as a measure of statistical significance. Testing of antioxidant activity is a useful preliminary step in identifying bioactivity with DPPH being the most popular *in vitro* antioxidant assay in use. However, there are problems associated with it in that DPPH does not exist naturally, is only active in organic solvents and is a poor measure for radical quenching *in vivo* [166]. In addition, it is recommended that all *in vitro* antioxidant assays be followed up with cell-based, *in vivo* and *ex vivo* assays to determine efficacy in biological systems [50].

4.5.2 The relationship of phytopharmacological studies to EM usage of *C. spinarum*

Ethnopharmacological studies on medicinal plants incorporate traditional usage into the phytopharmacological investigations. Do the examined studies provide useful scientific information on the use of *C. spinarum* root in TM?

Addressing the issues highlighted in the previous section would improve the relevance of phytopharmacological research to the study of *C. spinarum* in TM. Traditional preparation of the root, such as through pounding and mashing, can result in quantitative variations in the composition of the resultant extract with different levels of cell membrane rupture and release of metabolites [605]. In further methods of preparation such as occur with Chinese herbal medicines, including decoction, stir-frying, steaming, stewing, calcining and the addition of excipients, the chemistry varies since processing can induce structural transformations of constituents, reduce toxic components or enhance solubility of constituents of extracts [606].

Choice of solvent in studies is important given that water is the most common solvent used in the traditional usage of *C. spinarum*. While a few studies (12) examined the bioactivity of aqueous decoction and cold-water extracts of *C. spinarum* root, there were none investigating the aqueous infusion, the volatile preparation or the whole root preparation. Forty-eight studies examined the pharmacological activity of organic solvent *C. spinarum* extracts. Organic solvents extract a different range of metabolites to those extracted in water. They extract non-polar components which are often thought to be the most bioactive constituents of medicinal plants. However, they may also contribute to toxicity of extracts and may reduce synergisms that can occur in aqueous preparations [311]. The use of organic solvents has been favoured in phytochemistry due to the preferential extraction of less polar secondary metabolites, considered to be the active components of the plant and the elimination of bulk material such as carbohydrates, proteins and amino acids which create technical problems during analysis [311]. However, these

problematic bulk components of an aqueous decoction may be therapeutically important through improving the oral bioavailability of active components of the mixture and facilitating pharmacokinetic synergies of complex plant extracts [607].

Oral bioavailability of identified bioactive phytochemicals is a key aspect of phytotherapy and processes in EM therapy may affect bioavailability. All of the high yielding ubiquitous compounds selected (**9**, **10**, **11**, **12**) are known to have poor oral bioavailability (Table 24). Quercetin (**11**), for instance, has low bioavailability at 4% and undergoes extensive metabolism after ingestion. However, additional ingredients such as dietary fat and non-digestible fibre as well as the initial source of **11** are known to affect bioavailability [538, 608, 609]. TM preparations in which the whole root is ingested such as by cooking the root with meat/animal fat/in soup, or with other additives noted in this study such as mixing with alcohol/milk/sugar/black pepper/chili pepper/other species may lead to the absorption of bioactive constituents not normally bioavailable from the simple aqueous preparation [311, 610-612]:

*Decoction of boiled root mixed with sheep tallow
[424](Maasai, Kenya: STI)*

*Root boiled with water and goat fat and taken orally [325]
Maasai Kenya: STI)*

Root added to soup [613] Batemi Tanzania: cough

Phenolic and saponin components of medicinal and food plants can act as solubilisers, improving the partition coefficient of otherwise poorly soluble compounds. This effect has been noted with rutin (**10**) in St John's Wort preparations and with **11** [601]. Co-extraction with other species can be seen to significantly change the chemical composition of extracts as shown with Danggui Buxue Tang in TCM (Chapter 3) [311]. Rootbark paste, mixed with black pepper and sugar cane juice, was given orally in India to treat fever of long duration. In this example, the whole rootbark was ingested and the addition of black pepper could aid absorption of some constituents of the paste [169]. Sugar cane juice, itself, could be contributing to the bioactivity [170]. The phenomenon of polar matrix formation, formed principally from organic acids, amino acids and carbohydrates can improve dissolution of other ingredients. Knowledge is developing of the formation of NADES (natural deep eutectic solvent) – a viscous plant liquid medium consisting mainly of sugars, choline, amino acids and organic acids - that form a super-molecule. NADES can significantly increase the oral bioavailability of lipophilic substances such as **10** [601, 607]. Other key bioactive components such as **2**, **4**, **5**, **6** and **7**, are predicted to have moderate

to good water solubility and oral bioavailability by ADMET analysis (Table 24). Consequently, these compounds may be present in aqueous formulations and the activities and toxicities identified for them may be relevant to traditional usage of *C. spinarum* root decoctions, infusions, pastes and macerations.

In order to understand the relevance of the bioactivity of isolated compounds to the TM usage of a medicinal plant, it would be necessary to know if they are present in a traditional preparation such as an aqueous decoction or a plant boiled in soup. This could be done using hyphenated techniques coupled with a detection system such as described in section 4.3.1.2. Generating metabolic fingerprints of tested extracts would facilitate comparison between different studies. In addition, the fact that traditional preparations are often complex mixtures which interact, generating multiple, possibly conflicting, interactions with body systems means that bioactivities identified for individual phytochemicals may not have additive effects or the observed *in vitro* or *in vivo* effects when applied to the human body. In order to measure the quality, safety and efficacy of traditional medicines in a laboratory or clinical situation, it is these traditional preparations that should be tested.

4.6 Analysis of sample of *C. spinarum* root by direct volatilisation

A small sample (75 mg) of *C. spinarum* root was obtained from Château Pérouse Botanic Gardens in southern France. The root was directly infused into GC/MS and analysed using the headspace-GC/MS technique for the determination of volatile constituents in liquid or solid samples through the analysis of the vapour phase. The obtained chromatogram (Figure 57) shows almost equal volume of 2-HAP (**2**) (55%) and Limonene (**3**) (45%). This sample differs from literature reports which found 89.5-99% 2-HAP in distilled root oil **2** [466, 468-470] and only one of which identified limonene (**3**) (1.2%) from the distilled root oil [470]. This could relate to numerous factors including geographic and genetic variation as described (4.5.1). Given the volatility of constituents, it could also be related to storage conditions given the freshness of the headspace-GC/MS tested sample and the relatively greater volatility of limonene (**3**). This analysis is limited by the fact that it was poorly collected and had not been cleaned after harvesting. It was very small and it was not possible to repeat the analysis (i.e., $n = 1$) so the results of this experiment need to take these provisos into account.

4.7 Future research on *C. spinarum*

Improved EM research which records details on harvesting, storage, preparation, local therapeutic indications and outcomes of treatment can form a better basis for ethno-directed phytopharmacological investigations. This could entail

revisiting sites where it is used extensively to gather accurate details on usage such as in areas in Ethiopia (identified in Chapter 3) where it is a treatment of choice for STI; the investigation of the composition and absorption of ingredients from preparations of *C. spinarum* with fat, pepper and other additives; the development of antimalarial and anti-plasmodial models that can investigate complex EM preparations and plant/host interaction; further analysis of hepatoprotective activity which is relevant to several tropical and non-communicable diseases. The design of clinical studies that mimic traditional preparations and usage would be a valuable addition to the ethnopharmacological research on *C. spinarum*. The use of *C. spinarum* as a food additive such as among the Maasai in East Africa may reflect long-term health benefits that could guide more intensive investigation of antioxidant activity in biological systems. *C. spinarum*, having significant intra-species variation and a wide habitat range, is a particular candidate for metabolic fingerprinting of all tested extracts as a basic requirement of research. Toxicology, with the inclusion of selectivity indices, is also vital to ensure safety in practice, bearing in mind that extraction solvent may increase toxicity relative to EM preparations. To date there is one product, Zedupex™, identified containing *C. spinarum* as the primary ingredient, licenced to treat *Herpes simplex* infections in Kenya. With the widespread use of *C. spinarum* across continents in the treatment of multiple illnesses including infections, GI and respiratory diseases, it has great potential for further recognition as a medicinal species.

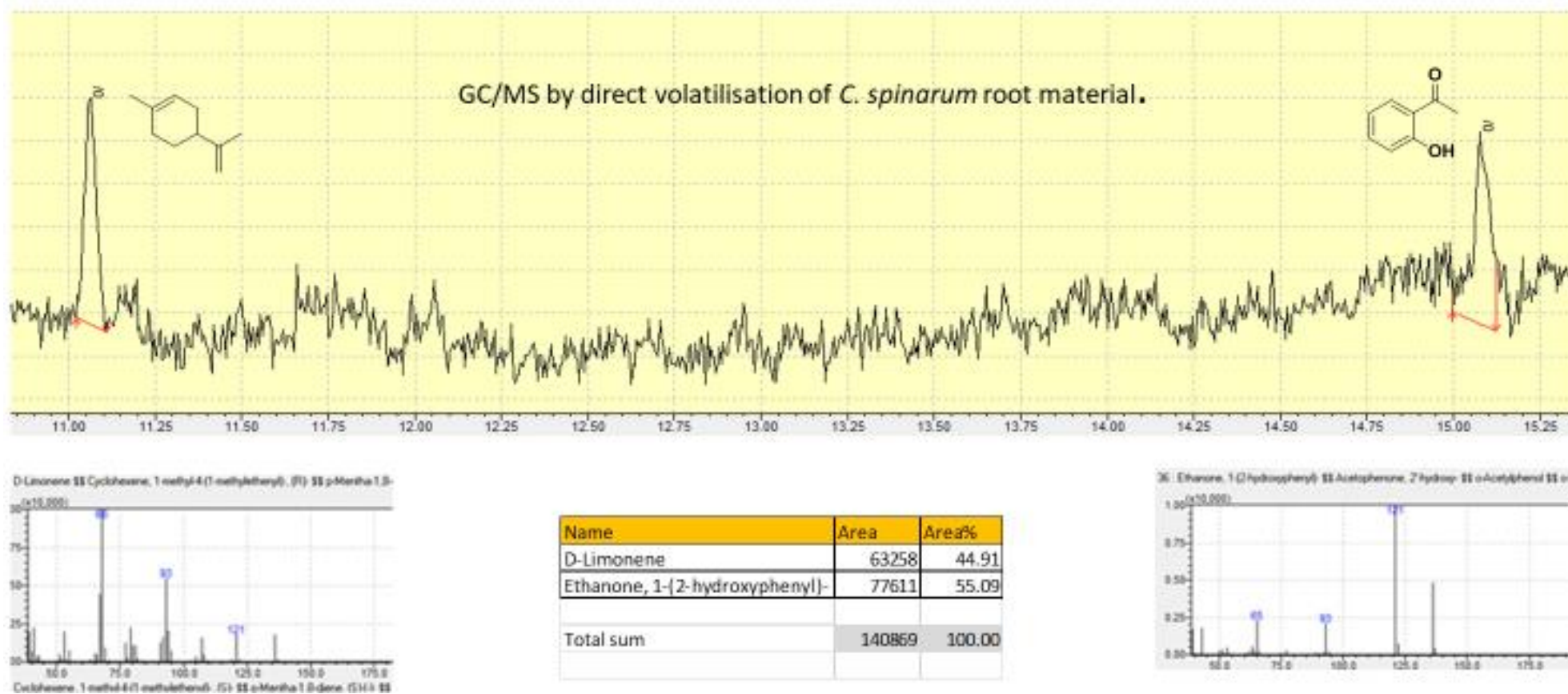


Figure 57: Analysis of *C. spinarum* root sample using Headspace-GCMS technique

Chapter 5: Analysis of 2'- Hydroxyacetophenone using Network Pharmacology

5.1 Introduction

The growing knowledge of the human genome forms the basis of systems biology and leads to an understanding of the complex relationships between disease phenotypes and genotypes [614]. The use of bioinformatics in systems biology enables the integration of large volumes of data from multiple sources. These sources include literature mining and gene expression data from microarray analysis. This results in an ever-improving understanding of the web of interactions involved in disease pathologies, many involving disorders of multiple proteins and pathways such as in cancer pathogenesis [615].

Drug development has historically been based on the “magic bullet” model of single compound/single disease. However, the developments in systems biology feed into changes in the nature of drug development with the growth of poly-pharmacology (single drug/multiple targets) and multi-drug/multi-target therapeutics [614]. Network Pharmacology (NP) is an *in silico* method, combining molecular biology, systems biology, and bioinformatics which has the potential to streamline the process of drug development. NP is a relatively new tool in the history of drug development, the term having been coined by Hopkins in 2007 [614]. It applies computational methods to data mined from multiple databases relating to ligands and disease processes. Online tools used in NP generate representations of complex networks of interaction and predict the activity of drug molecules in the human body.

NP has the potential to find new disease targets for drugs already in use through prediction of mechanisms of action (MOA) of drug ligands at the molecular level. In such drug repositioning, drugs with known safety profiles can be investigated for treatment of other diseases including rare diseases, which could be otherwise uneconomical to research. The development of personalised medicines, such as in the treatment of certain cancers, could also be improved through the use of NP techniques. NP applied to TM provides a bridge between TM and systems biology, can help to elucidate the MOA of traditional therapeutics, identify synergies in complex botanical formulae and enhance the potential for the development of future therapies for complex diseases. Traditional Chinese Medicine (TCM) is noted for its use of complex mixtures of species in the whole-system treatment of illness, intrinsically taking a multi-target approach to treatment of health and illness [616].

Recent *in silico* drug repositioning studies with the well-known drugs, metformin and pantoprazole, found that some predicted cancer signalling pathways are in agreement with *in vitro* and *in vivo* studies [617]. In a recent review, Wang *et al* [616] have reported on the combination of NP with experimental and clinical data to explore aspects of TCM. Reported studies have identified biomarkers of diseases and TCM syndromes such as Hot and Cold

Syndrome in rheumatoid arthritis and mechanisms of action of TCM herbal formulae such as Dengzhan Shengmai in vascular dementia.

This chapter explores the simple phenolic compound, 2-HAP (**2**), the primary component of *C. spinarum* volatile root oil using an NP approach. NP was used to augment the known bioactivity of **2** by predicting potential targets and diseases, comparing these to traditional uses of volatilised root of *C. spinarum* and predicting mechanisms of action. The primary NP process was conducted by this author and supervised by Dr. Junying Liu. Due to time constraints, Dr. Liu conducted and demonstrated the molecular docking, Dr. Shouli Yuan conducted the molecular dynamics, and Dr. Ismael Obaidi performed and demonstrated the cell biology studies.

5.2 Materials and Methods

5.2.1 Network Pharmacology of 2-HAP (**2**)

1. ID and Screen bioactive molecules

Pre-screen of 2: databases to identify ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profile (FAF-Drugs4, Discovery Studio).

2. ID gene lists of interest

Target ID Databases: to identify the protein targets of **2** (PharmMapper, SEA, SymMap, SwissTarget, BATMAN-TCM)

3. Identify target diseases from identified gene list for **2** (Metascape)

4. Disease Target ID: Analysis tools to identify targets, pathways, processes associated with disease (DisGeNet, GEO, GeneCards, DrugBank).

5. Overlapping targets of **2** and disease (Venn diagram)

6. Biological process analysis

KEGG pathways and GO enrichment: Databases to identify KEGG pathways, GO enrichment associated with particular disease(s) (Metascape)

7. Network construction and visualisation

- **Network construction** – PPI network using STRING
- **Network Visualisation** with Cytoscape

8. Molecular docking and molecular dynamics: computational simulation to validate the predictions of the NP analysis

9. *In vitro* and *in vivo*: design of experimental studies

The basic NP process involves several steps beginning with the identification and screening of bioactive metabolites from medicinal plants summarised in the box.

A preliminary step in the process is to examine the ADMET profile and, therefore druggability of **2**. This was done using the FAFDrugs4 program (Table 26). This includes several outputs on **2**, including oral bioavailability (OB) and drug-likeness (DL). Discovery Studio 4.5 was also used to predict ADMET.

Table 26: Databases and tools used for ADMET prediction

Function	Databases & Online Tools	Description
Ligand structure, bioactivity and more	PubChem	Public database with chemical structures, bioactivity & etc. (https://pubchem.ncbi.nlm.nih.gov/).
Pre-screen for ADMET	FAF Drugs4	FAFDrugs4 program includes a number of outputs on the test molecule including oral bioavailability (OB), drug likeness (DL) [618] (https://fafdrugs4.rpbs.univ-paris-diderot.fr/)
Pre-screen for ADMET	Discovery Studio	Discovery Studio 4.5 is a software tool for predicting ADMET including toxicity [619]

Online tools and databases were employed to identify potential targets of **2** include: BATMAN-TCM [620], PharmMapper [621], SEA [622], SymMap [623] and SwissTarget [624] and the output was put through STRING (Version 11.5) [625, 626] to eliminate duplications and standardise the gene names used. The molecular structure of **2** was downloaded from PubChem (ID 8375) [179]. The formats of SDF (for Pharammapper), SMILES (CC(=O)C1=CC=CC=C1O) (for SEA and SwissTarget), PubChem ID (for BATMAN-TCM) and CAS number (for SymMap) were copied from PubChem as required for the different online tools.

The combined target list was entered in Metascape [627] to identify key biological processes, pathways and diseases of **2**. Metascape enrichment analysis identifies GO terms and KEGG pathways, constructs PPI networks and uses the DisGeNet database to produce a predicted target disease list.

Target genes for Pneumonitis were identified using DrugBank 9 [628], GeneCards (*Homo sapiens*) [629], GEO dataset in NCBI (GSE20346) [630] and DisGeNet [631]. Analysis of the gene expression profile of the GSE20346 dataset was performed using the GEO2R facility on NCBI site. In addition, a search of Google Scholar with the search term “Pneumonitis single-cell RNA sequencing” was done to identify recent relevant research which could provide a gene list for Pneumonitis. The full list of Pneumonitis target genes was generated from the combined lists from each data source.

The intersecting target genes from the analyses for **2** and Pneumonitis were identified using an online Venn diagram tool (bioinformatics.psb.ugent.be).

To identify the relationships between the identified intersecting targets for Pneumonitis and **2**, PPI analysis was performed using STRING. The species was set at *Homo sapiens*, and the confidence score for interactions was set at 0.4 (medium) and hiding unconnected genes. The PPI network diagram was downloaded. The PPI network was imported from STRING into Cytoscape version 3.9.0 and analysed using the Analyze Network tool in Cytoscape (Cytoscape.org). The node table (of targets) was exported for further analysis and filtered for degree ≥ 4 . The network visualisation was modified to emphasise the most highly networked genes through the colour and size of the nodes.

The 24 core intersecting genes (core targets) filtered by degree (≥ 4) and closeness centrality (> 0.4) were imported to Metascape to generate heatmaps and networks of enriched terms, pathways and diseases. Further visualisation of enriched biological processes was conducted using the data from the Metascape analysis of core targets. Each GO term had an associated gene list in the Metascape analysis, and the top 9 GO terms (by logP) and 24 common genes were arranged in a matrix. The matrix was used to generate a plot of Biological Process enrichment using the Bioinformatics online CIRCOS plot tool (bioinformatics.com.cn). A similar process was followed for the analysis of KEGG enrichment.

5.2.2 Molecular Docking and Dynamics of 2-HAP (**2**)

For molecular docking simulation, the crystal structures of key targets were obtained from the Protein Data Bank (PDB) and processed by removing solvent and organics in Pymol software. The structure data file (SDF) of **2** was downloaded from PubChem (8375) and converted to PDB format. The docking pocket of each target protein was predicted using DeepSite. 2-HAP (**2**) and the corresponding targets were subsequently docked in AutoDock Vina software (version 1.1.2) to prepare PDBQT format files for target and ligand screening and grid and docking parameter files. The molecular dynamics simulations of these complexes were performed using Gromacs 2020.1, in which the charm36-jul2020 force field was chosen. The complex was solved in TIP3P water and immersed in a dodecahedron box extending to at least 1 nm of the solvent on all sides. Then, the system was neutralised by Na⁺ and Cl⁻ and 0.15 M NaCl was added. Using the steepest descent algorithm, the system was performed with energy minimised to make a maximum force of less than 1000 kJ/mol/nm. Then, it was equilibrated in a constrained NVT (number of particles, volume, temperature) and NPT (number of particles, pressure, temperature) running for 100 ps. Finally, molecular dynamics simulations of the complex were carried out for 100 ns. The Verlet cut-off scheme and a Leap-frog integrator with a step size of 2 fs were applied. The final analysis of molecular dynamics included the root

mean square deviation (RMSD) of protein and molecule and the interaction energy between protein and molecule, followed by calculation using GROMACS 2020.1.

5.2.3 Preliminary biological screening of 2-HAP (2)

Immortalised Bone Marrow Derived Macrophage (iBMDM) cells were used to examine the biological action of **2**. The cell line was generated with J2 recombinant retrovirus carrying v-myc and v-raf oncogenes. This cell line was a generous gift from Dr. Michael Carty, School of Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin. The iBMDM cells were grown in a cell culture medium which consisted of Dulbecco's Modified Eagle Medium (Gibco Life Technologies, Carlsbad, CA, USA), a medium which contains glucose, and was supplemented with Foetal Bovine Serum (FBS) 10%, Normocin™ 0.2% (antimicrobial formulation) and a mixture of penicillin/streptomycin at a concentration of 1%. FBS was used in order to provide growth factors, amino acid and proteins needed by the cells to grow, while Normocin™ and the penicillin/streptomycin mixture was used to prevent harm to the cells from microorganisms such as bacteria and fungi. The cell culture medium was prepared under aseptic conditions in a class 2 biological safety cabinet (BSC). Prior to preparing the medium, all components of the medium were warmed in a thermostatically controlled water bath set at 37°C to replicate human body temperature.

Cell Culture Methods

All cell culture techniques were demonstrated, supervised and assisted by Dr Ismael Obaidi. Given the short research period for this element and the limited time available to learn and practice cell culture techniques, Dr Obaidi performed many techniques himself.

Cell Maintenance

iBMDM cells were taken from liquid nitrogen storage at -196°C and were quickly thawed in a water bath at 37°C to prevent damage to cells that could occur if they were thawed slowly over time. After thawing, these cells were added to a flask containing 25mL of cell culture medium and these flasks were then incubated at 37°C to allow the cells to grow and divide. The cells taken from the liquid nitrogen storage had to be placed in a cell culture medium soon after thawing, as the cells were stored in 10% DMSO due to its cryoprotectant activity. DMSO is toxic to cells at this concentration; hence the cell culture medium had to be changed 24 hours after initial incubation until the cells were split for the first time. Cells were viewed under a light microscope at least every 24 hours in order to know the splitting time correctly.

Incubated cells were split approximately every 48 hours after stabilisation to prevent the cells' overcrowding and to provide fresh medium to the cells, assuming there was satisfactory confluence of cells within the flask. To split, flasks were first scraped to remove adherent cells and centrifuged to remove the cells from the cell culture medium. The pellet of centrifuged cells was then resuspended in 10mL of fresh cell culture medium, and 0.5mL of this cell broth was added to 25mL of cell culture medium in a flask and then incubated at 37°C.

Cell Seeding

To prepare a 96-well plate of cells for the experiments, the cells were split, resuspended and 10µl of the cell broth was then taken and mixed in an Eppendorf tube with 10µl of Trypan Blue stain and applied to a glass slide to count cells using a Bio Rad TC20 Automated Cell Counter. When the concentration of cells in each cell broth was accurately known, a mixture of cell broth and cell culture medium was made up to reflect a cell concentration of 0.5×10^6 cells/mL, and 200µL of this mixture was added to each well of the plate and the plate was then incubated at 37°C.

Cell Treatment

The stock solution of **2** (Sigma Aldrich) was prepared and then diluted to a stock solution of 1.1mg/mL in a 500µL Eppendorf tube. From this stock solution, additions were made to the wells containing cells suspended in culture medium so that each 96-well plate had 6 wells of each concentration of each sample, with the final concentration of **2** in the wells being 10, 30, 50, 75 and 100µg/mL, which was accomplished by adding 1.9, 6, 10, 15 and 20µL of stock solution to the wells respectively. The plates were then incubated at 37°C, and after two hours, an 8µl aliquot of 0.5% LPS solution was added to 3 of the 6 wells for each concentration of **2**, meaning that for each concentration of **2**, there were 3 wells with cultured cells plus **2** and 3 wells with cultured cells, **2** plus LPS. The plates were then incubated for 24 hours at 37°C.

Cell Freezing

Cells were frozen in order to preserve the iBMDM cell line used in the experiment. The cells were first split and resuspended in cell culture medium after centrifugation, and part of this cell suspension was then added to a 15mL tube containing FBS and DMSO to reflect a final concentration of 10% w/v of DMSO. 1mL aliquots of this mixture were then transferred using a P100 pipette to individual cryovials and placed in a Nalgene Cryo 1°C Freezing Container for freezing at -80° C for 24 hours. After 24 hours, the individual cryovials were transferred from the freezing container into liquid nitrogen storage at -196 °C for long-term storage.

Cell Viability Assay

A cell viability assay was used to measure whether **2** caused any cell cytotoxicity, and the assay was conducted using a GenieBlue Cell Viability Assay Kit. To quantify this, resazurin reagent was used, which measures the levels of cellular metabolic activity in the wells, and the assay is based on the colour change of resazurin (blue) to resorufin (red) when resazurin is reduced by dehydrogenase enzymes in metabolically active cells. The culture medium used to dilute resazurin solution at 1:10, was kept away from light until the supernatant had been harvested from the wells into a fresh 96-well plate. A volume of 100µl of diluted resazurin solution was added to each well of the 96-well plate, and the plates were incubated for 90 minutes at 37°C. The plates were wrapped in aluminium foil for incubation to protect the reagent from light. After the incubation period had elapsed, the fluorescence emitted by each well was measured using a Fluostar Optima plate reader at an excitation wavelength of 544nm and emission wavelength of 590nm.

ELISA assay

Supernatant from the cytotoxicity assay was used to test **2** for anti-inflammatory activity using the ELISA (enzyme-linked immunosorbent assay) method. Briefly, highly binding 96-well immunoplates were treated with captured antibodies and incubated at 4°C overnight. Next day, the plates were washed with 1X phosphate buffer saline (PBS) (Gibco Life Technologies, Carlsbad, CA, USA) containing 0.05% Tween 20®, then blocked with reagent buffer containing 1% bovine serum albumin (BSA) in PBS. Standard solutions were prepared by proper dilution of standard stock solutions according to the manufacturer's recommendations. IL6, RANTES and TNFα were diluted at 1:85, 1:190, and 1:100, respectively using reagent buffer to final top standard concentrations of 2000pg/mL, 4000pg/mL, and 4000pg/mL, respectively. IFNβ stock solution was diluted by 1:100 to 2000U/mL as the highest standard concentration. The standard solutions were then serially diluted into 1:2 using the reagent diluent. Plates were incubated for two hours at room temperature, followed by washing and further incubation with detection antibodies at a dilution of 1:60 for two hours. Finally, the plates were washed and incubated with Streptavidin-HRP for one hour before adding 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution. The reaction was then stopped using 10% sulfuric acid. The absorbance was measured at 450nm, and the readings were subtracted from the absorbance at 540nm.

Statistical analysis

A paired t-test was employed to analyse and compare between control and **2** treated groups. Data are expressed as mean ± SEM of three independent experiments. GraphPad prism was used to analyse the viability curves of both **2** and LPS + **2** treated groups. The non-linear regression function was selected.

Concentrations were transformed to the “log” function, then dose-response-inhibition/log(inhibitor) vs. normalized response – The Variable slope model was employed to calculate IC₅₀.

5.3 Results

The process and summary of the results of the initial analysis is presented in Figure 58 while the process of identification of a predicted mechanism of action of **2** in a specific disease is visualised in Figure 59 below.

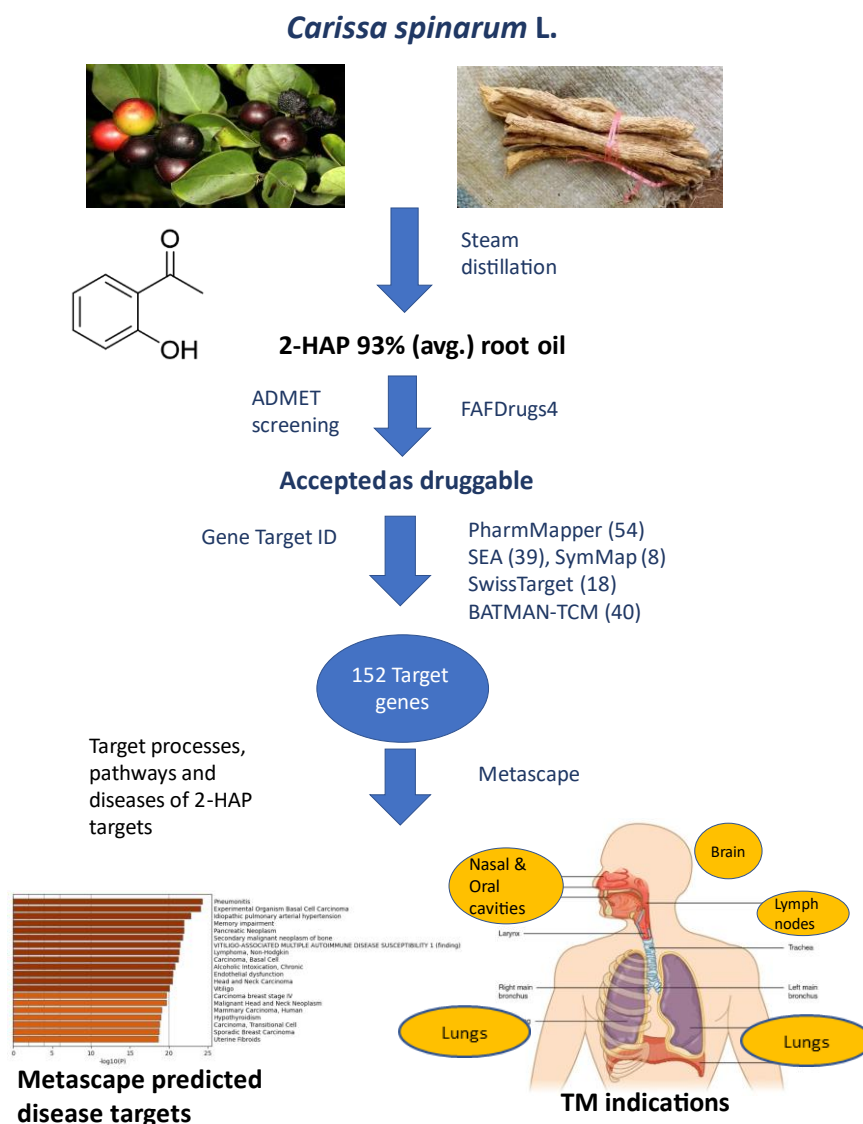


Figure 58: NP process to identify target diseases of **2**

Network Pharmacology process to ID 2-HAP MOA in Pneumonitis

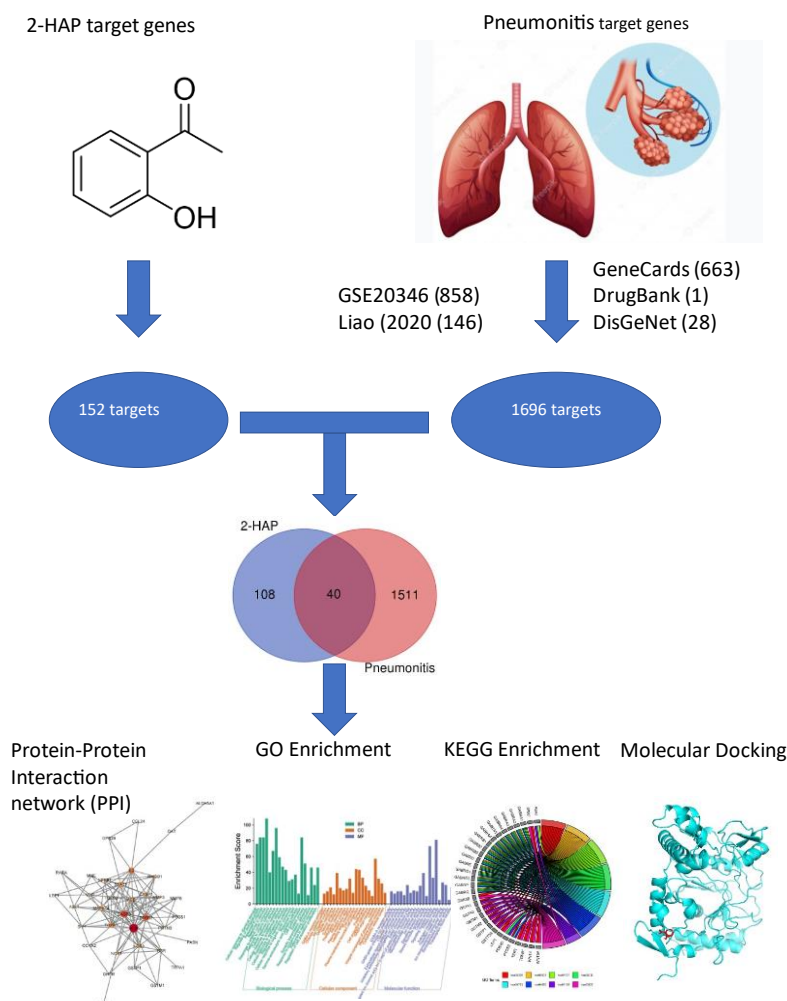


Figure 59: Network pharmacology process to identify mechanisms of action of **2** in pneumonitis

5.3.1 Network Pharmacology of 2-HAP (**2**)

5.3.1.1 Screening for Drug Likeness

The result of the FAF-Drugs4 analysis as a preliminary test for drug likeness revealed that **2** has a good druggability profile (Table 27). 2-HAP (**2**) has no Lipinski violations with a molecular weight of 136.15, one H-bond donor, 2 H-bond acceptors and a logP of 1.92 (Table 28) [632]. It has good oral bioavailability under the Veber and Egan rules and under GSK 4-400 test, **2** has a good druggability profile. 2-HAP (**2**) fails one of the Pfizer 3-75 rules with a total polar surface area (tPSA) of 37.3 (should be >75) which results in a potential toxicity warning in the FAF-Drugs4 screen. The final overall status from the FAF-Drugs4 analysis is that **2** is accepted as a druggable compound in this screening

Table 27: FAF-Drugs4 analysis of druggability of 2

Property	Value		Property	Value
Ligand	2'-HAP		Heavy Atoms	10
ID	8375		Carbon Atoms	8
SMILES	CC(=O)C1CCCCC1O		Hetero Atoms	2
MW	136.15	≤ 500	Ratio H/C	0.25
logP	1.92	≤ 5	Lipinski Violation	0
logD	1.88		Solubility (mg/l)	17104.78
logSw	-2.07		Solubility Forecast Index	Good
tPSA	37.3	< 140	Oral_Bioavailability_VEBER	Good
Rotatable B	1	< 10	Oral_Bioavailability_EGAN	Good
Rigid B	7		Traffic Lights	0
Flexibility	0.13		GSK 4-400	good
HBD	1	≤ 5	Pfizer 3-75	warning
HBA	2	≤ 10	Phospholipidosis	Non-Inducer
HBD_HBA	3		Fsp3	0.13
Rings	1		Stereo Centers	0
MaxSize Ring	6		PPI_Friendly	Not Computed
Charges	0		Result	Accepted
Total Charge	0		Alert	N/A

Table 28: Drug ADMET definitions

Defined Term	Description
Drug pre-screen terms	
Lipinski's Rule of 5	Rules for assessing the OB of small molecules. Molecular weight < 500, H bond donors ≤ 5, H bond acceptors ≤ 10 and a partition coefficient (log P) value < 5 [632]
Veber rules	Rotatable bond count ≤ 10, PSA ≤ 140 [633]
Pfizer rules	Likelihood of toxicity of compounds: Pfizer 3-75 rule, high log P (>3) and low tPSA (<75) are 2.5 times more likely to be toxic as clean
GSK 4-400	Rules for assessing druggability: logP < 4, MW < 400 Da

process. Discovery Studio 4.5 additionally predicted medium blood-brain barrier penetration, no predicted mutagenicity and two predicted toxicity measures - CYP4502D6 toxicity and a predicted rat oral LD₅₀ of 0.951808(g/kg) and calculated a slightly different tPSA of 38.116 compared to FAFDrugs4 (37.3) (Table 29, Supplementary file 7).

Table 29: Discovery Studio analysis of druggability of **2**

ADMET	Value	Comments
Name	2-HAP (2)	
PubChem MW	136	< 500
Solubility	-1.388 (Optimal)	OB
BBB	-0.347 (Medium)	
CYP2D6	-5.44952	CP450 2D6 toxicity
ADMET_EXT_PPB	-2.47923	Not plasma protein bound
ADMET_EXT_PPB Prediction	FALSE	
ADMET_Absorption_Level	0 (Good absorption)	
tPSA	38.116	
TOPKAT Ames Prediction	Non-mutagen	
TOPKAT Rat Oral LD ₅₀ prediction	0.951808g/kg body wt	

5.3.1.2 2-HAP (2) target and disease prediction

In target prediction for **2**, BATMAN-TCM identified 40 target genes, PharmMapper identified 54 targets, SEA (39 targets), SymMap (8 targets) and SwissTarget (18 targets). When duplications were removed, this resulted in 152 target genes/proteins (Supplementary file 8).

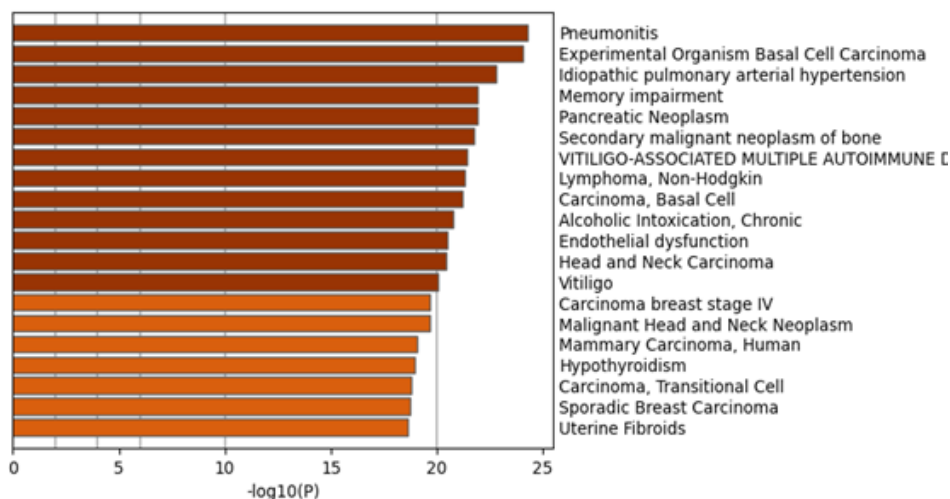


Figure 60: Heatmap for top 20 diseases for **2** from Metascape analysis using DisGeNet logP -24 to -19.

The Metascape analysis of 152 target genes for **2** resulted in the heatmap of disease targets (Figure 60). This identified the top 20 diseases associated with these target genes, those having a logP better than or equal to -20 included: Pneumonitis, several cancers, idiopathic pulmonary arterial

hypertension, memory impairment, alcoholic intoxication, chronic endothelial dysfunction, and vitiligo related disorders (Supplementary file 9). When this list of disease targets is compared to the traditional uses of the root volatile oil, there is limited overlap. Possible overlap occurs in the traditional treatment of chest congestion/respiratory conditions with the Metascape prediction of Pneumonitis. Traditional treatments of mental disorder, brain tension and stress overlap with the Metascape target predictions of memory impairment, mood disorder and depression which have poorer logP values (Figure 60 and Supplementary file 9). Pneumonitis was chosen as the most promising disease for more detailed investigation related to the traditional use of *C. spinarum*.

5.3.1.3 Pneumonitis target gene identification

The GEO dataset (GSE20346) relates to the blood of patients with Influenza A and bacterial pneumonia [634]. The differentially expressed genes (DEGs) are filtered for those with logFC (fold change) > 1 and < -1 indicating those genes which are significantly up- and down-regulated. The most significantly up - or down-regulated genes in the treatment group are represented in the volcano plot (Figure 61) where the most statistically significant genes with the largest fold changes are at the top of the graph on either side. Those in black are not significant. This resulted in 858 DEGs [634]

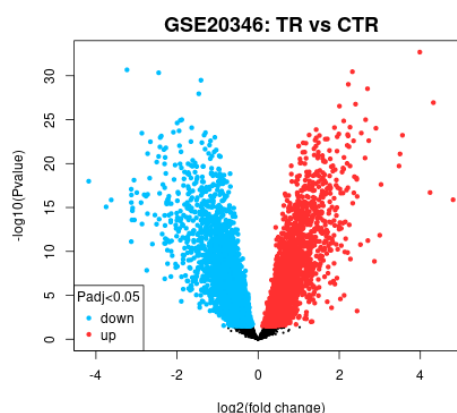


Figure 61: Volcano plot of GSE20346 data indicating significantly upregulated and downregulated genes.

(Supplementary file 11). A study characterising the bronchoalveolar lavage fluid (BALF) in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection through RNA sequencing yielded 148 DEGs [635]. The database searches for DEGs in Pneumonitis yielded: DrugBank (1), GeneCards (663) and DisGeNet (28). Lists of DEGs from each data source were combined and duplicates removed giving a total list of 1551 DEGs (Supplementary file 12).

5.3.1.4 Herb metabolite 2-HAP (2)-Targets - Disease network

The intersection between **2** and Pneumonitis, arising from the Venn diagram tool, resulted in a common target set of 40 genes, visualised in Figure 62 (Supplementary file 13). These intersecting target genes are: TGFB2, FASN, GSTP1, DHFR, LEF1, MAPK14, SHH, CASP1, TRPA1, PTGS2, TGFB1, MME, ARSA, CCNA2, STAT1, ALDH5A1, CCL24, CASP3, FPR1, VDR, ACE, GSR, RARA, MMP3, CCR6, MMP8, GSTM1, IL2, PRTN3, ABL1, NOS3, NOX1, NFKB1, ALB, PTGS1, BRAF, ENPP3, FBLN5, HMGB1 and OAT.

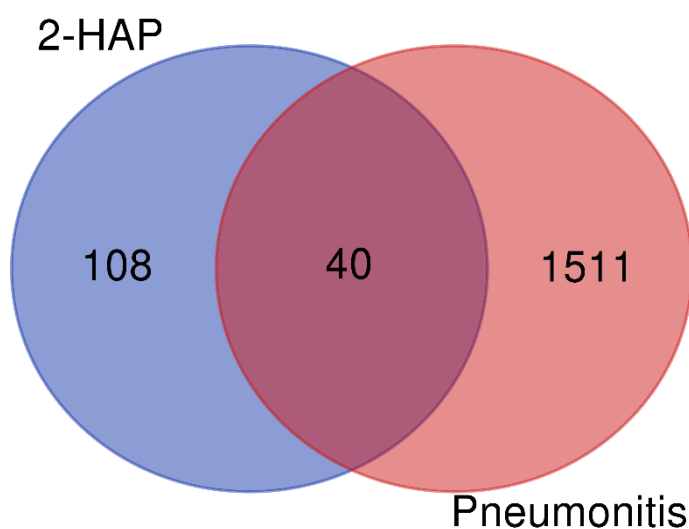


Figure 62: Venn diagram representing the intersection of target for **2** and Pneumonitis

5.3.1.5 Network analysis – PPI, GO and KEGG enrichment

PPI Network construction using STRING

The STRING database of genomes and encoded proteins was used to generate a PPI network of predicted interactions between the 40 common gene targets. The statistics relating to this dataset are: 40 nodes, 125 edges, average node degree = 6.25, avg. local clustering coefficient = 0.555, expected number of edges = 39, PPI enrichment p-value = < 1.0e-16.

Cytoscape Visualisation of 2-HAP (2) and Pneumonitis disease network

The PPI network based on the common targets of **2** and Pneumonitis was imported from STRING into Cytoscape and the network was constructed (Figure 63) with a bar chart (Figure 64) representing the key targets and their correlated degree. The data on Degree, Betweenness and Closeness centrality data of 24 core overlapping targets are listed with the most connected targets selected by

the criteria Degree ≥ 4 and Closeness centrality > 0.4 (Table 30) (Supplementary file 14). The centrality data provides a way of measuring the flow of information through a network and can identify nodes that are bridges between different areas of the network.

The network and chart illustrate that ALB is the most important node in the network. It is the largest and darkest node in the network. The other core genes with Degree ≥ 10 are CASP3, PTGS2/COX2, IL2, TGFB1, STAT1, MAPK14, ACE, NOS3 and CASP1 and are visualised in the central area of the graph as having the highest number of interactions. These targets are potentially involved in the activity of *C. spinarum* root oil in treating chest conditions. This needs to be verified through molecular docking techniques and the design of experiments based on these results.

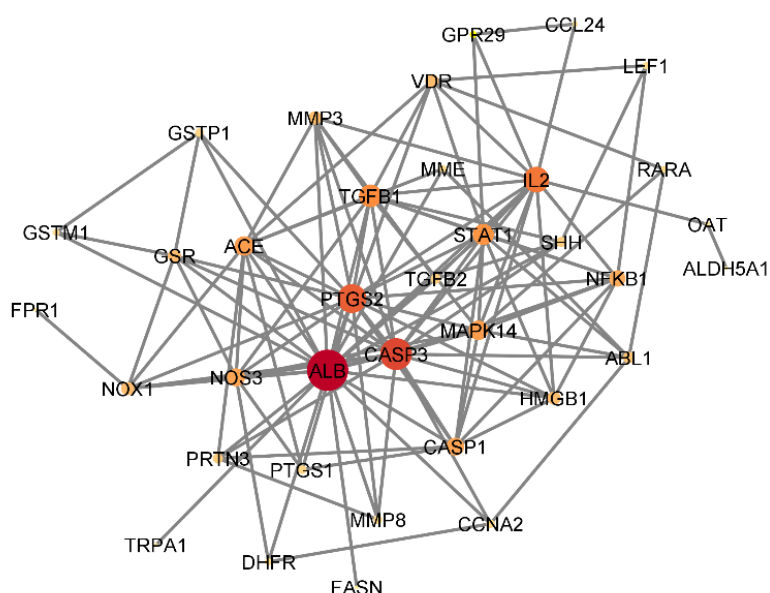


Figure 63: Cytoscape visualisation of PPI Network for 2 and Pneumonitis.

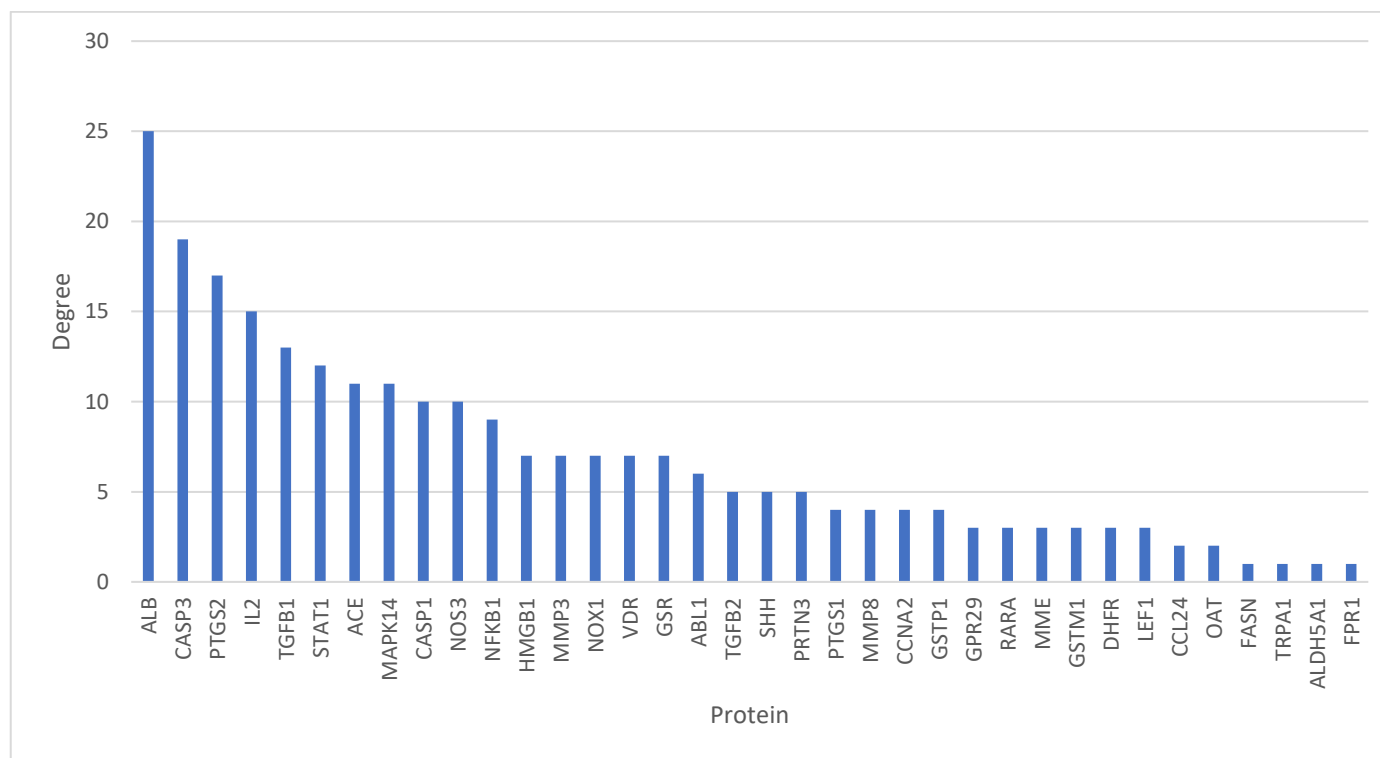


Figure 64: Bar chart represents the common targets of 2 and Pneumonitis and their correlated degrees.

Table 30: Information on 24 core genes after PPI network analysis¹⁸

#	Function (information from GeneCards- Stelzer <i>et al.</i> [629] (accessed 30 th November 2021) - and other stated sources)	Gene	Protein name	Degree	CC	BC
1	Most abundant human protein – homeostasis. Folate and Lipoprotein metabolic pathways	ALB	Albumin	25	0.760869565	0.358808614
2	Cysteine-aspartic acid protease 3 plays a central role in the execution-phase of cell apoptosis PubChem finds 2-HAP (2) inactive as inducer of CASP3 signalling pathway.	CASP3	Caspase-3	19	0.673076923	0.112109175
3	Prostaglandin-Endoperoxide Synthase 2 has a key role in prostaglandin biosynthesis and is involved in inflammation. Related pathways are G-Beta Gamma Signalling and Cytokine Signalling in Immune system both of which are elements of the immune response.	PTGS2/COX-2	Prostaglandin G/H synthase 2	17	0.648148148	0.079890478
4	The cytokine IL2 is produced by T cells and is important for the proliferation of T and B cells in the immune response to antigenic or mitogenic stimuli.	IL2	Interleukin-2	15	0.625	0.201197948
5	The protein encoded by this gene regulates the cell cycle and is involved in Interferon- γ (IFN γ) and tumour necrosis factor α (TNF α) activation. It is often present in tumour cells. PubChem finds 2-HAP (2) inactive as inducer of TGF beta signalling pathway.	TGFB1	Transforming growth factor beta-1 proprotein	13	0.593220339	0.0250007

¹⁸ Degree ≥ 4 , CC > 0.4m CC = Closeness Centrality, BC = Betweenness Centrality

#	Function (information from GeneCards- Stelzer <i>et al.</i> [629] (accessed 30 th November 2021) - and other stated sources)	Gene	Protein name	Degree	CC	BC
6	This is a transcription activator. The protein encoded by this gene is thought to be important for cell viability in response to various stimuli and is important in the immune response. It mediates the response to certain cytokines.	STAT1	Signal transducer and activator of transcription 1- alpha/beta	12	0.583333333	0.039060244
7	The protein encoded by this gene is involved in a range of cellular processes and is activated by pro-inflammatory cytokines and environmental elements.	MAPK14	Mitogen-activated protein kinase 14	11	0.573770492	0.040330347
8	The protein encoded by this gene is an enzyme involved in the conversion of Angiotensin 1 to Angiotensin 2 and has a role in controlling blood pressure and fluid-electrolyte balance. The homolog ACE2 is thought to be involved in regulating susceptibility to SARS-COV2 infection.	ACE1	Angiotensin-converting enzyme	11	0.546875	0.030689973
9	The protein encoded by this gene is involved in Nitric oxide production in the vascular epithelium. NO is a signalling molecule regulating vascular tone, platelet aggregation, angiogenesis, response to infection and inflammation. Damaging and healing effects of NO in airways have been demonstrated.	NOS3	Nitric oxide synthase	10	0.53030303	0.020168627
10	Cysteine-aspartic acid protease 1 involved in signalling pathways for apoptosis, pyroptosis and inflammation. It activates Interleukin 1 (IL1) (from IL1 β) and Interleukin-18 (IL18). It has an important function in cell immunity as an initiator of inflammation.	CASP1	Caspase-1	10	0.564516129	0.020144258

#	Function (information from GeneCards- Stelzer <i>et al.</i> [629] (accessed 30 th November 2021) - and other stated sources)	Gene	Protein name	Degree	CC	BC
11	This gene codes for a protein that is a transcription factor, present in almost all cell types. It is involved in the cellular response to environmental stressors and cytokines and has a key function in the immune and inflammatory response. PubChem finds 2-HAP (2) inactive as either agonist or antagonist of NFkB signalling pathway.	NFkB1	Nuclear factor NF-kappa-B p105 subunit	9	0.514705882	0.021264506
12	NADPH oxidases catalyse the production of reactive oxygen species (ROS). Activated NADPH oxidases have a central role in the immune response. They respond to bacteria, cytokines and other stimuli through the production of superoxides. NOX1 is predominantly expressed in colon, uterus and placenta.	NOX1	NADPH oxidase 1	7	0.5	0.057478992
13	This gene codes for the Vitamin D3 receptor which mediates the action of Vitamin D on cells. It is also a receptor for a bile acid, is involved in mineral metabolism and in other pathways involved in immunity and cancers. VDRs are mainly expressed in the intestine, kidney and thyroid. PubChem finds 2-HAP (2) inactive as either agonist or antagonist of VDR signalling pathway.	VDR	Vitamin D3 receptor	7	0.538461538	0.038232748
14	This gene codes for a proteinase which is involved in the breakdown of extracellular matrix and is important for growth and development. It is supposed to be involved in wound repair, in tumour growth and in atherosclerosis. It has both pro-and anti-inflammatory effects depending on level of expression and tissue involved [636].	MMP3	Matrix Metalloproteinase 3 or Stromelysin-1	7	0.52238806	0.003024622
15	Glutathione-Disulfide Reductase is a central enzyme in cellular antioxidant defence, maintaining high levels of the anti-oxidant Glutathione in the cytosol, contributing to the immune response.	GSR	Glutathione reductase	7	0.5	0.007415087

#	Function (information from GeneCards- Stelzer <i>et al.</i> [629] (accessed 30 th November 2021) - and other stated sources)	Gene	Protein name	Degree	CC	BC
16	The encoded protein regulates transcription and DNA. It plays a role in several cellular processes including inflammation, tumour migration and cell differentiation. It promotes the host immune response to stimuli.	HMGB1	High mobility group protein B1	7	0.53030303	0.001032371
17	This gene encodes a ubiquitously expressed protein tyrosine kinase. This kinase is involved in cell division, adhesion, differentiation and the response to stressors. A simple deletion creates an oncogene and so is implicated in some cancers. Liu <i>et al.</i> [637] found high immune cell infiltration where ABL1 expression is high in hepatocellular carcinoma.	ABL1	Tyrosine-protein kinase ABL1	6	0.460526316	0.013041216
18	This gene encodes neutrophil serine proteinase, PR3. PR3 is involved in triggering cytokine mediated inflammatory pathways, assists in the defensive role of neutrophils, degrades cellular proteins. It is thought to be involved in inflammatory disease processes including COPD (chronic obstructive pulmonary disease), asthma, interstitial lung disease, rheumatoid arthritis, crohn's disease and cystic fibrosis [638].	PRTN3	Myeloblastin/ Proteinase 3	5	0.479452055	0.001176471
19	This gene codes for a multi-functional protein that is involved in the processes of angiogenesis and heart development. PubChem finds 2-HAP (2) inactive as inducer of TGF beta signalling pathway.	TGFB2	Transforming growth factor beta-2 proprotein	5	0.5	0.002480992
20	This gene encodes a protein that is important in embryonic development. PubChem data says 2-HAP (2) inactive in sonic hedgehog signalling pathway, either as agonist or antagonist	SHH	Sonic hedgehog protein	5	0.479452055	0.014078862
21	This gene codes for the protein which is a member of the cyclin family which function as regulators of the cell cycle.	CCNA2	Cyclin-A2	4	0.472972973	0.004394886

#	Function (information from GeneCards- Stelzer <i>et al.</i> [629] (accessed 30 th November 2021) - and other stated sources)	Gene	Protein name	Degree	CC	BC
22	Prostaglandin-Endoperoxide Synthase 1 (COX-1) has a key role in prostaglandin biosynthesis and is involved in the inflammatory response.	PTGS1/COX1	Prostaglandin G/H synthase 1	4	0.432098765	6.72E-04
23	Matrix Metalloproteinase 8 (MMP8) codes for a proteinase which is involved in the breakdown of extracellular matrix and is important for growth and development. MMP8 has been shown to have a role in cancer development, either inhibiting or promoting cancer development depending on tissue of origin [639]. MMP8 associated with colorectal cancer and systemic inflammation and known to have both pro- and anti-inflammatory functions [640]	MMP8	Neutrophil collagenase	4	0.472972973	3.36E-04
24	Glutathione S-transferases have a role in detoxification through conjugation of reduced glutathione with target compounds. [641] reported an anti-inflammatory role for GSTP1 in an in-vitro macrophage model. In a mouse model of sepsis, the authors found that GSTP1 inhibited excessive inflammatory response and reduces sepsis-related damage and mortality [641].	GSTP1	Glutathione S-transferase P	4	0.466666667	9.53E-04

Metascape Visualisation of 2 and Pneumonitis intersecting targets

The key outputs from the Metascape GO enrichment analysis of 2 and Pneumonitis targets are visualised in Figure 65 (Supplementary file 15). The top 20 enriched GO terms include the GO Biological processes: response to LPS, tube morphogenesis, positive regulation of cytokine production, haemopoiesis, cellular oxidant detoxification, cellular response to organic cyclic compound, response to oxidative stress, positive regulation of cell death, response to inorganic substance, response to xenobiotic stimulus, regulation of cellular response to stress and regulation of ROS metabolic process. The enriched KEGG pathways are AGE-RAGE signalling pathway in diabetic complications, Pathways in cancer and Lipid and atherosclerosis.

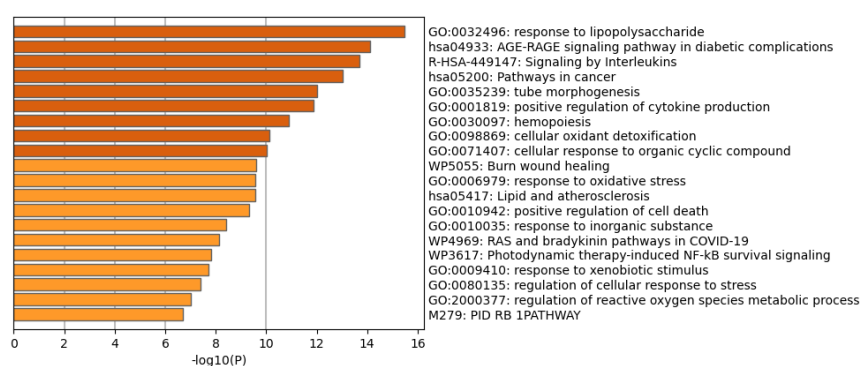


Figure 65: Metascape analysis of 24 common gene targets: Heatmap of enriched GO terms in common genes coloured by p-values

CIRCOS plot visualisation of KEGG pathways and GO processes

The CIRCOS plot for the top nine Metascape derived KEGG pathways (Figure 66) illustrates six genes that are involved in more than four pathways: CASP3, MAPK14, NFκB1, STAT1, TGFB1 and TGFB2 (dataset and CIRCOS matrix in Supplementary file 16). The KEGG pathways are: AGE-RAGE signalling pathway in diabetic complications (hsa04933); Pathways in cancer (hsa05200); Leishmaniasis (hsa05140); Hepatitis B (hsa05161); Tuberculosis (hsa05152); Chagas disease (hsa05142); Diabetic cardiomyopathy (hsa05415); C-type lectin receptor signalling pathway (hsa04625); Lipid and atherosclerosis (hsa05417). TGFB1, TGFB2, NFκB1 and MAPK14 are the most common targets in these pathways.

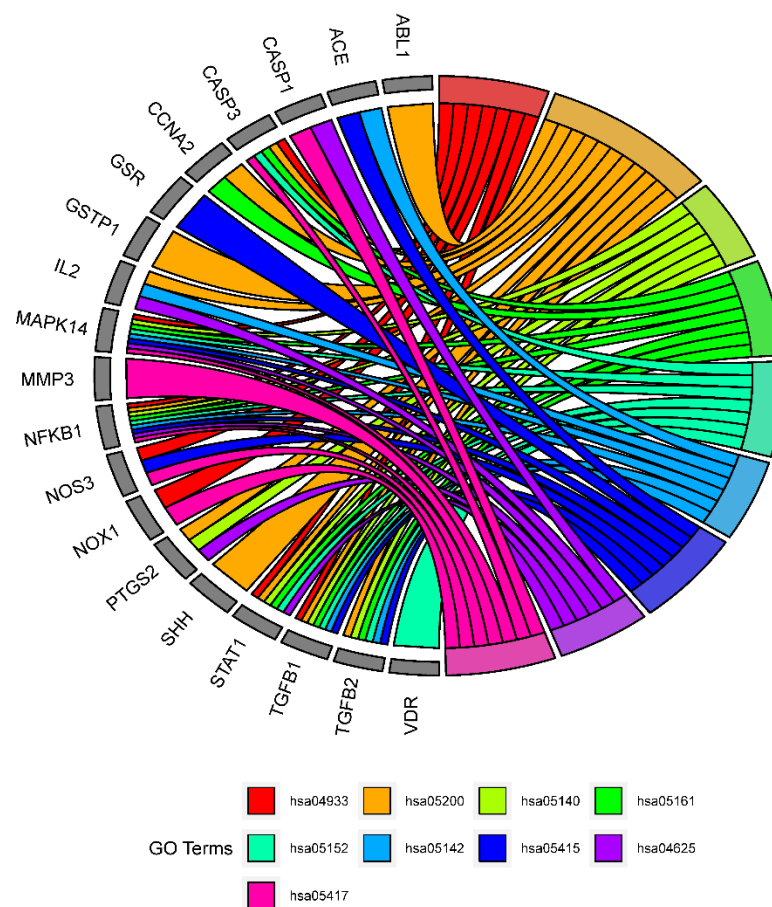


Figure 66: CIRCOS plot of Metascape derived top nine KEGG pathways filtered by logP

The top nine GO biological processes are: response to LPS (GOBP1), tube morphogenesis (GOBP2), positive regulation of cytokine production (GOBP3), haemopoiesis (GOBP4), cellular oxidant detoxification (GOBP5), cellular response to organic cyclic compound (GOBP6), response to oxidative stress (GOBP7), positive regulation of cell death (GOBP8) and response to inorganic substance (GOBP9). The CIRCOS plot visualising the intersection of common targets with these processes (Figure 67) illustrates that 13 genes are involved in 6 or more processes. These are: ABL1, CASP3, PTGS2/COX2, ACE, GSTP1, MAPK14, NFKB1, NOS3, STAT1, TGFB1, HMGB1, NOX1 and VDR. Response to LPS has the best logP (-15.49) of all Biological Processes in this analysis (Supplementary file 19).

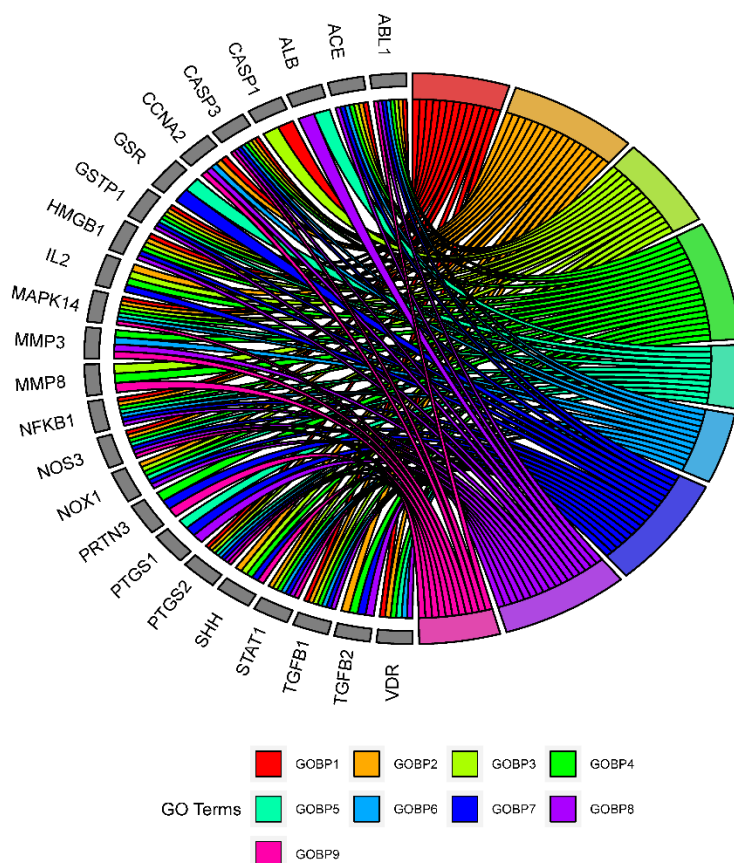


Figure 67: CIRCOS plot of Metascape derived top nine GO terms (all biological processes) filtered by logP.

The most promising targets to investigate by molecular docking were chosen because they were hub targets of the PPI network excluding NFkB1, TGFB1, TGFB2 and VDR as they had tested negative in screening of 2-HAP (2) [179]. CASP3, which had also tested negative in screening, was included in molecular simulations as control to contribute to validation of the molecular docking and dynamics for the other targets. IL18, PTGS1, CCNA2 and INS were also selected for docking from literature reports of their effect in respiratory conditions. The final list of targets for simulation studies was PTGS2/COX2, NOS3, ALB, MAPK14, ACE2, CASP3, STAT1, IL2, IL18, PTGS1/COX1, CCNA2 and INS.

5.3.2 Molecular docking and molecular dynamics simulations of receptor-ligand

Molecular docking is a computer simulation technique to predict the best binding of ligands in protein pockets and helps identify protein binding sites. Using the three-dimensional ligand structure, it identifies potential binding sites on the protein structure and scores the strength of the association in different poses by assigning binding energies (kcal/mol) to the complex [642]. The lower the

energy, the greater the possibility of receptor-ligand interaction [643] with ranges of < -7 kcal/mol predicting strong binding), < -5 kcal/mol predicting moderate binding and > -5kcal/mol predicting no binding [644]. Molecular dynamics is used alongside molecular docking to predict the stability of selected docking complexes. This technique simulates the behaviour of the protein-ligand complex over a specified time, measuring the root mean square deviation (RMSD) and interaction energy of the simulated complex.

5.3.2.1 Molecular Docking

The binding affinities of key intersecting targets of **2** and Pneumonitis were analysed and are listed in Table 31. The complementarity of the ligand and protein molecular surfaces are visualised in binding configurations in Figure 68 (A-G). Dashed lines in Figure 68 (A-G) represent hydrophobic bonds and solid lines represent hydrogen bonds. Binding affinities of docking patterns, with the exception of IL2-2HAP, were < -5 to -7 kcal/mol, indicating moderate to strong binding. The binding affinities (Table 31) include PTGS2-**2** (-7.0 kcal/mol), IL18-**2** (-6.7 kcal/mol), NOS3-**2** (-6.9 kcal/mol), ALB-**2** (-6.8 kcal/mol), PTGS1-**2** (-6.6 kcal/mol), CCNA2-**2** (-6.6 kcal/mol), MAPK-14-**2** (-5.3 kcal/mol), ACE2-**2** (-5.9 kcal/mol), CASP3-**2** (-5.7 kcal/mol), STAT1-**2** (-5.5 kcal/mol), INS-**2** (-5.3 kcal/mol) and IL2-**2** (-4.8 kcal/mol) (indicating no binding). 2-HAP (**2**) demonstrated the strongest binding with PTGS2 in the active site and formed hydrophobic bonds, mainly through the aromatic portion with amino acid residues such as LEU 390, LEU 391, TRP-387, TYR-385, ALA-202 and GLN-203 (Figure 68 A). 2-HAP (**2**) had good binding energy with IL18, forming H-bonds with ASP-143 and LYS-142 and hydrophobic bonds and π -stacking through the aromatic portion with TRP-163 (Figure 68 B). 2-HAP (**2**) interacted with NOS3, forming H-bonds through the hydroxy and ketone groups with amino acid residues GLN-464, THR-82 and GLN-437 and the aromatic portion forming hydrophobic bonds with amino acid residues LEU-433, VAL-467 and VAL-73 (Figure 68 C). 2-HAP (**2**) interacted with ALB, forming a H-bond with amino acid residue SER-448 and hydrophobic bonds mainly through the aromatic portion with LEU-406, LEU-429, PHE-402 and VAL-387 (Figure 68 D). 2-HAP (**2**) interacted with PTGS1, forming hydrophobic bonds with amino acid residues ALA-527, ILE-523, LEU-352, LEU-384, TYR-385 and TRP-387 (Figure 68 E). 2-HAP (**2**) interacted with CCNA2 forming hydrophobic bonds with the amino acid residues VAL-64, PHE-80, ASP-145, LEU-134 and VAL-18 and formed H-bonds with LYS-33 and GLU-51 (Figure 68 F). 2-HAP (**2**) interacted with amino acid residues of MAPK14 forming hydrophobic bonds with LEU-291, PRO-242, ILE-259, PRO-191 and GLU-192 in the aromatic portion and with formed hydrophobic

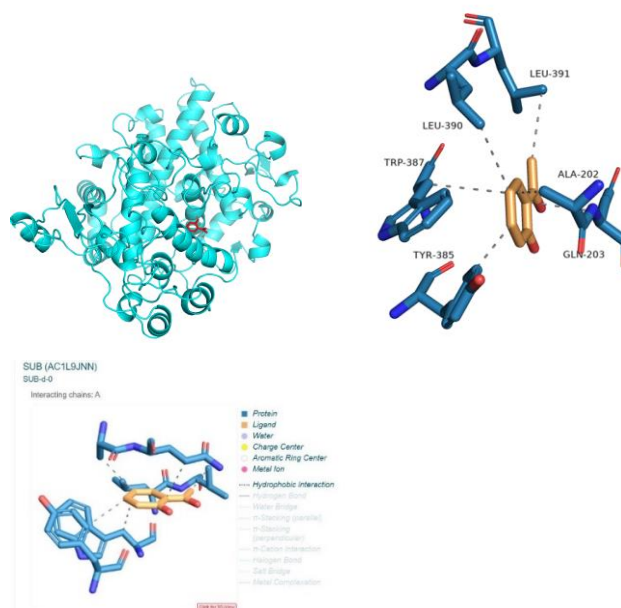
bonds through its methyl group with TRP-197 and ILE-250v (Figure 68 G). 2-HAP (**2**) interacted with ACE-2 forming H-bonds with

Table 31: 2-HAP (**2**) and protein target binding affinities (kcal/mol) and binding residues

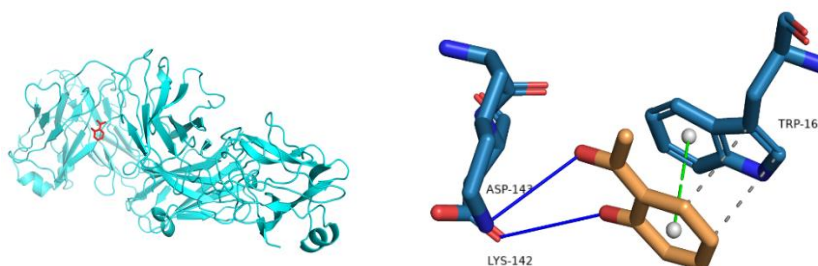
Ingredient-Target	Binding Affinity	Binding residues			Interaction energy kJ/mol
		H-bonds	Hydrophobic interaction	π -stacking	
PTGS2 - 2	-7.0	N/A	LEU 390, LEU 391, TRP-387, TYR-385, ALA-202 and GLN-203	N/A	-103.557
IL18 - 2	-6.7	ASP-143, LYS-142	TRP-163	TRP-163	72.6358
NOS3 - 2	-6.9	GLN-464, THR-82, GLN-437	LEU-433, VAL-467, VAL-73	N/A	-81.5752
ALB - 2	-6.8	SER-448	LEU-406, LEU-429, PHE-402, VAL-387	N/A	97.2789
PTGS1 - 2	-6.6	N/A	ALA-527, ILE-523, LEU-352, LEU-384, TYR-385, TRP-387	N/A	-90.1046
CCNA2 - 2	-6.6	LYS-33 and GLU-51	VAL-64, PHE-80, ASP-145, LEU-134, VAL-18	N/A	-133.49
MAPK14 - 2	-5.3	LEU-291, PRO-242, ILE-259, PRO-191, GLU-192, TRP-197, ILE-250	N/A	N/A	-88.5498
ACE2 - 2	-5.9	ASP-192, THR-155	N/A	N/A	
CASP3 - 2	-5.7	ASP-253	TRP-206, VAL-4, PHE-256	N/A	
STAT1 - 2	-5.5	ILE-136, LYS-135, SER-62	VAL-186, GLU-187, GLU-132, LYS-151	N/A	
INS - 2	-5.3	LYS-146	THR-80, TYR-84, TYR-123, TYR-116, LEU-81, TRP-147, THR-143	N/A	
2- IL2	-4.8	Docking not done	N/A	N/A	

ASP-192, THR-155. 2-HAP (**2**) interacted with CAPS3 forming H-bond with ASP-253 and hydrophobic bonds with TRP-206, VAL-4, PHE-256. 2-HAP (**2**) interacted with STAT1 and formed H-bonds with ILE-136, LYS-135, SER-62 and hydrophobic bonds with VAL-186, GLU-187, GLU-132, LYS-151. 2-HAP (**2**) interacted with INS, forming a H-bond with LYS-146 and hydrophobic bonds with THR-80, TYR- 84, TYR-123, TYR-116, LEU-81, TRP-147, THR-143.

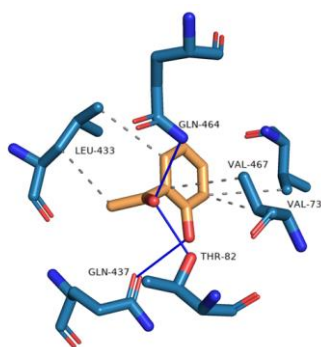
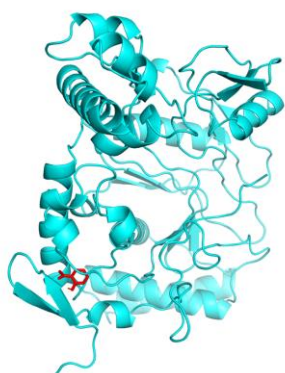
A: PTGS2-2HAP (-7.0 kcal/mol)



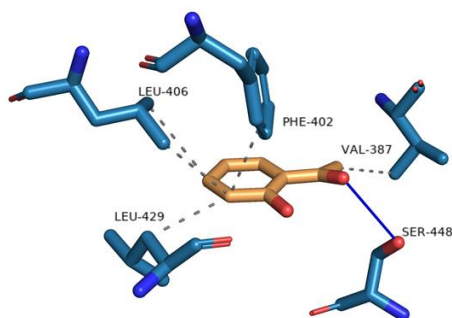
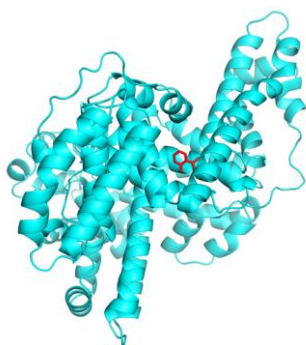
B: IL18-2HAP (-6.7 kcal/mol)



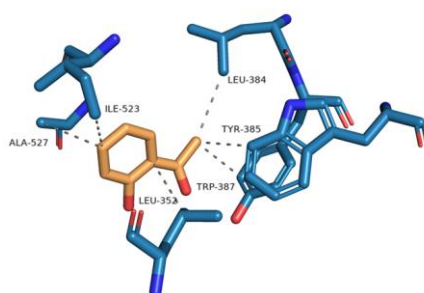
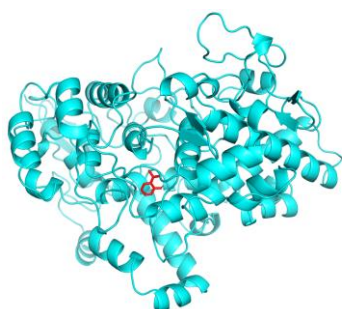
C: NOS3-2HAP (-6.9 kcal/mol)



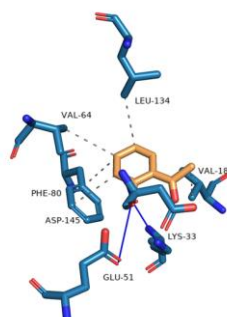
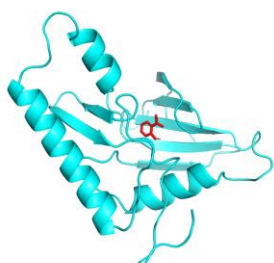
D: ALB-2HAP (-6.8 kcal/mol)



E: PTGS1-2HAP (-6.6 kcal/mol)



F: CCNA2-2HAP (-6.6 kcal/mol)



G: MAPK14-2HAP (-5.3 kcal/mol)

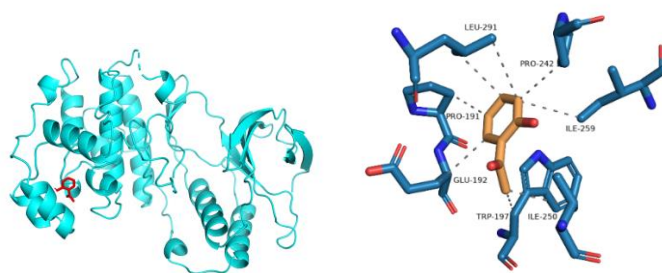


Figure 68 (A-G): Molecular docking of protein targets and **2** in pneumonitis

5.3.2.2 Molecular Dynamics simulation

In using molecular dynamics simulation to explore the binding stability of the protein-**2** docking complexes, the interaction energy and RMSD were calculated after 100ns (Figure 69, Supplementary file 20). The results showed that the protein and small-molecule RMSD in the PTGS2-**2** -complex, IL18-**2**, NOS3-**2**, ALB-**2**, PTGS1-**2**, CNA2-**2** and MAPK-14 – **2** were stable during simulation and that the average interaction energies were -103.557 kJ/mol, -72.6358 kJ/mol, -81.5752 kJ/mol, -97.2789 kJ/mol, -90.1046 kJ/mol, -133.49 kJ/mol and -88.5498 kJ/mol respectively (Figure 69 A-U). ACE2-**2**, CASP3-**2**, STAT1-**2**, INS-**2** and IL2-**2** did not form stable complexes and were not investigated further. 2-HAP (**2**) was reported to have tested negative for activity with CASP3 [179] and the molecular dynamics results validates this negative result.

The binding complexes of PTGS2-**2** and ALB-**2** were the most stable. There was slight instability in the interaction energy of PTGS2-**2** at 20 ns which corresponded with fluctuations in the RMSD of **2** in the docking complex at that time (Figure 69 A-C). The interaction energy of ALB-**2** stabilised at 20 ns (avg. -97.2789 kJ/mol), with instability from 0-20 ns corresponding with fluctuations in RMSD of **2** in the docking pocket but it was stable thereafter (Figure 69 J-L). The NOS3-**2** complex was stable with average interaction energy of -81.5752 kJ/mol over 100ns. It decreased to -150 kJ/mol at 20 and 80 ns corresponding with fluctuations in RMSD of NOS3 indicating temporary instability of the complex while at 10 ns, there is a brief increase in RMSD of **2** in the docking pocket with a corresponding change in interaction energy before it stabilises again (Figure 69 G-I). The interaction energy of IL18-**2** is relatively stable with a brief increase to zero kJ/mol at 20 ns before stabilising again around -70 kJ/mol (avg. -72.6358 kJ/mol). This increase at 20 ns corresponds with brief instability of **2** in the IL18 docking pocket where the RMSD rises from 0 to 12 nm before stabilising again around 0 nm until it increases sharply again around 80 nm. A similar instability is seen in the RMSD of IL18, lasting from 0 to 30ns and 70 to 100ns, though this is not reflected in the interaction energy. This shows that IL18 is predicted to have some instability in combination with **2** (Figure 69 D-F). *In vitro* experiments are needed to verify whether or not this instability features in the interaction of **2**

with IL18. The interaction energy of the PTGS1-**2** complex was relatively stable with an average of -90.1046 kJ/mol but increased to about -80 kJ/mol at 60 ns and reached equilibrium after that. 2-HAP (**2**) and PTGS1 showed modest instability in the first 10-20 ns of the docking, where both molecules undergo conformational change before quickly gaining equilibrium. The change in interaction energy around 60ns corresponds with instability of **2** in the docking pocket at 65 ns, with RMSD increasing from 0.4 nm to 1 nm before it reached a stable state again around 0.4 nm (Figure 69 M-O). The average interaction energy of CCNA2-**2** complex was the lowest at -133.49 kJ/mol but had more variability throughout, with instability of both CCNA2 and **2** reflected in significant RMSD variation throughout the 100 ns (Figure 69 P-R). The interaction energy of the MAPK14-**2** complex was relatively stable with an average of -88.5498 kJ/mol but decreased at 75ns and remained stable at the lower level from then to 100 ns. The RMSD of **2** in the MAPK14-**2** complex was relatively stable around 0.3 nm but increased to 0.5 nm after 75 ns and stabilised at the higher level, corresponding with the change in interaction energy (Figure 69 S-U).

In conclusion, we obtained the structures of target proteins - **2** docking complexes through computer simulation, and the molecular dynamics simulation results showed that six of the docking complexes were relatively stable including PTGS2-**2**, NOS3-**2**, MAPK14-**2** and ALB-**2** complexes being the most stable.

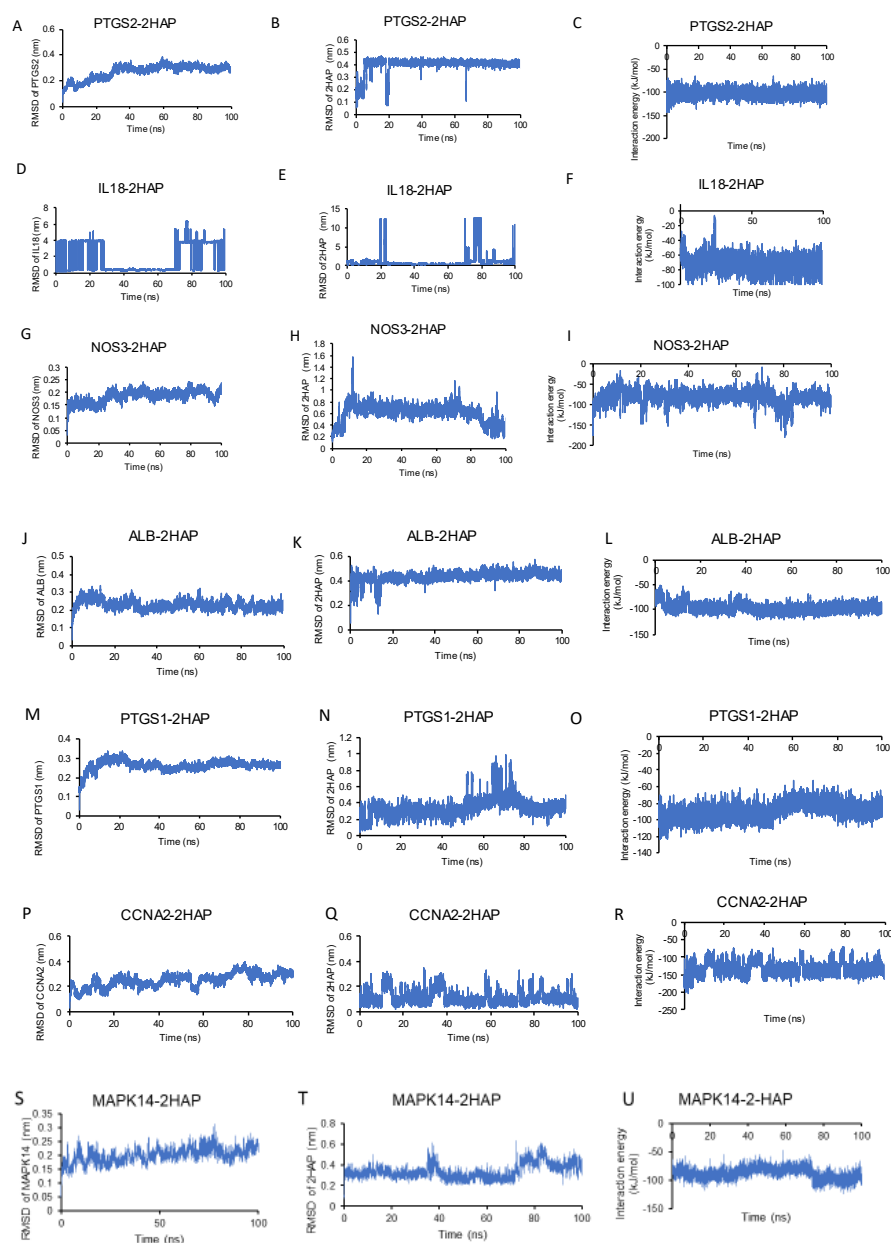


Figure 69: (A-U) Molecular dynamics simulations of protein - 2 docking complexes with RMSD and interaction energies during 100 ns simulations

5.3.3 Preliminary screening of 2-HAP for biological activity in murine iBMDM cells

Due to time and resource constraints, we have carried out a preliminary evaluation of the anti-inflammatory effects of **2** in murine iBMDM cells. These cells were used initially because the cells were robust, easy to maintain, express most of the innate immune pattern recognition receptors (PRRs) including Toll-like Receptor (TLR) 4 and can produce high levels of cytokines upon stimulation. Due to the fact that the PRR pathways are highly conserved in the mouse and human systems, the inhibition in the murine iBMDMs is highly predictive of inhibition in the human system including in THP1 macrophages.

The resazurin-based viability assay was employed to investigate the cytotoxicity of the **2** on iBMDM cells, with and without LPS. A dose response curve was used to estimate the subtoxic concentrations (IC_{10}) of the extract at six different levels: 0, 10, 30, 50, 75, and 100 μ M. No cytotoxicity was observed for **2** at the highest tested concentration (100 μ M) (Figure 70).

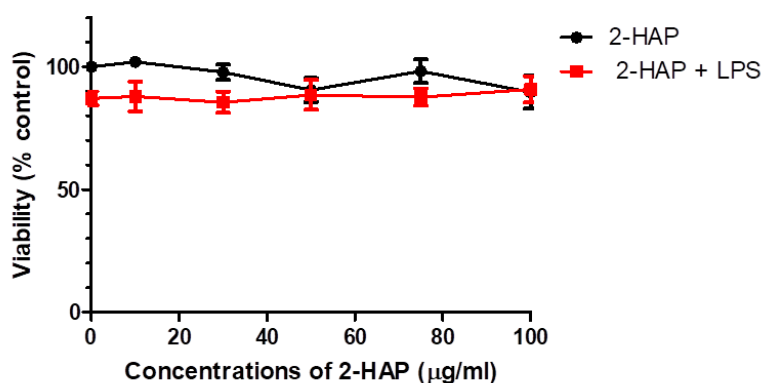


Figure 70: Effect of **2** on iBMDM cell viability with/without LPS

The ELISA technique was used to test the anti-inflammatory potential of **2** at 100 μ M against LPS-treated iBMDM cells. To this end, four different cytokines were investigated: beta interferon ($IFN\beta$), interleukin- 6 ($IL6$), CCL5/RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted) and $TNF\alpha$. Figure 71 illustrates the signalling cascade from LPS activation to the transcription of these cytokines. The results of these investigations are presented in Figure 72. Pre-treatment of iBMDM cells with **2** significantly inhibited LPS-induced $IFN\beta$ and $IL6$ ($p < 0.05$, $n = 3$). 2- HAP (**2**) also showed a clear but non-significant inhibition of RANTES/CCL5 and $TNF\alpha$ (Figure 72) (Supplementary file 21).

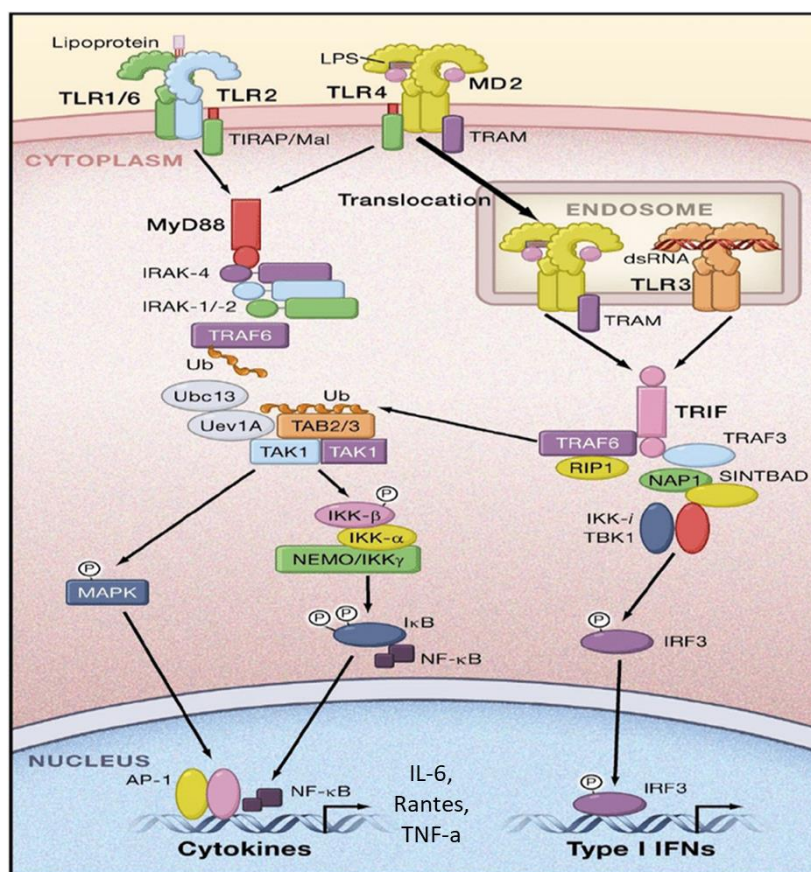


Figure 71: Pattern recognition receptors activated by LPS stimulate signalling cascade leading to innate and adaptive immune responses (adapted from [645])

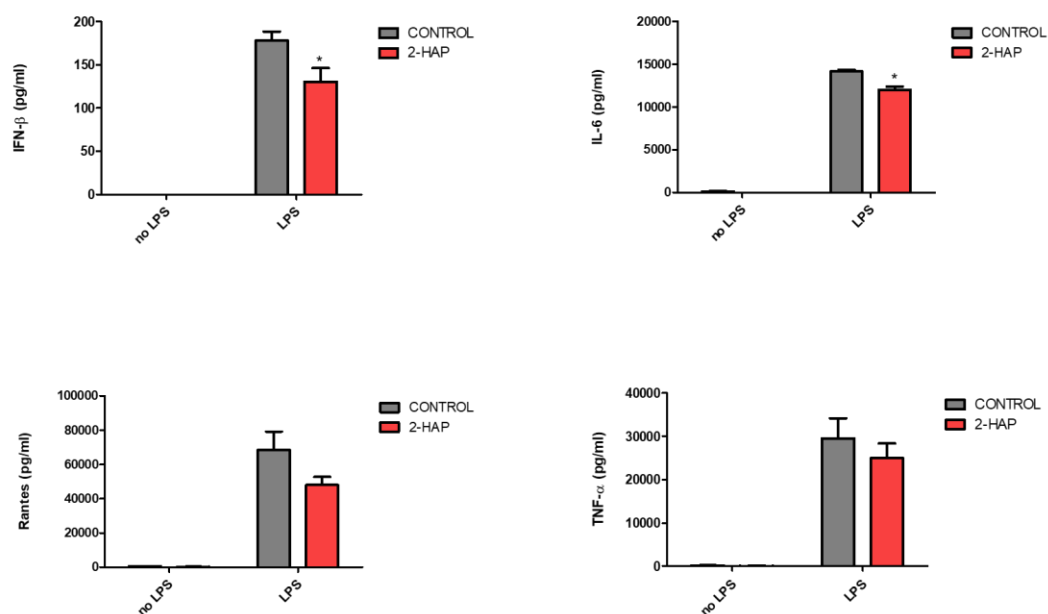


Figure 72: Effect of 2 on IFNβ, IL6, RANTES/CCL5 and TNFα. * means significance at $p < 0.05$

5.4 Discussion

5.4.1 *C. spinarum* – TM and relationship to Metascape predictions

The traditional use of *C. spinarum* root by direct and steam inhalation and by the application of untreated or warmed root as described in 4.2.2 indicates potential bioactivity of volatile components of the root. The results of this NP investigation have the potential to support the traditional usage of the inhaled and applied root and may suggest further potential uses of this simple phenolic compound.

Taken overall, the disease targets emerging from this NP analysis which best overlap with traditional therapeutic uses are respiratory and CNS disorders. Pneumonitis was the disease target with the best logP value, while CNS related disorders were also identified though with a poorer logP value. Cancers, identified by Metascape as disease targets of 2, are not reported as a traditional use of *C. spinarum* root by inhalation or application, though this NP analysis may point towards disease targets beyond traditional usage. Pneumonitis was selected as the best candidate for a case study, using NP methods to identify possible mechanisms of action of *C. spinarum* root volatile in the traditional treatment of respiratory disease.

5.4.2 Pneumonitis and airway inflammatory processes

Lung inflammation in the form of Pneumonitis emerged from the Metascape analysis as a target disease of 2. It refers medically to non-infectious lung inflammation while pneumonia refers to an infectious Pneumonitis [631, 646]. DisGeNet, used by Metascape for disease target identification, additionally uses the definition that Pneumonitis is “a lung disease that involves lung parenchyma or alveolar inflammation and abnormal alveolar filling with fluid (consolidation and exudation). It results from a variety of causes including infection with bacteria, viruses, fungi or parasites, and chemical or physical injury to the lungs”, and thus is wider than other definitions [631]. There are many potential causes for lung inflammation including infection (e.g., Influenza A, COVID 19, *Streptococcus pneumoniae*, *Haemophilus influenzae*), hypersensitivity to inhaled antigens such as smoke, moulds, bacteria, feathers, bird faeces, dust [647]; aspiration of gastric contents [648]; toxic substances including drugs, radiation therapy; inflammatory disorders and systemic diseases [646, 649]. Many of these causes of inflammation and symptoms experienced are potentially present in the context of traditional medicine usage. The symptoms of Pneumonitis include shortness of breath, difficulty breathing and cough with related symptoms of fatigue, reduced appetite and weight loss. The processes of airway inflammation, reviewed by Aghasafari *et al.* [650], are varied depending

on aetiology and the allergic inflammatory process of hypersensitivity Pneumonitis is qualitatively different that of asthma [651].

The process of airway inflammation is complex with the basic inflammatory process structure of an inducer/stimulus (e.g., pathogen) acting on a detecting sensor cell (e.g., epithelial cell) which then produces mediators (e.g., cytokines) to cause effector cells (e.g., macrophages) to eliminate the inducer/stimulus through a variety of mechanisms (e.g., phagocytosis, lysis). In the first step, the inflammatory stimulus - pathogen/antigen/toxin - is recognised by Pattern Recognition Receptors (PRRs) on immune cells such as dendritic cells, macrophages, mast cells and epithelial cells, activating the NF κ B signalling pathway, mainly via the IKK complex within these cells. The NF κ B signalling pathway is central to the immune response to infection and other foreign stimuli although its dysregulation can lead to inflammatory disorders such as COPD, cancers and autoimmune diseases [652]. NF κ B is a transcription factor which, when activated, moves from the cytoplasm to the cell nucleus where it induces many factors including cytokines (such as TNF α , IFN β , IL1 β , IL2, IL18), chemokines (such as IL8, RANTES), adhesion molecules and growth factors. It is involved in the transcription of other inflammatory proteins such as PTGS2/COX2, the differentiation of T-cells, the proliferation of macrophages with further production of cytokines and chemokines, the recruitment of neutrophils and the regulation of cell cycle and apoptosis e.g., through cyclins and caspases [652, 653]. Alveolar macrophages, on recognising the stimulus/pathogen, rapidly phagocytose debris and invading pathogens, dissolving or eliminating them upwards through the mucociliary escalator. In addition, the macrophages produce inflammatory cytokines such as TNF α in response to pathogens which further activates the NF κ B pathway and other immune cells and processes [653]. The alveolar macrophages also indirectly attack pathogens by promoting T-cell differentiation, further promoting inflammation and elimination of the stimulus. Cytokines lead to further activation of effector cells such as macrophages, mast cells and neutrophils and the recruitment of other cells which act directly against the inflammatory stimulus. This is facilitated by the release of chemokines which support the movement of inflammatory cells to the site of infection or attack such as: histamine which causes vasodilatation and smooth muscle contraction; heparin which causes swelling and inflammation; and bradykinin which causes an increase in vasodilatation, vascular permeability and pain.

Inflammatory mediators also travel to non-effector organs to indirectly support the elimination of the antigen/pathogen. This could include: signalling the brain to increase body temperature to aid elimination of a pathogen; signalling the liver to produce opsonins (e.g., C-reactive protein) which then opsonise pathogens, marking them for more efficient phagocytosis by

macrophages [654]; and signalling blood vessels to increase vascular permeability to facilitate the extravasation that allows the inflammatory molecules to reach the site of infection/attack [655].

5.4.3 Known activities of 2-HAP (2) and related molecules

To the best of our knowledge, there are no studies on the direct anti-inflammatory effects of **2**. However, a glycoside of **2**, acetophenone-2-*O*- β -xylopyranosyl-(1 $\rightarrow$ 6)-*O*- β -glucopyranoside (**43**) (Figure 73), isolated from the MeOH extract of *C. spinarum* rootbark was tested *in vitro* for PTGS2/COX2 inhibitory activity [487] and was found to have moderate activity (IC_{50} 0.6 μ M) which was lower than for the control, indomethacin (IC_{50} 1.1 μ M). Compound **43** was found to be inactive as an antioxidant in DPPH and Ferric Reducing Antioxidant Power Assay (FRAP) assays, the authors attributing this to the loss of the phenolic group [487].

In an *in vitro* study of XO inhibition described in 5.4.2, Saeed and Ahmed [464] demonstrated that **2** had inhibitory activity (IC_{50} of 11.66 ± 0.27 μ g/mL), comparing well to the XO inhibitor allopurinol (control) (IC_{50} of 2.74 μ g/mL). XO is generally investigated in relation to the treatment of raised uric acid levels in gout. In addition, XO is known to promote the production of ROS, which are pro-inflammatory [656], and are present in respiratory epithelial cells. Uric acid can be pro-inflammatory in the lungs via promoting inflammasome, NF- κ B activation and increasing pro-inflammatory cytokines. XO is implicated in influenza and in Acute Respiratory Distress Syndrome (ARDS) [657]; with allopurinol reducing the expression of proinflammatory cytokines in lung epithelial cells infected with respiratory syncytial virus (RSV) [657]. Additionally, in patients with severe COVID-19, allopurinol was shown to reduce viral inflammatory reactions, oxidative stress, inflammatory biomarkers and acute lung injury and improved oxygen levels [658].

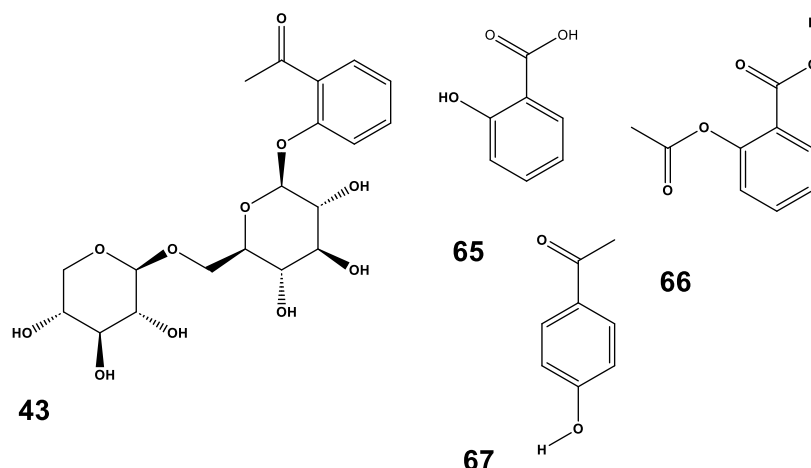


Figure 73: Some compounds related in structure to **2**

Some simple phenolic compounds have been tested for anti-inflammatory activity and may provide an indicator of similar activity in **2**. Salicylic acid (**65**) (Figure 73) is structurally similar to **2** whereby the methyl group of **2** is substituted by an - OH group in **65**, transforming it to an acid. Salicylic acid (**65**) is an active metabolite of acetylsalicylic acid (aspirin) (**66**) (Figure 73) in the human body and is responsible for some of its activity [659]. Choi *et al.* [659] demonstrated that human high mobility group box 1 (HMGB1) is a binding protein for **65** which then inhibits the HMGB1-induced increase in pro-inflammatory cytokines (IL6 and TNF α) and PTGS2/COX2 and chemoattractant activity. Salicylic acid (**65**) was shown to inhibit the activation of NF κ B, thus preventing the expression of several pro-inflammatory cytokines [660] and significantly reduced the IL1 β -induced expression of the inflammatory marker, C-reactive protein (CRP) in endothelial cells from human umbilical veins (HUVEC) [661]. It was found to inhibit the release of inflammatory mediators, histamine, IL-1 β and CCL3 from mouse peritoneal mast cells via blocking the purinergic receptor, P2X7R, in these cells [662]. On the other hand, in an *in vitro* study in human lung cells subjected to carbachol-induced bronchoconstriction, **65** had little effect on inflammatory mediators though it did potentiate the induced bronchoconstriction through a different mechanism [663]. Salicylate (**65**), like **2**, has been reported to be a XO inhibitor and was included as a standard in a search for XO inhibitors to treat oxidative stress in COPD [664, 665]. Aspirin (**66**) is a well-known inhibitor of COX1 and 2 [179]. In a systematic review of **66** in ARDS, Liang *et al.* [666] reported a reduced expression of inflammatory factors such as TNF α and IL1 in lung macrophages and reduced neutrophil activation, with the proviso that these results are not corroborated by other studies. Kwack *et al.* [667] found reduced activation of NF κ B and reduced expression of TNF α in BALF in C57BL/6 mice treated with **66** and omega-3 fatty acids in a lung injury model. 4'-hydroxyacetophenone (p-HAP) (**67**) (Figure 73) is a structural isomer

of **2** although the para orientation of the phenolic group would lead to different conformations in protein active sites and different H-bonding patterns. A study, investigating molecular mechanisms of action of **67** in carrageenan-induced paw oedema in ICR mice, found several changes observed in homogenised paw tissue including reduction in inflammatory cytokines such as TNF α and IL6, and reduced NF κ B, COX2 and NO levels. The authors also measured antioxidant activity of **67** in the liver. Levels of malondialdehyde (MDA), a marker of oxidative stress, were found to be reduced and the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase were increased, each effect indicating a contribution to an anti-inflammatory and protective effect of **67** [668] and supports the finding of COX2 inhibitory activity in **43** [487]. Table 32 summarises the known activities of **65**, **66** and **67** against the predicted targets and other inflammatory mediators [179]. Other phenolic compounds studied have shown anti-inflammatory activity which, though somewhat different to **2** in structure, may indicate comparable activities in **2**. In cell studies of inflammation, *p*-coumaric acid was found to inhibit the expression of iNOS, PTGS2/COX2, IL1 β and TNF α in LPS-stimulated RAW264.7 macrophage cells and was thought to act by blocking the MAPK and NF κ B signalling pathways [669]. Caffeic acid and its derivatives inhibited the production of iNOS in LPS-stimulated RAW 264.7 macrophages [670] and inhibited the release of a number of pro-inflammatory cytokines *in vitro* [179]. Some targets identified here show good potential for further *in vitro* and *in vivo* investigation of the activity of **2** in the inflammatory process. These include the PTGS2/COX2 and PTGS1/COX1 given that **65**, **66** and **67** inhibit PTGS2/COX2 and **65** and **66** inhibit PTGS1/COX1. 2-HAP (**2**) may have a role in NO regulation through the iNOS and NOS3 given that **65** and **66** inhibit iNOS and *p*-HAP reduces NO levels. The interleukins, IL18, IL1 β and IL6 are worth investigating further in relation to **2**: IL18 due to the predicted stability and strong interaction energy of the docking complex *in silico*; IL1 β is inhibited by **65**, **66** and **67**; IL6 is inhibited by **66** and **67**. These activities indicate that **2** may also be an inhibitor of these inflammatory mediators.

Table 32: Comparative activities of 2, p-HAP, salicylic acid and aspirin using data derived from PubChem¹⁹

Targets	2-HAP (2)	p-HAP (67)	Salicylic acid (65)	Aspirin (66)
PTGS2/COX2 inhibition	<i>In silico</i> (v. stable) (13 is inhibitor)	✓	✓ Weak [671]	✓
PTGS1/COX1 inhibition	<i>In silico</i> (stable)	-	✓ Weak [671]	✓
MAPK14	<i>In silico</i> (v. stable)	-	-ve	-ve
CCNA2	<i>In silico</i> (stable)	-	-	-ve
IL18	<i>In silico</i> (stable)	-	-	-
NOS3	<i>In silico</i> (v. stable)	-	-	-
iNOS inhibition or NO level change	-	NO reduced (weak)[672]	iNOS ✓	iNOS ✓
ALB	<i>In silico</i> (v. stable)	-	-	-
NFκB1 inhibition	-ve	✓	✓ [660]	✓
TNFα inhibition	-	✓	✓	✓
IL2 inhibition	<i>In silico</i> (unstable)	-	-	✓
IL1β inhibition	-	✓[673]	✓ [661]	✓
IL6 inhibition	-	✓	-ve [663]	✓
IL8 inhibition	-	-	✓[673] -ve [663]	✓
IFNβ inhibition	Inhibits (cell assay)	-	-	-ve
IFNγ	-	-	-ve	-
CASP3	<i>In silico</i> (unstable) -ve	-	-ve	-ve
TGFB1 inhibition	-ve	-	-ve	-ve
XO inhibition	✓	-	✓	-

¹⁹ - = not reported; ✓ = active; -ve = inactive from PubChem data [179] unless other source stated

5.4.4 Network pharmacology predicted 2-HAP (2) target genes in pulmonary inflammatory processes

The combination of NP, molecular docking and molecular dynamics with literature mining culminated in the prediction that PTGS2/COX2, NOS3, ALB, MAPK14/p38 α , IL18, PTGS1/ COX1 and CCNA2 form stable docking complexes with **2**, the first four predicted to form the most stable complexes.

The PTGS2/COX2 enzyme, induced by NF κ B and compounds including mitogens and endotoxins [674], is responsible for the production of prostaglandins. Prostaglandins generally have a significant pro-inflammatory role in the body including in the respiratory system [675]. In contrast, PGE2, a product of PTGS2/COX2, has both pro- and anti-inflammatory effects in the lung [676]. As an anti-inflammatory, PGE2 was shown to be protective against bronchoconstriction in murine models [677], reduced group 2 innate lymphoid cells (ILC2) activation [678] and was found to be reduced in patients with pulmonary fibrosis due to reduced capacity to upregulate PTGS2/COX2 [679] supporting a counter-inflammatory role for PGE2 via PTGS2/COX2 relative to other prostaglandins. It is not known whether **2** has PTGS2/COX2 activity although its glycoside and similar compounds (**14**, **15** and **16**) do inhibit PTGS2/COX2 as described above and may indicate the same for **2**. Nitric oxide (NO) is a regulator of homeostasis in the lungs and is produced by inducible Nitric Oxide Synthase (iNOS/NOS2). Its production is altered under inflammatory conditions such that NO levels increase through inflammatory mediators such as TNF α , IL1 β and IFN γ via NF κ B and Interferon regulatory pathways, leading to further pulmonary inflammation and contributes to poor outcomes in respiratory viral infections [680, 681]. However, iNOS is also shown to modify respiratory inflammation via NF κ B inhibition [682]. NOS3, which is predicted to be acted upon by **2**, is mostly found in the vascular rather than pulmonary smooth muscle. However, a study of airway inflammation in mice, found that NOS3 was essential for iNOS expression [683]. Thus **2** may have an impact on NOS2 modulation of airway inflammatory processes through NOS3. In addition, **14** and **15** have iNOS inhibitory activity and this may indicate similar activity for **2** (Table 32). Albumin (ALB) is the most abundant human protein. Its primary role is in the maintenance of plasma oncotic pressure although it has been shown to be involved in inflammatory processes. Studies of pulmonary epithelial cells demonstrated that ALB increases intracellular glutathione, regulates the activation of NF κ B and inhibits some TNF α pathways indicating a role in reducing inflammatory and oxidative stress damage. Limited clinical studies have also shown the benefit of ALB in treating infection [684, 685]. As such, **2** may have an immunomodulatory role via ALB which merits further

investigation. MAPK14/p38 α is an essential component of the MAPK14 signalling pathway. This is activated by LPS, cytokines, and other stressors and is thought to be involved in allergic airways inflammation [674]. It is activated by cell surface PRRs to further promote NF κ B-mediated expression of TNF α and to increase the inflammatory response [686]. PTGS2/COX2 expression and concomitant PGE2 synthesis is reported to be induced by MAPK14/p38 α in airway smooth muscle cells [674]. It is considered to be a potential drug target in several inflammatory diseases including COPD and COVID-19 [687, 688]. The NF κ B signalling pathway activates caspase 1 which stimulates the conversion of pro-IL18 to the active IL18, a pro-inflammatory cytokine involved in the response to pathogens. IL18 has been shown to be involved in host defence through innate and adaptive immunity pathways, acting on natural killer cells, natural killer T cells, Th1 cells and mast cells, acting in conjunction with other interleukins including IL2, IL3 and IL12 for its effect [689]. In pulmonary disease, it is implicated in tuberculosis [690] and was shown to induce airway inflammation, airway hyperresponsiveness and to be implicated in “difficult to treat” bronchial asthma in mice [689]. CCNA2 is a cycle regulator, cell cycle being important during both inflammatory and homeostatic processes. Though it is unclear what impact **2** activity on CCNA2 would have in relation to pulmonary disease, CCNA2 has been shown to be activated in pulmonary fibrosis [691, 692] and was found to be upregulated in GSE20346 [634], supporting a role in pulmonary inflammation.

These intersecting inflammatory processes are aimed at eliminating the antigen/pathogen, centring on the production of inflammatory cytokines through multiple cell types and feedback loops which overlap with the predicted activity of **2**. The targets MAPK14, PTGS2/COX2 and IL18 in particular, each intersect with the key NF κ B signalling pathway which is pivotal in innate and adaptive immune processes. To date, there has been no experimental data showing *in vitro* or *in vivo* activity of **2** against any of these targets.

5.4.5 Preliminary screening of 2-HAP (**2**) for bioactivity

The preliminary investigation of biological activity in murine iBMDM cells did not address the predicted targets of the NP analysis. However, the study did demonstrate inhibitory activity of **2** against all four cytokines: IFN β , IL6, RANTES/CCL5 and TNF α with inhibition of IFN β and IL6 being statistically significant. This supports a role for **2** and *C. spinarum* root in modulating inflammatory activity in response to infection and tissue damage.

The type I interferons, such as IFN β , play an integral role in the immune response to viral and other infections of the lung and provoke significant pulmonary inflammation in response to infection, aiding recovery [693]. However, dysregulation of IFN β expression is associated with impaired gaseous exchange

in the lungs and may prevent lung repair during the resolution phase [693, 694]. IL6 is involved in the immune response and inflammation. The IL6 signalling pathway regulates the balance of immuno-protection and immunopathology during infection and serves as a pyrogenic cytokine to regulate thermoregulation in response to tissue damage by viruses such as Influenza A. It may aggravate the pathogenesis of viral infections including Sars-COV-2 infection by increasing the expression of angiotensin-I and II receptors and creating redox imbalance in the system such that raised IL6 levels were shown to be a good predictor of severe COVID 19 [695, 696]. IL6 is also implicated in chronic inflammatory disorders [697].

The promising activity of **2** in inhibiting IFN β in particular is significant in constructing a potential mode of action of **2** in respiratory inflammatory disorders and in other pathologies.

5.4.6 Potential for toxicity of 2-HAP (**2**)

The reported assay in murine iBMDM cells for **2** to be non-toxic at 100 μ M. In addition, no specific data has been identified in the literature reporting significant toxicity of **2** with LD₅₀ in mice of 100mg/kg i.p. PubChem data states that it is a dermatotoxin causing skin burns although it is also reported to be used as a flavouring agent with no safety concerns at normal levels of intake [179]. However, the prediction of potential toxicity of **2** in FAFDrugs4 and Discovery Studio analyses needs to be investigated further, the latter having predicted hepatotoxicity. Appropriate cell models e.g., using liver cells, could be used to examine whether the ADMET predictions hold true in practice. In heated aqueous preparations, cyclisation or dimerisation may occur with further implications for knock-on effects on bioactivity in addition to other reactions with other metabolites present. In burned preparations such as for evil eye, **2** along with the other metabolites, would be subject to pyrolysis. It has been reported that **14** completely degrades first to phenol at 150-200°C and then further decomposes to 0% at higher temperatures [698], a process that could be replicated for **2**, although this would need to be verified by experimentation.

5.5 Future research – 2-HAP (**2**) and *C. spinarum*

There are several potential avenues for future research on **2**. Combining the results of NP with the known activities of related compounds provides a template for the potential additional targets to investigate including PTGS2/COX2, PGTS1/COX1, NOS3, iNOS, TNF α , IL1 β and IL8. Study in human cells such as THP1 cells would be a good initial model for investigation of **2** activity against TNF α , IL1 β and IL6 identified here and would additionally allow testing for IFN γ activity. Further study on activity against IFN γ would be interesting as it would

complement IFN β activity through its immunomodulatory functions [693]. Such studies could validate the traditional use of volatile preparations of CS root in respiratory disorders. Further investigation of the XO inhibitory activity demonstrated *in vitro*, and mirrored in salicylic acid (**14**), would be worthwhile given the wider application of XO inhibition in the present day beyond traditional indications for gout.

Pneumonitis was selected for detailed exploration with NP. However, CNS disorders could similarly have been selected due to its relationship with traditional use and may have an inflammatory component, linking well with the current analysis on pneumonitis. Such disorders bear a good correspondence to traditional usage of the volatile root given that it is used to treat evil spirits and evil eye, mental disorder, brain tension and stress (section 5.3.1.2). Other diseases identified through Metascape analysis that could be explored further are periodontal disease, periodontitis and hyperalgesia which correspond with the treatment of toothache and adenitis with warmed root. CNS, pain relieving and anti-inflammatory effects of **2** could be explored in a further detailed NP analysis based on these other traditional uses of the volatilised root.

2-HAP (**2**) is moderately soluble in water and is identified as an orally bioavailable molecule through FAF-Drugs4 screening. As such, it is possible that it is one of the components of traditional whole root formulations and water-based formulations of CS root and may contribute to overall bioactivity of these formulations, although only one study analysed the aqueous decoction and 2-HAP (**2**) was not identified. A wider NP analysis involving more of the known compounds of the metabolome, identified in Chapter 4, could be a productive study, used in combination with knowledge of traditional use as a basis for the NP analysis. The combined targets of these compounds could be analysed to identify the key target diseases of the combination, construct a PPI network, enrichment analyses and thus propose a mechanism of action for the whole plant formulations in traditional uses of *C. spinarum* root.

5.6 Potential and limitations of this NP analysis of 2-HAP and of network pharmacology more broadly

NP analysis provides a valuable tool which is time and cost-effective in screening for bioactivity before progressing to *in vitro* and *in vivo* studies. Prior to the availability of *in silico* techniques, the investigation of anti-inflammatory activity for instance, would have required mouse models of inflammation to test for activity and to determine pathways of action. In the present day, zebrafish models are more commonly used for phenotypic screening which bypasses some of the cost and ethical issues of mammalian models [699]. However, *in*

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silico studies provide a valuable, time and resource saving guide for pharmacological studies.

NP generally utilises a multi-drug-multi-target approach to its analyses given that traditional herbal formulations utilise plant extracts from one or several species. This analysis is unusual in NP in that it focuses on a single metabolite of *C. spinarum* root, **2**, as the literature has found it to be the near sole constituent of the root volatile oil. This information was combined with ethnobotanical studies which indicated that the volatilised form of the root is used in the treatment of some diseases including those of the respiratory tract. However, the headspace-GC/MS analysis conducted for this study found limonene (**3**) to be present in almost equal proportions in the root sample examined, although this sample was very small and not replicated due to lack of available material. This points to the potential for variability and the fact that, in ethnomedical practice, constituents other than **2** may have a role in the therapeutic effect of inhaled or absorbed *C. spinarum* root. It would have been useful to have included **3** in the NP and cell biology studies for a more complete representation of volatilised root preparations.

The small size of **2**, means that it is less suitable for NP analysis as there is often no clear cavity in the protein [700]. In addition, the binding affinities that were found were in the moderate to strong range with none having a very low (very strong) binding affinity.

In addition, time limitation has meant that this study does not illustrate the wider possibilities of NP in investigating complex herbal formulae a major strength of NP.

A key limitation of this study of **2** is the lack of available experimental data which would support the NP predictions, either in the literature or through laboratory experiments. It is not known whether **2**, in its proposed association with the disease targets in Metascape, is upregulating or downregulating these targets and thus whether it is protective or damaging in the affected processes. Time restrictions and limited access to viable cell lines limited this study to one very preliminary murine cell-based assay testing inflammatory mediators that were different to those predicted in the NP analysis. The finding that CASP3 was unstable in molecular dynamics simulations accords with the PubChem report that **2** was inactive against CASP3 [179]. This supports the molecular docking and dynamics simulation process for the other targets and helps to validate the study predictions.

The primary support for the NP predictions is in the traditional usage itself so that the NP can speak to the ethnopharmacology. However, the disease targets identified in DisGeNet and other databases are biomedically based, are usually very specific and work best for specific diseases e.g., Alzheimer's or

Diabetes mellitus. TM, like most primary care, is not normally this specific in its disease targets as such precision requires access to laboratory-based diagnostic techniques. Also, the nature of ethnobotanical research usually does not include clinical personnel who might be able to give a biomedical diagnosis. Another factor is that TM uses a holistic perspective of illness and disease compared to biomedical care and such as those from India and China, which date back over 5,000 years. Pneumonitis, as identified in this analysis, is biomedically understood as non-infectious lung inflammation. However, in the setting of TM usage, a significant proportion of lung inflammation is likely to be infectious in origin. Nonetheless, the inflammatory processes are overlapping to some degree, whatever the cause of the inflammation so that NP is still an appropriate approach to investigate mechanisms of action of **2** prior to *in vitro* and *in vivo* experimental design.

There are reported limitations to the NP approach more generally. The databases that are used, especially the free ones which are most commonly used, may be incomplete and skew the results. NP cannot mimic biological synergies and modulations that occur *in vivo* and clinically. In natural product NP, the exact bioactive metabolites are not fully known, making the NP analysis necessarily incomplete. The preparation methods and vehicles used in TM preparations may alter the chemistry of an extract, having an impact on the applicability of the ADMET and NP predictions [614]. While NP is a valuable and growing area of research, it needs to be recognised as a preliminary stage in research, requiring experimental and clinical research to validate its results. Wong *et al* [644] have also pointed to the need to standardise NP processes and agree evaluation indicators in order to improve the reliability of the research.

5.7 Concluding Remarks

Ethnopharmacology provides the basis for investigation of the bioactivity of *C. spinarum* root such that, in this instance, the root is inhaled to treat respiratory disorders. This analysis focuses on **2**, a single metabolite of *C. spinarum* root, as it is the near sole constituent of the root volatile oil. The NP process followed presents a step in investigating the mechanism of action of **2** in Pneumonitis and *C. spinarum* root in respiratory disorders. These are very preliminary but positive results and support the proposition that the activity of **2** is worth investigating further.

Chapter 6: Conclusions, Limitations and Future Directions

This research has been a journey from ethnomedical research conducted in West Pokot, Kenya, in the early 1990s through an ethnobotanical and phytopharmacological analysis of the medicinal species, *Carissa spinarum* L. (Apocynaceae) and to an *in silico* study of a metabolite of this species with reference to its traditional use.

The ethnomedical research in West Pokot, Kenya, demonstrates that there was a rich knowledge of local flora and pathological states of the body. As with all ethnomedical research, it is a snapshot in time and space and of a particular group of participants. The differences in *materia medica* reported herein and in other studies in the same community are an illustration of this partial picture. Of the fifteen selected prominent species used by participants, four are under-reported in the literature, presenting a valuable avenue for further study. The remaining eleven species are well-supported in the ethnomedical literature in East Africa with some also present across the continent and in Asia, indicating a significant health resource for study communities and a high likelihood of bioactive principles. Just four of the fifteen species were among the sixty voucher specimens retained by the East African Herbarium which correlates with the fact that the sixty specimens were not among the first tranche of samples brought for identification and thus logically, less frequently used. This also illustrates the interconnectedness of the data and the difficulty of a partial publication.

C. spinarum, one of the species used in West Pokot, among neighbouring communities in Kenya and Uganda and more widely through SSA, Asia and Australia, was analysed in detail through the ethnobotanical and ethnopharmacological records. The ethnobotanical literature showed a species, valued for many reasons but especially as a medicine. The phytochemistry of solvent extracts has been well-characterised, with substantial research on the pharmacology of these extracts and metabolites being described in the literature. The phytopharmacology has, to date, shown no outstanding *Carissa*-specific potent metabolites contributing to its bioactivity but it shows a metabolome which has the potential to contribute to broad anti-infective and anti-inflammatory activity. However, the phytochemical and pharmacological studies do not, in the main, mimic traditional formulations as the majority pertain to phytochemical studies, using organic solvent extracts. Such extracts differ qualitatively and quantitatively from the primarily aqueous decoctions reported in the literature. While knowledge of organic solvent extracts and of bioactive constituents is useful, it potentially bears little resemblance to therapeutic efficacy of plant extracts in clinical usage. To counter this, metabolic fingerprinting of tested extracts permits comparison between different bioactivity studies and correlation with traditional usage. The design of studies that use aqueous extracts in models

that mimic *in vivo* and clinical situations where possible would be a valuable advance in ethnopharmacological research. The incorporation of the real-life preparation such as with animal fats, spices and other species is also a valuable avenue of this research.

One of the metabolites of *C. spinarum* root, **2**, a simple phenolic compound with structural similarities to salicylic acid (**14**), was identified in the literature as having limited investigation of bioactivity. As the major ingredient of the root volatile oil and in the absence of sufficient root material for *in vitro* study, it was selected for *in silico* analysis which predicted anti-inflammatory activity. In parallel, a very preliminary cell biology study, was conducted which revealed immunomodulatory activity. These findings lend support to the Pokot and broader ethnomedical usage of *C. spinarum* and point to potential modes of action, albeit in relation to just one of the many components of the plant metabolome. However, it demonstrates the value of linking *in silico* analysis with ethnomedical knowledge, to help validate traditional uses and predict potential disease targets in a dynamic landscape of health and illness.

In silico analysis, as demonstrated here, allows us probe potential mechanisms of action without access to plant material. This is a sustainable approach to starting investigations, giving a pointer to further pharmacological investigations through the combination of the ethnomedical record, phytochemistry, pharmacology, knowledge of molecular biology and state of the art bioinformatics techniques. Obviously, the potential for synergistic interactions of metabolites is recognised and is extremely important to consider and investigate, and looking at only one molecule either *in vitro* or *in silico* can limit the predictions for human use. Ultimately, the aim is to use *ex vivo* tissue, with human biomarkers to identify mechanisms of action.

6.1 Limitations of the study

The limitations of the study have been highlighted in each individual chapter given the varied subject matter throughout this thesis. In summary, the inability to fully use the West Pokot research data through CBD and matters relating to traditional knowledge was the primary limitation of the study and the consequent limitations on returning the material to the community, although I am hopeful that this will be possible in the future. The many limitations of myself as researcher within an African community, have been described along with basic difficulties of translating emic into etic understandings of health and illness. However, these lived experiences have led to a greater understanding of the challenges and opportunities of ethnomedical field research and informed a critical appraisal of the ethnobotanical data on *C. spinarum*. Although substantial ethnobotanical data on *C. spinarum* is reported here, there is limited access to ethnomedical

data from Australia and the non-English language data from South Asia as described, which is a further limitation in the ethnobotanical research of this species. Given the critique of the reductionist approach of ethnomedical and ethnopharmacological research, this research, itself takes a reductionist approach in later chapters. In analysing the phytochemistry of this species, individual phytochemicals were investigated rather than a fuller exploration of traditional preparations. However, this was necessitated given the relative lack of laboratory data on the traditional formulations and points to the need to optimise methods of ethnopharmacological research that better reflect traditional medical knowledge. The space limitations of this thesis have required a circumscribed analysis of the phytopharmacology of *C. spinarum* which is compounded by the lack of supporting metabolic fingerprints of plant extracts. In relation to the analysis of *C. spinarum*, the obvious lack of plant material is the primary obstacle to this element of the research. A root fragment, kindly donated by Château Perouse in France, was only sufficient for a once-off headspace-GC/MS analysis. Nonetheless, this revealed interesting data contrasting with the literature in relation to the presence of limonene (**3**), altering the picture of the bioactivity of the root oil in practice.

6.2 Future Work

Future research on the activity of *C. spinarum* subsequent to *in silico* predictions of 2-HAP (**2**) would be a follow-on study of predicted cytokine release in human cell lines. NP predictions of the known wider metabolome, particularly of the high concentration secondary metabolites analysed in Chapter 4, would be a valid starting point for guiding studies of the plant extract in *in vitro* and *ex vivo* models to identify the modes of action of *C. spinarum* root. This may be assisted through further outreach through the Botanic Gardens Conservation International (BGCI) network or through collaboration with a Kenyan university in further research on this species following this model. In relation to further research in West Pokot, this study has highlighted some key Pokot species which are under-reported in the literature, including *Pavetta subcana* var. *longiflora* (Vatke) Bridson, *Vepris glomerata* (F. Hoffm.) Engl. var. *glabra* and *Manilkara butugi* Chiov. in particular in relation to malaria and CNS disorders which would merit further study. However, the initial research proposal for this thesis, based on my original research in West Pokot, was for a sociological study examining the impact of new regulations governing traditional medicine on rural practitioners in West Pokot. The project proposal was approved by TCD and NACOSTI in Kenya and a research network established with counterparts in Maseno University and local Pokot research

facilitators. This approval was predicated on a successful ethics application, which in itself establishes a workable protocol for a future study.

I was invited to attend a workshop in Nairobi in 2019 on the subject of legislation relating to traditional knowledge and intellectual property and the rights of marginalised communities. Through this workshop, I established valuable connections with people in this field of interest. John Harrington, Professor of Global Health Law and Director of the Centre for Law and Global Justice at Cardiff University, Wales, a legal researcher based in the UK but with substantial research experience in Kenya, was particularly interested in the perspective of someone from the field of traditional medicine practice. Connections were made and interesting discussions were held with people involved in traditional knowledge and intellectual property issues including lawyers, government representatives, land rights activists and Maasai traders arguing against the exploitation of Maasai artwork. Due to my underlying health issues, it has not been possible to proceed with this research, and my thesis had to be adapted to fit circumstances.

A recent IRC New Foundations Award was granted to Professor Sheridan and me, for a collaboration-building visit to Kenya. Unfortunately, taking up this grant was delayed due to COVID restrictions, but it opens up a range of possibilities to facilitate further in-country and cross-institutional research. This research could include an investigation of Pokot medicinal species such as *C. spinarum* as an exemplar of more appropriate phytopharmacological methods in ethnopharmacological research. The grant may also facilitate the conduct of cross-disciplinary research on the sociological aspects of changes in governance of traditional medicine in Kenya by this or another researcher building on the original proposal and consolidating established relationships for this interesting area of study, which would be a case study with relevance across global traditional communities.

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Appendix I

Interview schedules

Semi-structured interview schedule on plant use (modified from East African Herbarium):

Basic plant data
Flora of
Pokot Name
Economics
Locality
(Altitude –proxy by village)
Habitat
Description
Date

Detail asked on medicinal usage followed the following format:
What are the medicinal uses of this plant?
What part of the plant is used for this usage?
How much of the plant is used in the preparation?
How is it processed (raw, crushed, added to water, boiled)?
How is it administered? (Orally, topically, by inhalation)
What dosage of medication is given?
How many times a day?
For how long is it given?
Are there any known side effects?
Is there anyone it should not be given to?
Is there any situation in which it should be used with caution?
Interviews on particular illness categories:
Name of Illness
Signs and Symptoms of illness
Understanding of illness
Diagnosis ²⁰ (not all)
How it is treated, sequence of treatment if appropriate
Any treatment besides medicinal plants
Rationale for treatment
Illness treated -any restrictions in relation to age and pregnancy

²⁰ Diagnosis was discussed for a few categories where there was something specific and additional to visible physical symptoms (e.g., analysis of abdominal pulse, applying coloured soils). Illnesses with particular diagnostic techniques were Anaemia, Yomut, Ptoukuot, Dizziness, Cheptukon, Chemwaror.

How illness contracted (not all)²¹ /cause of illness
When to refer (not all)
Any follow-up
Other information
Illnesses treatable by TM and not treatable by TM
Transmission of traditional medical knowledge – who taught you about Pokot medicine

Treatment diary reporting:
Patient details – adult or child and the age of the patient if a child.
The symptoms of the patient
Illness category if appropriate
How long they had been ill for.
What treatment if any had been used up to that point?
What treatment they gave to the patient?
What was the outcome of treatment?
How long did it take to work?
Any further treatment/referral where relevant and where to?
If the patient attended clinic/dispensary/hospital, what was the clinical diagnosis if known

Interviews with particular participants regarding their practice (4 participants) and most common illnesses treated (13 participants):
Are you a Chepsaktian?
How long have you been practicing as a Chepsaktian?
Who taught you how to be a Chepsaktian?
Who do you treat (just family or other people also)?
How many people do you treat per week/month?
What illnesses do you treat? What illnesses do you know the treatments for?
Are there illnesses that you would not treat at all?
If not, why not?
What symptoms would you consider to be really serious?
What symptoms would you consider referring? To whom?

Appendix II

Sample printouts of NAPRALERT data (two different styles of printout, same type of data)

²¹ Cause was only discussed for a few illnesses – Chepkolomut, Chemwaror, Cheptukon, Korupoi, Yomut, Kakatan, Kuprirr, Nukeese, Termes.

Cyphostemma junceum (Webb) Desc. ex Wild & R.B.Drumm.

NAPRALERT PROFILE FOR CYPHOSTEMMA JUNCEUM

Listed below are two types of information that may be of interest to you relative to the species for which information is requested. First is an alphabetical list of common names for the species which occur in the NAPRALERT database. Second is a list of synonymous Latin binomials that have been found in the literature covered by NAPRALERT. For most articles where the synonym has been found, we have changed the name of the plant to that indicated as the preferred binomial. In this way, all ethnomedical, pharmacological and chemical information found in the literature for a given species, regardless of whether the information was published from a plant considered as synonymous, will be retrieved under one plant name. We do not have the capacity to check every Latin binomial for synonymy and thus if the user of this information feels that the information is incomplete, additional queries should be made of the database using each of the synonymous Latin binomials listed below.

NEITHER COMMON NAMES NOR SYNONYMS ARE AVAILABLE FOR THIS SPECIES

ETHNOMEDICAL INFORMATION ON CYPHOSTEMMA JUNCEUM (3part query for nap, 08/26/94)

NO INFORMATION

BIOLOGICAL ACTIVITIES FOR EXTRACTS OF CYPHOSTEMMA JUNCEUM (3part query for nap, 08/26/94)

CYPHOSTEMMA JUNCEUM (VITACEAE) DRIED ROOT ZIMBABWE

ANTIYEAST ACTIVITY * H2O EXT * * AGAR PLATE * * CONC USED NOT STATED / * INACTIVE * * CANDIDA ALBICANS * T10823
USING HOLE-PLATE DIFFUSION METHOD.
ANTIYEAST ACTIVITY * H2O EXT * * BROTH CULTURE * * CONC USED NOT STATED / * INACTIVE * * CANDIDA ALBICANS * T10823
USING TEST-TUBE DILUTION METHOD.
ANTIYEAST ACTIVITY * MECL2 EXT * * AGAR PLATE * * CONC USED NOT STATED / * INACTIVE * * CANDIDA ALBICANS * T10823
USING HOLE-PLATE DIFFUSION METHOD.
ANTIYEAST ACTIVITY * MECL2 EXT * * BROTH CULTURE * * CONC USED NOT STATED / * INACTIVE * * CANDIDA ALBICANS * T10823
USING TEST-TUBE DILUTION METHOD.
ANTIYEAST ACTIVITY * MEQH EXT * * AGAR PLATE * * CONC USED NOT STATED / * ACTIVE * * CANDIDA ALBICANS * T10823
USING HOLE-PLATE DIFFUSION METHOD.
ANTIYEAST ACTIVITY * MEQH EXT * * BROTH CULTURE * * CONC USED NOT STATED / * ACTIVE * * CANDIDA ALBICANS * T10823
USING TEST-TUBE DILUTION METHOD.
ANTIYEAST ACTIVITY * PET ETHER EXT * * AGAR PLATE * * CONC USED NOT STATED / * INACTIVE * * CANDIDA ALBICANS * T10823
USING HOLE-PLATE DIFFUSION METHOD.
ANTIYEAST ACTIVITY * PET ETHER EXT * * BROTH CULTURE * * CONC USED NOT STATED / * INACTIVE * * CANDIDA ALBICANS * T10823
USING TEST-TUBE DILUTION METHOD.

PRESENCE OF COMPOUNDS IN CYPHOSTEMMA JUNCEUM (3part query for nap, 08/26/94)

NO INFORMATION WAS RECOVERED

LITERATURE CITED

T10823 SCREENING OF EXTRACTS FROM ZIMBABWEAN HIGHER PLANTS II: ANTIFUNGAL PROPERTIES.
GUNDIDZA, M:
FITOTERAPIA 57 2: 111-113 (1986)
(DEPT PHARM FAC MED UNIV ZIMBABWE HARARE ZIMBABWE)

the end

Appendix III

Glossary of selected Pokot and Swahili words

Chemwaror: severe illness with fever, pain and GI symptoms

Chepsaktian (chepsaktis pl.): herbalist

Dawa: medicine

Homa: Swahili word for fever and for malaria

Kakatan: mostly malaria

Kama-echo: traditional birth attendant (TBA)

Kifafa: Swahili for seizure

Korupoi I: red body in neonate

Lapow: ritual washing ceremonies

Mzungu: Swahili word for European/western

Mzungu medicine: biomedicine

Ng'asat: hepatomegaly

Ptangtang: illnesses involving dizziness and fainting

Ptokuot: gastrointestinal (GI) illness with fever and black tongue

Sakt: medicine (medicinal plants)

Takat: lung - respiratory illness

Termes: splenomegaly

Tionypomut: illnesses involving dizziness

Tum: ritual part of healing

surrum: umbilicus and pain around the umbilicus

Yomut: a progressive paralysis

Appendix IV

Meetings with study participants

No	Date Location	People present
1	1/3/1993 OMH	Chepintany Loyoma (Kapromtim), KoChepkiror Muket (Kapromtim), Chemurkong Lokit (Kapchemogem) Chemurkong Yopokwang (Ortum) Lonah Chekee (Kasongwor) Mary Hachuka (Ortum) Rosebella Lokada (Sobukwo) Miriam Lomunyuny(Ywalateke) Josephine Lousot (Kasongwor) Cheparkasum Muket (Kapromtim) Chepskak Lokonwa (Sobukwo) Monica Kimani (Ortum) Koagnes Ngoriareng (Sebit) Elizabeth Pkukat (Chepareria) Rosemary Lokosyown (Kapromtim)
2	21/4 /1993 Sebit	Veronica Lingareng at her home
3	24/4/1993 Sebit	Koagnes N'goriareng, Veronica Lingareng and Mary Julius Loruama
4	19/5/1993 Kasongwor	Lonah Chekee and Josephine Lousot
5	4/6/1993 Kasongwor/Mongorion	Lonah Chekee and Josephine Lousot
6	4/5/1993 Kasongwor	Lonah Chekee and Josephine Lousot
7	21/6/1993 Chepkorniswo/Kapromtim	Rosemary Lokosoywon
8	22/6/1993 Sobukwo	Rosebella Lokada and Cheposkak Lokonwa
9	25/6/1993 Chepkorniswo	Cheparkasum Muket, Miriam Lomunyuny Rosemary Lokosoywan
10	2/7/1993 Chepkorniswo	Chemurkong Lokit, Chemurkong Yopokwang, Paulina Kirakwong, Cheposumbaya Lodung'oyit, Chepokaptul Adowuyangiro.
11	6/7/1993 Kapromtim	Elizabeth Pkukat and Rosemary Lokosyown
12	31/7/1993 Kasongwor	Lonah Chekee, Josephine Lousot
13	10/8/1993 Kapchemogem	Chemurkong Lokit, Chemurkong Yopokwang, Paulina Kirakwong, Cheposumbaya Lodung'oyit, Chepokaptul Adowuyangiro and Mukulata Matthew, Koblima
14	24/8/1993 Kapromtim	Elizabeth Pkukat and Rosemary Lokosoywan
15	6/11/1993 Parua (upland specimen gathering)	Veronica Lingareng

16	11/9/1993 Kasongwor	Lonah Chekee
17	14/9/1993 Kapromptim	Elizabeth Pkukat, Rosemary Lokosyown and Priscillah Long'ura
18	28/9/1993 Sebit	Veronica Lingareng, Kophilip Siwanoi
19	5/10/1993 Kapromptim	Rosemary Lokosoywon, Priscillah Long'ura, Dorcas Steven, Sabina Muket
20	9/10/1993 Kasongwor	Lonah Chekee
21	23/10/1993 Kapromptim	Mamachepto, Priscillah Longura, Elizabeth Pkukat, Rosemary Lokosyown and Sabina (Koitum) Muket
22	2/11/1993 Kapchemogem	Koblina, Mukulata, Veronica Lingareng, Chemurkong (elder)
23	13/11/1993 Kasongwor	Veronica Lingareng
24	16/11/1993 Kapromptim	Priscillah Longura, Elizabeth Pkukat and Sabina (Koitum) Muket and Dorcas Steven
25	18/11/1993 Sebit	Kophilip Siwanoi
26	23/11/1993 Chepokoriong	Kamalaripo
27	23/11/1993 Sebit	Veronica Lingareng
28	24/11/1993 Sebit	Kophilip Siwanoi
29	4/12/1993 Chepokoriong	Kamalaripo
30	6/12/1993 Kapchemogem	Paulina, Chemurkong, Mukulata
31	7/12/1993 Kapromptim	Priscillah Long'ura
32	11/12/1993 Kasongwor	Lonah Chekee
33	21/12/1993 Chepokoriong	Kochepkemoi
34	24/12/1993 Kapromptim	Rosemary Lokosyown
35	1/1/1994 Chepokoriong	Kochepkemoi
36	4/1/1994 Sebit	Kophilip Siwanoi, Kamalaripo

Appendix V

Key Pokot medicinal species with selected supporting literature-based ethnomedical and pharmacological information

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
Cheripar (14) <i>Pavetta subcana</i> var. <i>longiflora</i> (Vatke) Bridson (Rubiaceae) (IUCN not listed)	Pokot Usage: Tionypamut and Cheptukon Backache Mental disturbance Emesis as treatment High blood pressure/pulse rate Chemwaror/"Typhoid" Barren Biomedical Interpretation: <i>Dizziness and fainting</i> <i>Convulsions</i> <i>Pain</i> <i>Psychiatric disorder</i> <i>Malaria</i> <i>GI disorder</i> <i>Infertility</i> <i>Hypertension or</i> <i>tachycardia</i>	Very little ethnomedical information GUI: Kenya (Narok) gonorrhoea [424], Uganda (QE reserve) – used in STDs [701] Snakebite: Southern Ethiopia, Zay people use leaf orally for snakebite [702]. RTI: Kenya Maasai use in EVM; give infusion of plant to calves for coughing; (Narok) cough EVM [424] Skin infection: Kenya Maasai apply ash to calves with fleas[122]. Kenya	No bioactivity information on this species. Other species of <i>Pavetta</i> have shown good antiprotozoal including antiplasmodial, antimicrobial, antitumor, anticonvulsant, hepatoprotective and anthelmintic activity [187, 188] Other <i>Pavetta</i> species have shown good antiprotozoal including anti-plasmodial, antimicrobial, antitumor, anticonvulsant, hepatoprotective and anthelmintic activity [187, 188]	No phytochemical information identified. Glycosides, alkaloids, phytosterols, saponins, tannins, and phenolic and flavonoids have been isolated from <i>P. indica</i> Linn. [703]

²² See section 2.2 for descriptions of emic Pokot therapeutic indications and section 2.3.7 for BM interpretations

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
Kamil/Parasinde (9) <i>Combretum molle</i> R.Br. ex G.Don (Combretaceae) (IUCN Least concern)	Pokot Usage: Cough Chepkolomut Chemwaror Jaundice Blangmukulogh Ng'asat Biomedical Interpretation: Cough Malaria Oral thrush GI disorder Jaundice Hepatomegaly Splenomegaly	Respiratory: Kenya (Loitoktok) (respiratory) [145], Uganda (TB) [704], Uganda (asthma, cough) [146, 705, 706], Uganda (Mpigi) (TB, general infections) [707], East Africa [122], Tanzania (influenza) [708], Ethiopia (TB) [709, 710], Ghana [711], Ivory Coast (respiratory disorders, bronchial complaints [712-715], Mozambique (respiratory) [716], Swaziland (asthma), South Africa(coughs, colds and TB) [355, 717-719], Swaziland (asthma) [720] Malaria/fever: East Africa (fever) [122], Kenya (Loitoktok) (backache) [145], Kenya (Embu and Mbeere) (typhoid) [721], Kenya (Loita Maasai) (backache) – Nankaya 2019, Kenya (Ilkisonko Maasai) (backache) [722], Uganda (malaria) [327], Uganda (Mpigi) (malaria) [707], Tanzania [216], Ethiopia (malaria) [709, 710], Ghana [711], Burkina Faso [723], Mali (malaria and pain) [713], Ivory Coast (fever) [714], Mozambique	Antibacterial activity <i>in vitro</i> [457, 708, 714, 728-735] Antimycoplasmal activity <i>in vitro</i> [710, 719, 736], Anti-trypanosomal activity <i>in vitro</i> [737] Anthelmintic activity <i>in vitro</i> [738] Anti-plasmodial activity <i>in vitro</i> [723, 739] Antimalarial <i>in vivo</i> [740, 741] Antifungal activity <i>in vitro</i> vs <i>C. albicans</i> [730, 735, 742-746] Anti-inflammatory activity <i>in vitro</i> [747], inactive in COX-2 inhibition [748], Anti-inflammatory triterpenoids present [722] Anticholinesterase activity <i>in vitro</i> [712, 749]	Iridoid glycosides [175]; oleanane-type pentacyclic triterpenoids and oleanane-type triterpene saponin, β -D-glucopyranosyl 2 α ,3 β ,6 β -trihydroxy-23-galloylolean-12-en-28-oate isolated with <i>in vivo</i> anti-inflammatory activity [177].

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		(fever, pain, headache, stomach ache)[716], Zambia (fever) [37], South Africa (fever) [717], South Africa (fever) [719], Saudi Arabia (malaria) [320] GI: Uganda (Mpigi) (diarrhoea, haemorrhoids) [707], Ethiopia (stomach ache, gut parasites) [724], Saudi Arabia (stomach ache, digestive disorders) [320, 725], Nigeria (animal diarrhoea) [726]. Jaundice: Ethiopia (liver diseases) [709], Ivory Coast [713] Splenomegaly or hepatomegaly: Uganda (Mpigi) (cancers) [707], Tanzania [37], Tanzania (oedema) [708], Sudan (swellings) [727] Inflammation: Uganda (Mpigi) (inflammatory disorders, skin/tissue infections) [707] Wound/skin: Uganda (wounds, skin infection) [146, 707]	Breathing reducing bronchial constriction and cough <i>in vivo</i> [750] Oxidative stress – reduced <i>in vivo</i> [751] Pancreatic protection <i>in vivo</i> [751] Toxicity – non-cytotoxic [211, 752]	

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
Kapkopf (9) <i>Cyphostemma bambuseti</i> (Gilg & M.Brandt) Desc. ex Wild & R.B.Drumm. (Vitaceae) (IUCN not listed)	Pokot usage: Kakatan/malaria Seme wa cho Fever Biomedical Interpretation: Malaria Fever Malaria with anaemia	Very little ethnomedical information on this species. RTI: Kenya (Samburu) (TB) [120] Pain: Kenya (Samburu) (bone pain) [120]	No bioactivity information on this species.	Phenolic compounds, flavonoids and terpenoids identified in phytochemical screen of another <i>Cyphostemma</i> sp [753]
Kamugh/Kurion (17) <i>Vepris nobilis</i> (Delile) Mziray (syn <i>Teclea nobilis</i> Delile) (Rutaceae) (IUCN Least concern)	Pokot Usage: Cheptukon and Tionypamut Chemwaror/"Typhoid" Vomiting in pregnancy Barren Eye red Kakatan/fever group Back Pain Biomedical Interpretation: <i>Dizziness and fainting</i> <i>Convulsions</i> <i>Malaria</i> <i>GI disorder</i> <i>Vomiting in pregnancy</i> <i>Infertility</i> <i>Conjunctivitis</i> <i>Pain</i>	Convulsions: Tanzania [754] Malaria: Kenya (Pokot) (fever) [87], Kenya (Sangurur) (invigorant, immunostimulant, blood cleanser) [423], Kenya (malaria)(Nakuru, Embu, Samburu, Maasai) [120, 121, 721, 755, 756], Tanzania (fever) [37], Uganda (Kibale) [757], Uganda (fever) [162], Ethiopia (fever, malaria)[162] Pain: Kenya (Narok) (arthritis) [424], Kenya (Sangurur) (backache, arthritis) [423], East Africa [122], Uganda [138], Uganda (dental caries) [758], Ethiopia [759]	Anti-inflammatory [759, 761, 762] Antipyretic activity <i>in vivo</i> [759, 762] Analgesic activity <i>in vivo</i> [759, 762] Anti-plasmodial activity [757] Antibacterial activity weak [758, 763]	Alkaloids isolated including furanoquinoline alkaloids: skimmianine, kokusaginine and maculine, monrifoline and acridone alkaloid: arborinine which have anti-plasmodial activity at μM concentrations [162] Skimmiamine and kokusaginine have antibacterial and anti-leishmanial activity at μM and sub μM levels [163]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		GI disorder: Ethiopia (Stomach ache) [760]		
Kerelwo (16 URs) <i>Croton dichogamus</i> Pax. (Euphorbiaceae) (IUCN Least concern)	Pokot usage: Chepkolomut/Cheposkut Sore throat Fever in child Dysuria Dysmenorrhoea Fever with fast pulse and back pain Chemwaror Biomedical Interpretation: <i>Malaria</i> <i>Oral thrush</i> <i>GUI</i> <i>Dysmenorrhoea</i> <i>Fever</i> <i>Pain</i> <i>GI disorder</i>	Malaria: (Kenya (Samburu, Nandi, Kamba, Suiei Ndorobo)[120, 165, 166, 764] Oral thrush: Kenya (Marakwet) (oral thrush); Kanya (Maasai (toothbrush) [121, 164]	Antibacterial, antiplasmodial and anti-inflammatory activity which support its ethnomedical usage [167].	Crotofolane diterpenoids (Crotoxiide A and B) isolated from leaf [765]
Kinyotwo (14) <i>Ximenia americana</i> L. (Olacaceae) (IUCN Least concern)	Pokot Usage: Chepkolomut and Cheposkut Diarrhoea Pulse – irregular or fast Painful and absent menstruation Kakatan	Malaria: Kenya (Meru) [203], Kenya (Luo) [292], Kenya (Loita Maasai)(body strength) [756], Ethiopia [766], Ghana (headache) [711], West Africa (headache with fever) [29], Nigeria (headache) [767], Nigeria (malaria) [768], Burkina Faso [769], Mali [713],	Antipyretic activity <i>in vivo</i> [205] Analgesic activity <i>in vivo</i> [205] [795] Anti-inflammatory <i>in vitro</i> [795-797]	Toxicity issues – active and toxic doses too close [203-205]. Hepatotoxicity [207], HCN in fruit kernel and leaf [37, 206], higher concentrations of HCN, saponins and oxalates in roots than other plant parts [207]. Good safety profile <i>in vivo</i> [800] Roots contain HCN, oxalates and saponins which contribute to toxicity [791]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
	Fever Cannot urinate Termes Biomedical Interpretation: <i>Malaria</i> <i>Fever</i> <i>Oral thrush</i> <i>Diarrhoea</i> <i>Oliguria</i> <i>Dysmenorrhoea and amenorrhoea</i> <i>Splenomegaly</i> <i>Pulse fast or irregular</i>	Ivory Coast [770], Mali (malaria, fever, headache, pain)[771], Mozambique (body pains) [716], Namibia (malaria) [772], Sudan (pain) [391], Brazil [773, 774], Brazil (anti-inflammatory) [775], Brazil (pain, back pain) [776]. Splenomegaly: Brazil (internal organ inflammation) [776], Brazil (headache) [777] Fever: Tanzania [29], West Africa [29], West Africa [205], Senegal [29], Brazil (fever) Brazil [773, 774], Brazil (fever) [777] Oral thrush (Tanzania [744], Mali (vaginal thrush, gingivitis, toothache, throat infection) [771], Namibia (oral thrush) [778], South Africa (mouth ulcers) [779] Diarrhoea (+ GI Disorders) (Kenya (Marakwet) (abdominal pain) [119], Kenya (Ilkisonko Maasai) (stomachache, constipation) [722], Kenya (Loitoktok) (stomach ache) [145],	Antibacterial activity <i>in vitro</i> [715, 773, 776, 798-801] Antifungal activity (<i>C. albicans</i>) [160] Enhances antibiotic activity [776] Anti-leishmanial [776] Anti-salmonella <i>in vivo</i> [800] Anti-plasmodial activity [203, 802] Anthelmintic activity [803] Antiviral [771, 800] Antioxidant activity [801, 804, 805] Anti-gastric ulcer activity [161]	Riproximin isolated from fruit kernels has anti-neoplastic activity in ng/mL concentrations [159]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		East Africa (vermifuge, stomach ache, diarrhoea) [122], Kenya (Luo) GI problems [293], Ethiopia (Omo) [780], Ethiopia (Oromia) (gastritis) [781], Ethiopia (stomach ache) [410], Tanzania (Meru, north) (diarrhoea with blood) [782], Tanzania [29], Ghana [711], Senegal (colitis, helminthiasis) [29], Burkina Faso [769], Ivory Coast [715, 783], Mali (gastric ulcer, gastric pain, haemorrhoids, dysentery, vomiting) [771], Angola (diarrhoea, stomach ache) [392], Mozambique (stomach ache) [784], Mozambique (worm infection) [716], South Africa (blood in stool) [785], South Africa [786], South Africa (dysentery) [779], South Africa (diarrhoea) [355], South Africa (bloody diarrhoea EVM) [37], Zimbabwe (anthelmintic) [783], Bolivia [787], Brazil [773], Brazil (constipation) [776], Brazil (gastritis) [788]. Oliguria/ Urinary problems: Ethiopia (EVM – haematuria) [766], Burkina Faso [769], Mali		

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		(urinary schistosomiasis) [789], Mali (UTI) [771], Brazil [773] Dysmenorrhoea and amenorrhoea (+ Menstrual problems) (Kenya (Pokot) [87], Kenya (Maasai) (uterine bleeding) [145], Kenya (Embu and Mbeere) (gonorrhoea) [721], Ethiopia (menstruation, contraceptive) [401], Ghana [711], Burkina Faso [769], Mali (dysmenorrhoea, amenorrhoea, vaginitis, sterility, uterine prolapse) [713, 771], Namibia (gonorrhoea) [772], Zambia (STI) [790], South Africa (menorrhagia) [779], Mozambique (anti-abortion, menstrual cycle, women's fertility) [784], South Africa (menorrhagia) [785], Brazil [791], Brazil (uterine pain) [792], Brazil (gynaecological inflammation) [776], Brazil (uterine pain) [788], Brazil (uterine inflammation, ovary inflammation) [777]. Swelling: Mozambique (swelling) [716]		

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		Wound healing: Ethiopia (Berbere) [380], Ethiopia (Gojjam) (wounds) [793], Uganda (Bukoba) (rash)[794] , Ethiopia (wound) [409], Mali [771], Mozambique (wound) [784], Brazil (wounds) [788] Cardiovascular: Mozambique (stabbing heart) [784]		
Lagetetwo (13) <i>C. spinarum</i> (syn. <i>C. edulis</i> (Forssk.) Vahl (Apocynaceae) (IUCN Least concern except in Seychelles 1998 endangered)	Pokot Usage: Cheptukon and Tionypamut Chemwaror/"Typhoid" Constipation Kakatan/fever group Amenorrhea Emesis as treatment Biomedical Interpretation: Dizziness and fainting Convulsions Malaria GI disorder Constipation Amenorrhoea	Dizziness (Kenya – [806], Kenya (Marakwet) (malaise) – [164]; Convulsions (Kenya – [423]; Zimbabwe – [807]; Ethiopia (brain tension, stress) – [409], Malawi – [808]). Malaria (Kenya- [329], [136, 322, 806, 809]; Kenya (Loita)(backache) [756], Kenya (Machakos) (typhoid)[810], Uganda – [353]; Tanzania (Meru, north) (headache, lack of strength) [782], Tanzania (backache)[811], Ethiopia (pain/toothache)[724] –, Benin [251]; Burkina Faso [259], Mozambique (malaria, pain)[716].	Anti-convulsant activity [580, 582] Anti-plasmodial activity [172, 325, 329]. Antimalarial in vivo [570] Antispasmodic activity [461] Antibacterial activity [467, 596] Further information on phytochemistry and pharmacology of <i>C. spinarum</i> in Chapter 4	Lignans – nortrachelogenin has anti-inflammatory, antifungal and antibacterial activity in µg/mL concentrations [171] [173], Kebenei <i>et al.</i> [498] Eudesmane sesquiterpene, carissone, with anti-inflammatory activity in µg/mL concentrations [174]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		Constipation (+ other GI) (Kenya – [325], Kenya (Machakos) (diarrhoea)[810], Kenya (Loita)(diarrhoea)[756], Kenya (Amoebiasis)[294], Uganda [575]; Ethiopia [400, 812], Ethiopia (stomach ache) [410], Ethiopia (stomach ache) [378], Angola (diarrhoea, stomach ache) [392], Mozambique (dysentery) [716], South Africa (constipation) [355], Saudi Arabia (parasitic worms, colic) [320], India [813-815] Menstrual problems (+ women's health) (Kenya [321]– Delbanco 2017, Kenya (Loita)(pelvic pain) [756], Ethiopia (breast cancer) [724], Saudi Arabia (dysmenorrhoea)[320], South Africa (menorrhagia, infertility) [355] (Further information on ethnomedicine of <i>C. spinarum</i> in Chapters 3 and 4)		
Lopotwokanyrput (9) <i>Solanum</i> sp. (Solanaceae)	Pokot Usage: Pkata group Diarrhoea Headache	Genus only	Genus only	

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
	Chepkolomut and Cheposkut Urinary symptoms Ptangtang Eye pain Biomedical Interpretation: GUI Diarrhoea Pain Malaria Dysuria Dizziness with headache Eye pain			
Manempelion (16) <i>Vepris glomerata</i> (F.Hoffm.) Engl.var <i>glabra</i> (Rutaceae) (IUCN not listed)	Pokot Usage: Cheptukon and Tionypamut Kakatan/fever group Eye pain/red/gritty Rash Body swelling below knees Chemwaror/"Typhoid" Korupoi Biomedical Interpretation: Dizziness and fainting Convulsions Malaria	Malaria (Kenya (Pokot) fever – emetic [87], Tanzania [189], Ethiopia (malaria) [250] Headache (Kenya (Pokot) [87] Convulsions (+CNS) (Tanzania (psychosis, epilepsy, stroke) [189] Eye disease (Tanzania [816]) Body swelling (Tanzania [163])	Very little information on this species. Anti-malarial: Inactive <i>in vivo</i> anti-malarial [189] Toxicity: Low brine shrimp toxicity [216]	Compounds –flavonoid and furoquinoline alkaloids isolated with antioxidant activity and antibacterial activity of isolated compounds [817]. The flavonoid, hesperetin isolated which has antioxidant and anti-inflammatory activities [163] [191]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
	Fever Eye infection Rash Oedema GI disorder Cancer	GI: Ethiopia (abdominal colic) [250]		
Mukurkong (11) <i>Harrisonia abyssinica</i> Oliv. (Rutaceae) (IUCN Least concern)	Pokot Usage: Pkata Nyowas Painful urination Amenorrhoea Barren Rash Biomedical Interpretation: GUI Amenorrhoea Infertility Skin rash	GUI (Kenya (Machakos and Kitui) (syphilis) [818], Kenya (Loitokitok (STD) [145], Kenya (Ilkisonko Maasai) (STD)[722], East Africa (swelling of testicles, gonorrhoea) [122], Tanzania (Tarime) [819], Tanzania (Korogwe) [820], Tanzania (Bagamoyo) [821], Tanzania (Tanga, Korogwe) (vaginal thrush) [744], Uganda (Bulamogi)(syphilis) [316], Ghana [711] Amenorrhoea (+ menstrual) (Kenya (Luo) (EVM – expel afterbirth) [336], Kenya (Embu and Mbeere) (blood clotting) [721], Tanzania (Bagamoyo) (menorrhagia) [821] Infertility (Tanzania (Tarime) [819]	Antibacterial - [783, 818, 819, 823-829] Anti-plasmodial –[824] Antiviral - [824] Antifungal - [823, 824, 826, 830, 831] (<i>C. albicans</i>) Antioxidant - [832] Neuroprotective – [833] Compounds: antibacterial and antifungal compounds from rootbark [834, 835] Interacts with Cytochrome system [194] Toxicity – rootbark and stembark demonstrate brine shrimp toxicity [213]	Tetranortriterpenes isolated obacunone, harrisonin and acetoxyharrisonin; tetranortriterpenoid [178] Obacunone has exhibited antiproliferative and neuroprotective activity in nM concentrations [179]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		Skin rash (East Africa (wounds) [122], Tanzania (abscess) [37, 822], Uganda (Karamoja) (EVM skin conditions) [335], Uganda (Bulamogi) (wound, incisions) [316]) Malaria (Kenya (malaria) – [185, 823], Tanzania (malaria, fever) [822])		
Posion (10) <i>Manilkara butugi</i> Chiov. (Sapotaceae) (IUCN not listed)	Pokot Usage: Termes and Ngasat Diarrhoea Pain in leg joints Ptangtang Vaginal bleed in pregnancy Biomedical Interpretation: <i>Hepatomegaly</i> <i>Splenomegaly</i> <i>Diarrhoea</i> <i>Vaginal bleed in pregnancy</i> <i>Arthrosis</i>	GI: Ethiopia (Stomach ache) [760]	No information on this species. Other species within the genus have demonstrated analgesic, anti-inflammatory, anti-diarrhoeal, anthelmintic, hepato-protective, antifungal and antibacterial activities [836]	
Putapuk (9) <i>Aerva lanata</i> (L.) Juss.	Pokot Usage: Cheposkut Chepkolomut Cheseriketch Teething	Oral thrush (Kenya (Pokot) (mouth ulcers) [87], India (sore throat) [837])	Antibacterial activity [180, 840, 846-848] Anti-plasmodial [180]	High TPC, Ecdysones, betulin, Acylated flavone glucosides, Ferulic acid, sulfonoquinovosyldiacylglyceride, alkaloids [180]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
(Amaranthaceae) (IUCN not listed)	Seme wa kagh Fever Malaria Biomedical Interpretation: <i>Oral thrush</i> <i>Tropical ulcer</i> <i>Teething</i> <i>Leprosy</i> <i>Fever</i> <i>Malaria</i>	Tropical ulcer/wound (Kenya (Somali) – wound EVM [336], Uganda (skin infections) [146], East Africa (snakebite) [122], Saudi Arabia (scorpion stings) [320], India (Ayurveda) (skin infections, snakebite, boils) [838] Ramachandra, Shilali et al. 2011, India (skin diseases) [837, 839-841]) Teething/pain (Tanzania (Meru, north) (pain) [782], India (Ayurveda) (toothache) [842], India (anti-inflammatory) [839], Sri Lanka (body pain) [843]) Leprosy (India (skin diseases) [840]) Malaria (+ symptoms) (East Africa [122], Philippines (fatigue) - [180], India (malaria) [844], India (headache) [180, 841], Sri Lanka (body pain) [843], Bangladesh (headache) [845]) Cough (India [180, 837], Muthu 2006, Bangladesh (cough) [845])	Antiviral (HIV) – [849]Gujjeti 2014 Immunomodulatory <i>in vivo</i> [850, 851] Antioxidant [180] Anti-inflammatory [840, 852] Anti-inflammatory <i>in vivo</i> [852, 853] Analgesic <i>in vivo</i> [852] Antifungal [840, 847, 854] Hepatoprotective [180] Antidiabetic <i>in vivo</i> [217] Toxicity non-toxic in acute study <i>in vivo</i> given orally but moderate toxicity intraperitoneally, possible long-term toxicity in tissue damage and modified haematological parameters [217]	Indole alkaloids including canthin-6-one which has antifungal and benzodiazepine receptor binding activity in μM levels [179] Sulfonylquinoxalylglyceride has antibacterial and anti-HSV activity in $\mu\text{g/mL}$ concentrations and Feruloyltyramine anti-inflammatory activity at μM levels [179]

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Siria/Siriowo (14) <i>Searsia natalensis</i> (Bernh. ex C.Krauss) F.A.Barkley (syn. <i>Rhus natalensis</i> Bernh. ex C.Krauss) (Anacardiaceae) (IUCN Least concern)	Pokot Usage: Diarrhoea/bloody diarrhoea Stomach pain Vomiting Kakatan/fever group Cheposkut and Cheposkut Prevention Chemwaror/"Typhoid" Measles Cough Bleed in pregnancy Biomedical Interpretation: <i>GI disorder</i> <i>Malaria</i> <i>Oral thrush</i> <i>Habitual abortion, PV bleed in pregnancy</i> <i>Bleeding gums</i> <i>Cough</i> <i>Measles</i>	GI disorder/ GI Infection (Kenya [215], Kenya (Sangurur) (liver and spleen diseases, peptic ulcer) [423], Kenya (Ilkisonko Maasai) (stomachic) [722], Kenya (stomach ache) [294], Kenya (Mbeere and Embu) (diarrhoea) [855], Uganda (Jaundice) [146], Tanzania [37], Tanzania [856], Tanzania [857], Kenya (Nandi) [455], Kenya (Luo) [91], Kenya (Marakwet) [119], Ethiopia (stomach disorders, bad breath) [250], Ethiopia (diarrhoea, vomiting, food poisoning) [378] Malaria (Kenya (Kilifi, Meru, Kikuyu, Maasai)[136, 203, 215, 722], Uganda [858, 859], Tanzania [860], Namibia (meningitis) [778] Habitual abortion, Kenya (Loitoktok) (endometritis)[145], Uganda (EVM – retained placenta) [861], Tanzania (PV bleed in pregnancy) [37]	Anti-plasmodial activity <i>in vitro</i> [215, 859, 863] Antimalarial activity <i>in vivo</i> [215, 863] Anti-measles virus activity <i>in vitro</i> [864] Protease inhibition in periodontopathic bacteria [862] Antinociceptive activity (mild) <i>in vivo</i> [865](Kariuki 2012)	Several flavanones and flavones isolated including 7-O-Methyl isokaemferide, 7-O-methyl naringenin and eriodictyonone and genkwanin which had antioxidant activity and the biflavonoid rhuschromone which is active against <i>S. aureus</i> [158]

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		Bleeding gums (Kenya (chewing stick, toothbrush) [143, 862], Uganda (Bukoba) (Herpes simplex) [794]) Cough (+ respiratory)(Kenya (Kilifi, Luo, Nandi [215, 455, 805], Kenya (Ilkisonko Maasai) (respiratory disorders) [722], Kenya (Mbeere and Embu) (influenza)[855], Tanzania [37]) Measles (Uganda for rash [857], Uganda (Bukoba) (Herpes simplex, skin infection) [794], Tanzania (chicken pox) [811]) Wounds/skin Uganda (wounds, skin infections) [146], Ethiopia (swelling, wounds) [413], Ethiopia (toothache) [378], Ethiopia (boils) [380], Ethiopia (snakebite orally) [724]) Strengtheners (Kenya (Ilkisonko Maasai) (strengtheners) [722])		
Simoyowo (11) <i>Hoslundia opposita</i> Vahl	Pokot Usage: Pkata Painful urination Irregular menstruation	Dizziness (+ CNS) (Uganda (epilepsy) [706], Ghana (vertigo) [711], Ivory Coast (convulsions) – Malan 2015)	Analgesic <i>in vivo</i> [880] Anti-inflammatory <i>in vivo</i> [880]	Abietane-type esters isolated including 3-O-Benzoyl-hosloppone which has antiparasmodial in µg/mL concentrations [870]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
(Lamiaceae) (IUCN not listed)	Chepkolomut Body swelling Tionypamut Biomedical Interpretation: <i>GUI</i> <i>Irregular menstruation</i> <i>Malaria</i> <i>Oral thrush</i> <i>Dizziness</i>	GUI (Uganda(west) (vaginal infections) [701], Uganda (syphilis, gonorrhoea) [146], Tanzania (North and Bunda) (vaginitis) [822], Tanzania (Tanga and Korogwe) (male genital painful swellings) [866], Tanzania (gonorrhoea) [867], Ghana [711] Irregular menstruation (+ menstrual issues) (Uganda (post-natal care) [146], Uganda (painful uterus) [705], Tanzania (Tanga and Korogwe districts) (menorrhagia, irregular menstruation, dysmenorrhoea) [866, 867], Tanzania (Meru, north) (persistent barrenness) [782]–, Tanzania (cleanse womb, facilitate conception) [811]) Malaria (+ related symptoms) (Kenya (Nandi, Coast, Kilifi) [455, 868], Kenya (Kilifi, Embu and Mbeere)(colds and flu) [215, 721], Uganda (back pain, arthritis) [146], Uganda (Sango Bay, western, central) (malaria) [138, 328, 705,	Antipyretic <i>in vivo</i> - [880] Anticonvulsant <i>in vivo</i> - [881]Olajide, Awe et al. 1999, <i>in vitro</i> [882] Anti-plasmodial <i>in vitro</i> (low to moderate with poor selectivity) [872], <i>in vitro</i> (moderate with mild cytotoxicity) [215], antiplasmodial [875, 883, 884] Antimalarial <i>in vivo</i> [215] Antiviral activity <i>in vitro</i> (moderate) [830] Antibacterial <i>in vitro</i> (good) [885], antibacterial [867, 869, 875, 886, 887], inactive vs oral pathogens [876], active vs <i>H. pylori</i> [878] Antifungal <i>in vitro</i> [888], inactive vs oral pathogens [876], inactive vs <i>C. albicans</i> [869]	Two 6-furanoflavones and four methylpyranoflavonic analogues isolated showing anti-leishmanial activity [181]

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		858], Uganda (Kiohima) (fever) [757], Uganda (south) (childrens seizures) [869], Tanzania (malaria) [757, 822, 870, 871], Tanzania (North, Dar es Salaam, Bunda, Tanga and Korogwe districts) (fever, convulsions, childhood convulsions, epilepsy) [754, 822, 866], Tanzania (Shambala, Bunda) (fever) [37, 822], Ethiopia (Northwest)(Dek island) (febrile illness- <i>mich</i>) [332], Ivory coast (malaria) [872], Burkina Faso (malaria) [769], Nigeria (fever) [873], Zimbabwe (seizures) [808], South Africa (malaria) [874] Oral thrush (Kenya (Marakwet, Kilifi) (oral thrush/oral wounds) [164, 215], Uganda (oral wounds) [758], Tanzania (oral wounds) [875], Nigeria (chewing stick) [876] Wounds/skin – Uganda (Skin infections, Wounds, Pressure sores, Boils)[146, 861, 877], Ghana (deep wounds) [878], Nigeria (ulcer) [879]	Toxicity – leaf extract poor cytotoxicity to antiplasmodial ratio [872], leaf extract significant cytotoxicity [757], leaf extract mild cytotoxicity, root extract poor selectivity (antiplasmodial to cytotoxicity ratio) [215], root extract non-toxic in brine-shrimp assay [214]. Leaf extract no acute toxicity in mice [889]	

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
Songowo (24) <i>Zanthoxylum chalybeum</i> Engl. (Rutaceae) (IUCN Least concern)	Pokot Usage: Kakatan Fever Chemwaror/"Typhoid" Tionypamut Headache and cough Chepkolomut and Cheposkut Headache Rash Stomach pain with headache Sore eyes with pus Biomedical Interpretation: <i>Malaria</i> <i>Fever</i> <i>GI disorder</i> <i>Fainting/dizziness</i> <i>URTI</i> <i>Oral thrush</i> <i>Pain</i> <i>Rash</i> <i>Eye infection</i>	Malaria (Kenya malaria (Pokot, Marakwet, Machakos, Mutomo, Digo, Meru, Kikuyu, Kilifi and Tharaka, Kwale, Msambweni, Giriama) [87, 136, 164, 166, 184, 185, 326, 810, 890, 891] [868], Uganda malaria (Sango Bay, Mpigi, Karamoja, Budiope)[138, 327, 707, 858, 892], Tanzania (Urumwa) (malaria, headache) [893]. Fever (Kenya (Pokot, Turkana, Suiei Ndorobo, Gabbra, Sangurur) [87, 165, 423, 894, 895] Tanzania [37], Ethiopia (Typhus – febrile illness) [724] Fainting/dizziness (+ CNS) (Kenya (Pokot) [87], Kenya (Sangurur) (epilepsy, psychotic disorders) [423], Uganda (Mpigi) (epilepsy) [707], Uganda (epilepsy, psychosis) [252, 896], Tanzania (epilepsy, convulsions) [754, 816] RTI (Kenya (Pokot, Samburu, Turkana, Mutomo, Southern Kaijiado, Gabbra, Suiei Ndorobo)	Anti-plasmodial activity <i>in vitro</i> [156, 212, 754, 883, 891, 909-912] Low-moderate activity and with poor selectivity index i.e., toxicity [211] Antimalarial <i>in vivo</i> [156, 209] Antifungal activity <i>in vitro</i> [901, 913, 914]. Inactive <i>in vitro</i> antifungal [915] Antibacterial activity <i>in vitro</i> [734, 758, 914, 916-920], inactive <i>in vitro</i> antibacterial [915] Anti-mycobacterial (moderate) [921] Anthelmintic activity [922] Anti-inflammatory activity <i>in vitro</i> [917, 923] anti-inflammatory activity via COX-2/PGH ₂ pathway [748] Anti-diabetic activity <i>in vivo</i> [924, 925]	Quaternary alkaloids – nitidine has strong anti-plasmodial activity in ng/mL concentrations. Also has antimicrobial, anti-inflammatory and analgesic activity [156]. Benzo[c]phenanthridines alkaloids including chelerythrine which has antibacterial and antifungal activity [157]. Isoquinoline alkaloids including protoberberine. Benzo[c]phenanthridinealkaloids from <i>Zanthoxylum</i> species have anticholinesterase activity [926] Toxicity of extracts thought to be due to quaternary alkaloids including candicine [156].

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		[87, 165, 166, 336, 895, 897], Kenya (Machakos, Luo) (pneumonia) [805, 810] Uganda (General RTI) (Mpigi, Mabira) [705, 707], Uganda (TB) (Mpigi, Butambala, Nakapiripirit, Lake Victoria) (TB) [257, 704, 707, 898, 899]. Tanzania (cough) [820, 900, 901], Tanzania (asthma, lung inflammation, chest pains) [816, 820, 893, 902], Ethiopia (common cold) [378] Oral thrush (+ other oral symptoms) (Kenya (thrush) (Sangurur) [423], Kenya (gum problem, tonsillitis) [903], Uganda (thrush, sore throat)(Karamajong) [707, 892], Tanzania (oesophageal candidiasis) [744]) Pain (Kenya (pain, rheumatism) (Pokot, Marakwet, Machakos, Embu and Mbeere) [87, 164, 388, 721], Kenya (Mutomo Hill) (toothache) [166], Uganda (Mpigi, Karamajong, Sango Bay, Budiope) (inflammatory disorders/pain, toothache)[138, 146, 327, 335,	Anti-oxidant activity [748] Toxicity: Low to moderate selectivity index [204], low toxicity in brine shrimp assay [209], nontoxic in long term low-dose, toxic in very high doses in rats [210], toxic intraperitoneal dose in mice, toxic to lung fibroblasts, weakly mutagenic [156], non-cytotoxic [211], cytotoxicity of stem bark in brine shrimp assay [921], non-toxic [212]	

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		707, 758, 892], Tanzania (dysmenorrhoea, rheumatism) [816, 820], Tanzania (Urumwa)(headache, toothache, stomach ache) [893], Ethiopia (toothache, back pain) [724], Somalia (dysuria) [904], Burundi (headache) [905], Ghana (pain) [875] Rash (Uganda (Mpigi) (skin cancer) [707], Tanzania (Bagamoyo) (skin diseases, urticaria) [816], Ghana (skin infections) [875], Uganda (wound disinfection) [707] GI (Kenya (Pokot) (EVM), Marakwet, Luo) [87, 91, 164, 906], Uganda (GI disorders, nausea, liver cancer) [707], Uganda (Karamajong) (diarrhoea, jaundice, stomach ache) [335, 705, 892], Tanzania (stomach pain) [816, 820], Tanzania (intestinal worms) [816, 902], Somalia (stomach problems) [904], Ethiopia (amoebiasis, abdominal discomfort) [250, 724], Burundi		

Pokot name (URs) Species (Family) (IUCN status)	Pokot Indication ²² and <i>BM interpretation</i>	Selected supporting EM usage	Selected supporting pharmacological activity	Selected supporting phytochemical information
		(intestinal worms and GI problems)[905, 907], DRC (EVM – diarrhoea) [908], Ghana (stomach ache) [875], Zimbabwe (diarrhoea and vomiting) [807]		
Tiyin (11) <i>Dichrostachys cinerea</i> (L.) Wight & Arn. (Fabaceae) (IUCN Least concern)	Pokot Usage: Chemwaror/"Typhoid" Kakatan/fever group Headache Chepkolomut and Cheposkut Tionypamut group Constipation Painful urination Biomedical Interpretation: <i>Malaria</i> <i>GI disorder</i> <i>Oral thrush</i> <i>Dizziness and fainting</i> <i>Constipation</i> <i>Dysuria</i> <i>Pain</i>	Malaria (+ related symptoms) (Kenya [868], Tanzania [927], Benin [928], Niger [929], Nigeria (fever) [930], Namibia (malaria) [772] Pain (Kenya (Pokot, Narok) [87, 424], Tanzania [927], DRC [37], Congo-Brazzaville [929], Liberia [37], Ghana (headache) [931], Nigeria (toothache, headache) [879], Ivory Coast [715], Senegal [932], Angola [933], Zimbabwe [807], Mozambique (skeletal disorders) [784], South Africa [779, 934] Tonga [37] STI Kenya (Narok) (gonorrhoea) [424], DRC [935], Liberia [37], Ghana [931], Botswana [936], Namibia [937], Zimbabwe [807], Zambia [790], South Africa (STI, erectile dysfunction) [779]	Anti-inflammatory activity <i>in vitro</i> and <i>in vivo</i> [934, 944] Analgesic activity <i>in vivo</i> [945] Diuretic activity <i>in vivo</i> [946] Antibacterial activity <i>in vitro</i> [219, 733, 947-952] Antimycobacterial – inactive [953] Analgesic activity <i>in vivo</i> [945, 949] Anticonvulsant activity <i>in vivo</i> [945, 954] Anti-plasmodial <i>in vitro</i> [218] Antimalarial <i>in vivo</i> [218]	The hydroxycinnamic acid amide, clovamide is active vs Influenza A and against HIV at μM levels [169, 179] Meroterpenes (flavono-labdanes, dichrostachines) isolated from root and stembark exhibited potential anti-cancer activities [168]. Tannins extracted from the root exhibited antibacterial activity <i>in vitro</i> [170]

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		Epilepsy (Tanzania (Urumwa) (dizziness, epilepsy) [893], South Africa [299]) Oral thrush (Tanzania [744], Namibia [778]) Urinary problems (Burkina Faso [769], Mali [938], India [939, 940]) GI: (Sudan [727, 941], Sudan (jaundice) [391], Tanzania [893, 927], DRC [935], Congo-Brazzaville, Niger, Benin, Ivory Coast [929], Burkina Faso [769], Mozambique [784, 942], Zimbabwe [451, 807], Namibia (stomach ache) [772], South Africa [37, 264, 934], India [940, 943])	Toxicity – most extracts non cytotoxic [218], root extracts non-toxic <i>in vivo</i> in rats [220], mild cytotoxicity in brine shrimp assay [219]	