Insecticidal Activity of Individual and Mixed Monoterpenoids of Geranium Essential Oil Against *Pediculus humanus capitis* (Phthiraptera: Pediculidae)

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ABSTRACT The major components of geranium (*Geranium maculatum* L.) oil and their mixtures were tested against female *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae). Chemical analysis by gas chromatography coupled to mass spectrometry revealed four major constituents: citronellol (38%), geraniol (16%), citronellyl formate (10.4%), and linalool (6.45%) (concentration expressed as percentage of total). Topical application demonstrated that the most potent component was citronellol and geraniol, with LD_{50} values 9.7 and 12.7 μg/insect, respectively. Linalool and Citronellyl formate were less toxic with LD_{50} values 24.7 and 38.5 μg/insect, respectively. Toxicity of these four major constituents in the same proportion as the natural oil, was greater than whole oil and each individual component. Removal of any four constituents produced a decreased in effectiveness. The absence of citronellol caused the greatest decrease in toxicity (DL_{50} from 2.2 to 10.9 μg/insect), leading us to conclude that this constituent is the major contributor to oil toxicity. The knowledge of the role of each constituent in the toxicity of the whole oil gives the possibility to create artificial blends of different constituents for the development of more effective control agents.

KEY WORDS geranium oil, *Geranium maculatum*, artificial mixture, synergism, head lice

Human pediculosis is a condition whereby lice (Phthiraptera: Pediculidae) infest the head (*Pediculus humanus capitis* De Geer) and body of human hosts (*Pediculus humanus humanus* L.). Where conventional pediculicides fail as the result of insecticide resistance (Picollo et al. 1998; Mougabure Cueto et al. 2008, Burgess 2004, Kasai 2009), investigations continue to focus on the development of selective human louse control alternatives. Plant essential oils have been suggested as an alternative to control a variety of insect pests because they constitute a rich source of bioactive chemicals (Isman 1999).

Plant essential oils are the odorous constituents and secondary metabolites than can be separated from plants through steam distillation. Most products are mixtures of monoterpenes, sesquiterpenes, and monophenols. They are often quite volatile and are commonly used as fragrances and food flavoring additives (Isman 2000).

Considering that plants usually present defenses as a suite of compounds, other workers have looked at the efficacy of individual terpenoids compared with whole oil against human lice. Weston et al. (1997) showed that components of cypress oil such as terpineol, camphene, and α-pinene were more toxic than the whole oil. Toloza et al. (2006) demonstrated that the essential oil from the native *Myrcianthes cisplatensis* Cambess, was most effective than it components 1,8-cineol, limonene, α-terpineol, linalool, and α-pinene.

We assumed that an essential oil is more than the sum of its parts, and the interactions between the components are a vital part of the insecticidal activity. Therefore, we evaluated geranium (*Geranium maculatum* L.) oil, and its major components, for pediculicidal activity and synergy against female *P. humanus capitis.*

Materials and Methods

Insects. Female *P. humanus capitis* were used in this study and obtained using a fine-toothed comb from heads of infested children (6–12 yr old) from elementary schools in several areas of Buenos Aires, Argentina. The study was carried out according to a protocol approved by ad hoc committee of the Research Centre of Pests and Pesticides (Buenos Aires, Argentina) (Research Centre of Pests and Pesticides, unpublished data); briefly, the required permission was received from parents, schools authorities, and the Ministry of Education of Buenos Aires City. After collection, insects were transported to our laboratory using the method of Picollo et al. (2000). The time period between head louse collection and the start of the experiments was <2 h. Lice were examined carefully,

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before testing, with a stereoscope microscope and any damaged ones discarded.

Chemicals. Pure compounds (geraniol, citronellol, linalool, citronellyl formate) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Geranium oil was obtained from Fritzsche Saica (Buenos Aires, Argentina). Analytical grade acetone (Merck, Buenos Aires, Argentina) was used as the carrier. Compounds were dissolved in acetone for topical application.

Chemical Analysis of Geranium Oil. The chemical composition of geranium oil was analyzed by gas chromatography coupled to mass spectrometry using a GCMS-QP 5050A (Shimadzu, Japan) equipment. A capillary polar column CP Wax 25 m × 0.25 mm × 0.25 μm was used. An aliquot of 1 μl of a hexane solution of geranium oil (1 mg/ml) was injected. The analytical conditions were: injector and interface temperatures of 250 and 280°C, respectively; initial temperature of 60°C during 2 min; programmed temperature of 250°C (10°C/min.); and helium as carrier gas (30 kPa). The electron impact voltage was set at 70 ev. The identification of components was done by comparison of their retention index with chemicals purchased from Sigma-Aldrich Company, where possible; and by comparison of the mass spectra with available National Institute of Standards and Technology or Wiley mass spectral library resident in the GCMS. The quantification was performed by peaks area normalization.

Bioassays. Acute toxicity of geranium oil, major constituents, and mixtures was determined by topical application on adult females. Five mixtures were used: the first contained all major constituents, the remaining four contained all constituents except one. In all cases, constituents were combined in the same ratio as the geranium oil.

In all tests, groups of 10 insects were treated with 0.1 μl of acetone solution of the compounds. Doses were applied to the dorsal abdomen using a repeating topical dispenser attached to a 5 μl Hamilton syringe (Hamilton Company, Reno, NV). Doses ranged from 0.2 to 20 μg/insect. Each dose was replicated at least three times. Topical application with acetone alone was made for controls. All treated head lice were placed in plastic boxes lined with 55 mm diameter filter paper disc (Whatman Ltd., Maidstone, United Kingdom) moistened with 0.5 ml water, and held for 18 h in a growth chamber (18 ± 1°C, 97 ± 2% RH, 12:12 L:D photoperiod) (Gallardo et al. 2009).

Statistical Analysis. Mortality data were corrected using Abbott formula (Abbott 1925). Dose-mortality values were subjected to probit regression analysis (Litchfield and Wilcoxon 1949) using POLO-PC Software (LeOra Software 1987). Mean lethal dose values (LD_{50}) and confidence intervals are expressed as micrograms per insect. To establish the type of interaction that occurred in each mixture the Combination Index (CI) was calculated (Chou 2006). The CI was determined based on the LD_{50} of each constituent and mixture by using CompuSyn (Chou and Martin 2005). A CI of <1, = 1, or >1 indicates synergism, an additive effect, and antagonism, respectively (Chou 2006).

Table 1. Chemical composition of geranium oil expressed as relative percentage on total area in the chromatogram

Oil constituents[a]	%
Citronellol	38.0
Geraniol	16.0
Citronellyl formate	10.4
Linalool	6.5
Isomenthone	5.6
Epi-γ-eudesmol	4.9
Geranyll formate	3.8
Menthone	2.2
Cis-rose oxide	1.5
Geranyl acetate	<1
Citronellyl acetate	<1
α-pinene	<1
α-terpineol	<1
Trans-caryophyllene + β-candinene + β-bourbunene	6.0
Geranyl, citronellyl isobutyrate	3.0
Geranyl + citronellyl butyrate, valerate, and tiglate	<1
Unidentified	<1

[a] Main constituents of essential oil, determined by CG-MS. Unidentified compounds: <90% similarity index.

Results

Chemical analysis of the geranium oil revealed four major constituents (concentration expressed as percentage of total): citronellol (38.0%), geraniol (16.0%), citronellyl formate (10.4%), and linalool (6.5%) (Table 1). These components constituted 70.9% of the geranium oil, and isomenthone, epi-y-eudesmol, geranyl formate, menthone, *cis*-rose oxide, trans-caryophyllene + β-candinene + β-bourbunene, and geranyl, citronellyl isobutyrate, accounted for 29.2% of the volatile substances constituents of the oil. Of the four major components from geranium oil, the two most toxic were citronellol and geraniol (Table 2).

Contact pediculicidal activity of the mixture with the four major constituents was more toxic than the natural geranium oil and more toxic than all the other mixtures tested (Table 3). The most toxic three-component mixture was geraniol: citronellol:citronellyl formate mixture while the least toxic was geraniol: citronellyl formate:linalool mixture. Combination indices for all mixtures demonstrated synergistic effects among the constituents (CI< 1).

Discussion

We demonstrated the insecticidal activity of geranium oil and its major constituents against *P. humanus*

Table 2. Toxic activity by topical application of individual major constituents of geranium oil against female *P. humanus capitis*

Oil constituents[a]	n	χ^2	Slope ± SE	LD_{50}[a] (95% CL)
Geraniol	99	0.633	3.186 ± 0.714	12.70 (9.86–17.47)
Citronellol	105	1.887	0.587 ± 0.170	9.71 (2.70–190.57)
Linalool	105	0.173	2.369 ± 0.786	24.73 (16.80–99.93)[b]
Citronellyl formate	120	0.666	0.740 ± 0.209	38.47 (12.64–826.48)[b]

[a] Mean lethal dose expressed in micrograms per insect.
[b] Extrapolated value.

Table 3. Toxic activity by topical application of mixtures of major constituents of geranium oil against female *P. humanus capitis*

Mixture of major constituents[a]	n	χ^2	Slope ± SE	LD_{50}[b] (95% CL)	CI[c]
Geraniol:citronellol:citronellyl formate:linalool	120	0.083	0.73 ± 0.18	2.21 (0.83–6.13)	0.15
Geraniol:citronellol:citronellyl formate	90	1.275	0.84 ± 0.19	3.24 (1.53–7.42)	0.22
Geraniol:citronellol:linalool	105	0.936	0.57 ± 0.19	8.18 (2.41–133.9)	0.56
Geraniol:citronellyl formate:linalool	90	0.014	0.50 ± 0.19	10.95 (3.93–175.9)	0.66
Citronellol:citronellyl formate:linalool	111	0.007	0.94 ± 0.21	7.11 (3.31–22.34)	0.46

[a] Constituents mixed in the same proportion than natural oil.
[b] Mean lethal dose expressed in micrograms per insect.
[c] Combination index (CI) < 1 indicates synergism.

capitis. In addition, we demonstrated synergistic interactions in artificial mixtures of those components.

Clearly, the toxic effect of synthetic mixtures of selected constituents of the geranium oil in the natural ratio revealed that some ingredients are major contributors to the toxicity of the artificial blend. In fact, it was frequently noted that complex mixtures of monoterpenoids are considerably more efficacious than the pure compounds. For example, the investigation of single and joint fumigant insecticidal action of citruspeel oil components, established that in artificial mixtures, several pure components potentiated their individual fumigant action against *Callosobruchus maculatus* (Don-Pedro 1996).

Similarly, the comparative toxicity of essential oils of *Litsea pungens* and *Litsea cubeba* and blends of their major constituents against cabbage lopper *Trichoplusia ni,* indicated a synergistic effect among constituents, and demonstrated that the major constituent (1,8-cineol) was the major contributor to the toxicity of the artificial blend (Jiang et al. 2009). The described results confirmed a previous report that 1,8-cineol was responsible for the major toxicity of rosemary oil against the twospotted spider mite *Tetranychus urticae* (Miresmailli et al. 2006). In our study, the absence of citronellol from the artificial mixture caused the highest decrease in toxicity (DL_{50} from 2.2 to 10.9 μg/insect), leading us to conclude that this constituent is the major contributor to geranium oil toxicity. Conversely, the removal of linalool from the mixture had no important effect on the toxicity of the blend (DL_{50} from 2.2 to 3.2 μg/insect).

The toxicity of mixtures based on the major constituents of geranium oil in our study revealed the presence of synergism. Just as importantly, we found some constituents could reduce the toxicity of the mixture (DL_{50} of natural oil compared with DL_{50} of synthetic mixture). We believe that toxicity reduction was produced by a dilution of the more effective constituents or an antagonistic interaction.

Although conventional pesticides are more toxic, botanical oils and its constituents may have a role in the development of pediculicides especially in developing countries and in areas where resistance renders commercial products ineffective. In this regard, this work shows that the search for synergy between components of essential oils is a promising strategy to improve the pediculicidal activity of natural compounds.

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