

Accepted Manuscript

Title: Identification of a new antinociceptive alkaloid isopropyl N-methylantranilate from the essential oil of *Choisya ternata* Kunth

Authors: Niko S. Radulović, Ana B. Miltojević, Michael McDermott, Steve Waldren, John Adrian Parnell, Mariana Martins Gomes Pinheiro, Patricia Dias Fernandes, Fabio de Sousa Menezes

PII: S0378-8741(11)00171-1
DOI: doi:10.1016/j.jep.2011.03.035
Reference: JEP 6666

To appear in: *Journal of Ethnopharmacology*

Received date: 23-12-2010
Revised date: 26-2-2011
Accepted date: 13-3-2011

Please cite this article as: Radulović, N.S., Miltojević, A.B., McDermott, M., Waldren, S., Parnell, J.A., Pinheiro, M.M.G., Fernandes, P.D., de Sousa Menezes, F., Identification of a new antinociceptive alkaloid isopropyl N-methylantranilate from the essential oil of *Choisya ternata* Kunth, *Journal of Ethnopharmacology* (2010), doi:10.1016/j.jep.2011.03.035

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Identification of a new antinociceptive alkaloid isopropyl N-methylantranilate from the essential oil of *Choisya ternata* Kunth

Niko S. Radulović^{a,*}, Ana B. Miltojević^a, Michael McDermott^b, Steve Waldren^c, John Adrian Parnell^c, Mariana Martins Gomes Pinheiro^d, Patricia Dias Fernandes^d and Fabio de Sousa Menezes^b

^a*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*

^b*Panoz Institute, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin 23, Westland Row, Dublin 2, Ireland*

^c*Department of Botany, School of Natural Sciences, Trinity College, Dublin 2, Ireland*

^d*Laboratório de Farmacologia da Inflamação e do Óxido Nítrico, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil*

* Corresponding author. Tel.: +38118533015; fax: +38118533014.
E-mail address: vangelis0703@yahoo.com (Niko S. Radulović)

Abstract

Ethnopharmacological relevance: Mexican people employed infusion of leaves of *Choisya ternata* Kunth for their antispasmodic and “simulative properties”.

Aim of the study: In the present study the detailed GC and GC-MS analyses of the essential oil of *Choisya ternata* Kunth (Rutaceae) were performed. The presence of a minor constituent isopropyl *N*-methylantranilate (**1**) was revealed among other identified volatiles. A synthesis of **1** was undertaken in order to corroborate this find and obtain gram quantities that would allow the testing of its biological activity (peripheral and central antinociceptive activity).

Material and methods: The oils were investigated by GC and GC-MS. Synthesized compounds were spectrally characterized (UV-Vis, IR, 1D and 2D NMR, MS). The obtained synthetic samples of compounds were assayed for peripheral and central antinociceptive activity in two models (effects on acetic acid induced writhing in mice and the hot plate test for nociception).

Results: Detailed GC and GC-MS analyses of the essential oil of *Choisya ternata* Kunth (Rutaceae) among 157 other identified volatiles revealed the presence of a minor constituent isopropyl *N*-methylantranilate (**1**). Compound **1**, named ternanthranin, is therefore detected as a natural product for the first time with a very restricted occurrence (samples of several citrus oils were screened for the presence of **1**). The antinociceptive activities were assayed for ternanthranin, the two other synthetic analogs, methyl and propyl *N*-methylantranilate, as well as the essential oil and the crude ethanol extract of the leaves. The results clearly demonstrate a very high (even significant at 0.3 mg/kg) dose dependent activity for the anthranilates (and the extracts). Isopropyl *N*-methylantranilate showed the highest, while methyl *N*-methylantranilate showed the lowest activity (with the methyl ester at 3 mg/kg still better than acetylsalicylic acid, at 200 mg/kg, in the first, or comparable with morphine, at 5 mg/kg, in the second test).

Conclusion: This study once again revealed that detailed investigations of plant species with ethnopharmacologically documented activity may yield new natural compounds – a new alkaloid (ternanthranin), a volatile simple anthranilate that can be considered responsible for the antinociceptive activity of the crude plant extracts.

Keywords: *Choisya ternata*, essential oil, synthesis, esters of *N*-methylantranilic acid, isopropyl *N*-methylantranilate, peripheral and central antinociceptive activities.

1. Introduction

Mexican orange (*Choisya ternata* Kunth, Rutaceae, subfamily Rutoideae, Bayer et al., 2009) is a small evergreen ornamental shrub with leaves which when bruised give off a strong and pungent smell. The abundantly produced and highly fragrant flowers make Mexican Orange an important and popular horticultural plant. Despite its popularity, the volatile oils of this species have only been investigated in detail on one previous occasion (Respaud et al., 1997). Other previous phytochemical studies indicated the importance of its non-volatile anthranilate-derived alkaloid constituents. In particular, it has previously been shown to contain seven quinoline alkaloids (some of which widespread in the family Rutaceae): skimmianine, kokusaginine, 7-isopentenylxyloxy-c-fagarine, evoxine, choisyine, platydesminium methosalt and balfourodinium methosalt (Boyd et al., 2002; Grundon et al., 1974; Johns et al., 1967). Recently, Boyd et al. (2007) have isolated a range of seventeen quinoline alkaloids, involving several types of oxidations during their biosynthetic pathways, from leaves of *C. ternata*.

In 1895, Boudoresques studied this species and undertook the first pharmacological test by experimenting the effects of aqueous effects of leaves on himself: he found them tonic and appetizing. On the other hand, the oil is strongly repulsive. In 1923, Standley reported that Mexican people employed infusion of leaves for their antispasmodic and “simulative properties”; *C. ternata* was registered in the 4th and 5th edition of the Mexican Pharmacopoeia. As far as we know, the present use of *C. ternata* is not widespread. However, it may be recalled that some

Rutaceae species, such as *Ruta graveolens* and *Ptelea trifoliata*, which have same chemical constitution, are still used pharmaceutically (Creche et al., 1993).

The antispasmodic properties of the leaves are probably due to evoxine, which is known as sedative and spasmolytic. Kokusaganine has gangioplegic effects. The main tertiary alkaloids, skimmianine, is antidiuretic and hypothermic. Balfourodinium has been found to produce a slight spasmolytic effect on isolated rat duodenum (Creche et al., 1993).

Volatiles play a significant role in plant-plant, plant-insect and other relationships conveying important messages that usually require minute quantities of volatile secondary metabolites that are part of an inhomogeneous group of compounds (commonly jointly termed as essential oils). Many plants emit volatiles to attract pollinators or repel herbivores, especially useful at night when visual clues become insufficient. Essential oil constituents can be biosynthesized by several pathways, wherein benzenoids, isoprenoids and fatty acid derivatives are their most typical chemical classes (Knudsen et al., 1993).

Natural substances derived from plants have played an extremely important role in the development of analgesic drugs and in the understanding of the complex mechanisms involved in pain transmission and pain relief (Yunes et al., 2005). For example, salicin, a glycoside obtained from the bark of *Salix* species, was the lead compound for the synthesis of aspirin based on its activity and structural properties. Recently discovered antinociceptive substances include alkaloids, terpenoids and flavonoids (Calixto et al., 2000). Such findings have opened new possibilities for research into new potent analgesic drugs based on structure-activity relationships.

Due to the obvious lack of detailed studies of the volatile chemistry of this *Choisya* species we set our goal to determine the chemical composition of the leaf essential oil of *C. ternata*. The second aim of this study was to assess the activity of the essential oil, crude ethanol extract, and selected constituents of the oil in chemical (acetic acid-induced visceral pain) and thermal (hot-plate test)

models of nociception in mice and to possibly collate these results to the ethnomedical uses of this plant.

2. Material and methods

2.1. General

UV spectra (in acetonitrile) were measured using a UV-1650 PC Shimadzu spectrophotometer (Tokyo, Japan). The IR measurements (ATR-attenuated total reflectance) were carried out using a Thermo Nicolet model 6700 FTIR instrument (Waltham, USA). The NMR spectra were recorded on a Varian Gemini 200 (^1H at 200 MHz, ^{13}C at 50 MHz) spectrometer, using CDCl_3 as the solvent. Chemical shifts are expressed in δ (ppm) using TMS (Me_4Si) as an internal standard. 2D experiments (^1H - ^1H COSY, NOESY and HETCOR) and DEPT were run on the same instrument with the usual pulse sequences. Preparative medium-pressure liquid chromatography (MPLC) was performed with a pump module C-601 and a pump controller C-610 Work-21 pump (Büchi, Switzerland) and was carried out on pre-packed column cartridges (40x75 mm), Silica-gel 60, particle size distribution 40-63 μm , Büchi. Silica gel 60 on Al plates, layer thickness 0.2 mm (Kieselgel 60 F254, Merck) was used for thin layer chromatography (TLC). The spots on TLC were visualized by UV light (254 nm) and by spraying with 50% (v/v) aqueous H_2SO_4 followed by heating. Microanalysis of carbon, hydrogen and nitrogen were carried out with a Carlo Erba 1106 microanalyser; their results agreed favorably with the calculated values. All the reagents and solvents were obtained from commercial sources (Aldrich, USA; Merck, Germany; Fluka, Germany) and used as received, except the solvents were purified by distillation.

2.2. Plant material

Leaves of *C. ternata* were collected on the 20th October 2008 from the Trinity College Botanical Gardens, Dartry, Dublin (living plant accession number 19850023). One batch of plant material was dried at room temperature for one week. Another portion of the material was immediately submitted to hydrodistillation. Voucher specimens were deposited in the Herbarium of Trinity College Dublin (TCD) under the collection number SW 10-52.

2.3. Test animals

All experiments were performed with male Swiss mice (18-25 g) obtained from our own animal facilities (Laboratório de Farmacologia da Inflamação e do Óxido Nítrico). Animals were maintained in a room with controlled temperature 22 ± 2 °C for 12 h light/dark cycle with free access to food and water. Twelve hours before each experiment animals received only water, in order to avoid food interference with substances absorption. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), approved by the Biomedical Science Institute/UFRJ, Ethical Committee for Animal Research, and received the number DFBCICB-015.

Tested compounds (at 0.3, 1, and 3 mg/kg) were re-suspended in sterile corn oil and administered by oral gavages (p. o.) to mice. Essential oil and ethanol leaf extract were tested at 10, 30, and 100 mg/kg doses.

2.4. Isolation of essential oils and preparation of the ethanol extract

Fresh or air-dried, to constant weight, plant material (two batches of about 250 g) was subjected to hydrodistillation with *ca.* 2.5 L of distilled water for 2.5 h using the original Clevenger-type apparatus to produce the yellowish essential oils (about 0.25 ml per batch) in the yield (% v/w, typical values): 0.08% essential oil 1 (from fresh leaf tissue), and 0.10% essential oil 2 (from dried leaves). The obtained oils were separated by extraction with diethyl ether (Merck, Germany), dried over anhydrous magnesium sulphate (Aldrich, USA) and stored at -18 °C until analysis. Ground air-dried leaves of *C. ternata* were macerated for three days with ethanol (100 mL of ethanol per one gram of plant material) in the dark. The obtained extract was evaporated to dryness *in vacuo* at ambient temperature. The resulting oily residue was used in the antinociception assays.

2.5. Gas chromatography-mass spectrometry (GC-MS) analyses

Analyses of the oils were carried out by GC and GC/MS. The GC/MS analyses (three repetitions for each sample) were performed on a Hewlett-Packard 6890N gas chromatograph equipped with fused silica capillary columns HP-5MS and DB-5MS (5% phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 µm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 ° and 290 °C, respectively. Oven temperature was raised from 70° to 290 °C at a heating rate of 5 °C/min and then isothermally held for 10 min. As a carrier gas helium at 1.0 ml/min was used. The samples, 1 µl of the oil solutions in diethyl ether (1:100), were injected in a pulsed split mode (the flow was 1.5 ml/min for the first 0.5 min and then set to 1.0 ml/min throughout the remainder of the analysis; split ratio 40:1). Mass selective detector was operated at the ionization energy of 70 eV, in the 35–500 amu range and scanning speed of 0.34 s. GC (FID) analysis was carried out under

the same experimental conditions using the same columns as described for the GC/MS. The percentage composition was computed from the GC peak areas without the use of correction factors. Qualitative analyses of the essential oil constituents were based on the comparison of their linear retention indices relative to retention times of C_7 – C_{31} *n*-alkanes on the HP-5MS (van den Dool and Kratz, 1963) with those reported in the literature (Adams, 2007), and by comparison of their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils, and wherever possible, by co-injection with an authentic sample (as indicated in Table 1).

2.6. Reductive methylation of methyl anthranilate

Reductive methylation was accomplished following a literature method (da Silva et al., 2007) employing it for the first time on an *ortho* substituted aniline. A mixture of 1.51 g of methyl anthranilate (10 mmol), 2.40 g of glacial acetic acid (40 mmol), 0.92 g of 35% aqueous formaldehyde (11 mmol), 1.31 g of zinc dust (20 mmol) and 20 ml of dioxane was stirred and heated at 50-60 °C for the duration of 4 h. After completion of the reaction (monitored by TLC) aqueous ammonia was added and the reaction mixture was extracted with chloroform, followed by a usual work-up (drying with $MgSO_4$ and solvent evaporation) and gave 1.66 g of the crude product (consisting of *ca.* 80% of the wanted product, 8% of methyl *N,N*-dimethylantranilate and 12% of the starting ester, as inferred from a GC-MS analysis). A portion of this product, 0.77 g, was directly subjected to the transesterification procedure as described below. The yield (76%) of the methylation step was determined gravimetrically after an MPLC fractionation using a

gradient of hexane and diethyl ether. This also gave an analytical sample to be utilized for spectral characterization.

2.7. Alkaline transesterification of methyl *N*-methylantranilate

The same procedure was exploited for the synthesis of both isopropyl (**1**) and propyl *N*-methylantranilates (**3**). A solution of the corresponding alkoxide was prepared by dissolving 1.50 g of metallic sodium (80 mmol) in refluxing rigorously anhydrous alcohol (40 ml, 1- or 2-propanol) and cooled to room temperature. The crude methyl *N*-methylantranilate (6.5 mmol, calculated according to the results of a GC-MS run) was added to this solution and brought to reflux (CaCl₂ tube) and quenched with excess ice after 20 min. This was followed by immediate extraction of the reaction mixture with Et₂O, the solvent evaporated under reduced pressure and the residue chromatographed (MPLC, gradient Et₂O : hexane, from pure hexane to pure diethyl ether with an increment step of 5%). In the case of the isopropyl ester (**1**) 0.70 g of the pure product was obtained (a 56% conversion of the methyl to isopropyl ester was achieved), while the other one (**3**) was obtained in the yield of 66% (0.82 g).

2.8. Isopropyl *N*-methylantranilate

Isopropyl 2-(methylamino)benzoate; yellowish liquid; UV (CH₃CN) $\lambda_{\max}(\log \epsilon)$ 353 (3.62), 256 (3.78), 222 (4.24) nm; FTIR (neat) ν_{\max} 3374.7 (N-H), 2978.4 (C-H), 2818.8 (NCH₂-H), 1672.8 (C=O), 1605.7, 1579.0, 1517.1 (C=C), 1256.3 (C-O, asym.), 1230.9 (C-O, sym.), 746.1, 701.8 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; EIMS *m/z* 193 [M]⁺ (53.8), 105 [M – H₂C=CH-CH₃ – H₂O – CO]⁺ (100.0), 151 [M – H₂C=CH-CH₃]⁺ (62.3), 133 [M – H₂C=CH-CH₃ – H₂O]⁺ (37.3),

104 [M – H₂C=CH-CH₃ – H₂O – CO – H]⁺ (57.6), 77 [M – (CH₃)₂CHO – CH₂=NH – CO]⁺ (34.7), 134 [M – (CH₃)₂CHO]⁺ (25.5), 132 [M – H₂C=CH-CH₃ – H₂O – H]⁺ (22.2), 106 [M – COOCH(CH₃)₂]⁺ (17.4); Anal. C 68.30, H 7.85, N 7.19%, calcd for C₁₁H₁₅NO₂, C 68.37, H 7.82, N 7.25, O 16.56%; Rt 17.057 min, RI (HP-5MS) 1515.

2.9. Methyl *N*-methylantranilate

Methyl 2-(methylamino)benzoate; yellowish liquid; UV (CH₃CN) λ_{max}(log ε) 354 (3.73), 256 (3.89), 219 (4.32) nm; FTIR (neat) ν_{max} 3379.3 (N-H), 2948.6 (C-H), 2818.1 (NCH₂-H), 1678.3 (C=O), 1606.0, 1577.2, 1518.1 (C=C), 1233.5 (C-O, asym.), 1258.5 (C-O, sym.), 746.7, 702.0 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; EIMS *m/z* 165 [M]⁺ (100.0), 105 [M – CH₃OH – CO]⁺ (85.8), 104 [M – CH₃ – H₂O – CO]⁺ (84.4), 132 [M – CH₃ – H₂O]⁺ (54.4), 77 [M – C₃H₆O₂N]⁺ (47.9), 133 [M – CH₃OH]⁺ (29.7), 91 [M – C₃H₆O₂]⁺ (10.0); Anal. C 65.52, H 6.69, N 8.47%, calcd for C₉H₁₁NO₂, C 65.44, H 6.71, N 8.48, O, 19.37%; Rt 14.584 min, RI (HP-5MS) 1412.

2.10. Propyl *N*-methylantranilate

Propyl 2-(methylamino)benzoate; yellowish liquid; UV (CH₃CN) λ_{max}(log ε) 354 (4.42), 256 (4.02), 219 (3.86) nm; FTIR (neat) ν_{max} 3376.1 (N-H), 2965.6 (C-H), 2818.4 (NCH₂-H), 1677.0 (C=O), 1606.1, 1578.8, 1518.4 (C=C), 1256.7 (C-O, asym.), 1234.3 (C-O, sym.), 747.4, 702.0 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; EIMS *m/z* 193 [M]⁺ (83.3), 105 [M – H₂C=CH-CH₃ – H₂O – CO]⁺ (100.0), 151 [M – H₂C=CH-CH₃]⁺ (20.0), 133 [M – H₂C=CH-CH₃ – H₂O]⁺ (39.0), 104 [M – H₂C=CH-CH₃ – H₂O – CO – H]⁺ (61.6), 77 [M – C₃H₇O – CH₂=NH – CO]⁺ (39.1), 134 [M – C₃H₇O]⁺ (30.6), 132 [M – H₂C=CH-CH₃ – H₂O – H]⁺ (43.2), 106 [M – COOC₃H₇]⁺

(20.4); Anal. C 68.39, H 7.81, N 7.21%, calcd for C₁₁H₁₅NO₂, C 68.37, H 7.82, N 7.25, O 16.56%; Found.: Rt 18.734 min, RI (HP-5MS) 1586.

2.11. Isopropyl *N, N*-dimethylantranilate

Isopropyl 2-(dimethylamino)benzoate; EIMS *m/z* 207 [M]⁺ (38.0), 164 [M – (CH₃)₂CH]⁺ (100.0), 148 [M – (CH₃)₂CHO]⁺ (56.1), 132 [M – H₂C=CH-CH₃ – CH₃OH – H]⁺ (58.0), 118 [M – H₂C=CH-CH₃ – H₂O – CO – H]⁺ (42.7), 105 [M – H₂C=CH-CH₃ – CH₃OH – CO]⁺ (18.9), 77 [M – (CH₃)₂CHCOO – C₂H₅N]⁺ (28.9), 133 [M – H₂C=CH-CH₃ – CH₃OH]⁺ (20.9), 104 [M – H₂C=CH-CH₃ – CH₃OH – CO – H]⁺ (21.4); Rt 16.731 min, RI (HP-5MS) 1500.

2.12. Isopropyl anthranilate

Isopropyl 2-aminobenzoate; EIMS *m/z* 179 [M]⁺ (30.2), 119 [M – (CH₃)₂CHOH]⁺ (100.0), 137 [M – H₂C=CH-CH₃]⁺ (32.9), 92 [M – COOCH(CH₃)₂]⁺ (29.9), 120 [M – (CH₃)₂CHO]⁺ (25.5), 91 [M – (CH₃)₂CHOH – CO]⁺ (5.2); Rt 15.532 min, RI (HP-5MS) 1451.

2.13. Methyl *N, N*-dimethylantranilate

Methyl 2-(dimethylamino)benzoate; EIMS *m/z* 179 [M]⁺ (64.0), 164 [M – CH₃]⁺ (100.0), 148 [M – CH₃O]⁺ (88.1), 132 [M – CH₃OH – CH₃]⁺ (97.1), 77 [M – C₄H₈O₂N]⁺ (57.6), 91 [M – C₄H₈O₂]⁺ (56.4); Rt 14.445 min, RI (HP-5MS) 1406.

2.14. Methyl 2-(methyleneamino)benzoate

EIMS m/z 163 $[M]^+$ (30.3), 148 $[M - CH_3]^+$ (100.0), 132 $[M - CH_3O]^+$ (33.8), 105 $[M - C_2H_2O_2]^+$ (40.9), 77 $[M - C_3H_4O_2N]^+$ (57.1); Rt 13.372 min, RI (HP-5MS) 1363.

2.15. Methyl 2-(hydroxymethylamino)benzoate

EIMS m/z 181 $[M]^+$ (0.1), 119 (100.0), 148 (75.6), 92 (62.0), 151 (60.0), 77 (45.6), 120 (34.3), 105 (33.0), 65 (28.2), 132 (24.8), 163 (22.9), 181 (0.1); Rt (broad peak, apex) 15.150 min, RI (HP-5MS) 1435.

2.16. Propyl *N,N*-dimethylantranilate

Propyl 2-(dimethylamino)benzoate; EIMS m/z 207 $[M]^+$ (51.4), 148 $[M - C_3H_7O]^+$ (100.0), 164 $[M - C_3H_7]^+$ (97.6), 132 $[M - H_2C=CH-CH_3 - CH_3OH - H]^+$ (70.4), 118 $[M - H_2C=CH-CH_3 - H_2O - CO - H]^+$ (54.7), 105 $[M - H_2C=CH-CH_3 - CH_3OH - CO]^+$ (29.1), 91 $[M - C_6H_{12}O_2]^+$ (51.3), 77 $[M - C_3H_7COO - C_2H_5N]^+$ (41.5), 133 $[M - H_2C=CH-CH_3 - CH_3OH]^+$ (21.3), 104 $[M - H_2C=CH-CH_3 - CH_3OH - CO - H]^+$ (31.6); Rt 18.224 min, RI (HP-5MS) 1565.

2.17. Propyl anthranilate

Propyl 2-aminobenzoate; EIMS m/z 179 $[M]^+$ (39.8), 119 $[M - C_3H_7OH]^+$ (100.0), 137 $[M - H_2C=CH-CH_3]^+$ (13.4), 92 $[M - COOC_3H_7]^+$ (29.9), 120 $[M - OC_3H_7]^+$ (29.4), 91 $[M - C_3H_7OH - CO]^+$ (4.9); Rt 17.140 min, RI (HP-5MS) 1518.

2.18. Acetic acid-induced abdominal writhing test

Mice were treated according to Whittle (1964) and adapted by Matheus et al. (2005). Briefly, the total number of writhing following intraperitoneal administration of 2% (v/v) acetic acid (AA) was recorded over a period of 20 min, starting 5 min after AA injection. Mice were pre-treated with the test substances or acetylsalicylic acid (ASA - 200 mg/kg) or vehicle, 60 min before administration of AA.

2.19. Hot-plate test

Mice were tested according to the method described by Sahley and Berntson (1979) and adapted by Matheus et al. (2005). Animals were placed on a hot plate (Insight equipments, Brazil) set at 55 ± 1 °C. Reaction times were recorded when the animals licked their fore- and hind-paws and jumped at several intervals of 30 min after oral administration of the test substances, vehicle or morphine (5 mg/kg). Baseline was considered as the mean of reaction time obtained at 60 and 30 min before administration of the substances, vehicle or morphine and defined as normal reaction of animal to the temperature. Increase in baseline (%) was calculated by the formula: $((\text{reaction time} \times 100)/\text{baseline}) - 100$. Antinociception was quantified as area under the curve (AUC) of responses from 30 to 180 min after drug administration. The following formula, based on the trapezoidal rule, was used to calculate the AUC: $\text{AUC} = 30 \times \text{IB} [(\text{min}30)/2 + (\text{min} 60) + \dots + (\text{min} 150) + (\text{min} 180)/2]$, where IB is the increase in baseline (in %).

2.20. Statistical analysis

All experimental groups were composed of 6-10 mice. The results are presented as mean \pm S.D. The AUC was calculated by Prism Software 5.0 (GraphPad Prism Software). Statistical significance between groups was determined by the ANOVA analysis of variance followed by Bonferroni's test. P values less than 0.05 ($p < 0.05$) were used as the significance level (* when comparing a treated group with a vehicle-treated group and # when comparing a treated group with an ASA/morphine-treated group).

3. Results and Discussion

A portion of the plant material was extracted for essential oils immediately upon collection. The other portion was allowed to air dry in a dark unheated room and after one week the sample was extracted. This provided two oils from this plant, oil 1 (fresh material) and 2 (after drying). In general, the two samples of *C. ternata* gave rather poor yields of their respective leaf oils (0.08% v/w[1] and 0.10% v/w[2]).

Component identification along with their percent composition, RI values and methods of identification are summarized in Table 1. The GC and GC-MS analyses of the hydrodistillate from *C. ternata* leaves revealed a complex mixture of compounds of which 157 were identified (the extent of identification of components being more than 98% of the total detected GC peak areas). The main difference between the two samples (1 and 2) was the fact that sample 2 showed a slightly high concentration of sesquiterpenoids. Sample 2 had a total of 157 compounds identifiable including 0.2% hemiterpenoids, 83.8% monoterpenoids, 13.6% sesquiterpenoids, 0.2% diterpenoids and 0.9% other unclassified molecules (Table 1). The essential oils from leaves of *C. ternata* show sabinene (*ca.* one third of the oils), terpinen-4-ol (around 10%), myrcene (7.8-8.3%), β -phellandrene (5.4-6.6%) and γ -terpinene (4.2-4.7%) as the major oil

components. In contrast, the previously investigated leaf oil of *C. ternate* (Respaud et al., 1997) showed the presence of α -phellandrene (64-71%) and myrcene (11.5%) as the predominant components. The previously mentioned study (Respaud et al., 1997) on the oil of this species resulted in the identification of only eighteen components (all of which were also detected by us).

Table 1

Percentage composition of the essential oil of *Choisya ternata* Kunth (samples 1 and 2).

Rt/min§	RI	%1	%2	IdenMeth	Class	Component
2.256	765	0.1	0.1	a,b	HT	3-Methyl-2-buten-1-ol (syn. prenilol)
2.343	778	tr	tr	a,b	HT	3-Methyl-2-butenal (syn. prenal)
2.424	800	tr	tr	a,b,c	O	Octane
2.439	802	tr	tr	a,b,c	GL	Hexanal
2.882	847	tr	tr	a,b,c	HT/E	Ethyl isovalerate
2.920	851	tr	tr	a,b	GL	(<i>E</i>)-2-Hexenal
2.931	852	tr	tr	a,b	GL	(<i>E</i>)-3-Hexen-1-ol
3.026	862	tr	tr	a,b	GL	(<i>E</i>)-2-Hexen-1-ol
3.038	863	tr	tr	a,b,c	GL	1-Hexanol
3.317	892	tr	tr	a,b,c	HT/E	Isopropyl isovalerate
3.751	923	tr	tr	a,b	M	Tricyclene
3.826	928	0.5	0.5	a,b	MT	α -Thujene
3.954	936	1.0	1.0	a,b,c	MP	α -Pinene
4.083	945	tr	tr	a,b,c	HT/E	Propyl isovalerate
4.196	953	0.1	0.1	a,b,c	M	Camphene

4.370	964	tr	tr	a,b,c	O	Benzaldehyde
4.448	969	tr	tr	a,b,c	HT/E	Isoamyl propionate
4.628	980	36.5	35.1	a,b,c	MT	Sabinene
4.677	984	2.7	2.8	a,b,c	MP	β -Pinene
4.724	987	tr	tr	a,b	O	Methyl 5-methylhexanoate
4.808	993	7.8	8.3	a,b,c	MA	Myrcene
4.861	996	tr	tr	a,b	MM	Dehydro-1,8-cineole
4.912	999	tr	tr	a,b,c	O	Mesitylene
5.026	1005	tr	tr	a,b,c	HT/E	Isobutyl isovalerate
5.040	1006	tr	tr	a,b	GL	(<i>E</i>)-3-Hexenyl acetate
5.083	1008	tr	tr	a,b	MM	<i>p</i> -Mentha-1(7),8-diene (syn. pseudolimonene)
5.086	1008	0.2	0.2	a,b	MM	α -Phellandrene
5.199	1014	tr	tr	a,b	M	Δ^3 -Carene
5.309	1019	3.2	2.8	a,b,c	MM	α -Terpinene
5.457	1027	0.3	0.2	a,b,c	MM	<i>p</i> -Cymene
5.552	1031	2.3	2.0	a,b,c	MM	Limonene
5.555	1034	5.4	6.6	a,b	MM	β -Phellandrene
5.615	1035	3.0	2.2	a,b,c	MM	1,8-Cineole
5.790	1043	tr	tr	a,b,c	HT/E	Butyl isovalerate
5.855	1047	0.1	0.1	a,b	MA	(<i>E</i>)- β -Ocimene
6.132	1060	4.7	4.2	a,b,c	MM	γ -Terpinene
6.315	1069	1.2	1.3	a,b	MT	<i>cis</i> -Sabinene hydrate

6.570	1082	tr	tr	a,b	O	4-Methylhexyl acetate
6.762	1091	1.3	1.1	a,b	MM	Terpinolene
6.885	1098	tr	tr	a,b	GL	(Z)-3-Hexenyl propionate
6.961	1101	1.2	1.3	a,b,c	MA	Linalool
6.984	1102	0.8	0.8	a,b	MT	<i>trans</i> -Sabinene hydrate
7.053	1105	tr	tr	a,b,c	HT/E	Isopentyl isovalerate
7.121	1108	tr	tr	a,b,c	HT/E	2-Methylbutyl isovalerate
7.291	1115	tr	tr	a,b	MM	1,3,8-p-Menthatriene
7.313	1116	tr	-	a,b	M	(E)-4,8-Dimethyl-1,3,7-nonatriene
7.316	1116	tr	tr	a,b	HT/E	3-Methyl-3-butenyl 3-methylbutanoate
7.513	1124	0.8	0.7	a,b	MM	<i>cis</i> -p-Menth-2-en-1-ol
7.922	1142	0.5	0.4	a,b	MM	<i>trans</i> -p-Menth-2-en-1-ol
7.941	1142	tr	tr	a,b	GL	(Z)-3-Hexenyl isobutanoate
8.052	1147	tr	tr	a,b	MA	Ipsdienol
8.065	1148	tr	tr	a,b	MA	(E)-Myroxide
8.078	1148	tr	tr	a,b	HT/E	3-Methyl-3-butenyl valerate
8.280	1157	tr	0.1	a,b	MA	2,6-Dimethyl-1,5,7-octatrien-3-ol* (syn. Ocimenol)
8.409	1162	tr	tr	a,b	GL	(Z)-3-Heptenyl propionate
8.595	1170	0.1	0.1	a,b	MM	δ -Terpineol
8.714	1175	tr	-	a,b,c	GL	1-Nonanol
8.896	1183	10.5	9.9	a,b,c	MM	Terpinen-4-ol
9.022	1188	tr	tr	a,b	MM	p-Cymen-8-ol

9.048	1189	tr	tr	a,b,c	O	Naphthalene
9.103	1192	tr	tr	a,b	MM	Cryptone
9.167	1194	1.7	1.6	a,b,c	MM	α -Terpineol
9.281	1199	0.2	0.2	a,b	MM	<i>cis</i> -Piperitol
9.420	1205	tr	0.1	a,b	HT/E	Isohexyl 2-methylbutyrate
9.560	1210	0.3	0.2	a,b	MM	<i>trans</i> -Piperitol
9.649	1214	tr	tr	a,b	HT/E	Isoamyl isohexanoate
9.988	1228	tr	tr	a,b,c	MA	Citronellol
10.027	1229	tr	tr	a,b,c	MA	Nerol
10.087	1232	tr	-	a,b	GL	(<i>Z</i>)-3-Hexenyl 2-methylbutanoate
10.172	1235	tr	tr	a,b	GL	(<i>Z</i>)-3-Hexenyl 3-methylbutanoate
10.371	1243	tr	tr	a,b,c	MM	Ascaridole
10.661	1255	tr	tr	a,b	O	Chavicol
10.661	1255	tr	tr	a,b,c	MA	Geraniol
10.717	1257	tr	tr	a,b	O	9-Methyl-2-decanone
10.844	1262	tr	tr	a,b,c	GL	(<i>E</i>)-2-Decenal
11.089	1272	tr	tr	a,b,c	MA	Geranial
11.277	1279	tr	tr	a,b	GL	(<i>Z</i>)-2-Hexenyl valerate
11.506	1289	tr	tr	a,b,c	M	Isobornyl acetate
11.628	1293	0.1	0.1	a,b,c	O	2-Undecanone
11.643	1294	-	tr	a,b	MA	<i>trans</i> -Thio rose oxide
12.148	1314	tr	-	a,b,c	O	1-Methylnaphthalene
12.207	1317	tr	tr	a,b,c	O	p-Vinylguaiacol

12.238	1318	tr	tr	a,b	GL	(2 <i>E</i> ,4 <i>E</i>)-2,4-Decadienal
12.319	1321	tr	-	a,b,c	O	Isoamyl benzyl ether
12.388	1324	tr	tr	a,b	GL	(<i>Z</i>)-3-Hexenyl tiglate
12.576	1331	tr	tr	a,b	MM	p-Mentha-1,4-dien-7-ol
12.900	1344	tr	tr	a,b	MM	<i>exo</i> -2-Hydroxycineole acetate
13.106	1355	tr	tr	a,b,c	MA	Citronellyl acetate
13.238	1358	tr	tr	a,b	O	10-Methyl-2-undecanone
13.286	1360	0.6	0.8	a,b,c	O	Eugenol
13.390	1364	tr	tr	a,b,c	MA	Neryl acetate
13.762	1379	tr	tr	a,b,c	M	Isobornyl propanoate
13.793	1380	tr	tr	a,b	S	α -Copaene
13.869	1383	tr	tr	a,b,c	MA	Geranyl acetate
13.939	1386	tr	tr	a,b,c	O	Methyl (<i>E</i>)-cinnamate
13.974	1387	tr	tr	a,b	O	(<i>E</i>)- β -Damascenone
14.024	1389	0.1	tr	a,b,c	S	β -Bourbonene
14.169	1395	0.1	tr	a,b	S	β -Elemene
14.340	1402	-	tr	a,b,c	O	(<i>Z</i>)-Jasmone
14.421	1405	tr	tr	a,b,c	O	Methyl eugenol
14.566	1412	tr	tr	a,b,c	O	Methyl <i>N</i> -methylantranilate
14.885	1424	0.3	0.2	a,b,c	SCAR	β -Caryophyllene
15.107	1434	tr	tr	a,b	S	β -Copaene
15.475	1449	tr	-	a,b,c	S	Aromadendrene
15.620	1455	tr	tr	a,b	SCAD	<i>cis</i> -Muurolo-3,5-diene

15.714	1458	0.2	0.2	a,b,c	SCAR	α -Humulene
15.897	1466	tr	tr	a,b	SCAR	9- <i>epi</i> -(<i>E</i>)-Caryophyllene
15.935	1468	tr	tr	a,b	SCAD	<i>cis</i> -Cadina-1(6),4-diene
16.026	1472	tr	tr	a,b	SCAD	<i>cis</i> -Muurolo-4(14),5-diene
16.169	1477	tr	tr	a,b	SCAD	<i>trans</i> -Cadina-1(6),4-diene
16.235	1480	tr	tr	a,b	SCAD	γ -Muurolole
16.378	1486	1.8	1.4	a,b,c	SGER	Germacrene D
16.645	1497	tr	-	a,b	SCAD	<i>trans</i> -Muurolo-4(14),5-diene
16.685	1499	tr	0.1	a,b	S	<i>epi</i> -Cubebol
16.742	1501	0.1	tr	a,b	SGER	Bicyclogermacrene
16.797	1503	0.1	0.2	a,b	SCAD	α -Muurolole
16.810	1504	-	tr	a,b	SCAD	Epizonarene
16.914	1508	tr	tr	a,b	S	(<i>E,E</i>)- α -Farnesene
16.956	1510	tr	tr	a,b	SGER	Germacrene A
17.044	1514	tr	tr	a,b,c	O	Isopropyl <i>N</i> -methylantranilate
17.143	1518	0.1	0.1	a,b	SCAD	γ -Cadinene
17.178	1520	tr	tr	a,b	S	Cubebol
17.347	1527	1.0	1.2	a,b	SCAD	δ -Cadinene
17.380	1528	tr	tr	a,b	O	Chavibetol acetate
17.406	1530	tr	tr	a,b	SCAD	Zonarene
17.562	1536	tr	tr	a,b	SCAD	<i>trans</i> -Cadina-1,4-diene
17.689	1542	tr	tr	a,b	SCAD	α -Cadinene
17.953	1553	tr	tr	a,b	SGER	Hedycaryol

18.025	1556	tr	tr	a,b	S	Mintoxide
18.148	1561	tr	tr	a,b	SCAD	<i>cis</i> -Muurool-5-en-4 β -ol
18.222	1565	tr	tr	a,b,c	S	(<i>E</i>)-Nerolidol
18.402	1572	tr	tr	a,b	S	1,5-Epoxysalvial-4(14)-ene
18.599	1581	1.1	1.5	a,b	SGER	Germacrene D-4-ol
18.660	1583	tr	tr	a,b,c	S	Spathulenol
18.788	1589	0.1	0.1	a,b,c	SCAR	Caryophyllene oxide
18.898	1594	tr	-	a,b	S	4- <i>epi</i> -Cubebol
18.973	1597	tr	-	a,b	S	Globulol
19.030	1599	tr	tr	a,b	S	Salvial-4(14)-en-1-one
19.251	1609	0.1	0.1	a,b	S	Ledol
19.365	1614	0.5	0.6	a,b	S	β -Oplopenone
19.491	1620	tr	0.1	a,b	SCAD	1,10-di- <i>epi</i> -Cubenol
19.523	1621	tr	tr	a,b,c	O	(<i>E</i>)-Isoeugenyl acetate
19.783	1633	0.1	0.1	a,b	SCAD	1- <i>epi</i> -Cubenol
20.073	1646	0.5	0.5	a,b,c	SCAD	<i>epi</i> - α -Cadinol (syn. τ -cadinol)
20.097	1647	1.1	1.5	a,b	SCAD	<i>epi</i> - α -Murrolol (syn. τ -muurolol)
20.181	1651	0.3	0.4	a,b	SCAD	α -Muurolol (syn. torreyol)
20.247	1654	tr	-	a,b	S	β -Himachalol
20.396	1661	3.2	4.0	a,b	SCAD	α -Cadinol
20.962	1686	tr	tr	a,b	S	Eudesma-4(15),7-dien-1 β -ol
21.087	1692	0.6	0.9	a,b	SGER	Germacra-4(15),5,10(14)-trien-1 α -ol
21.199	1697	0.3	0.4	a,b	S	Shyobunol

22.214	1744	0.1	-	a,b	S	Mint sulphide
22.726	1769	tr	-	a,b,c	O	Benzyl benzoate
27.402	2003	0.1	0.1		D	Unidentified diterpene 1**
27.702	2020	0.1	0.1		D	Unidentified diterpene 2***
27.832	2027	tr	tr		D	Unidentified diterpene 3****
29.415	2116	tr	tr	a,b,c	D	(<i>E</i>)-Phytol
38.788	2700	tr	tr	a,b,c	O	Heptacosane
41.566	2900	tr	tr	a,b,c	O	Nonacosane
44.153	3100	tr	tr	a,b,c	O	Hentriacontane
<hr/>						
		99.0	98.5			Total identified
		0.1	0.2			Hemiterpenoids (HT)
		tr	0.1			Esters (HT/E)
		86.4	83.8			Monoterpenoids (M)
		66.1	65.0			Hydrocarbons
		20.3	18.8			Oxygenated
		39.0	37.7			Thujane (MT)
		3.7	3.8			Pinane (MP)
		9.1	9.8			Acyclic (MA)
		34.5	32.4			Menthane and related (MM)
		11.8	13.6			Sesquiterpenoids (S)
		3.8	3.3			Hydrocarbons
		8.0	10.3			Oxygenated

0.6	0.5	Caryophyllane and related (SCAR)
6.4	8.1	Cadinane and related (SCAD)
3.6	3.8	Germacrane and related (SGER)
0.2	0.2	Diterpenoids (D)
tr	tr	“Green leaf” volatiles (GL)
0.7	0.9	Others (O)

§ Compounds listed in order of elution on HP-5MS column (Rt-retention time (in min) and RI- experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C₇-C₃₁)

*correct stereoisomer not determined

** MS, 70 eV, 230 °C, *m/z*(rel. int.): 39(16), 40(3), 41(62), 43(19), 53(26), 55(56), 57(7), 65(13), 67(32), 68(11), 69(47), 77(34), 78(8), 79(52), 80(35), 81(49), 82(5), 83(9), 91(50), 92(18), 93(100), 94(26), 95(36), 96(3), 105(40), 106(15), 107(73), 108(26), 109(29), 115(1), 117(4), 119(45), 120(18), 121(39), 122(19), 123(20), 131(5), 133(24), 134(31), 135(30), 136(25), 137(20), 145(9), 147(23), 148(14), 149(9), 150(2), 159(12), 160(2), 161(20), 162(7), 173(5), 175(2), 187(3), 189(10), 201(2), 203(1), 229(14), 244(6), 257(12), 272(14).

*** MS, 70 eV, 230 °C, *m/z*(rel. int.): 39(24), 41(84), 43(25), 44(6), 53(43), 55(66), 56(3), 57(6), 65(20), 66(2), 67(69), 68(19), 69(38), 77(54), 78(11), 79(89), 80(17), 81(86), 82(8), 83(8), 91(84), 92(20), 93(92), 94(20), 95(48), 96(5), 97(3), 105(78), 106(24), 107(73), 108(24), 109(35), 117(14), 118(15), 119(89), 120(34), 121(100), 122(22), 123(25), 131(29), 132(24), 133(83), 134(47), 135(40), 136(12), 145(36), 146(20), 147(36), 148(18), 149(12), 159(37), 160(5), 161(51), 162(9), 173(40), 175(26), 187(23), 189(15), 201(25), 203(2), 229(28), 243(10), 257(61), 258(10), 272(18).

**** MS, 70 eV, 230 °C, *m/z*(rel. int.): 39(21), 41(75), 43(31), 53(43), 55(58), 67(55), 68(18), 69(38), 77(61), 79(76), 80(6), 81(78), 91(89), 92(11), 93(86), 95(47), 105(92), 106(27), 107(88), 108(38), 109(51), 119(97), 120(41), 121(100), 122(26), 123(15), 133(100), 134(80), 135(68), 136(26), 145(15), 147(34), 148(19), 159(18), 161(50), 162(30), 175(16), 189(28), 229(6), 257(66), 272(6).

tr- trace (<0.05%); syn. – synonym;

IdenMeth – identification method:

- a- constituent identified by mass spectra comparison;
- b- constituent identified by retention index matching;
- c- constituent identity confirmed by co-injection of an authentic sample.

Class:

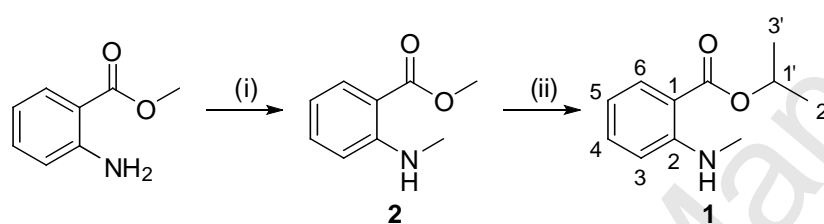
HT - HemiTerpenoids, HT/E - Hemiterpenoid Esters, M - Monoterpenoids, MT - Thujane Monoterpenoids, MP - Pinane Monoterpenoids, MA - Acyclic Monoterpenoids, MM - Menthane and related Monoterpenoids, S – Sesquiterpenoids, SCAR - Caryophyllane and related Sesquiterpenoids, SCAD - Cadinane and related Sesquiterpenoids, SGER - Germacrane and related Sesquiterpenoids, D- Diterpenoids, GL – “Green leaf “ volatiles, O – otherwise unclassified constituents (Others).

However, our attention was mostly focused on the minor components detected in the oils of this species. Two peaks in the GC chromatograms (detected in both samples 1 and 2) that were tentatively identified (based on mass spectral comparison with the MS from the Wiley-NIST data base) as methyl (**2**) and isopropyl (**1**) *N*-methylantranilates were of great interest since nitrogen volatiles are known as potent floral volatiles that are difficult to detect and are frequently missed out during the analyses (Jabalpurwala et al., 2009). A literature survey on these two compounds gave the following results: the methyl ester of *N*-methylantranilic acid (**2**) was previously reported from a number of Rutaceae species (belonging to one of the two sister subfamilies, Rutoideae and Aurantioideae, namely the genera *Zanthoxylum* (Detoni et al., 2009; Dreyer and Brenner, 1980), *Citrus* (Brophy et al., 2001; Jabalpurwala et al., 2009; Lota et al., 2000; Tomi et al., 2008), *Fortunella* (Quijano and Pino, 2009a, 2009b; Umano et al., 1994) and *Murraya* (Imai et al., 1989), respectively) with some sporadic (most frequently singular) occurrence (in low amount) in the unrelated genera *Chamaecyparis* (Thomas et al., 1966), *Corydalis* (Olesen and Knudsen, 1994), *Jasminum* (Calvarano, 1966; Cheng and Chao, 1979; Ito et al., 2002; Kaiser, 1988; Toda et al., 1983), *Mangifera* (Pino et al., 2005), *Michelia* (Kaiser, 1991), *Siphonochilus*

(Viljoen et al., 2002), and *Trifolium* (Honkanen et al., 1969). However, the isopropyl ester (**1**) has never been detected in a sample of natural origin. Methyl *N*-methylantranilate (**2**) is regarded as the marker compound of *Citrus recutita* (mandarin) essential oil, and the value of its isotopic $^{14}\text{N}/^{15}\text{N}$ ratio has been proposed as a means of natural sample authentication (Faulhaber et al., 1997). This compound was found to be important in the aroma of mandarin peel oil and the flavor of mandarin juice (Fanciullino et al., 2006, Wilson and Shaw, 1981), has been attributed with the attractiveness to Mexican fruit flies (*Anastrepha ludens*, Massa et al., 2008) and thrips (*Thrips coloratus*, Imai et al., 2001), as well as the repellency in bird species (e.g. *Sturnus vulgaris*, Clark et al., 1991; other species, Schafer et al., 1983), to possess the inhibitory effect on the L-alanine-induced initiation of spore germination in *Bacillus subtilis* (Prasad, 1974), and induce a chemotactic response of *Escherichia coli* (Ohba and Hayashi, 1979). It is also claimed to be phototoxic (Api, 1997) and to show an acute and short-term toxicity in rats (Gaunt et al., 1970). The *N*-demethylated ester (methyl anthranilate) is also a characteristic feature of the citrus oils (Jabalpurwala et al., 2009) but was not identified as the constituent of the currently investigated *C. ternata* oils. Also noteworthy is the fact that it serves an important biological role in plant defense as a bird repellent. The chemosensory irritation caused by this compound has been employed to not only protect crops (Avery et al., 2001; Curtis et al., 1994) but also prevent bird-aircraft accidents (Engeman et al., 2002).

Having all of this in mind we decided to corroborate our tentative structure assignments by comparing the chromatographic properties of the two oil components (supposedly methyl and isopropyl *N*-methylantranilates, **2** and **1**) to that of authentic material - synthetic samples of **1** and **2**. Isolation from the at hand oil samples has been dismissed as an option due to the complex oil matrix and their low relative abundance in the oils. We have undertaken a synthetic effort to produce gram quantities of the mentioned two esters, and since the procedure already present in

the literature (Staiger and Miller, 1959, Dembele et al., 1988) was not convenient (low yields and complex reaction mixtures) a new strategy was envisaged. The commercially available methyl anthranilate was the starting material and a two step transformation (*N*-methylation and transesterification) of this molecule to isopropyl *N*-methylantranilate (**1**) has been achieved in 43% overall yield. The reductive methylation, followed by transesterification with the isopropoxide was the preferred order of synthetic events (as depicted in Scheme 1).



Scheme 1. Synthesis of isopropyl *N*-methylantranilate (**1**); Reagents and conditions: (i) 1.1 eq HCHO(aq), 2 eq Zn, 4 eq AcOH(aq), stirring at 50 °C, 4 h, yield 76%; (ii) 10 eq Na⁺ ⁻OiPr, iPrOH, 20 min of reflux, yield 56%.

The reversed order gave a poor yield probably due to the steric hindrance of the isopropyl group rendering the necessarily transient *ortho* iminium cation unreactive to the *in situ* generated reducing agent (Zn + AcOH, da Silva et al., 2007). This relatively recent approach to the reductive methylation of primary and secondary amines was used since the classical ones gave unsatisfactory results (formaldehyde (aq) and NaBH₄ - the main isolated product were the imine - methyl *N*-methylenanthranilate and aminal - *N*-(hydroxymethyl)-derivative, while the direct methylation with MeI gave an irresolvable mixture of the mono- and dialkylated products along with a significant amount of the unreacted methyl anthranilate). In order to exclude the possibility that compound **1** is a propyl rather than the isopropyl ester we employed an analogous reaction

sequence and obtained propyl *N*-methylantranilate (**3**) in 50% total yield. To obtain pure samples of the three prepared anthranilates final reaction mixtures, after the usual workup, were subjected to a gradient MPLC (100% hexane to 100% Et₂O, with the increment step of 5%). All reported yields take this purification step into account. After the co-injection of these three synthesized compounds with the oil of *C. ternata*, the originally proposed hypothesis was corroborated and compounds **1** and **2** were proved to be isopropyl- and methyl *N*-methylantranilates.

The structural assignment of the synthesized compounds was achieved by spectral means (UV-Vis, IR, 1 and 2D NMR and MS). The ¹H NMR spectra of compounds **1**, **2** and **3** all displayed two doublet of doublets (dd 6.66-6.67, 7.92-7.96 ppm) and two ddd (6.59-6.62, 7.38-7.39 ppm) signals in the chemical shifts range of aromatic protons. This pattern was indicative of an aromatic core *ortho* substituted with one electron donating group and an electron withdrawing carbonyl group (due to the anisotropic deshielding of the *ortho* H to the C=O group). Additional common features of the three compounds were the signals originating from the NHMe protons. The *N*-methyl group unambiguously resonated at 2.90-2.92 ppm as a singlet. The exchangeable protons attached to the nitrogen atom pointed out to a subtle distinction between the methyl and propyl esters (appearing at δ 7.68 as a broad singlet for both **2** and **3**) on one side and the isopropyl (δ 7.75) on the other suggesting a somewhat different in strength intramolecular hydrogen bonding (again probably as a consequence of the more sterically demanding isopropyl group that tips the carbonyl of the ester outside of planarity with the aromatic ring). This is also evident from the corresponding IR vibrations of the N-H (3375, 3376, 3379 cm⁻¹, respectively for **1**, **3** and **2**). Thus, the spectra are consistent with the anthranilic acid core as the base structural fragment and the alcohol moieties making up the rest of the spectra. The ester groups appeared at δ 168.2-169.0 in the ¹³C NMRs, and gave strong IR absorptions at 1672.8-1678.3 cm⁻¹ confirming the connection modes of the alcohols and the acid, i.e. the presence of a conjugated

and strongly hydrogen bonded ester group. This is also strengthened by the fragmentation patterns visible in the MS spectra (with intense m/z corresponding to M^+ ions) of **1**, **2** and **3** that all possessed an important contribution of an ion corresponding to the loss of the OR groups, and the McLafferty fragmentation of the propyl and isopropyl esters giving the $[M-C_3H_6]^+$ ion of *N*-methylantranilic acid. The usual multiplicity and chemical shifts of hydrogens of the methyl, propyl and isopropyl esters of carboxylic acids can be readily seen from the 1H NMRs. All data on the 1H NMR of these three compounds are summarized in Table 2. The ^{13}C NMRs contained the expected number of signals for compounds **2** (nine signals) and **3** (eleven signals), but in the case of the isopropyl derivative it appeared to have one carbon atom signal missing (nine instead of ten). This situation was resolved by simple comparison of the spectrum of **1** with those of **2** and **3**, that suggested that the signal at δ 110.5 was arising from an overlap of two Cs (these are at 109.8 and 110.6 ppm for **2**, and 110.2 and 110.7 ppm for **3**). The assignment of all proton and carbon NMR signals was made possible only by the use of 2D NMR (1H - 1H COSY and HETCOR spectra) as well as comparison with literature data (Yoshikawa et al., 1994). Correlations observed in these spectra give rise to the assignments given in Table 2. For example, the overlapping signals at 110.5 ppm for **1** corresponded to C-1 and C-3.

Table 2NMR Spectroscopic Data (200 MHz, CDCl₃) for compounds **1**, **2** and **3**.

position	Isopropyl <i>N</i> -methylantranilate (1)		Methyl <i>N</i> -methylantranilate (2)		Propyl <i>N</i> -methylantranilate (3)	
	δ_C , mult.	δ_H (<i>J</i> in Hz)	δ_C , mult.	δ_H (<i>J</i> in Hz)	δ_C , mult.	δ_H (<i>J</i> in Hz)
1	110.5 ^a C		109.8 C		110.2 C	
2	152.0 C		152.0 C		152.1 C	
3	110.5 ^a CH	6.67, dd (1.0, 8.7)	110.6 CH	6.66, dd (1.0, 8.6)	110.7 CH	6.66, dd (1.2, 8.6)
4	134.3 CH	7.39, ddd (1.7, 7.1, 8.7)	134.5 CH	7.38, ddd (1.6, 7.1, 8.6)	134.5 CH	7.38, ddd (1.6, 7.2, 8.6)
5	114.1 CH	6.62, ddd (1.0, 7.1, 8.0)	114.2 CH	6.60, ddd (1.0, 7.1, 8.0)	114.3 CH	6.59, ddd (1.2, 7.2, 8.0)
6	131.4 CH	7.96, dd (1.7, 8.0)	131.5 CH	7.92, dd (1.6, 8.0)	131.5 CH	7.93, dd (1.6, 8.0)
7	168.2 C		169.0 C		168.8 C	
1'	67.2 CH	5.23, septet (6.2)	51.1 CH ₃	3.85, s	65.7 CH ₂	4.21 t (7.1)
2'	21.8 CH ₃	1.38, d (6.2)	-	-	22.0 CH ₂	1.77, sextet (7.1)
3'	21.8 CH ₃	1.38, d (6.2)	-	-	10.4 CH ₃	1.02, t (7.1)
N-CH ₃	29.3 CH ₃	2.92, s	29.3 CH ₃	2.90, s	29.4 CH ₃	2.90, br s
N-H		7.75, s		7.68, s		7.68, s

^a Overlapping signals.

The obtained synthetic samples of compounds **1-3**, as well as the essential oil and the ethanol extract of leaves were assayed for peripheral and central antinociceptive activity in two models used to evaluate the potential analgesic activity of drugs. The results for the first time show that the essential oil and crude ethanol leaf extract of *C. ternata*, and all of the anthranilates, when given orally, produce dose-related and significant antinociception according to assessment of the abdominal constrictions elicited by acetic acid and the hot plate test (Figure 1). The tested natural product isopropyl *N*-methylantranilate (at 3 mg/kg) was more potent and efficacious than aspirin (at 200 mg/kg) in the acetic acid induced writhings assay and all the anthranilates were, at 3

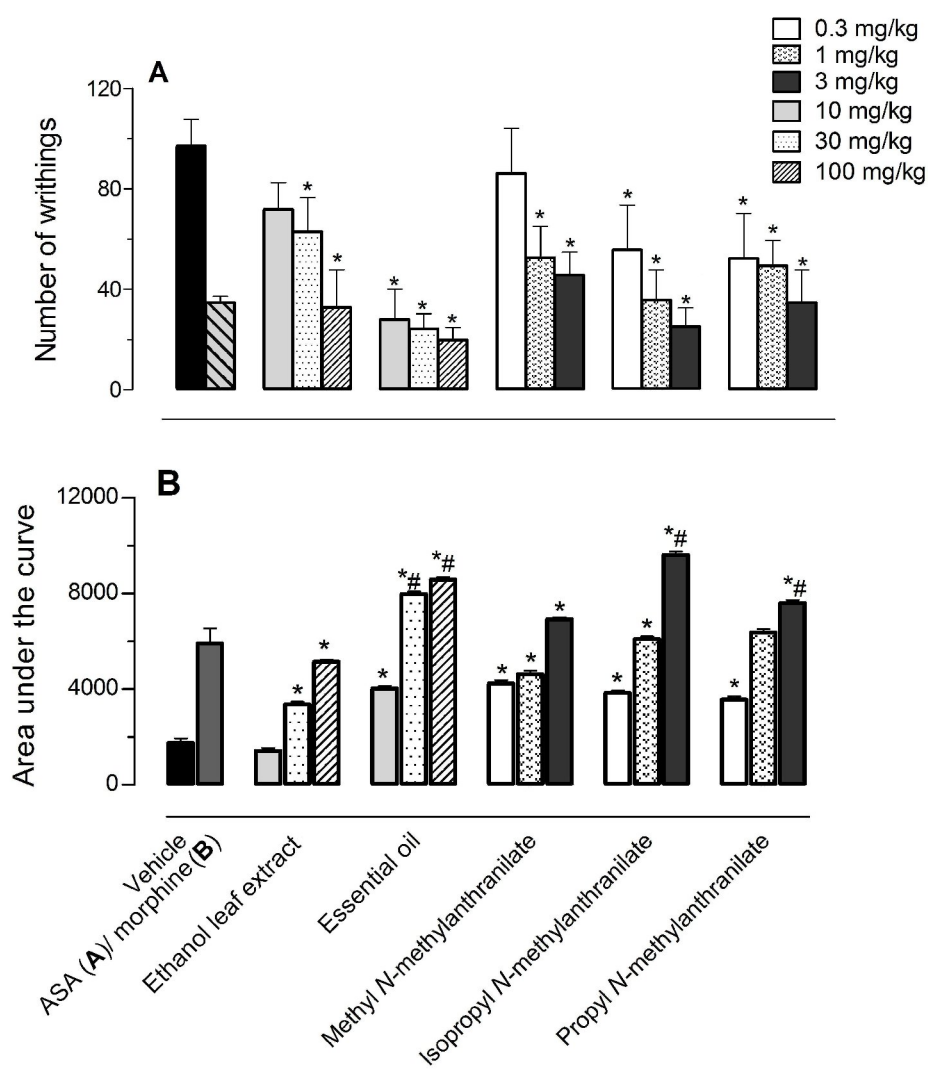
mg/kg, more potent, and at a dose of 0.3 mg/kg, of a similar efficacy in relation to morphine (at 5 mg/kg) in inhibiting the heat-induced nociceptive response. The results of the first model (acetic acid induced writhing) showed that the activity was dependent on the substitution pattern in the following crescent order: **2** < **3** < **1** (Figure 1). The responses to the thermal stimuli in the hot plate test (the second model) revealed that the substitution pattern also plays an important role in this case (the same crescent order: **3** < **1** again with methyl *N*-methylantranilate displaying the least activity (Figure 1)). The maximum response for isopropyl *N*-methylantranilate was achieved at 60 minutes after the administration of the drug (at 10 mg/kg) and the effect goes down up to 120 minutes when no effect can be seen. With morphine the maximum effect is shown at 90 minutes after the administration of the drug and although it goes down with time it is still high and significant after 180 minutes.

Another interesting result of this current study was the fact that p.o. administration of the essential oil of the leaves of *C. ternata* exhibited one hundred times less potency and efficacy when compared with isopropyl *N*-methylantranilate in preventing the acetic acid-induced pain. This fits nicely with idea that the antinociceptive activity of the oil (that can be roughly regarded as a 100-1000 fold dilution of the anthranilates) is caused by the presence of the two anthranilates (the summed content of the methyl and isopropyl *N*-methylantranilates is *ca.* 0.1%). Possibly the higher activity of the ethanol extract could be linked to other non-volatile (alkaloid) constituents of *C. ternata* leaves.

Since the nociceptive neurons are sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and to narcotics and other centrally acting drugs (Yunes et al., 2005; Calixto et al., 2000), and although the anthranilates possess a high degree of structural similarity to the known antinociceptive molecule ASA and have a different time dependent nature of action when

compared with morphine, a deeper evaluation of these simple compounds (anthranilates), using antagonists and antibodies, is necessary in order to understand their mechanism of action.

Figure 1. A – Effects of the anthranilates **1-3**, essential oil and ethanol leaf extract of *C. ternata* on acetic acid-induced writhings in mice; **B** – Hot-plate test results. The vehicle and acetylsalicylic acid (ASA, 200 mg/kg) or morphine (5 mg/kg) were p. o. administered to the control groups. * $p < 0.05$ when comparing a treated group with a vehicle-treated group and # $p < 0.05$ when comparing a treated group with an ASA/morphine-treated group.



4. Conclusion

Hence, these arguments confirm **1** as a new and biologically/ecologically interesting natural product. It is our proposal that this compound should be named ternanthranin for its very restricted occurrence in the natural world. We have screened commercially available samples of various oils of *Citrus* sp. for the presence of methyl and isopropyl *N*-methylantranilates. Neither of the analyzed oils had the new alkaloid as its constituent, and only the oil of mandarin possessed a certain quantity (less than 1%) of the methyl ester (The volatile oils from peel of grapefruit, bergamot, lemon and orange contained neither the methyl nor the isopropyl esters.). This constituent is, thus, a strong marker compound, and might have additional chemotaxonomical implications as new investigations provide further information on its natural distribution.

Yet another aspect of the natural occurrence of this compound deserves comment. It is by no means an accident that the plant species that represents a rich source of anthranilate-derived quinoline alkaloids would be the one to produce two volatile alkaloids that probably originate from the commencement of the same pathway (the enzyme that is considered to perform the *N*-methylation step has been isolated and characterized, Maier et al., 1995) either as a side product or as, and this is a mere speculation, a regulatory molecule of this metabolism. One other possibility is that **1** is merely an artefactual compound formed during hydrodistillation from the mentioned quinoline alkaloids or **2**, but this seems unlikely due to two facts: 1) the methyl ester is an established natural product (whose biosynthesis has been investigated, Maier et al., 1995) and 2) the acid catalyzed (trans)esterification of *N*-methylantranilic acid/esters (mimicking the hydrodistillation conditions) with isopropanol was entirely unsuccessful (this was our first attempt in the synthesis of **1**) giving not even trace amounts of the desired ester.

In summary, the results of the present study demonstrate for the first time that the ethanol extract and essential oil, as well as one of its minor alkaloid constituents from the leaves of *C. ternata* produce dose-related antinociceptive action in chemical (acetic acid-induced visceral pain) and thermal (hot-plate test) models of nociception in mice. The mechanism by which the isopropyl *N*-methylantranilate produces antinociception still remains unclear, but pharmacological studies are continuing so as to characterize the mechanism(s) responsible for the antinociceptive action. Furthermore, the antinociceptive action demonstrated in the present study supports, at least partly, the ethnomedical uses of this plant.

Acknowledgements

The authors are grateful to the Botany Department, Faculty of Natural Sciences, Trinity College Dublin as well as to the Staff of the Botanic Gardens, TCD. The authors from Brazil thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant support and fellowship to PDF), Fundação Carlos Chagas Filho de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ, grant support to PDF), and Conselho de Administração de Pessoal de Ensino Superior (CAPES, fellowship to MMGP). FSM is grateful to the Start up grant No. 51002.516.R01336 from Trinity College Dublin. The authors from Serbia acknowledge funds received from the Ministry of Science and Technological Development of Serbia (Project 172061).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

References

- Adams, R.P., 2007. Identification of essential oil components by gas chromatography and mass spectrometry, fourth ed. Allured Publishing Corporation, Carol Stream, Illinois USA.
- Api, A.M., 1997. *In vitro* assessment of phototoxicity. *In Vitro Toxicology*. 10, 339-350.
- Avery, M.L., Tillman, E.A., Laukert, C.C., 2001. Evaluation of chemical repellents for reducing crop damage by Dickcissels in Venezuela. *International Journal of Pest Management*. 47, 311-314.
- Bayer, R.J., Mabblerley, D.J., Morton, C., Miller, C.H., Sharma, I.K., Pfeil, B.E., Rich, S., Hitchcock, R., Sykes, S., 2009. A molecular phylogeny of the orange subfamily (Rutaceae: Aurantioideae) using nine cpDNA sequences. *American Journal of Botany*. 96, 668-685.
- Boyd, D.R., Sharma, N.D., Loke, P.L., Malone, J.F., McRoberts, W.C., Hamilton, J.T.G., 2002. Absolute configuration assignment and enantiopurity determination of chiral alkaloids and coumarins derived from O- and C-prenyl epoxides. *Chemical Communications*. 24, 3070-3071.
- Boyd, D.R., Sharma, N.D., Loke, P.L., Malone, J.F., McRoberts, W.C., Hamilton, J.T.-G., 2007. Synthesis, structure and stereochemistry of quinoline alkaloids from *Choisya ternata*. *Organic & Biomolecular Chemistry*. 5, 2983-2991.
- Brophy, J.J., Goldsack, R.J., Forster, P.I., 2001. The leaf oils of the Australian species of *Citrus* (Rutaceae). *Journal of Essential Oil Research*. 13, 264-268.
- Calixto, J.B., Beirith, A., Ferreira, J., Santos, A.R.S., Filho, V.C., Yunes, R.A., 2000. Naturally occurring antinociceptive substances from plants. *Phytotherapy Research*. 14, 401-418.
- Calvarano, M., 1966. Composition of Calabria jasmine essences. II. Gas-chromatographic analysis. *Essenze, Derivati Agrumari*. 36, 237-255.

- Cheng, Y.-S., Chao, Y.-L., 1979. Absolute of Siu-eng flower (*Jasminum odoratissimum*). International congress of essential oils, [Pap.] 7th. 7, 467-469.
- Clark, L., Shah, P.S., Mason, J., Russell, J., 1991. Chemical repellency in birds: relationship between chemical structure and avoidance response. *Journal of Experimental Zoology*. 260, 310-322.
- Creche, J., Tremouillaux-Guiller, J., Groeger, D., Chenieux, J.C., Rideau, M., 1993. *Choisya ternata* (Mexican orange): in vitro culture and the production of alkaloids, in: Bajaj, Y.P.S. (Ed.), *Biotechnology in Agriculture and Forestry 24 (Medicinal and Aromatic Plants V)*. Springer, Berlin Heidelberg New York, pp. 107-121.
- Curtis, P.D., Merwin, I.A., Pritts, M.P., Peterson, D.V., 1994. Chemical repellents and plastic netting for reducing bird damage to sweet cherries, blueberries, and grapes. *Horticultural Science*. 29, 1151-1155.
- Dembele, A., Deshayes, H., Pete, J.P., 1988. Reduction and photoreduction of carbonic acid derivatives: effect of hexamethylphosphorotriamide. *Bulletin de la Societe Chimique de France*. 4, 671-680.
- Detoni, C.B., Cabral-Albuquerque, E.C., Hohlemweger, S.V., Sampaio, C., Barros, T.F., Velozo, E.S., 2009. Essential oil from *Zanthoxylum tingoassuiba* loaded into multilamellar liposomes useful as antimicrobial agents. *Journal of Microencapsulation*. 26, 684-691.
- Dreyer, D.L., Brenner, R.C., 1980. Chemotaxonomy of the Rutaceae. Part XIII. Alkaloids of some Mexican *Zanthoxylum* species. *Phytochemistry*. 19, 935-939.
- Engeman, R.M., Peterla, J., Constantin, B., 2002. Methyl anthranilate aerosol for dispersing birds from the flight lines at Homestead Air Reserve Station. *International Biodeterioration & Biodegradation*. 49, 175-178.

- Fanciullino, A.-L., Tomi, F., Luro, F., Desjobert, J.M., Casanova, J., 2006. Chemical variability of peel and leaf oils of mandarins. *Flavour and Fragrance Journal*. 21, 359-367.
- Faulhaber, S., Hener, U., Mosandl, A., 1997. GC/IRMS Analysis of mandarin essential oils. 1. $\delta^{13}\text{C}_{\text{PDB}}$ and $\delta^{15}\text{N}_{\text{AIR}}$ values of methyl *N*-methylantranilate. *Journal of Agricultural and Food Chemistry*. 45, 2579-2583.
- Gaunt, I.F., Sharratt, M., Grasso, P., Wright, M., 1970. Acute and short-term toxicity of methyl-*N*-methylantranilate in rats. *Food and Cosmetics Toxicology*. 8, 359-368.
- Grundon, M.F., Harrison, D.M., Spyropoulos, C.G., 1974. Biosynthesis of aromatic isoprenoids. Part II. Aromatic hydroxylation in the biosynthesis of the furoquinoline alkaloids, skimmianine, evoxine, and choisyne. *Journal of the Chemical Society Perkin Transactions 1*. 2181-2184.
- Honkanen, E., Moisio, T., Karvonen, P., 1969. Volatile flavor substances in some clover species. *Suomen Kemistilehti B*. 42, 448-451.
- Imai, F., Itoh, K., Kishibuchi, N., Kinoshita, T., Sankawa, U., 1989. Constituents of the root bark of *Murraya paniculata* collected in Indonesia. *Chemical & Pharmaceutical Bulletin*. 37, 119-123.
- Imai, T., Maekawa, M., Murai, T., 2001. Attractiveness of methyl anthranilate and its related compounds to the flower thrips, *Thrips hawaiiensis* (Morgan), *T. coloratus* Schmutz, *T. flavus* Schrank and *Megalurothrips distalis* (Karny) (Thysanoptera: Thripidae). *Applied Entomology and Zoology*. 36, 475-478.
- Ito, Y., Sugimoto, A., Kakuda, T., Kubota, K., 2002. Identification of potent odorants in Chinese jasmine green tea scented with flowers of *Jasminum sambac*. *Journal of Agricultural and Food Chemistry*. 50, 4878-4884.
- Jabalpurwala, F.A., Smoot, J.M., Rouseff, R.L., Russell, L., 2009. A comparison of citrus blossom volatiles. *Phytochemistry*. 70, 1428-1434.

- Johns, S.R., Lamberton, J.A., Sioumis, A.A., 1967. Alkaloids of *Choisya ternata*, The structure of choisyine. Australian Journal of Chemistry. 20, 1975-1981.
- Kaiser, R. 1988. New volatile constituents of *Jasminum sambac* (L.) Aiton, in: Lawrence, B.M., Mookherjee, B.D., Willis, B.J. (Eds.), Flavors and fragrances: a world perspective. Proceedings of the 10th International congress essential oils. Elsevier Sci. Publ., Amsterdam, pp. 669-684.
- Kaiser, R., 1991. New volatile constituents of the flower concrete of *Michelia champaca* L. Journal of Essential Oil Research. 3, 129-146.
- Knudsen, J.T., Tollsten, L., Bergstroem, L.G., 1993. Floral scents. A checklist of volatile compounds isolated by head-space techniques. Phytochemistry. 33, 253-280.
- Lota, M.-L., de Rocca Serra, D., Tomi, F., Casanova, J., 2000. Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco. Biochemical Systematics and Ecology. 28, 61-78.
- Maier, W., Baumert, A., Groeger, D., 1995. Partial purification and characterization of S-adenosyl-L-methionine:anthranilic acid *N*-methyltransferase from *Ruta* cell suspension cultures. Journal of Plant Physiology. 145, 1-6.
- Massa, M.M, Robacker, D.C., Patt, J., 2008. Identification of grape juice aroma volatiles and attractiveness to the Mexican fruit fly (Diptera: Tephritidae). Florida Entomologist. 91, 266-276.
- Matheus, M.E., Berrondo, L.F., Vieitas, E.C., Menezes, F.S., Fernandes, P.D., 2005. Evaluation of antinociceptive properties from *Brillantaisia palisotti* stems extracts. Journal of Ethnopharmacology. 102, 377-381.
- Ohba, M., Hayashi, H., 1979. Studies on bacterial chemotaxis. III. Effect of methyl esters on the chemotactic response of *Escherichia coli*. Journal of Biochemistry. 85, 1331-1338.

- Olesen, J.M., Knudsen, J.T., 1994. Scent profiles of flower color morphs of *Corydalis cava* (Fumariaceae) in relation to foraging behavior of bumblebee queens (*Bombus terrestris*). *Biochemical Systematics and Ecology*. 22, 231-237.
- Pino, J.A., Mesa, J., Munoz, Y., Marti, M.P., Marbot, R., 2005. Volatile components from mango (*Mangifera indica* L.) cultivars. *Journal of Agricultural and Food Chemistry*. 53, 2213-2223.
- Prasad, C., 1974. Initiation of spore germination in *Bacillus subtilis*. Relation to inhibition of L-alanine metabolism. *Journal of Bacteriology*. 119, 805-810.
- Quijano, C.E., Pino, J.A., 2009a. Volatile compounds of kumquat (*Fortunella margarita* (Lour.) Swingle) leaf oil. *Journal of Essential Oil Research*. 21, 194-196.
- Quijano, C.E., Pino, J.A., 2009b. Volatile compounds of round kumquat (*Fortunella japonica* Swingle) peel oil from Colombia. *Journal of Essential Oil Research*. 21, 483-485.
- Respaud, M.-J., Moulis, C., Fouraste, I., Bessiere, J.-M., 1997. Essential oil composition of *Choisya ternata* Kunth (Rutaceae) leaves. *Journal of Essential Oil Research*. 9, 475-476.
- Sahley, T.L., Berntson, G.G., 1979. Antinociceptive effects of central and systemic administrations of nicotine in the rat. *Psychopharmacology*. 65, 279-283.
- Schafer Jr., E.W., Bowles Jr., W.A., Hurlbut, J., 1983. The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds. *Archives of Environmental Contamination and Toxicology*. 12, 355-382.
- da Silva, R.A., Estevam, I.H.S., Bieber, L.W., 2007. Reductive methylation of primary and secondary amines and amino acids by aqueous formaldehyde and zinc. *Tetrahedron Letters*. 48, 7680-7682.
- Staiger, R.P., Miller, E.B., 1959. Isatoic anhydride. IV. Reactions with various nucleophiles. *Journal of Organic Chemistry*. 24, 1214-1219.

- Thomas, A.F., Stoll, M., Palluy, E., 1966. Hinoki leaf Oil. Riechstoffe, Aromen, Koerperpflegemittel. 16, 42-48.
- Toda, H., Mihara, S., Umamo, K., Shibamoto, T., 1983. Photochemical studies on jasmine oil. Journal of Agricultural and Food Chemistry. 31, 554-558.
- Tomi, F., Barzalona, M., Casanova, J., Luro, F., 2008. Chemical variability of the leaf oil of 113 hybrids from *Citrus clementina* (Commun) x *Citrus deliciosa* (Willow Leaf). Flavour and Fragrance Journal. 23, 152-163.
- Umamo, K., Hagi, Y., Tamura, T., Shoji, A., Shibamoto, T., 1994. Identification of volatile compounds isolated from round kumquat (*Fortunella japonica* Swingle). Journal of Agricultural and Food Chemistry. 42, 1888-1890.
- Van den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography. 11, 463-471.
- Viljoen, A.M., Demirci, B., Baser, K.H.C., van Wyk, B.-E., 2002. The essential oil composition of the roots and rhizomes of *Siphonochilus aethiopicus*. South African Journal of Botany. 68, 115-116.
- Whittle, B.A., 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. British Journal of Pharmacology. 22, 246-253.
- Wilson, C.W., III, Shaw, P.E., 1981. Importance of thymol, methyl *N*-methylantranilate, and monoterpene hydrocarbons to the aroma and flavor of mandarin cold-pressed oils. Journal of Agricultural and Food Chemistry. 29, 494-496.
- Yoshikawa, K., Taninaka, H., Kan, Y., Arihara, S., 1994. Antisweet natural products. XI. Structures of sitakisosides VI-X from *Stephanotis lutchuensis* Koidz. var. *japonica*. Chemical & Pharmaceutical Bulletin. 42, 2455-2460.

Yunes, R.A., Cechinel F.V., Ferreira, J., Calixto, J.B., 2005. The use of natural products as sources of new analgesic drugs, in: *Studies in Natural Products Chemistry (2005)*, 30 (Bioactive Natural Products (Part K)). Publisher: Elsevier B.V., pp. 191-212.

Accepted Manuscript

Identification of a new antinociceptive alkaloid isopropyl *N*-methylantranilate from the essential oil of *Choisya ternata* Kunth

Niko S. Radulović^a, Ana B. Miltojević^a, Michael McDermott^b, Steve Waldren^c, John Adrian Parnell^c, Mariana Martins Gomes Pinheiro^d, Patricia Dias Fernandes^d and Fabio de Sousa Menezes^b

^aDepartment of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

^bPanoz Institute, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin 23, Westland Row, Dublin 2, Ireland

^cDepartment of Botany, School of Natural Sciences, Trinity College, Dublin 2, Ireland

^dLaboratório de Farmacologia da Inflamação e do Óxido Nítrico, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

