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Insecticidal effect of *Cryptocarya alba* essential oil on the housefly, *Musca domestica* L

[Efecto insecticida del aceite esencial de Cryptocarya alba en la mosca doméstica, *Musca domestica* L.]

Dania DI COSMO[1], Rocío SANTANDER[1], Alejandro URZÚA[1], Sara M. PALACIOS[2] & Yanina ROSSI[2]

[1]Laboratory of Chemical Ecology, Faculty of Chemistry and Biology, Universidad de Santiago de Chile, Casilla 40, Correo 33, Santiago, Chile
[2]Laboratory of Fine Chemicals and Natural Products, Faculty of Chemical Sciences. Catholic University of Córdoba, Camino a Alta Gracia Km 10, (5000) Córdoba, Argentina
Contactos | Contacts: **Rocío SANTANDER - *E-mail address:*** **rocio.santanderm@usach.cl**
Contactos | Contacts: Dania DI COSMO - E-mail address: dania.dicosmo@usach.cl

Abstract: The composition of the essential oil (EO) from *Cryptocarya alba* obtained by hydro distillation of fresh leaves was analyzed using gas chromatography (GC) and gas chromatography/mass spectrosmetry (GC/MS). The insecticidal effect of the oil on the house fly *Musca domestica* was evaluated by placing flies in a sealed glass jar containing a piece of EO-treated cotton yarn. The dose necessary to kill 50% of flies (LC_{50}) in 0.5 and 1 h was determined at 26 ± 1 °C. The essential oil from *Cryptocarya alba* showed significant insecticidal properties [33.56 (7.06-159.50) mg/dm^3 (0.5 h) and 15.07 (5.29-42.91) mg/dm^3 (1 h)]. According to GC and GC/MS analysis a total of 38 compounds were identified. The composition of the *Cryptocarya alba* essential oil reported in this study is different to that reported in other publications with 4-terpineol (17.48%) ; 4- (3,3-dimethyl-but-1-ynyl) -4-hydroxy-2,6,6-trimethylcyclohex-2-enone (12.84%); 1,8-cineole (7.90%); p-cymene (7.11%) and sabinene (6.80%), accounting for 52.13% of the EO. The EO from *Cryptocarya alba* appears promising as a natural insecticide against houseflies.

Keywords: Musca domestica; *Cryptocarya alba*; essential oil composition; GC-MS; NMR; natural insecticide.

Resumen: La composición del aceite esencial (AE), obtenido por hidrodestilación de hojas frescas de *Cryptocarya alba* 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 colocando las moscas en un frasco de vidrio sellado con un trozo de hilo de algodón tratado con diferentes cantidades de AE. La dosis necesaria para matar el 50% de las moscas (LC_{50}) en 0,5 y 1 hora se determinó a 26 ± 1 ºC. El aceite esencial de *Cryptocarya alba* mostró un buen efecto insecticida [33,56 (7,06-159,50) mg/dm^3 (0,5 h) and 15,07 (5,29-42,91) mg/dm^3 (1 h)]. La composición del aceite esencial de *Cryptocarya alba* encontrada en este trabajo es diferente al informado en otras publicaciones, con 4-terpineol (17,48%); 4-(3,3-dimetil-but-1-inil)-4-hidroxi-2,6,6-trimetilciclohex-2-enona (12.84%); 1,8-cineole (7,90%); p-cimeno (7,11%) y sabineno (6,80%), lo que representa el 52.13% del AE. El AE de *Cryptocarya alba* parece prometedor como un insecticida natural contra la mosca doméstica.

Palabras clave: *Musca domestica*; *Cryptocarya alba*; composición del aceite esencial; CG-EM; RMN; insecticida natural.

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INTRODUCTION

In our continuing interest in the potential of essential oils (EO's) from Chilean flora as insecticides against *Musca domestica* (Urzúa *et al.*, 2010a; Urzúa *et al.*, 2010b; Urzúa *et al.*, 2011; Urzúa *et al.*, 2013), we present an evaluation of the insecticidal property of a widespread species endemic to the sclerophyllous forest in Central Chile, *Cryptocarya alba* (Mol.) Looser (Lauraceae) (Riedeman & Aldunate, 2001). In addition to being a medicinal plant with several curative properties (Muñoz *et al.*, 1981), a key factor in its selection was that the spices-scented leaves of *C. alba* and their essential oil shows insecticidal properties against the maize weevil; *Sitophilus zeamais* Motschulsky (Guerrero, 2013).

EXPERIMENTAL

General

cis-β-Terpineol; 1-terpineol; α-pinene; β-pinene; α-terpineol and 4-terpineol, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl-2,2-dichlorovinyl phosphate (DDVP) was provided as a gift by Professor H. Masuh from the Center of Investigation on Pests and Insecticides, CONICET, Argentina. The essential oil component analysis was performed using gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). Qualitative analysis was performed using a Thermo Scientific Trace GC Ultra linked to a ISQ quadrupole mass spectrometric detector with an integrated data system (Xcalibur 2.0, Thermo Fisher Scientific Inc. USA); quantitative analysis was carried out using a Shimadzu GC-9A gas chromatograph fitted with a FID-9 detector (Shimadzu Corporation, Kyoto, Japan). The same capillary column (Rtx-5MS, film thickness 0.25 μm, 60m x 0.25 mm, Restek Corporation, Bellefonte, PA. USA) was used in both instruments.

Plant material

Leaves of *Cryptocarya alba* were collected at Cuesta Lo Prado (33°28´S, 70°56´W, 750 m above sea level) 15 km west of Santiago, during the flowering season, November 2010. Voucher specimens were deposited in the Herbarium of the National Natural History Museum, Santiago, Chile.

Essential oil extraction and analysis

Essential oil was extracted from 300 g of fresh leaves for 4 h by hydro distillation (2.5 L, H_2O) in a Clevenger-type apparatus. The EO was dried over anhydrous sodium sulfate and stored at -20º C until analysis. The EO component analysis was performed by gas chromatography (GC) and gas chromatography/mass spectrosmetry (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 1.25 ml/min; oven temperature program: 40 ºC for 5 min, increase to 260º C at 5º C/min, and then 260º C 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 40 to 400 amu. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST08 library database, and by comparison of their retention index with those reported in the literature (Adams, 2007), for the same type of column or those of commercial standards, when available.

Fly collection and maintenance

The colonies of *M. domestica* used in this study originated from adults collected in the experimental field of the Universidad Católica of Córdoba, in Córdoba, Argentina, using a sweep net. The flies were transferred to a small cage and then reared in entomological cages (30×30×30 cm) at 26 ± 1° C under a 12:12 light: dark cycle and 70% humidity. Adult flies were provided with water and fed a 1:1 (v/v; approximately) mixture of granulated sugar and powdered milk. Bran and milk were prepared at a weight ratio of 1:3 and 100 g of this mixture was placed on a plastic plate as an oviposition site.

Bioassay

The bioassay was designed so the flies would have high probability of coming into contact with volatile compounds within the one hour test period; therefore, the flies were allowed access to the total space within the exposure vessel. Ten 4-5 day old adult house flies, of both sexes, were placed in a glass jar (1.2 dm^3) fitted with a screw cap that had a 7-cm length of cotton yarn suspended from the center of its inner face. 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 sealed tightly and maintained at temperature of 26 ± 1° C. Each test was repeated three times. The assay was also conducted with the cotton yarn enclosed in a breathable cloth bag to prevent direct contact. Dimethyl 2, 2-dichlorovinyl phosphate (DDVP), a

volatile organophosphate, was used as a positive control. Mortality in each group was assessed after one hour of exposure.

Data analysis

The mean mortality data of the three repeated assays per dose (4-6 doses) was used to calculate the LC_{50}. Probit analysis (Harvard Programming; Hg1, 2) was used to analyze the dose-mortality response.

RESULTS AND DISCUSSION

From the fresh leaves of *C. alba* (300 g), 0.5 g (0.17%) of EO was obtained. The composition of the EO is listed in Table 1. 4-Terpineol (17.48%); 4-(3,3-dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethyl-cyclohex-2-enone (12.84%); 1,8-cineole (7,90%) [**3**]; p-cymene (7.11%) and sabinene (6.80%) , were the principal components of *C. alba* EO.

Table 1
Composition of the essential oil of leaves of *Cryptocarya alba*

Compound	RI	%	Identification	Compound	RI	%	Identification
hexanal *	802	0.37	RI,MS	α-terpineol *	1203	1.04	RI,MS,Co-I
3-hexenol *	862	0.42	RI,MS	myrcenal *	1213	0.27	RI,MS
α-thujene*	931	0.32	RI,MS	*trans*-piperitol	1220	0.17	RI,MS
α-pinene*	940	2.30	RI,MS,Co-I	α-cubebene	1366	0.40	RI,MS
camphene*	958	0.29	RI,MS	α-copaene	1395	0.48	RI,MS
sabinene *	981	6.80	RI,MS	β-elemene	1409	0.58	RI,MS
β-pinene *	985	1.53	RI,MS,Co-I	methyleugenol	1414	0.53	RI,MS
β-myrcene *	994	0.46	RI,MS	Caryophyllene	1445	0.14	RI,MS
p-cimene	1034	7.11	RI,MS	α-bergamotene	1452	0.55	RI,MS
1,8 cineol	1042	7.90	RI,MS,Co-I, NMR.	β-farnesene	1465	0.42	RI,MS
linalol *	1104	1.71	RI,MS	α-caryophyllene	1480	0.19	RI,MS
cis-β-terpineol	1133	0.33	RI,MS,Co-I	α-muurolene	1522	0.33	RI,MS
1-terpineol	1152	0.20	RI,MS,Co-I	α-amorphene	1539	0.21	RI,MS
trans-pinocarveol *	1155	0.25	RI,MS	calamalene	1548	2.67	RI,MS
2-nonenal	1167	0.68	RI,MS	viridiflorol	1599	0.30	RI,MS
1,4 cineol	1179	2.05	RI,MS	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	1613	12.84	RI,MS,NMR
β-terpineol	1185	3.15	RI,MS	caryophyllene oxide	1617	1.19	RI,MS
4-terpineol *	1192	17.48	RI,MS,Co-I, NMR.	cubenol	1657	0.60	RI,MS
ρ-cimen-8-ol	1196	0.40	RI,MS	α-cadinol	1670	0.65	RI,MS

RI: Retention index; MS: Mass spectrum; Co-I: Standard; NMR: Nuclear magnetic resonance;
***Also identified in Montes *et al*., 1988.**

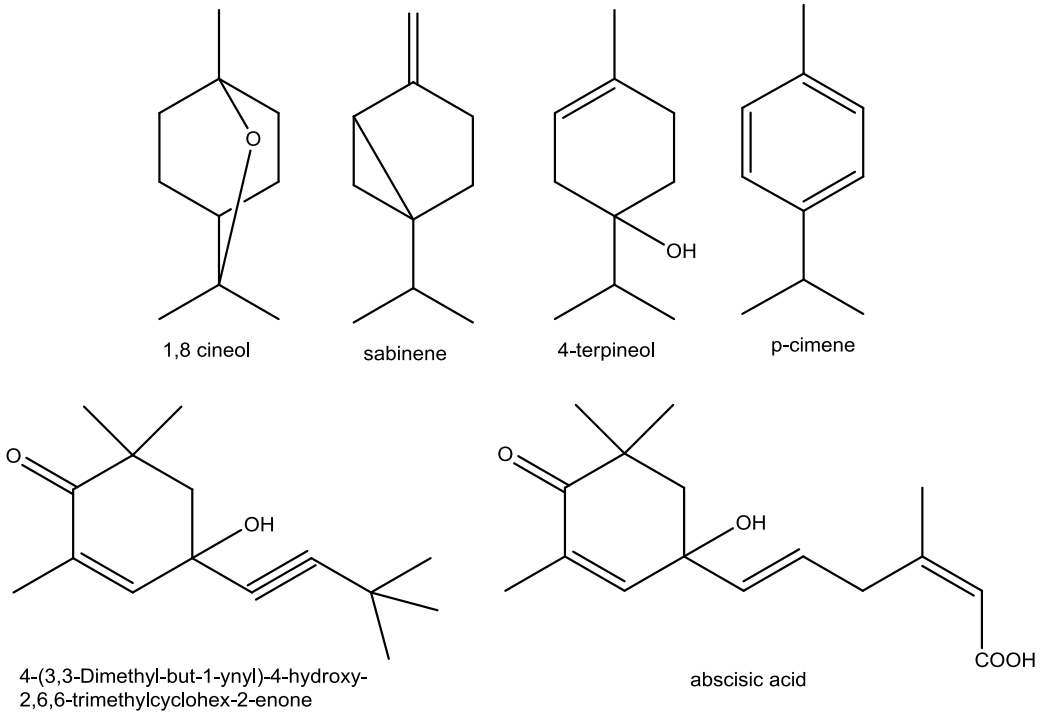

Figure 1
Principal terpenoids in the essential oil of *Cryptocarya alba* and abscisic acid

As far as we can determine, the composition of only one EO sample from *C. alba* have been investigated. Montes *et al.*, 1988, reported 72 compounds with 4-terpineol; 1, 8-cineole; *p*-cymene; α-pinene; limonene and β-pinene as the major compounds. The most important differences with our results is the absence of 4- (3, 3-dimethyl-but-1-ynyl) -4-hydroxy-2, 6, 6-trimethylcyclohex-2-enone. In addition a direct comparison is difficult as Montes *et al.*, 1988 did not report retention indices (RI) and the component identification of essential oils using only the GC-MS equipment database should be considered tentative (Adams, 2007).

Additionally, using ^{1}H and ^{13}C NMR studies of the EO was possible to confirm the three major components: 4-terpineol; 4- (3, 3-dimethyl-but-1-ynyl) -4-hydroxy-2, 6, 6-trimethylcyclohex-2-enone and 1, 8-cineole. For 4-terpineol and 1, 8-cineol, the assignment was performed using spectroscopic data published in the literature (Swigar & Silverstein, 1981). For 4- (3, 3-dimethyl-but-1-ynyl) -4-hydroxy-2, 6, 6-trimethylcyclohex-2-enone, the experimental chemical shifts were compared with spectroscopic data published in the literature for abscisic acid and derivatives (Milborrow, 1984).

The fumigant effects of EO against adult *M. domestica* were evaluated by determining the LC_{50} values, which are presented in Table 2.

Table 2
LC_{50} of *Cryptocarya alba* essential oil on *Musca domestica*

Time (h)	Mean LC_{50} in mg/dm^3 (95%CI)
0.5	33.56 (7.06 - 159.50)
1.0	15.07 (5.29 - 42.91)

Time: 1 h; t: 26 ± 1º C

The insecticidal properties of some monoterpenoids have been determined using the same bioassay, and the LC_{50} in a 0.5 h experiment were 3.35 mg/dm^3 for 1,8-cineol, 12.1 mg/dm^3 for α- pinene and 36.8 mg/dm^3 for 4-terpineol (Palacios *et al.,* 2009). Although the insecticidal properties of an essential oil may be related in principle to its individual components, in this case no correlation was possible because the *C. alba* EO contains many components in low proportion.

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