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Insecticidal properties of *Heliotropium stenophyllum* essential oil on the House fly, *Musca domestica* L.

[Propiedades insecticidas del aceite esencial de *Heliotropium stenophyllum* en la mosca doméstica, *Musca domestica* L.]

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Abstract
The composition of the essential oil (EO) obtained by hydro distillation from dry leaves of *Heliotropium stenophyllum* (Heliotropiaceae) was analyzed using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The insecticidal activity of the oil against the house fly *Musca domestica* was evaluated and the dose necessary to kill 50% of flies (LC_{50}) in 2 h was determined at 25 ± 1 °C. The essential oil from *Heliotropium stenophyllum* showed potent insecticidal properties (LC_{50} = 1.09 mg/dm^3) in comparison with other essential oils, in which at shorter times, the same bio-assay was used. According to GC and GC/MS analysis, junenol (19.08%); longiborneol (9.34%); (E, Z)-geranyl linalool (6.81%); selina-3,11-dien-6-α-ol (6.70%); α-cedrene epoxide (6.60%); heliofolen-12-al D (6.23%) and β-*epi*-bisabolol (4.83%) were the principal components of the EO. The *Heliotropium stenophyllum* essential oil, made up exclusively of sesquiterpenes, showed a composition very different from the EOs of the other species of *Heliotropium*, studied, and present a great potential as a natural insecticide against houseflies.

Keywords: *Heliotropium stenophyllum* essential oil composition; sesquiterpenoids; vapour pressure; *Musca domestica*; natural insecticide

Resumen
La composición del aceite esencial (AE) obtenido por hidrodestilación de hojas secas de *Heliotropium stenophyllum* (Heliotropiaceae) se analizó mediante cromatografía de gases (CG) y cromatografía de gases/espectrometría de masas (CG/EM). La actividad insecticida del aceite contra la mosca doméstica *Musca domestica* se evaluó y la dosis necesaria para matar el 50% de las moscas (LC_{50}) en 2 h se determinó a 25 ± 1 ºC. El aceite esencial de *Heliotropium stenophyllum* mostró potentes propiedades insecticidas (LC_{50} = 1,09 mg/dm^3) en comparación con otros aceites esenciales, en el que en tiempos más cortos, se utilizó el mismo bio-ensayo. De acuerdo con los análisis de CG y CG/EM, junenol (19,08%); longiborneol (9,34%), (E, Z)-geranil linalool (6,81%); selina-3,11-dien-6-α-ol (6,70%); epoxido de α-cedreno (6,60%); heliofolen-12-al D (6,23%) y β-*epi*-bisabolol (4,83%) fueron los componentes principales identificados en el AE. El aceite esencial de *Heliotropium stenophyllum*, formado exclusivamente por sesquiterpenos, mostró una composición muy diferente al de los AEs de otras especies de *Heliotropium*, estudiadas, y muestra un gran potencial como insecticida contra la moscas doméstica.

Palabras Clave: Composición del aceite esencial de *Heliotropium stenophyllum*; sesquiterpenos; presión de vapor, *Musca domestica*; Insecticida natural

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INTRODUCTION

In our continuing interest in the potential of essential oils (EOs) as insecticides against *Musca domestica* (Urzúa *et al.*, 2010a; Urzúa *et al.*, 2010b), we present an evaluation of the insecticidal properties of a species endemic to arid and semi-arid regions of Chile, *Heliotropium stenophyllum* Hook.et Arn. (Heliotropiaceae) (Johnston, 1928). The choice of this specie was motivated by field observations of their apparent resistance to insect aggression and a key factor in its selection was that the cuticle components of leaves, obtained by CH_2Cl_2 extraction, showed antiffedant properties against larvae of *Pieris brassicae* (Villarroel *et al.*, 1991).

We previously reported that *Heliotropium stenophyllum* contains a mixture of 2-geranyl-4-hydroxyphenyl acetate and several known flavonoids but the composition of essential oil from *Heliotropium stenophyllum* was never been investigated (Villarroel *et al.*, 1991).

In addition, as far as we are concerned, the composition of essential oils from only two species of *Heliotropium*, species have been investigated.

The essential oil of *Heliotropium indicum* was obtained in 0.004% and the major identified constituents were phytol (49.1%); 1-dodecanol (6.4%) and *β*-linalool (3.0%). In addition, long chain alcohols and hydrocarbons gave account of 11.8% of the components. The EO showed significant activity against *Mycobacterium tuberculosis* (Machan *et al.*, 2006).

The essential oil of *Heliotropium europaeum* was obtained in 0.1% and the major constituents were phytol (28.7%); *cis*-linoleic acid methyl ester (7.3%); silphiperphol-6-en-5-one (7.1%); geranyl acetone (6.3%); (E)-β-ionone (4.8%); phytol acetate (4.3%) and alloaromadendrene epoxide (3.8%). In addition, long chain alcohols and hydrocarbons gave account of 19.2% of the components. The essential oil show low antimicrobial activity (Saeedi and Morteza-Semnani, 2009).

MATERIAL AND METHODS

General

The essential-oil component analysis was performed by gas chromatography and gas chromatography/ mass spectroscopy (GC/MS). Qualitative analyses were performed in a Hewlett-Packard 6890 gas chromatograph linked to a Hewlett-Packard 5972 A mass spectrometric detector with an integrated data system (Hewlett Packard, Palo Alto, CA, USA); quantitative analyses were carried out with a Shimadzu GC-9A gas chromatograph fitted with a FID-9 detector (Shimadzu Corporation, Kyoto, Japan). The same capillary column (HP-5 MS, film thickness 0.25 μm, 30 m x 0.25 mm, Agilent, USA) was used in both instruments.

Plant material

Leaves of *H.stenophyllum*, were collected in November 2010, in Los Vilos, IV Region Chile (32º52'S, 71º29'W). Voucher specimens (ST-2560) were deposited in the Herbarium of the National Natural History Museum, Santiago, Chile

Essential oil extraction and analysis

Essential oils were extracted from 100 g of dry leaves for 4 h by hydro distillation in a Clevenger-type apparatus. The EO was dried over anhydrous sodium sulfate. The EO component analysis was performed by gas chromatography and gas chromatography/mass spectroscopy (GC/MS) using the instrumentation described above. The operating conditions were as follows: on-column injection; injector temperature, 250 °C; detector temperature, 280 °C; carrier gas, He at 1.0 ml/min; oven temperature program: 60 ºC increase to 260 ºC at 4 ºC/min, and then 260 ºC for 5 min. The mass detector ionization employed an electron impact of 70 eV. Recording conditions employed a scan time of 1.5 s and a mass range of 41 to 450 amu. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST2008 library database, and by comparison of their calculated retention index with those reported in the literature.

Fly collection and maintenance

Adult flies were obtained from 12 h pupae commercially obtained. Pupae of *M. domestica* were transferred to entomological cages (30×30×30 cm) at 25 ± 1 °C under a 12:12 light: dark cycle and 65% humidity. Emerged adult flies were provided with water and fed 1:1 (v/v; approximately) mixture of sugar and powdered milk.

Bioassay

The bioassay was designed such that the flies would have high probability of coming into contact with the volatile compounds within two hour test period; therefore, the flies were allowed access to the total

space within the exposure vessel. Ten 3 day-old adult house flies, of both sexes, were placed in a glass jar (1.0 dm^3) fitted with a screw cap with a 6-cm length of cotton yarn suspended from the center of the internal face of the cap. Different dosages of pure EO (without solvent) were applied to the yarn. The control vessel had no compound on the cotton yarn. The jars were then sealed tightly. The jars were maintained in a room at 25 ± 1 °C. Each test was replicated three times. The assay was also run with the cotton yarn enclosed in a bag made of breathable cloth so that the flies could not contact the yarn. Dimethyl 2, 2-dichlorovinyl phosphate (DDVP), a volatile organophosphate, was used as a positive control. Mortality in each group was assessed after a two our exposure.

Data analysis

The mean mortality data of the three replicates per doses (5 doses) was used to calculate the LC_{50}. Probit analysis (Harvard Programming; Hg1, 2) was used to analyze the dose-mortality response.

Table Nº 1
Essential oil Composition of leaves of *Heliotropium stenophyllum*

Compound	RI	%	Identification
α-Gurjunene	1409	0.462	RI, MS
β-Dihydroagarofuran	1497	0.901	RI, MS
α-Amorphene	1512	0.444	RI, MS
γ-Cadinene	1513	0.842	RI, MS
α-Agarofuran	1542	0.602	RI, MS
Silphiperfol-5-en-3-ol	1550	2.21	RI, MS
Spathulenol	1578	4.35	RI, MS
α-Cedren epoxide	1589	6.59	RI, MS
Longiborneol	1598	9.34	RI, MS
Heliofolen-12-al-D	1607	6.23	RI, MS
cis-Isolongi- folanone	1611	1.49	RI, MS
10-*epi*-γ-Eudesmol	1617	1.32	RI, MS
Junenol	1622	19.08	RI, MS
2-*epi*-α-Cedren-3-one	1627	1.92	RI, MS
Cedren-9-one	1633	3.10	RI, MS
α-Eudesmol	1636	4.10	RI, MS
α-Muurolol	1643	3.95	RI, MS
Agarospirol	1645	3.70	RI, MS
Selina-3,11-dien-6-α-ol	1650	6.70	RI, MS
β-*epi*-Bisabolol	1669	4.83	RI, MS
Eudesma-4(15)-7-dien-1-β-ol	1679	3.56	RI, MS
Cedroxyde	1708	1.56	RI, MS
(E)-Nuciferal	1726	1.24	RI, MS
Eremophilone	1734	1.29	RI, MS
Aristolone	1764	1.34	RI, MS
Bisabolol acetate	1797	1.18	RI, MS
β-Chenopodiol	1810	1.30	RI, MS
(E,Z) Geranyl linalool	1988	6.81	RI, MS

RI: retention index

Table Nº 2
LC_{50} of EO of *Heliotropium stenophyllum* on *Musca domestica*

Time	Mean LC_{50} in mg/dm^3 (95% CI)
½ h	13.21 (4.69 – 21.34)
1 h	5.26 (2.31 – 10.34)
2 h	1.09 (2.69 – 14.54)

RESULTS AND DISCUSION

In this communication from the dry leaves of *H. stenophyllum* (100 g), 0.57 g (0.57%) of EO was obtained. The composition of the EO (%) is listed in Table Nº 1, 28 compounds were identified : junenol (19.08%); longiborneol (9.34%); (E, Z)-geranyl linalool (6.81%); selina-3,11-dien-6-α-ol (6.70%); α-cedren epoxide (6.59%); heliofolen-12-al D (6.23%) β-epi-bisabolol (4.83), were the major constituents identified in the EO.

The *H. stenophyllum* essential oil, made up exclusively of sesquiterpenes, showed a composition very different from the EOs of the other species of *Heliotropium*, studied in which phytol is the characteristic major constituent, with long chain hydrocarbons and alcohols.

Recently, the insecticide activity of 12 EOs from edible plants, were screened against housefly adults and the dose necessary to kill 50% of flies (LC_{50}) in 30 min was determined at 26 ± 1°C. The EO from *Citrus sinensis* was the most potent insecticide (LC_{50} = 3.9 mg/dm^3), followed by EO from *C. aurantium* (LC_{50} = 4.8 mg/dm^3). Limonene (92.47%) and linalool (1.43%), were the principal components of *C. sinensis* EO. Limonene was also the principal constituent (94.07%) of *C. aurantium* EO (Palacios *et al.*, 2009a). More recently, using the same bio-assay, the LC_{50} of 9 EOs isolated from medicinal plants from Argentina was determined. The EO from *Minthostachys verticillata* (LC_{50} = 0.5 mg/dm^3) and *Hedeoma multiflora* (LC_{50} = 1.3 mg/dm^3) were the most potent and in both EOs the principal active component was identified as (4R) (+)-pulegone (Palacios *et al.*, 2009b).

The examination of the literature on the insecticide activity of EOs against the housefly shows that the principal components of the active oils are monoterpenoids and little is known on the insecticide activity of EOs in which the sesquiterpenoids are the principal components (Palacios *et al.*, 2009b).

Sesquiterpenoids are the main components of the essential oils of *Teucrium leucocladum* and *Pogostemon cablin*, both potent topical insecticides against *M. domestica* (El-Shazly and Hussein, 2004; Pavela, 2008). The insecticidal activity of the EO of *Pogostemon cablin* , with patchouli alcohol (42.70%), α-selinene (16.20%) and β-patchoulene (12.30%) as principal components has significantly higher impact on the mortality of *M. domestica* in the topical application than in the fumigant application (Pavela, 2008). The EO of *Teucrium leucocladum* also contains as principal component patchouli alcohol (31.24%), and with this EO mortality only in the topical application was recorded (El-Shazly and Hussein, 2004).

In the vapor phase test, the effective concentration of the compounds, in the exposure vessel needs to be sufficient to produce the toxic effect. In this context, the vapor pressure of the components of the EOs should be a fundamental parameter when working in short time bioassays. Other parameters such as lipophilicity, molecular weight and steric effects are not significantly correlated with the observed insecticidal activity of monoterpenes against *Pediculus humanus capitis* Yang *et al.*, 2004).

The insecticide activity of the essential oil of *H. stenophyllum* is due to the content of hydroxylated sesquiterpenes, compounds with estimated low vapor pressure. This fact explains that the strongest insecticidal effect is obtained at 2 h and not at 30 min (Table 2) as with monoterpenes (Palacios *et al.*, 2009a; Palacios *et al.*, 2009b). The study of mixtures of EOs with active monoterpenoids and sesquiterpenoids is a good combination to ensure insecticidal activity for a longer period of time in the field.

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