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Composition of essential oils isolated from three plant species and their molluscicidal activity against *Theba pisana* snails

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ABSTRACT

The essential oils of three plant species grown in Saudi Arabia, *Eucalyptus camaldulensis* Dehnh. (Myrtaceae), *Lavandula dentata* L. (Labiatae) and *Ruta chalepensis* L. (Rutaceae), were isolated by steam distillation. The constituents of these oils were identified by GC-MS. The main compounds in these three oils were Eucalyptole, α-Pinene, γ-terpinene, 4-terpineol, p-Menth-1-en-8-ol, 1-H-Cycloprop{e}azulene, 1-H-Cycloprop{e}azulene, decahyd, Dodecane and (-)-Globule, in *E. camaldulensis;* Camphor, Fenchone, Fenchol, Borneol, cis-linaloxide and β-Pinene, in *L. dentata* and 2-Nonanone, 2-Nonanol, Camphor, 2-Undecanone, Butylatedhydroxytoluene, 3,4-Methylenedioxybenzylacetone, in *R. chalepensis*. The isolated three oils were tested for their molluscicidal activity against the land snail, *Theba pisana*. The volatile oil of *L dentata* was the most effective one while that of *E camaldulensis* was the least effective oil with LD_{50} values of 238.2 and 658 μg/g body weight, respectively. The possible applications and uses of these volatile oils in the field of plant protection are discussed.

Key words: Essential oils, volatile oils, *Eucalyptus camaldulensis*, *Lavandula dentata*, *Ruta chalepensis*, molluscicidal activity, *Theba pisana*.

INTRODUCTION

Land mollusks (snails and slugs) are serious pests that cause severe economic damage to crops and ornamental plants (Barry, 1969; Miller *et al*., 1988 and South, 1992). The main current control method for these animal pests is the chemical method using pesticides. However, molluscicidal baits cause many environmental problems; metaldehyde is toxic to vertebrates and poultry (Homeida and Cooke, 1982). Methiocarb pellets caused hazards to non-target organisms (Bieri *et al*., 1989 and Buchs *et al*., 1989). Many alternative control methods have been tried in this field; some plant-derived compounds and extracts were found to be active against these pests, as molluscicides (Hussein *et al.*, 1994, 1999 and El-Zemity and Radwan 2001), antifeedants (Hussein and El-Wakeel, 1996; Hagele *et al.*, 1998 and Chen *et al.*, 2000, 2001) and repellents (Vokou *et al.*, 1998). Volatile oils from *Ruta* and *Lavandula* species were reported by several workers (Hassan *et al.*, 1976; Gamez *et al.*, 1990; Bagchi *et al.*, 2003 and Dob *et al*, 2005), but their effects against land snails have not been tested. The aim of this work was to report the composition of essential oils isolated from three plant species namely *Ruta chalepensis*, *Lavandula dentata* and *Eucalyptus camaldulensis*. The molluscicidal activity of these oils against *T. pisana* snails was also investigated. This work is a continuation to our previous efforts to find promising natural molluscicides (Hussein *et al.*, 1994, 1999 and Hussein and El-Wakil, 1996).

MATERIALS AND METHODS

Plants: Samples of fresh whole *Lavandula dentata* and *Ruta chalepensis*, were kindly provided by Dr. Ahmed Al-Ghamdy. Samples were collected from Araar, Saudi Arabia. Leaves of *E camaldulensis* were collected from *Eucalyptus* trees grown in the area of King Saud University, Riyadh. Plants were identified by the Botany Department, College of Science, King Saud University, Riyadh.

Isolation of the volatile oils: Leaves of *E camaldulensis, L. dentata* or *R. chalepensis* were cut into small pieces, and were directly steam distilled; the distillates were extracted with diethylether. The ether extracts were dried over anhydrous sodium sulfate. The solvent was evaporated under vacuum at 40 ^{0}C , using a rotary evaporator. The oily products were kept in glass vials with Teflon screw caps at – 20 ^{0}C

Instrumental Analysis: Agilant Technologies 6890 N Net work gas chromatograph system with Agilant 5973 mass selective detector was used for MS identification of the three isolated oils constituents. The gas chromatograph was equipped with Agilant 7683 injector. A HP-1 MS, 30 m x 0.25 mm i.d and film thickness 0.25 µm capillary column was used in combination with the following oven temperature programme: initial temperature 40 ^{0}C, held for 2 min, 2 ^{0}C / min ramp to 230 ^{0}C, held for 10 min. The flow rate of carrier gas (helium) was in constant flow mode at 1ml / min. Splitless injection of 1 µl sample, dissolved in methanol, was carried out at 250 ^{0}C. The mass spectrometer was operated in electron ionization mode with a transfer line temperature of 280 ^{0}C, ion source 230 ^{0}C and selected ion-monitoring mode.

Molluscicidal Activity:

Test snails: Adult specimens of *T. pisana* were collected from Sabahia Agricultural Experimental Station, Alexandria, Egypt. Snails were kept in cages at room temperature, provided with lettuce to feed on, three days before treatment. Weight of test snails was from 0.7 – 0.9 g.

Toxicity test: The molluscicidal activity of the tested oils was carried out according to our previous method (Hussein *et al.*, 1994). Tested oils were dissolved in dimethylsulfoxide (DMSO) at the range of 500-1000 μg/g of body weight for *Eucalyptus* oil and 100-400 μg/g for *L. dentata* and *R. chalepensis* oils. They were topically applied on the surface of the body of test snails inside the shell. Three replicates, with 10 snails each, were used for each dose. Control animals were treated with DMSO. Dead animals were counted after 48 hours and percentage mortality was corrected according to Abbott (1925). Probit analysis was carried out according to Finney (1971).

RESULTS AND DISCUSSION

Chemical Analysis:

Table 1 indicates the results of the MS analysis of the three volatile oils, which could be summarized as follows:

Oil of *E camaldulensis*: There were 131 compounds in this oil; the percentages of them ranged from 0.01 to 39.46 %. The main components were 1,8-cineole [eucalyptole (39.46 %)], alpha pienine (7.39 %), 1,4-Cyclohexadiene (5.98 %), 1-H- cycloprop[e]azulene (13.9 %) and 1-H-cycloprop[e]azulene, decahyd (11.86 %) and p-menth-1-en-8-ol (3.7%). These results agree well with those of Oyedeji *et al.* (1999 & 2000) who found that the volatile oil of *E. camaldulensis* consisted of eucalyptole (32.8-70.4 %) and alpha-pienine (8.8 %) and with Barton *et al.* (1989) who mentioned that the oil of *E. kochii* consisted mainly of monoterpenoids, with eucalyptole dominant.

Table 1. Volatile oils composition of *E. camaldulensis, R. chalepensis* and *L. dentata*

Plant	Compound	Retention time	%
E. camaldulensis	α-Pinene	11.50	7.39
	Eucalyptole	17.70	39.46
	γ-terpinene	19.55	5.98
	(+)-4-Carene	21.37	1.18
	4-terpineol	28.10	2.63
	p-Menth-1-en-8-ol	29.21	3.70
	Dodecane	29.76	2.30
	Tetradecane	43.64	1.21
	1-H-Cycloprop(e)azulene	43.85	13.90
	1-H-Cycloprop(e)azulene,decahyd	45.90	11.86
	(-)-Globule	54.76	2.12
L. dentata	α -Pinene	11.42	0.94
	Camphene	12.16	0.49
	β-Pinene	13.84	3.07
	Cis-Linaloxide	20.86	1.83
	Fenchone	21.89	15.00
	Fenchol	24.48	13.00
	Camphor	26.42	49.00
	Borneol	27.54	12.00
	Unknown	28.08	0.76
	Bicyco[3.1.1] hypt-2-ene-2-carb	29.39	1.28
	2-Naphthalenementhol	58.50	0.60
	Bis (2-ethylhexyl) phthalate	101.17	1.08
R. chalepensis	3-hexen-1-ol	8.18	0.50
	2-Nonanone	22.79	15.00
	2-Nonanol	23.55	3.95
	Fenchol	23.72	0.98
	Camphor	25.77	4.26
	Borneol	27.20	0.59
	2-Decanone	29.49	1.17
	Unknown	33.00	8.25
	2-Undecanone	37.38	24.00
	Unknown	37.53	1.17
	2-Dodecanone	41.37	1.39
	2-Tridecanone	49.76	0.83
	Butylatedhydroxytoluene	50.69	3.14
	3,4-Methylenedioxybenzylacetone	55.77	2.99
	Unknown	64.09	2.05
	2-H-furo[2,3-H]-1-benzopyran-2-one	68.24	0.97
	Unknown	78.87	1.00
	Phytol	82.72	0.61
	Unknown	85.28	5.90

Oil of *R. chalepensis*: The main compounds found in this oil were 2-undecanone (24 %), 2-nonanone (15%), 2-nonanol (3.95%), butylated hydroxytoluene (3.14%) and 3,4-methylenedioxybenzylacetone (2.99 %). Analysis of the oil isolated from *R. chalepensis* in India showed that the major constituents were 2-undecanone and 2-nonanone (Bagchi *et al.*, 2003). Also, similar results were recorded for the oil of *R chalepensis* from Turkey, with 2-undecanone and 2-nonanone which accounted as 66.49 and 26.24 %, respectively (Baser *et al.*, 1996). However, analysis of the oil of *R chalepensis* from Greece showed different pattern, with beta-phellandrene (10.7 %) and 2-methyloctyl acetate (44 %) as main constituents (Tzakou and Couladis, 2001).

Oil of *L. dentata*: The major constituents of *L dentata* volatile oil were Camphor (49 %), Fenchone (15 %), Fenchol (13 %), Borneol (12%), cis-linaloxide (1.83 %) and β-Pinene (3.07 %). These results agree well with those of Hassan *et al.* (1976) who found that the major constituent in the Saudi *L dentata* volatile oil was camphor; However in the present study, Fenchone, Fenchol, Borneol and β-Pinene represent other major constituents in *L. dentata* volatile oil that were identified using GC-MS. Gamez *et al.* (1990) identified the components of the volatile oil from the French *L dentata*, using GC-MS. The main constituent in the French oil was 1,8-Cineole (50.6 %), which was totally absent in the Saudi lavender, and the major constituent in the Saudi plant, Camphor, represents only less than 0.5 % of the French oil. Other constituents were commonly present in both Saudi and French Lavander oil, although at different ratios, β-Pinene, and Borneol; the essential oil of *L dentata* from Algeria consisted mainly from 1,8-cineole, cic-verbenol and p-cymene-8-ol (Dob *et al*, 2005). The differences in the constituents and their ratios among *L dentata* species from different countries may be attributed to environmental and geographical factors.

Molluscicidal activity: The relative potencies of tested oils against adults of *T pisana* are shown in Table 2. The oil of *L. dentata* was the most effective against *T. pisana* with LD_{50} and LD_{95} of 238.2 and 358.2 μg/g body weight, respectively. Camphor was the main constituent of this oil (49 %), this compound was shown to have many biological effects. El-Zemity *et al.* (2001) found that camphor was the most effective volatile compound against the land snail, *Helix aspersa*, with LD_{50} of 332.8 μg /snail. The LD_{50} of camphor against *T pisana* was 279.4μg/snail (El-Zemity and Radwan, 2001), which is very close to our results of this oil. The oil of *R chalepensis* came in the second order with LD_{50} and LD_{95} of 286.2 and 697.6 μg/gm of body weight, respectively. The main consituents of this oil were 2-nonanone (15%) and 2-undecanone (24%). Both constituents have not been tested against land snails before. Moreover, this oil contained a relatively high ratio of camphor (4.26%).

Table 2. Probit analysis of molluscicidal activity of tested volatile oils.

Source of oil	LD_{50}, µg/g b.wt. (95% CL)	LD_{95}, µg/g b.wt. (95% CL)	Slope ± SE
L. dentata	238.2 (227-249.8)	358.2 (330.8-387.9)	9.3 ± 0.8
R. chalepensis	286.2 (263.9-310.2)	697.6 (533.4-912.7)	4.25 ± 0.4
E. camaldulensis	658 (638.9-677.7)	798.3 (757.3-841.5)	19.6 ± 7.05

The oil of *E. camaldulensis* was the least effective one with LD_{50} and LD_{95} of 658 and 798.3 µg/g body weight, respectively. These results are in a good agreement with El-Zemity and Radwan (2001). They found that eucalyptole (which is the main component in the essential oil of Eucalyptus, 39.46%) was the least effective compound against *Helix aspersa,* and *T. pisana*, with LD_{50} of > 750 µg /snail. They reported that the LD_{50} of Eucalyptus oil against *T. pisana* was 535.73 µg /snail, which is very close to our results (658 µg/g body weight).

It could be concluded that the volatile oils of *L. dentata* and *R. chalepensis* are potent molluscicides. These oils are volatile and may disappear from treated surfaces (plants, crops, soils, etc.) within a short period leaving no residues, which render them safer than conventional pesticides. In addition, some constituents of these volatile oils are effective against many other pests, like insects (Mazyad and Soliman, 2001),fungi and bacteria (Aligiannis *et al.*, 2001 and Alvarez *et al.*, 2001), that is, they have a wide range of biological activities.

The present study suggested that these oils could be used against land snails in closed areas, like green houses, where their effect could last longer than in open fields. More effort is needed to formulate these oils in commercial forms to prolong the duration of their effects.

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مكونات الزيوت المتطايرة المعزولة من ثلاثة أنواع من النباتات والنشاط الابادى ضد القواقع الارضية

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تم عزل الزيوت المتطايرة من ثلاثة انواع من النباتات النامية في المملكة العربية السعودية عن طريق التقطير بالبخار وهى الكافور (العائلة الكافورية)، الحتحات (العائلة الشفوية)، الشذب (العائلة الشذبية). تم التعرف على مكونات هذه الزيوت بالتحليل الكروماتوغرافى الغازى الطيف كتلى. وكانت المركبات الاساسية في زيت الكافور هي يوكاليبتول، الفا- باينين، جاما-تربينين، 4-تربينيول، بارا-1-ميثيلين-8-اول، 1-يد-سيكلوبروب أزيولين-ديكا هيد، دوديكان، -(-)-جلوبيول-بينما كانت المكونات الرئيسيه في زيت الحتحات هي الكافور، الفينشون، الفينشول، البورنيول، سيس-لينالوكسايد وبيتا-بينين- اما في زيت الشذب فكانت المركبات السائدة هي 2-نونانون، 2-نونانول، كامفور، 2-أونديكانون، بيوتيل هيدروكسي طولوين و3، 4-ميثيلين دايوكسي بنزيل-اسيتون. كما تم دراسة النشاط الابادي للزيوت الثلاثه ضد القوقع الارضي تيبا بيزانا وكان الزيت المعزول من الحتحات أكثرها تاثيرا بينما كان زيت الكافور اقلها تاثيرا حيث كانت الجرعات القاتله لـ 50% من قوقع الاختبار هي 2، 238، 658 ميكروجرام / جرام من وزن الجسم على التوالي. تم مناقشة التطبيقات والاستخدامات المحتمله لهذه الزيوت في مجال وقاية النبات.