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PESTICIDAL ACTIVITY OF THREE SAUDI PLANTS

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ABSTRACT

The three plant species *Calotropis procera* (Ait) R Br, *Lavandula dentata* L. and *Juniperus procera* grow wild in different locations in the Kingdom of Saudi Arabia, extracts from the three plants were tested for their molluscicidal, bactericidal, fungicidal and insecticidal activity. Although extracts from *C procera* were highly toxic to the land snail *Theba pisana* Muller with LD_{50} of 8.11 mg/ kg and to *Culex pipiens* larvae with LC_{50} of 16.01 ppm, they did not show bactericidal or fungicidal activity. The crude oil of *J procera* and one of its fractions showed larvicidal activity with LC_{50} values of 84.35 and 42.01 ppm, respectively. Volatile oil of *Lavandula dentata* was not active against test organisms at tested concentrations.

INTRODUCTION

Recently, much interest has developed using natural chemicals from plants, microorganisms and venomous arthropods as insecticides (Arnason et al, 1989; Crombie, 1990). These organisms offer many potential new sources of pesticidal compounds with unique modes of action. Extensive research is underway to discover potential pesticides from these sources, particularly from plants which produce chemicals to defend themselves from pest attack (Jacobson, 1990; Powell, 1989). The three plant species *Calotropis procera* (Ait) R Br, *Lavandula dentata* L. and *Juniperus procera* grow wild in different locations in the Kingdom of Saudi Arabia, the first plant was shown to have some promising effects against many pest species (Morsy , 1997; Morsy et al., 2001; Latif et al, 1999; Awadh et al, 2001; Hussein et al, 1994; Hussein and El-Wakil,1996; Al-Rajhi et al, 2000). *Lavandula dentata* L. and *Juniperus procera* were shown to produce essential oils that contain many constituents (Adams, 1990 and Dob et al., 2005); therefore, this work was carried out to test the pesticidal activity of different extracts from the above mentioned three plants.

MATERIALS AND METHODS

Isolation of essential oils from *Lavandula dentata* L. and *Juniperus procera*:

Samples of *Lavandula dentata* L. and *Juniperus procera* were collected in January, 2003, from Abha, Saudi Arabia. Leaves were air dried ground and subjected to steam distillation; water distillate was extracted with diethyl ether, 2 x 200 ml, the organic layer was dried over anhydrous sodium sulfate and solvent was evaporated under reduced pressure to get the crude volatile oils which were analyzed with GC-MS. A portion of *J procera* oil was fractionated on silica gel column.

Fractionation of *J procera* oil: A portion of the crude oil of *J procera* was subjected to low pressure column chromatography (60 x 2.5 cm, filled with 150 gm silica gel for tlc); elution was carried out using 8 solvent systems: pet.ether, pet.ether-ether (75/25), pet.ether-ether (50/50), pet.ether-ether (25/75), ether, ether-acetone (80/20), ether-acetone (50/50) and acetone, 200

ml each. Fractions were chromatogramed on 20 x 20 silica gel plates, with chloroform-benzene as developing system. Spots were revealed by spraying with $H2SO_4$ /vanillin reagent and subsequent heating at 100 ^{0}C for 5 min, similar fractions were pooled and solvent was evaporated under reduced pressure. Fractions F1-F13 were pooled as J1, F14-F19 as J2, F20-F26 as J3, F27-F33 as J4 and F34-F38 as J5.

Instrumental Analysis

Agilant Technologies 6890 N Net work gas chromatograph system with Agilant 5973 mass selective detector was used for MS identification of the components of the three isolated oils. The gas chromatogram 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 carrier gas (helium) flow rate was in constant flow mode at 1.1ml / min. Splitless injection of one µl volume of the 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.

Extracts of *C procera*: Latex of *C procera* was collected from plants grown in Jeddah in brown glass bottles containing ethanol, the latex: ethanol ratio was 1:1; after filtration of 800 ml of collected latex on Buchner funnel, precipitate was washed with methanol and the methanol wash was added to the first filtrate. Filtrate was concentrated to 500 ml and 40 ml lead acetate (50 %) was added, followed with 10 ml of ammonium sulfate (30 %); filtrate was adjusted to pH 7 with 5 % sodium bicarbonate. The aqueous solution was extracted with chloroform 3 x 300 ml; the chloroform layer was washed with distilled water and dried with anhydrous sodium sulfate and evaporated to dryness to obtain the crude cardenolide as extract P.

Fractionation of extract P: Four gm of extract P was chromatogramed on silica gel column (150 gm, 70-230 mesh) with 7 solvent systems, 200 ml each: chloroform, chloroform-EtOAc (3:1), chloroform-EtOAc (1:1), chloroform-EtOAc (1:3), EtOAc, MeOH- EtOAc (2:98) and MeOH- EtOAc (5:95). Similar fractions were pooled after tlc on silica gel plates with EtOAc-MeOH (97:3) as developing system. Detection was carried out by spraying with Kedde reagent. Fractions 1-4 were pooled as Fraction P1, F10-14 as P2, F15-24 were concentrated to 200 ml and 400 ml pet.ether was added causing precipitation of 635 mg granular material (P3), solvent was evaporated to give 45 mg of a white powder (P4). Fractions eluted with 2-5 % MeOH- EtOAc were concentrated to 150 ml and kept at 5 0C for 48 h to give a yellow precipitate (P5), solvent was evaporated to give a yellow powder (P6).

Molluscicidal Activity

Test snails: Specimens of *T pisana* snails were kept at room temperature, provided with lettuce to feed on, three days before treatment. Average weight of test snails was 0.8 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 10 % aqueous dimethylsulfoxide (DMSO) and 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 same solvent. 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.

Mosquito colony rearing

A susceptible strain of *Culex pipiens* was reared at 25 ± 2 °C in wire-screened cages (45 x 45 x 45 cm). Adult females were blood- fed on a pigeon to get egg masses, the colony was provided with 10 % sucrose solution to feed on; hatched larvae were fed on finely powdered mouse feed.

Larvicidal activity: Twenty mosquito larvae (late 2nd and early 3rd instars) were placed in a 200 ml glass beaker containing 100 ml of distilled water. Stock of Test concentrations, dissolved in 0.3 ml (1 % tween 80 in ethanol) were added to give the required final concentrations and stirred quietly with a glass rod. Each concentration was replicated 3 times. Three controls that received only the solvent were maintained during the test. Dead larvae were counted 48 h after treatment; larvae that did not move when touched with a thin needle were considered dead.

Bactericidal and fungicidal tests: Bactericidal tests were performed on *Xanthomonas translucens, Pseudomonas corrugata, Escherichia coli* and *Bacillus subtillus*, using the disc diffusion method according to Lannett (1985). Fungicidal tests were carried out by the poisoned food technique on seven fungi species: *Fusarium oxysporium* (soy bean), *Pythium altimum* (bean), *Rhizoctonia solani* (egg plant), *Dothorella mangifera* (mango) , *Cholora porodoxa* (date palm) , *Fuzarium proliteratum* (date palm) and *Phoma glomerata* (date palm).

RESULTS AND DISCUSSION

GC-MS analysis of the tested oils

Table 1 shows the components of tested oils, the major compounds in *J procera* oil were α –Pinene, 3-carene, α –humulene, germacrene D and elemol. These results agree with Adams (1990). The major compounds of *L dentata* oil were α –fenchone, linalool and camphor; Sudria et al. (1999) reported that camphor was a major compound in the oil of *L dentata*.

Molluscicidal activity: Fraction P3 isolated from *C procera* was the most potent extract against the test snails (Table 2), it showed LD_{50} value of 8.11 mg/kg of body weight, followed by lannate which showed LD_{50} value equal to 34.18 mg/kg of body weight. This means that the *C procera* extract is 4.2 times more toxic to the test snails than the commercial molluscicide lannate (methomyl). This result is in good agreement with Hussein et al. (1996) and Hussein et al. (1994) who isolated a similar active extract from *C procera* against the same species and identified uscharin as the most potent molluscicidal compound tested against land snails. Fractions P5 and P6 had LD_{50} values more than 28.5 and 19 mg/kg, respectively. The crude oils of *J procera* and *L dentata* were not active up to 250 mg/kg. Compounds in fraction P3 should be isolated and tested individually on this species.

Table 1: GC-MS analysis of *J procera* and *L dentata* (retention time and percentage of components)

Plant	Compound	R time	%
J procera	α -Pinene	14.29	22.76
	1-β-Pinene	16.82	1.22
	β-myrcene	17.85	1.66
	3-carene	19.21	21
	B-phalandrene	20.24	1.23
	α- terpinoline	24.17	2.84
	caryophyllene	47.38	10.22
	α -humulene	49.62	12.41
	germacrene D	51.31	9.73
	elemol	55.29	3.42
	2-Naphthalenementhol	58.5	0.6
	α -eudesmol	61.65.	1.15
L dentate	linalool oxide	24.91	1.17
	α –fenchone	25.88	13.46
	trans- linalool oxide	26.06	1.18
	camphor	29.93	45.78
	B-eudesmol	61.56	1.6
	α –santalol	67.05	1.53

Table 2: Probit analysis of tested materials toxicity to *Theba pisana* [a]

Material	LD_{50} (95 % FL)	LD_{95} (95 % FL)	Slope ± SE
Fraction P3	8.11 (7.39-8.91)	22 (18.62-26.05)	3.8 ± 0.12
Methomyl	34.18 (29.86-39.19)	188.67 (142.1-251.36)	2.22 ± 0.04
Fraction P5	> 28.5		
Fraction P6	> 19		
J. procera oil	> 250		
L. dentata oil	> 250		

a: Values are in mg/kg of body weight

Insecticidal activity: The most active extract against *C pipiens* larvae was again fraction P3 isolated from *C procera* with LC_{50} equal to 16.01 ppm (Table 3), while fractions P1, P5 and P6 did not cause 50 % mortality up to 100 ppm. Fraction J2 isolated from Juniperus oil showed high insecticidal activity against mosquito larvae with LC_{50} of 42.01 ppm, the major constituent of this fraction was elemol; Ranaweera and Dyananda (1996) reported on the mosquitocidal activity of *Ceylon citronella* oil which contained elemol as the major constituent. Juniperus oil showed reasonable lowicidal activity, its LC_{50} was 82.55 ppm. Lavander oil was not active against mosquito larvae up to 1000 ppm. Girdhar et al. (1984) obtained 100 % mortality in mosquito larvae using the latex of *C procera* at 1 % (10000 ppm). Al-Rajhy et al. (2000) isolated an active extract against *C pipiens* 4th instar larvae with LC_{50} value of 27 ppm.

Table 3: Probit analysis of tested materials toxicity to *C pipiens* larvae[a]

Material	LC_{50} (95 % FL)	LC_{95} (95 % FL)	Slope ± SE
Fraction P3	16.01 (13.75-18.66)	77.62 (58.17-104.09)	2.4 ± 0.06
Fraction J2	42.01 (38.54-45.79)	118.05 (101.03-138.02)	3.67 ± 0.08
J. procera oil	84.35 (78.71-90.40)	176.3 (144.4-215.33)	5.1 ± 0.32
L. dentata oil	> 1000		

a: values are in ppm

Bactericidal and fungicidal activity: The crude extract of *C procera* (P) and the crude oil of *J procera* did not cause 50 % inhibition in the bactericidal tests up to 1000 ppm (40 µg/disk) and up to 1000 pppm in the fungicidal tests. The crude oil of *L dentata* was not active against test bacteria: however, at 1000 ppm, it caused 69.4 and 76 % inhibition in the growth of the two fungi, *Pythium ultimum* and *Rhyzoctonia solani.*

Conclusion:

C procera has higher molluscicidal activity than methomyl. it is a good source of highly active molluscicides, Hussein et al. (1994) identified one of these compounds, but the rest of its components should be isolated, identified and tested against land snails, the plant has also insecticidal activity against mosquito larvae, but its molluscicidal activity is much more higher than its insecticidal activity. The oil of *L dentata* has insecticidal activity against mosquito larvae, however, the effective concentrations are considered high when compared to the commercial insecticides

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النشاط الابادى لثلاثة نباتات سعودية

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تنمو نباتات العشار والجتجات والعرعر بالرياض بمناطق مختلفة من المملكه العربية السعودية- وتم اختبار مستخلصات النباتات الثلاثه ضد القواقع والبكتريا والفطريات والحشرات .وبرغم النشاط القوى لمستخلصات العشار ضد قوقع االتيبا بيسانا (الجرعه القاتله النصفيه = ٨,١١مجم/كجم) وضد يرقات بعوض الكيولكس بيبينز (التركيز القاتل النصفى = ١٦,٠١ جزء فى المليون) الا انه لم يظهر نشاط ضد البكتريا او الفطريات. وأظهر الزيت الخام لنبات العرعر وأحد أجزاءه نشاطا ضد يرقات الكيولكس (التركيز القاتل النصفى = ٨٤,٣٥ و ٤٢,٠١ جزء فى المليون على التوالى). لم يظهر الزيت المتطاير لنبات الجتجات أى نشاط ضد الآفات المذكورة عند التركيزات المختبرة.