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Schistosomicidal and molluscicidal activities of two *Junipers* species cultivated in Egypt and the chemical composition of their essential oils

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In the present study, *in vitro* bioassay screening of total methanolic extracts of two *Juniperus* species (*Juniperus horizontalis* Moench. and *Juniperus communis* L.) cultivated in Egypt for schistosomicidal and molluscicidal activities was carried out. *Schistosoma mansoni* Sambon worms and *Biomphalaria alexandrina* (Ehrenberg) snails were used. The screening results showed that both tested extracts had almost similar schistosomicidal activity (LC50 ≈ 91 µg/ml, in 3 days) while *J. communis* extract possess more potent molluscicidal activity than *J. horizontalis* (LC_{50} = 22.9 and 38.9 ppm, after one day, respectively). Analysis of the chemical composition of the essential oils obtained by hydro-distillation of the aerial parts of the two *Junipers* species was done by GC/MS. Sixty seven (94.32%) components were identified in *J. communis* oil with homogeraniol (36.95%) being the major constituent, while sixty (95.39%) components were identified in *J. horizontalis* oil with the main component, bronyl acetate representing 41.17%.

Key words: Essential oils, *Juniperus*, molluscicidal, schistosomicidal.

INTRODUCTION

The Cupressaceae or Cypress family is a conifer family of cosmopolitan distribution. The family includes about 70 genera (17 monotypic) with about 130-142 species. One of the important genus is *Juniperus* which has been well known as a source of cedarwood oil and widely used in folk medicine (Seca et al., 2007; Gumral et al., 2015). In the present study, the chemical composition of the essential oils of two *Juniperus* species was investigated: *Juniperus horizontalis* Moench known as creeping juniper and *Juniperus communis* L. known as juniper berry cultivated in Egypt and the antiparasitic effect of the methanolic extract of these two species were investigated. Several biological activities has been tested for both species under investigation such as hepatoprotective (Manvi et al., 2010), anti-inflammatory (Tunon et al., 1995), analgesic (Banerjee et al., 2012), antibacterial (Sati et al., 2010; Eryiğit et al., 2014), anti-hypercholesterolemic (Akdogan et al., 2012), antioxidant Stoilova et al., 2014) and antimalarial (Milhau et al., 1997). These biological activities may be attributed to the

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diversity of chemical constituents found in this two species e.g. volatile oils (Hoferl et al., 2014; Chandra et al., 2007), flavonoids (Ilyas et al., 1990; Lamer 1975) glycosides, sterols and di and triterpenes (Souravh et al., 2014). One of the biggest challenges in Egypt is the control of bilharzias or Schistosomiasis. Schistosomiasis is a parasitic disease caused by the digenetic trematodes of the genus *Schistosoma* which are commonly known as blood flukes. It is well documented that *Schistosoma haematobium* was endemic in Ancient Egypt. It was first diagnosed in mummies by Ruffer in 1910 (Rashida, 2013). Schistosomiasis comes after malaria among parasitic diseases with regard to the number of people infected and those at risk of infection (Chitsulo et al., 2000). In the continuous search for a control of this parasitic infection, the total methanolic extract of the aerial parts of both *J. horizontalis* Moench and *J. communis* L were screened for their schistosomicidal and molluscicidal activities.

MATERIALS AND METHODS

Plant

Non-flowering aerial parts of *J. communis* L. and *J. horizontalis* Moench were collected on April 2013 from the International Garden at Cairo, Egypt. Identification of the plants was confirmed by Dr. Therese Labib, specialist of plant identification in El Orman Garden, Cairo, Egypt. Two voucher specimens (No. JH-36 *J. horizontalis* and JC-37 *J. communis*) were deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University.

Preparation of plant extracts and oil extraction

Fresh aerial parts of both *J. horizontalis* and *J. communis*, 200 g each, were suspended in twice their volumes of distilled water and subjected to steam distillation for 6-8 h using volatile oil distillation apparatus. Prior to distillation, the samples were chopped into about 2 cm long pieces. The distillate was allowed to cool at room temperature, volatile oil was allowed to separate from water and each essential oil sample was weighed on analytical scale (1.5 ml (0.75% v/w) from *J. horizontalis* and 1.7 ml (0.85 % v/w) from *J. communis*). The oils were collected, dried over anhydrous Na_2SO_4 and kept in a freezer at -5°C until the GC-MS analysis can be performed. 10 g of the concentrated total alcoholic extract of the aerial part of each of *J. horizontalis* and *J.communis* prepared in 80% methanol were reserved for schistosomicidal and molluscicidal study.

Schistosomicidal activity (WHO, 1985)

The schistosomicidal *in vitro* effect of each plant was determined according to Yousif et al. (2007). The schistosome material used in the present study was supplied by the Schistosome Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute, Cairo, Egypt. Adult male and female *Schistosoma mansoni* Sambon worms were collected 7 weeks post exposure of laboratory bred Syrian golden hamsters (*Mesocricetus auratus*) to cercariae by perfusion technique. Worms were cleaned from blood in small sieves of 20 µ mesh size using phosphate buffer (pH 7.4). A stock solution (500 µg/ml) of each plant extract was prepared in 100% dimethyl sulfoxide (DMSO) and 0.1 ml of this solution was made to 2 ml with RPMI 1640 containing 20% fetal calf serum, 300 mg streptomycin, cc 300 units pencillin and 160 µg gentamicin/100 ml medium. The obtained stock solution was diluted by the same medium to give the following concentrations: 100, 80, 60, 40 and 20 µg/ml. Three pairs of worms, males and females equally represented were placed in each well using sterilized forceps, they were exposed to these concentrations for 3 days and two replicates were done for each concentration. Praziquantel, the most effective schistosomicidal drug was used as a positive control (0.2 µg/ml) and a clean medium was used as a negative one to allow critical comparison of the effect. Test and control wells were maintained in an incubator at 37°C examined daily for 3 days for worm viability using stereomicroscope. Worms which did not show any sign of motility for one minute were considered dead. The activity of the plant extract was measured by calculating the number of dead worms relative to the total number of worms and compared with the negative (DMSO) and positive (praziquantel) controls.

For determination of LC_{50} (lethal concentration that kills 50% of the worms) and LC_{90} (lethal concentration that kills 90% of the worms), the same experiment was repeated six times. The worm mortality was recorded in each case and the LC50 and LC90 was determined using SPSS statistical program (version 20, Chicago, IL, USA).

Molluscicidal activity

The molluscicidal efficacy of the plant extracts was determined against the snails using standard method (WHO, 1965; El Bardicy et al., 2012). Adult *Biomphalaria alexandrina* (Ehrenberg) (Planorbidae) snails were obtained from the laboratory colony at the Schistosome Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute, Cairo, Egypt. A stock solution of 1 L of the dechlorinated water with a concentration 100 ppm of each extract was prepared and the following concentrations (20, 30, 40, 50 and 60 ppm) were tested and ten snails were added to each concentration. They were maintained in the solution for 24 h at room temperature (25 ± 1°C). After the exposure period, the snails were washed thoroughly with dechlorinated water and maintained in fresh water for another 24 h for recovery. In each case, two replicates were performed and two groups of snails were used as negative and positive control groups. The conventional molluscicide (niclosamide) at the same concentrations was used as a positive control; dead snails were counted in each case. Snails that were killed either during exposure or recovery period were counted and recorded.

For determination of LC molluscicidal effect, the same method using descending concentration was performed and LC_{50} and LC_{90} were determined by SPSS statistical program (version 20).

Chemical composition study

Gas chromatography/mass spectroscopy [GC/MS] analysis was performed using a Shimadzu GC-17A gas chromatograph equipped with a DB5-MS fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm) and coupled to GCMS-QP 5050 mass analyzer. Operating conditions were: carrier gas helium, flow rate 0.9 ml/min; oven temperature program: 40-240°C at 3°C/minute; sample injection port temperature, 240°C; detector temperature, 230°C; ionization voltage and ionization current were according to tuning result; scanning speed, 0.5 s; split, 1:54. Essential oil components peaks were first deconvoluted using AMDIS software. Compounds were identified by their Kovates indices (KI) relative to n-alkanes (C6-C20) and through matching mass spectra and retention indices with those deposited in the NIST, WILEY library database and

Table 1. Results of screening of schistosomicidal activity of total alcoholic extracts of both *J.communis* and *J. horizontalis* on *schistosoma mansoni worms*.

Parameter	*J. communis*	*J. horizontalis*	Praziquantel
LC_{50} (µg/ml)	91.4	91.7	0.27
LC_{90} (µg/ml)	127.4	143.2	0.37

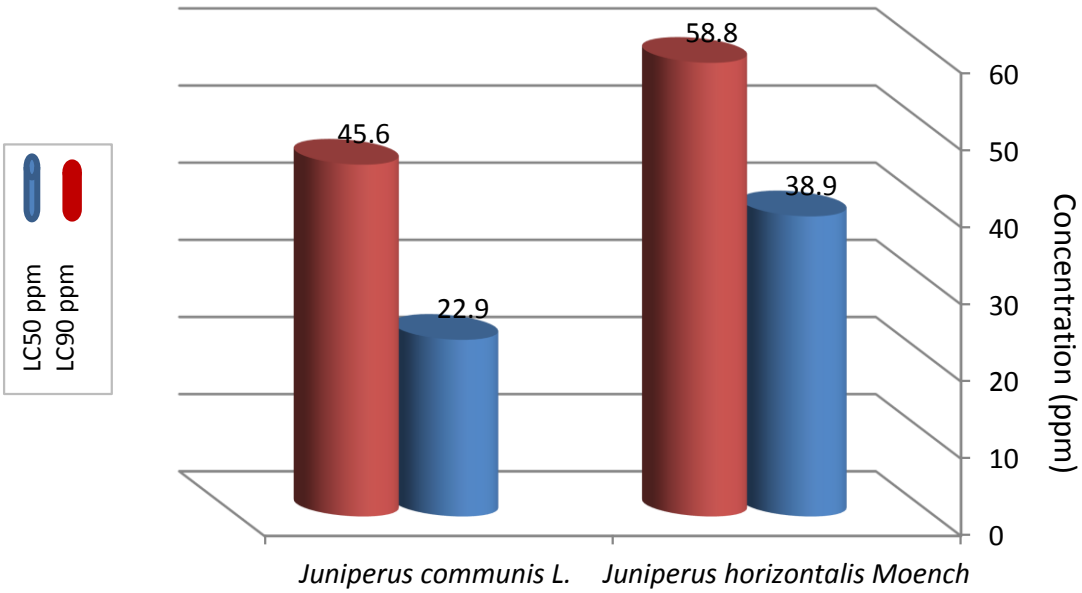

Figure 1. Results of screening of the molluscicidal activity of the methanolic extracts of both *J. horizontalis* and *J. communis.* Y-axis represent the concentration in ppm, X-axis represent LC_{50} and LC_{90} (% mortality) for different test fractions

reported in the literature. The distillation apparatus used was (VWR Scientific, catalog no.26319-008) rotatory evaporator (Buchi, G. Switzerland).

RESULTS

The screening results of schistosomicidal and molluscicidal activities of the total methanolic extracts of the two *Juniperus* species under investigation showed nearly equivalent schistosomicidal activity after 3 days exposure (Table 1). However this activity is less than that of praziquantel used as a positive control but still under 100 ppm. As for the molluscicidal activity against *Biomphalaria alexandrina* snails, *J. communis* extract showed higher activity with an LC50 equivalent to 22.9 ppm as compared to 38.9 ppm after one day exposure for *J. horizontalis* (Figure 1).

The chemical composition of the oils of the two *Juniperus* species under investigation analyzed by GC/MS (Table 2) showed a total of 60 compounds for *J. horizontalis* oil and 67 components for *J. communis* oil. The percentage of component identification for both plants was more than 90%.

DISCUSSION

Screening of the biological activity of the methanolic extracts of the two *Juniperus* species under investigation (Table 1 and Figure 1), showed that the schistosomicidal activity against *Schistosoma monsoni* worms of the total alcoholic extract of both species is nearly equivalent as seen in their LC_{50} values which is equal to 91.4 and 91.6 µg/ml for *J. communis* and *J. horizontalis*, respectively. In both cases, it is less than 100 ppm and consequently considered to posses schistosomicidal activity according to the recommendation of the world health organization (WHO, 1985). However, their effect is lesser than that of praziquantel (standard positive control used) showing an LC_{50} equal to 0.27 µg/ml. In the case of molluscicidal activity against *B. alexandrina* snails, *J. communis* extract showed higher activity than that of *J. horizontalis* with an LC_{50} = 22.9 ppm. These results are similar to that of

Table 2. Chemical composition of *J. communis and J. horizontalis* oils analyzed using GC/MS.

Identified compound	KI	*J. communis*	*J. horizontalis*
		area %	area %
1-Butene, 2-ethyl-3-methyl-	859.3	…	0.08
Tricyclene	924.7	0.46	0.4
α-Thujene	927.7	0.38	
α-Pinene	936.2	0.81	0.73
Vinylsulfonamide	946.3	0.25	….
Camphene	953.7	0.53	0.42
Sabinene	976.3	4.5	5.51
β-Pinene	981.9	0.19	….
β-Myrcene	990.4	5.15	5.94
Cyclobutane, 1,2-diethenyl-3,4-dimethyl	1002.1	0.21	….
N-Acrylonitrylaziridine	1002.2	…	0.03
α-Terpinene	1020.5	0.7	0.41
o-Cymene	1028.3	…	0.07
p-Cymene	1028.4	0.16	….
D-Limonene	1034	3.46	3.21
Bicyclo[4.1.0]heptane, 7-methylene	1048.1	…	0.14
Spiro[4.4]non-1-ene	1048.2	0.07	…
γ-Terpinene	1062.9	1.14	0.84
4-thujanol	1078.2	0.69	0.34
2,4(8)-p-Menthadiene	1090.5	…	0.55
α- Terpinolene	1090.7	0.62	…
Linalool	1102.1	0.61	0.25
2-Dimethylamino-3-methylpyridine	1109.5	…	0.15
Cyclopenta[c]pyran-1,3-dione, 4,4a,5,6-tetrahydro-4,7-dimethyl-	1110.6	0.19	…
6-Nonen-1-ol, (E)-	1114.9	0.5	…
Thujone	1126.3	1.98	4.8
β-Thujene	1133.3	…	0.2
α-Fenchene	1134	0.29	….
1H-Pyrrole-2-carbonitrile	1146	0.44	0.02
Butanenitrile	1148.3	…	0.11
Benzene, azido-	1149	0.15	…
α-Phellandrene	1151.7	…	0.18
(+)-2-Bornanone	1159.8	…	0.46
Camphor	1160.5	0.45	…
2-Fenchanol	1168.8	0.28	0.33
2-(1-Cyclopentenyl)furan	1179.2	…	0.39
2H-1-Benzopyran, 3,4-dihydro	1179.8	0.44	…
Ethanone, 1-(2-furanyl)	1184.2	…	0.17
5,5-Dimethyl-1,3-hexadiene	1184.9	0.2	…
Terpinen-4-ol	1191.1	3.82	2.88
Cyclohexanemethanol, α,α,4-trimethyl-	1204.2	…	0.29
α-Terpineol	1204.9	0.19	…
Urea, phenyl-	1218.4	…	0.16
2-Pyridinamine, N-nitro-	1219.2	0.05	…
Benzene, 1-methoxy-2-(1-methylethenyl	1226.9	0.92	1.01
1,6-Octadiene, 3,7-dimethyl-, (S)-	1229.7	…	0.21
1,6-Heptadiene, 3,3-dimethyl-	1231	0.41	…
1,2-Cyclooctadiene	1245.6	0.26	…
Methyl citronellate	1257.9	…	0.38
diazoadamantane	1259.5	0.45	…

Table 2. cont'd

cis-Geraniol	1265.7	…	0.08
4-Hepten-2-one, (E)-	1285.1	…	0.25
(Z) -8-Hydroxygeraniol	1286.4	0.41	…
Bornyl acetate	1295.4	…	41.17
Homogeraniol	1296.7	36.95	….
3-Phenylbut-1-ene	1316.6	…	0.05
Dispiro[2.2.2.2]deca-4,9-diene	1317.3	0.05	…
(E)-Geranic acid methyl ester	1324.1	0.09	0.09
1H-Pyrrole, 2,5-dihydro-	1330.1	…	0.07
Isobutyl 3-methylbut-3-enyl carbonate	1330.9	0.06	…
6,7-Dihydro-[1,2-e]-5H-pyrrolotetrazole	1357.8	…	0.06
Borane, ethylisopropylmethyl-	1358.6	0.09	….
5-Isopropenyl-1,2-dimethylcyclohex-2-enol	1382.2	…	0.08
4,4-Dimethyl-1,1a,3a,4,5,6-hexahydrocyclopropa[c]pentalene	1383.1	0.07	….
20-Carboethoxy-20-demethylvincadifformine	1397.2	…	0.1
2-Methoxycarbonylspiro[2.3]hexane	1398	0.14	…
3-Morpholino-1,2-propanediol	1401.4	…	0.15
Methanamine, N,N-difluoro-	1454.7	0.08	0.04
Silanamine, N-(dimethylsilyl)-1,1,1-trimethyl-, N-methyl	1467.5	0.07	…
4(3H)-Quinolinone, 3-hydroxy-	1481.6	0.04	…
D-Alanine, N-(4-butylbenzoyl)-, isohexyl ester	1488.1	…	0.14
Thiazole, 4-phenyl-	1489.1	0.14	…
(+)-Epi-Bicyclosesquiphellandrene	1501.3	…	0.15
cis-muurola-4(14),5-diene	1501.9	0.46	0.51
Tricyclo[6.2.1.0(2,6)]undeca-2(6),3-diene, 11-methyl-5,11-diaza-	1513.1	…	0.46
Cadina-3,9-diene	1531.2	…	1.68
Cadine-1,4-diene	1532.1	1.59	…
3-[Tetrahydro-3-thienyl]-2-oxazolidinone-S,S-dioxide	1537.8	…	0.08
Urea,1-furan-2-ylmethyl-3-[2-[(furan-2-ylmethylmethylamino) methyl]phenyl]-1-methyl-,	1538.6	0.1	…
N-(2-Phenylethenyl) acetamide	1547.3	…	0.06
10-Epi-elemol.	1563.5	9.45	8.44
3-Hexen-1-ol, benzoate, (Z)-	1581.9	0.1	…
9,9'-Bi-9H-fluorene, 9,9'-dimethoxy-	1588	0.08	…
1-Butyne, 3,3-dimethyl-	1598.3	0.24	…
6-(3-Methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol	1610.4	…	0.35
verbenyl acetate	1611.1	0.45	…
trans-β-Ionone	1624	2.25	1.93
1,3,4-Oxadiazole-2-thiol, 5-(3-pyridinyl)-	1633.8	0.28	…
α-Cubebene	1645.4	1.19	0.95
Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	1649.3	…	0.74
10-epi-γ-Eudesmol	1650.1	1.03	…
α-Cadinol	1659.2	…	1.96
(Cyclopropyl)trivinylsilane	1660.2	2.4	…
tau.-Cadinol	1672.1	….	2.41
tau.-Muurolol	1673.4	3	…
α-Eudesmol	1677.3	1.3	1.12
4-(Phenylmethyl)benzenemethanamine	1745.4	0.01	…
N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine	1779.6	0.03	…
Bergamotol, Z-α-trans-	1793.3	…	1.25
Lanceol, cis	1794	0.86	…
Ditrifluoromethyl(fluorocarbonyloxy)amine	1826.9	…	0.17

Table 2. cont'd

3,4-Dihydro-2,7-dimethylpyrimido[4,5-d]pyrimidine	1833.3	0.05	…
1,3-Di-n-Propyladamantane	1851.5	0.08	…
Ethanone, 1-(9-anthracenyl)-	1854.7	0.23	…
Acetamide, N-ethyl-N-(phenylmethyl	2888.4	…	0.19

previous study done on the genus *Juniperus* (*J. brevifolia*), showing high molluscicidal activity (Teixeira et al., 2012). Two other plants grown in Egypt belonging to family Cupressaceae were screened *in vivo* for schistosomicidal activity, namely *Chamaecyparis lawsoniane* and *Cupressus lasitanica* Mill. The extract of the bark of the former plant proved to show considerable effect with an LC50=59.6 ppm (Yousif et al., 2007).

It is worth mentioning that molluscicidal and schistosomicidal activity of several plants rich in volatile constituents were previously studied, such as *Thymus capitatus Marrubium vulgare* and *Chrysanthemum viscidehirtum*, showing promising activities against different parasites (Khallouki et al., 2000; Salama et al., 2012).

The oil content obtained by hydro-distillation of the aerial parts of *J. horizontalis* representing 0.75% v/w was analyzed using GC/MS (Table 2) showing a total of 60 compounds with a percentage of 95.39% of identified compounds. Bornyl acetate was seen as a major component representing 41.17% followed by 10-epi-elemol representing 8.44% and β-myrcene 5.94%, sabenine 5.51% and thujone 4.8%. This data greatly differs from that previously published on *J. horizontalis* essential oil composition (Eryiğit et al., 2014) and also differed from the composition stated by Cantrell et al. (2014) who showed that the major constituents present in the oil of *J. horizontalis* were alpha-pinene, sabinene and limonene. As for *J. communis* essential oil composition showed 67 component representing 0.85% v/w of which 1,7-Nonadien-4-ol, 4,8-dimethyl (homogeraniol) (36.95%) was the major component followed by 10-epielemol (9.45%) and β-Myrcene (5.15%), sabenine (4.5%), also this data differs from that already published on *J. communis* obtained from other sources mainly predominated by monoterpene constituents (Stoilova et al., 2014). Differences in essential oil composition may be expected due to geographical occurrences, climatic differences, the source of the oil either of commercial or of natural origin and may be due to the well proved relation between the time of collection and the percentage of active constituents and finally may be due to genetic variability among species (Khanzadi et al., 2015; Mammen et al., 2010). The high content of oxygenated compounds in the two species under investigation may explain the strong characteristic odor of these plants as referred to by Lund et al. (1981). However, Bornyl acetate was found to be absent in the aerial part of *J. communis*, while homogeraniol was found to be absent in the aerial part of *J. horizontalis*. It is worth mentioning that several compounds identified in the oil composition of the two studied species by the GC/MS analysis belongs to other classes such as hydrocarbons, fatty acids, minor alkaloids, amides and coumarins.

Conclusion

Both plants are good candidates for further studies on their schistosomicidal activity to determine the best effective doses to be used in the control of such dangerous parasite. The chemical composition of the essential oils of the two tested species of *Juniperus* differs greatly from the same species previously studied in different world zones and analyzed by similar analytical technique (GC/MS) which may be attributed to the difference in both climatic conditions and genetic characters of the studied species.

Conflict of interests

The authors have not declared any conflict of interests.

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