Insecticidal Activity of the Essential Oil and Extracts of *Gutierrezia mandonii* and *G. repens* (Asteraceae) Growing in Argentina

Sandra V. Clemente and Graciela Mareggiani,
Cátedra de Zoología Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453 (C 1417 DSE), Buenos Aires, Argentina

Berta E. Juárez and María E. Mendiondo,
Instituto M. Lillo, Miguel Lillo 251 (4000) Tucumán, Argentina.

Catalina M. van Baren, Paola Di Leo Lira, Adriana M. Broussalis, Arnaldo L. Bandoni and Graciela E. Ferraro,
Cátedra de Farmacognosia - IQUIMEFA (UBA-CONICET), Facultad de Farmacia y Bioquímica, Junín 956, 2° piso, (C 1113 AAD) Buenos Aires, Argentina; abandoni@ffyb.uba.ar

Abstract

The insecticidal activities of essential oils (EO), the remaining water phase (RW) from the hydrodistillation, and the dichloromethane and methanolic extracts from *Gutierrezia repens* Griseb. and *Gutierrezia mandonii* (Sch. Bip.) Solbrig were evaluated. The GC and GC/MS analyses of the oils resulted in the identification of 52 compounds from *G. mandonii* and 17 compounds from *G. repens* comprising 88.7% and 98.5% of the oils, respectively. Sabinene (0–13.1%), β-pinene (6.4–17.8%) limonene (0.7–13.3%), (E)-β-ocimene (1.3–7.0%), terpinen-4-ol (0.7–4.1%), spathulenol (0–4.1%) and, the isomers (6R,7R)-bisabolone (6.6–58.0%) and (6S,7R)-bisabolone (0–1.6%) were the main components of the oils. Mortality and development delays of *Ceratitis capitata* larvae, reared using a treated artificial diet, were recorded. *Gutierrezia mandonii* methanolic extracts and the oil produced lethal effects ($p \leq 0.05$). Methanolic and dichloromethane extracts, the oil and the remaining water from distillation (RW) produced sublethal effects. Meanwhile dichloromethane and methanolic extracts, the oil and RW from *G. repens* resulted in significant mortality ($p \leq 0.05$), and all causing also significant development delays. The required concentration of *G. repens* oil to avoid development in 50% of *C. capitata* larvae (EC_{50}) was significantly lower than *G. mandonii*.

Key Word Index

Gutierrezia repens; Gutierrezia mandonii; Asteraceae; essential oil composition; sabinene; β-pinene; (6R,7R)-bisabolone; *Ceratitis capitata*, insecticidal activity.

Introduction

At present, the use of natural agrochemicals is well accepted because of the necessity of new compounds to control pests without environmental deleterious effects (1). Many plants have been used for centuries to keep crops free from insect damage by interculture or foliar plant materials applications. However, scientific evidence to support these control methods is not always available (2). Higher plants offer an excellent source of biologically active natural compounds. There are many examples of plants natural products which demonstrate efficacy as insecticides (3).

The genus *Gutierrezia* (Asteraceae, tribe Astereae) is native from America. It is represented by about 20 species (4) and eight of them occur in Argentina (5). This genus has been studied chemically by many scientific groups, especially for flavonoids, labdanes, clerodanes, alicyclic diterpenes and some bisabolenes that were already reported (6 and lit. cited therein, 7–9). Only the volatiles from the species *G. sarothrae* have been studied previously (10,11).

Gutierrezia mandonii (Sch. Bip.) Solbrig and *Gutierrezia repens* Griseb. grow in the northwest of Argentina (Catamarca, Jujuy, La Rioja, Salta and Tucumán provinces) at an altitude of 1000 to 4000 m (12). *Gutierrezia mandonii* is known under the

*Address for correspondence

Received: December 2006
Revised: March 2007
Accepted: April 2007

1041-2905/08/0003-0276$14.00/0—

common name of "canchalagua" and it is used in folk medicine against flu and fever, in rheumatic pains, as digestive, tonic and emmenagogue (13).

Some *Gutierrezia* are widespread weeds in the western farms of North America, and are recognized as poisonous to livestock. A bionomic attack of this genus with some special lepidoptera was proposed for *Gutierrezia* spp. (14,15). However, this genus should contain some phytochemicals which might have a negative activity against generalist or polyphagous pests.

For this reason, the oil, the remaining water after distillation (RW) and dichloromethane and methanolic extracts of *G. mandonii* and *G. repens*, growing in the northwest of Argentina, were both analyzed and tested for insecticidal activity.

Experimental

Plant material: The aerial parts of *G. mandonii* and *G. repens* were collected in Tafí del Valle, Tucumán, Argentina, when flowering (March/April), the first one in Dique "La Angostura", and the second one in "El Rincón". Voucher specimens have been deposited at the Herbarium of the Instituto Miguel Lillo, San Miguel de Tucumán, Argentina (LIL 606395 and LIL 606817).

Preparation of extracts: Powered plant material from both species (30 g of *G. mandonii* and 10.4 g of *G. repens*) was extracted by maceration with dichloromethane three times for 24 h. A second extraction with methanol following the same procedure was done. Both extracts were taken to dryness under vacuum and freeze dried.

Isolation of the essential oil: The essential oils were separately obtained from the air-dried parts of plant materials (100 g of *G. mandonii* and 46 g of *G. repens*) by hydrodistillation for 3 h, using a Clevenger-type system (16). The oils were dried over anhydrous sodium sulfate and stored at -18°C until they were analyzed. The remaining water phase from each hydrodistillation was saturated with sodium sulfate and extracted with a minimal quantity of pentane. Only traces of oil were obtained from each aqueous extract. Subsequently they were freeze dried, constituting the respective fractions (RW).

GC/Analysis: GC analysis was performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with two flame ionization detectors (255°C), two fused capillary columns of different polarity: 5% phenyl, 95% methyl silicone and polyethylene glycol (HP-5 and HP-Wax, 60 m x 0.25 mm, film thickness 0.25 µm) were used simultaneously, injector (255°C), split ratio 1:150. Temperature program: from 110–220°C with a rate of 3°C/min using N_2 as carrier gas at a working flow rate of 0.8 mL/min. Quantitative data were obtained from FID area values without considering the respective responses factors.

GC/MS analysis: Mass spectra were obtained on a Hewlett Packard 5890 series II coupled to a HP 5972 mass selective detector at 70 eV. Range of masses: 40–300 Da. Scan time: 0.1 s Analytical conditions: capillary column with 5% phenyl, 95% methyl silicone previously cited stationary phases (HP-5 60 m x 0.25 mm, film thickness 0.25 µm), Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 230°C and 180°C, respectively. Column temperature was initially at 60°C, then gradually increased to 220°C at a rate of 3°C/min and held for 12 min. Diluted samples were injected (1 µL) using a split injector (ratio 1:60).

Identification of constituents: The oil components were identified by: 1) Determination of their retention indices (RI) in relation to a homologous series of n-alkanes (C_8 to C_{20}), in the two columns previously cited and comparison with those reported in the literature (17,18), 2) By comparison of their mass spectra with MS data reported in the literature (17) and with data stored in a library built up from authentic samples of standards.

Insecticide activity assay: *Ceratitis capitata* Weid. larvae (Diptera, Tephritidae), from an established laboratory colony reared in Cátedra Zoología Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Argentina, with a Terán artificial diet and environmental standard conditions (25 ± 2°C, 60 ± 5% RH, in darkness), were assayed. Cohorts of 10 one day old larvae were reared in plastic vessels containing the artificial diet previously mixed with ethanolic solutions of *G. mandonii* and *G. repens* extracts, the oil, the remaining water after hydrodistillation (RW) and the standards: α-pinene, ß-pinene and limonene, depending upon the treatment, to obtain a final concentration of 500 ppm. Untreated larvae were used as controls. Four replicates of each treatment were assayed. Each replicate was put inside a larger plastic vessel containing sterilized sand for pupation. Mortality until adult emergence (%) was recorded. EC_{50} -concentration needed to avoid development in 50% of larvae- was calculated assaying three concentrations of the oils and the standard limonene. Percentage of puparia number, expressed in relation to the number of exposed larvae, was used to calculate PT_{50} - pupating time or time needed to pupate 50% of larvae- and EC_{50} by Probit analysis computer program (19). Statistical differences in mortality ($p \leq 0.05$) were calculated with ANOVA and Tukey multiple range test.

Results And Discussion

Extracts and oils yields: The dichlorometane and methanolic extracts of *G. mandonii* afforded a residue, after vacuum dryness, of 4.1% and 6.8% (W/W) of the dried plant material, respectively. The corresponding values for *G. repens* were 7.3% and 5.2% (w/w) of the dried plant material. The yields of the oils obtained from the aerial parts of *G. mandonii* and *G. repens* were 0.5% and 0.9% (v/w of dry material) of oil, respectively. The waters remaining after hydrodistillations (RW) afforded 10.0% (*G. mandonii*) and 12.4% (*G. repens*) of residue after freeze dried. These aqueous extracts contained only traces of oil.

Chemical composition of the oil:. Analysis of the oils resulted in the identification of 52 compounds from *G. mandonii* and 17 compounds from *G. repens* (Table I), comprising 88.7 % and 98.5 % of the oils, respectively. Sabinene (13.1%), limonene (13.3%), terpinen-4-ol (4.1%), spathulenol (4.1%) and (6R,7R)-bisabolone (6.6 %) were the main components of the oil from *G. mandonii*, meanwhile β-pinene (17.8%), (E)-β-ocimene (7.0%) and (6R,7R)-bisabolone (58.0%) were the main components of the oil from *G. repens*. The chromatographic profile of the traces of oils extracted from the waters remaining after hydrodistillations (RW) of each species was identical in the most important peaks (the only detected constituents) to

the respective pure oils.

Insecticidal activity: Figure 1 shows that the methanolic extract and oil of *G. mandonii* produced significant mortality (60% and 43%, respectively) when were added to *C. capitata* diet. Similar lethal effects were observed for the standard limonene. This compound is one of the main compounds of *G. mandonii* oil (Table I), then it could be possible that the insecticidal activity here observed could be due to limonene, a compound with reported lethal activity (20,21). The two other standards, α- and β-pinene, did not produce significant mortality.

Sublethal effects of *G. mandonii*, expressed as PT_{50} (days), were also observed (Figure 2). The oil, methanolic, dichloromethane extracts and the remaining water after hydrodistillation (RW) applied to the diet produced significant development delays. Sabinene could have been responsible of this action in the oil, taking into account previous reports (22).

Figure 3 shows that the dichloromethane and methanolic extracts, the oil and RW from *G. repens* produced significant mortality ($p \leq 0.05$).

Sublethal effects of *G. repens*, expressed as PT_{50} (Figure 4) shows that the highest development delays were caused by the dichloromethane extract, the oil and RW. These results could be attributed to the high concentration of bisabolone isomers (Table I) in the oil.

Both species *G. mandonii* and *G. repens* have a noticeable insecticidal activity in methanolic extract and oil, with similar sublethal effects. However, the more straightforward extraction of the oil implies a significant advantage. The required concentration of the oil of *G. mandonii* to avoid development in 50% of *C. capitata* larvae was 1138 ppm while the EC_{50} for *G. repens* was 248 ppm. This low value shows that *G. repens* could be more promising source of insecticidal compounds.

Acknowledgments

This work was carried out with the financial support of the University of Buenos Aires (Projects UBACyT B 034, B 019, B 046 and G 062).

References

1. G.I. Guzmán Casado, M. Gonzalez de Molina and E. Sevilla Guzmán, *Introducción a la agroecología como desarrollo rural sostenible.* 529 pp., Mundi Prensa. Barcelona. Spain. (2000).
2. G. Mareggiani, *Manejo de insectos plaga mediante sustancias semioquímicas de origen vegetal.* Manejo integrado de plagas (Costa Rica) **60,** 22–30 (2001).
3. S. Clemente, G. Mareggiani, A. Broussalis, V. Martino and G. Ferraro, *Insecticidal effects of Lamiaceae species against stored products insects.* Bol. San. Plagas. **29,** 421–426 (2003).
4. J. Grau,. *Astereae-systematic review.* In: *The Biology and Chemistry of the Compositae* Edits., V.H. Heywood, J.H. Harborne and B.L.Turner, pp 539–565, Academic Press, London, UK (1977).
5. F.G Zuloaga and O. Morrone, *Catálogo de las plantas vasculares de la República Argentina II.* 202 pp., Missouri Botanical Garden Press, St Louis, MO (1999).
6. C. Zdero, F. Bohlmann and M. Niemeyer, *Labdane derivatives and alicyclic diterpenes from* Gutierrezia espinosae. Phytochemistry **29,** 567–571 (1990).
7. F. Bohlmann, M. Grenz, A.K. Dahr and M. Goodman, *Labdane derivatives and flavones from* Gutierrezia dracunculoides. Phytochemistry **20**, 105–107 (1981).
8. J.N. Roitman and L.F. James, *Chemistry of toxic range plants. Highly oxygentaed flavonol methyl esthers from* Gutierrezia microcephala. Phytochemistry **24**, 835–848 (1985).
9. R. Li, N. Fang and T.J. Mabry, *Flavonoids and a coumarin from* Gutierrezia sphaerocephala. Phytochemistry **27**, 1556–1559 (1988).
10. W.W. Epstein and J.L. Seidel, *Monoterpenes of* Gutierrezia sarothrae. J. Agric. Food Chem. **37**, 651–654 (1989).
11. M.E. Lucero, Ed L. Fredrickson, R.E. Estell, A.A. Morrison and D.B. Richman, *Volatile composition of* Gutierrezia sarothrae *(Broom Snakeweed) as determined by Steam Distillation and Solid Phase Microextraction.* J. Ess. Oil Res. **18**, 121–125 (2006).
12. A.L. Cabrera, *Flora de la Provincia de Jujuy, República Argentina. Parte X-Compositae.* Pp.160–163, Colección Científica del INTA,. Buenos Aires, Argentina (1978).
13. S.E. Freire and E. Urtubey, *Compuestas Medicinales de la Provincia Biogeográfica Pampeana: Claves para su Determinación e Iconografías. Parte I: Compuestas espinosas (grupo 1) y Compuestas con tallos alados (grupo 2).* Acta Farm. Bonaerense **18**, 191–199 (1999).
14. H.A. Cordo, C.J. De Loach and D.H. Habeck, *Biology of* Heilipodus ventralis *(Coleoptera: Curculeonidae) an Argentine weevil for biological control of Snakeweeds (*Gutierrezzia *spp.) in the United States.* Biological Control. **15,** 210–227 (1999).
15. J. Calderón, C. Céspedes, R. Rosas, F. Gómez-Garibay, J. Salazar, L. Lina, E. Aranda and I. Kubo, *Acetylcholinesterase and insect inhibitory activities of* Gutierrezia microcephala *on fall armyworm* Spodoptera frugiperda. J.E. Smith. Z. Naturforsch, **56c**, 382–394 (2001).
16. European Pharmacopoeia, P. 121, Council of Europe, Strasbourg, France (2005).
17. R.P. Adams. *Identification of Essential Oils Components by Gas Chromatography / Quadrupole Mass Spectroscopy.* Allured Publishing Corporation, Carol Steam, IL. (2001).
18. N.W. Davies, *Gas Chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases.* J. Chromatogr. **503,** 1–24.(1990).
19. J. Robertson and H. Preisler, *Pesticides bioassays with Arthropods.* pp. 17–31, CRC Press, Boca Raton, FL (1992).
20. H. Prates, J. Santos, J. Waquil, J. Fabris, A. Oliveira and J. Foster, *Insecticidal activity of monoterpenes against* Rhyzopertha dominica *(F.) and* Tribolium castaneum *(Herbst).* J. Stored Prod. Res. **34,** 243–246 (1998).
21. I. Park, S. Lee, D. Choi, J. Park and Y.Ahn, *Insecticidal activities of constituents identified in the essential oil from leaves of* Chamaecyparis obtusa *against* Callosobruchus chinensis *(L.) and* Sitophilus oryzae *(L.).* J. Stored Prod. Res. **39,** 375–384 (2003).
22. M. Tsoukatou, C. Tsitsimpikou, C. Vagias and V. Roussis, *Chemical intra-mediterranean variation and insecticidal activity of* Crithmum maritimun. *Sect C.*, Biosci., **56**, 211–215, 20 (2001).