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GC-MS Analysis, Free-Radical-Scavenging and Insecticidal Activities of Essential Oil of *Scrophularia oxysepala* Boiss.

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ABSTRACT

Background: *Scrophularia oxysepala* Boiss. is endemic to western and central regions of Iran, and belongs to the genus *Scrophularia* (family: Scrophulariaceae), which has long been used in traditional medicines. In the present work, chemical composition of the essential oil of the aerial parts of this species was analyzed. ***Methods:*** The air-dried ground aerial parts of *S. oxysepala* were subjected to hydro-distillation. The resulting oil was analyzed, for the first time, by the gas chromatography/mass spectrometry (GC-MS) and gas chromatography/flame ionization detection (GC-FID). Free-radical-scavenging activity of the essential oil was determined by the DPPH assay. Adults of *Oryzeaphilus mercator* were used for contact toxicity insecticidal assay. ***Results:*** A total yield of 0.1 ml of essential oil per 100 g of plant dry mass was obtained, and 15 compounds were identified, representing 91.2% of total oil. The essential oil was characterized by a high content of aromatic compounds and phytol. The main constituents were phytol (25.3%), methyl benzyl alcohol (9.3%), dehydrodieugenol (6.7%), methyl benzaldehyde (5.3%) and eugenol (1.3%). The low level of free-radical-scavenging activity of the essential oil was demonstrated by the RC_{50} value of 1.852mg/ml, and the insecticidal activity was also found to be low. ***Conclusion:*** The insecticidal activity of this essential oil was not remarkable, but was time and concentration-dependent. The low free-radical-scavenging activity was probably due to its low percentage of phenolic compounds.

Introduction

The genus *Scrophularia* L. (Scrophulariaceae) comprises about 200 species of herbaceous flowering plants, commonly known as 'figwort' and *Scrophularia oxysepala* Boiss. is one such species that is endemic to western and central regions of Iran.[1] Species of this genus are distributed throughout the northern hemisphere, but concentrated in Asia with only a few species in Europe and North America. Plants from this genus have long been used in traditional medicines around the world, *e.g.*, Ningpo figwort or Chinese figwort (*S. ningpoensis*), for the treatment of a form of tuberculosis. These species also have been found to possess antibacterial, antiprotozoal, antitumor, anti-inflammatory and diuretic properties, and have been used in the treatment of mental, nervous and gastrointestinal disorders. *Scrophularia nodosa*, also known as figwort, is a perennial herb that grows in Central Europe, Central Asia, and North America and has been used in traditional medicine to treat eczema, wounds, ulcers, fistulae, cancer, and also as a diuretic and anthelminthic agent. In modern herbal medicine *S. nodosa* is used to treat eruptive skin[2-8] diseases, eczemas, psoriasis, pruritis, ulcers and also as a purgative agent. Numerous species of the *Scrophularia* have been studied phytochemically showing the presence of biologically active phenylethanoids, phenylpropanoids, flavonoids, iridoids, iridoid glycosides and terpenoids.[9-12]

Essential oils make a major contribution to the biology of plants and also play an important role in offering pharmacological properties of plants. Numerous studies have been conducted on plant essential oils, their chemical compositions and pharmacological properties. Considering these points and as part of our on-going studies on Iranian medicinal plants, we have studied the aerial parts of *Scrophularia oxysepala* Boiss., commonly known as 'Shah Billi' in Iran. To the best of our knowledge, no research has been conducted on

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pharmacological activates or chemical composition of the essential oil of *S. oxysepala*. We now report on the free-radical-scavenging and insecticidal activities of the essential oil of this plant, as well as on the analysis of its essential oil by the GC-MS and the GC-FID techniques leading to the identification of 15 individual components.

Materials and Methods

Plant material

The aerial parts of *S. oxysepala* were collected from East Azerbaijan province 30 kilometer to Kalibar town, Gareh Dagh mountain during the flowering period and a voucher specimen (2821) has been deposited at the Herbarium of the Research Center for Agriculture and Natural Resources East Azerbaijan, Iran.

Distillation of plant materials

The air-dried ground aerial parts of *S. oxysepala* (100 g) were subjected to hydro-distillation for 4h using a Clevenger-type apparatus. The resulting oil was dried over anhydrous sodium sulfate and kept in a refrigerator at 4°C.

GC-MS and GC-FID analyses

The essential oil was analyzed using a Shimadzu GCMS-QP5050A gas chromatograph-mass spectrometer (GC-MS) fitted with a fused methyl silicon DB-1 column (60 m x 0.25 mm i.d., 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1.3 ml/min. The column temperature was kept three min at 50°C, increased to 260°C at a rate of 3°C/min, and finally kept 5 min at 260°C. The injector temperature was 240°C and split ratio was adjusted at 1:33. The injection volume was 1 µl (10 mg/ml in *n*-hexane) for analysis. The mass spectral (MS) data were obtained at the following conditions: ionization potential 70 eV; ion source temperature 200°C; quadrupole temperature 100°C; solvent delay 2 min; resolution 2000 amu/s and scan range 30-600 amu; EM voltage 3000 volts. Identification of compounds was based on direct comparison of the Kovats Indices (KI) and MS data with those for standard compounds, and computer matching with the NIST NBS54K Library, as well as by comparison with references.[13-15]

For quantitation (area %), the GC analyses was also performed on a Shimadzu GCMS-QP5050A gas chromatograph fitted with a FID detector. The FID detector temperature was 300ºC. To obtain the same elution order as with GC-MS, injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms obtained from the GC-FID analysis of the essential oil of the aerial parts of *S. oxysepala.*

Assay for free-radical-scavenging activity

The *in vitro* free-radical-scavenging activity of the essential oil was determined by the DPPH assay.[16] 2,2-Diphenyl-1-picrylhydrazyl, molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Sigma-Aldrich (Germany). DPPH (4 mg) was dissolved in MeOH (50 ml) to obtain a concentration of 80 µg/ml. The essential oil of *S. oxysepala* was dissolved in MeOH to obtain a concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625, 0.001953125, 0.000976563, 0.000488281 mg/ml. Diluted solutions were mixed with DPPH (1 ml) and allowed to stand for 30 min for any reaction to occur. The UV absorbance of sample and blank (without sample) was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control, quercetin. Percent inhibition of the free radical DPPH (I %) was calculated in the following way: I% = [(AB–AA)/AB] x 100, where AB: absorbance of blank; AA: absorbance of test sample. Concentration providing 50% inhibition (RC_{50}) was calculated from the graph plotting inhibition percentage against test sample concentrations.[16,17]

Contact toxicity insecticidal assay

Adults of *Oryzeaphilus mercator* were collected from a laboratory culture, and reared on a mixture of whole wheat and maize flour at the ratio 1:1 in glass containers containing 0.5 kg of the mixture. All insect were reared at 27±2°C, 12% moisture content in continuous darkness for about 3 weeks without exposing to insecticides. Adults used in the experiments were 1-3 week old and of mixed sex. The essential oil was dissolved in a suitable solvent to obtain concentrations of 1, 5, 10 and 15 mg/ml. The control was treated with pure solvent. After the solvent evaporation, 10 adults of *O. mercator* (Silvanidae) were placed in 20 ml capacity glass vials, and maintained at 27±2°C, 12 % moisture content and a 12h photo phase. The experimental design was completely randomized, with three replicates. Insect mortality was evaluated after 4, 8, 24, 48h of exposure. The procedure as described by Isman and coauthors[18] was adopted to evaluate the responses to treated vial versus control, which were converted to "percentage of mortality".

Results

The ground aerial parts of the flowering plant *S. oxysepala* was subjected to hydro-distillation for 4h using a Clevenger-type apparatus to provide odorous pale yellow oil with a yield of 0.1 % (v/w). The chemical composition of the essential oil, as determined by the GC-MS analysis and identified based on direct comparison of KI and MS data with those for standard compounds, and computer matching with the NIST, NBS54K Library, as well as by comparison with references,[13-15] is listed in Table 1. This is the first report on the analysis of the essential oil of *S. oxysepala*. A total of 15 compounds,

representing about 91.2% of the total essential oil, were identified in the essential oil of *S. oxysepala* and could be divided, based on chemical structure, to aromatic compounds, such as methyl benzyl alcohol (9.3%), dehydrodieugenol (6.7%), methyl benzaldehyde (5.3%) and eugenol (1.3%), which constitute over a quarter of the essential oil, alcohols, e.g., linalool (2.0%), isolauryl alcohol (0.6%), 1-octen-3-ol (2.0%) and phytol (25.3%) that constitute approximately a third of the essential oil, and other chemical groups including long chain alkanes and fatty acid esters, e.g., 6,10,14-trimethyl-2-pentadecanone (11.4%), palmitaldehyde diallyl acetone (3.3%), 2,4-dimethyl-eicosane (2.6%) and others (Figure 1; Table 1).

Table 1. GC-MS and GC-FID data of the components of the essential oil of *S. oxysepala*.

	Retention time (min)	Compounds	K. I.	Real % Area	Molecular formula
1	15.01	Benzaldehyde	940	0.6	C_7H_6O
2	16.41	1-Octen-3-ol	975	2.0	$C_8H_{16}O$
3	19.59	Methyl benzaldehyde	1057	5.3	C_8H_8O
4	20.13	Methyl benzyl alcohol	1071	9.3	$C_8H_{10}O$
5	21.52	Linalool	1107	2.0	$C_{10}H_{18}O$
6	30.94	Eugenol	1351	1.3	$C_{10}H_{12}O_2$
7	36.99	Pentadecane	1506	6.3	$C_{15}H_{32}$
8	42.02	Isolauryl alcohol	1667	0.6	$C_{17}H_{36}O$
9	46.94	6,10,14-trimethyl-2-pentadecanone	1839	11.4	$C_{18}H_{36}O$
10	48.81	Eicosane	1906	6.3	$C_{20}H_{42}$
11	50.17	Dehydrodieugenol	1958	6.7	$C_{20}H_{22}O_4$
12	53.58	2,4-Dimethyl eicosane	2134	8.6	$C_{22}H_{46}$
13	53.92	Phytol	2112	25.3	$C_{20}H_{40}O$
14	54.55	Palmitaldehyde,diallyl acetone	2237	3.3	$C_{22}H_{42}O_2$
15	58.82	Hexacosane	2595	6.6	$C_{26}H_{54}$
Total identified				91.2	

K.I. = Kovats index in DB1

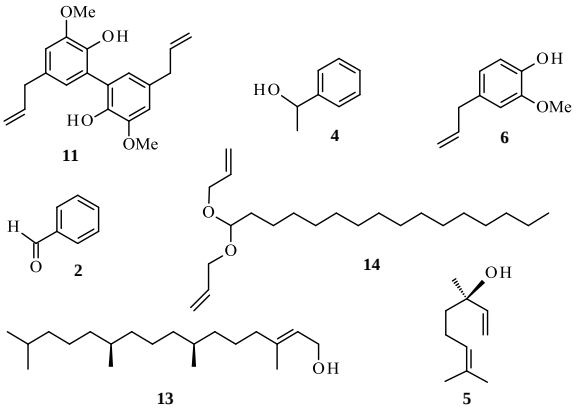

Figure 1.Structures of some chemical constitution of essential oil of *S.oxysepala.*

Discussion

Unlike *S. amplicaulis*, which has high free-radical-scavenging activity (RC_{50}= 4.41 x 10^{-3}mg/ml),[15] the free-radical-scavenging activity of *S. oxysepla* (RC_{50}=1.852 mg/ml) was relatively low, probably due to its low percentage of phenolic compounds. The phenolic contents of the essential oils of *S. amplicaulis* and *S. oxysepla* are, respectively, 53.8% and 8%, which might explain the differences in free-radical-scavenging potencies of these species. This is the first report on the insecticidal activity of the essential oil of the *Scrophularia* genus. Although the insecticidal activity of the essential oil of *S. oxysepala* was not found to be remarkable, the activity was time and concentration-dependent (Table 2). The essential oil of this plant was clearly rich in aromatic compounds. A few studies conducted on the essential oils of species of this genus and its family revealed a significant chemical diversity. However, some compounds such as anethole, anisaldehyde, eugenol, benzaldehyde, eugenol acetate are common in the *Scrophularia*. On the other hand, the presence of aromatic compounds in various genera of the *Scrophulariaceae* is one of the characteristics of this family.[19,20] Also, the presence of terpenoids, e.g., humulene, caryophyllene oxide, phytol, linalool and non-terpenoidal compounds, e.g., 1-octen-3-ol, 6,10,14-trimethyl-2-pentadecanone, pentadecanone are common in this genus. For example, 1-octen-3-ol, linalool and phytol were found in *S. striata*[21] and 1-octen-3- oxide and phytol in *S. amplexicaulis*.[15]

Table 2. Contact toxicity assay of the essential oil of *S. oxysepala*

Hours	Concentration (mg/mL)	% Mortality
4	0 (control)	0
4	1	0
4	5	3.3
4	10	0
4	15	3.3
8	0 (control)	0
8	1	3.3
8	5	0
8	10	3.3
8	15	3.3
24	0 (control)	0
24	1	6.6
24	5	3.3
24	10	6.6
24	15	3.3
48	0 (control)	0
48	1	6.6
48	5	3.3
48	10	6.6
48	15	6.6

Conclusion

The results of present and previous works have shown that the essential oils of *Scrophularia* species are rich in chemical diversity, and have aromatic and terpenoidal compounds at high percentage, which are probably responsible for various pharmacological activities.

Ethical issues

Not applicable in this research.

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