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Chemical Compounds, Antibacterial and Insecticidal Activity of Lipophilic Extract of *Scrophularia amplexicaulis* Benth and *Scrophularia oxysepala* Boiss

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ABSTRACT

Chemical compounds of the hydrophobic extracts (n-hexane-ether extract) of the aerial parts of Scrophularia amplexicaulis and Scrophularia oxysepala were analyzed for the first time by the Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detection (GC-FID). Total 36 compounds were identified which were 84.7% and 79.8% of total compounds of then-hexane-ether extract. The main constituents were n-Dotriacontane (15.1%), 1-Docosanol (13.6%), Eicosane (11.6%) in S. oxysepala and n-Pentatriacontane (32.9%), n-Tetrateracontane (16.8%) and Gamma-Sitostrol (8.7%) in S. amplexicaulis. The antibacterial was tested using the inhibition of broth dilution with shaking (BDS) method and contact toxicity assay indicated by calculation of percentage mortality of insects based on Isman method.

Keywords: *Scrophularia amplexicaulis*, *Scrophularia oxysepala*, Hydrophobic compounds, Antibacterial activity, Contact toxicity

INTRODUCTION

Resistance to antibiotics and anti-parasitic drugs caused to researchers on novel compounds with antibacterial and anti-parasitic activities converse to the major need in the world health. Recently, lipophilic extracts of plants attract alot of attentions for finding new active compounds specially, antibacterial and antiparasitic structures [1,2]. Methodical investigation about antibacterial and anti-parasitic activity of terpenealcohols with aliphatic carbon chain andlong chain fatty acids show that these hydrophobic compounds have suitable antibacterial/anti-parasitic potential [3-6]. Earlier works reported that the antimalarial properties of n-3 and n-6 polyunsaturated fatty acids, where fatty acids such as 22:6 (n-3) and 20:5 (n-3) were the most potent in these studies [7]. In another side, besides to the common fatty acids, fatty acid derivatives also show edantimicrobial potential. Thesecompounds class are mainly found in microorganisms, algae, or plants, surface barrierswhichmay mediate chemical defense against other invasive microorganisms. In many cases indicated that long-chain unsaturated fatty acids can be show suitable bactericidal against important pathogenic microorganisms, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacteria* [8,9]. Synchronous tothese activities, low toxicity of them presented these groups of the natural compounds as very generous potent compounds for screening [10]. The folklore remedies are a brilliant source for a finding of new potent active compounds, among these remedies, seed and aerial part oils have formed a massive part of traditional therapeutic. These oils can be extracted by various methods such as cold compress, liquid extraction by nonpolar solvents. The genus *Scrophularia* L. (*Scrophulariaceae*) commonly known as 'figwort', containing about 200 species of herbaceous flowering plants. This genus distributed throughout the northern hemisphere, concentrated in Asia with some species in Europe and North America, have share square stems, opposite leaves and open two-lipped flowers forming clusters at the end of their stems. Figwort has been known as a famousremedy in traditional medicine of themany nations especially in Chinese medicines (Xuan Shen) and Iranian medicine (Sendertis) to treat the infectious diseases also used as a treatment of nervous and gastrointestinal disorders [11,12]. Besides to these historical uses, many laboratory studies indicated antibacterial, antiprotozoal, antitumor, anti-inflammatory and diuretic activities in *Scrophularia* genus. Some chemical class e.g., Phenylethanoids, phenylpropanoids, flavonoids, iridoids, iridoid glycosides, and terpenoids have been identified in some species of *Scrophularia* [13-15]. This study is the first investigation on the lipid profile of *Scrophularia* genus. Therefore this research focused on lipophilic compounds of twofigworts, *S. amplexicaulis* and *S. oxysepala* which are two endemic herbs in western and central regions of Iran. In this Study, we investigated chemical compositions of the *n*-hexane-ether extracts of the aerial parts of *S. amplexicaulis* and *S. oxysepala* by GC-MS, GC-FID analyses, contact toxicity, and antibacterial activity of them.

MATERIAL AND METHODS

Plant material

The aerial parts of *S. amplexicaulis* and *S. oxysepala* were collected from Sahand Mountain, Gharegolvillage, 30 kilometers to Kalibar town, Garehdagh Mountain respectively in East Azerbaijan province, Iran, in April 2010. Voucher specimens 2817 (*S. amplexicaulis* Benth.) and 2821 (*S. oxysepala* Boiss.) were deposited at the Herbarium of the Researches center for agriculture and natural resources, East Azerbaijan, Iran.

Extraction, GC-MS and GC-FID analyses

The dried ground aerial parts of *S. amplexicaulis* and *S. oxysepala* (1800 g) were extracted with *n*-hexane-ether (1:1, v/v) at room temperature and the yield were found to be 1.28, 3.45% of the dry plants material. The extracts were dried over anhydrous sodiumsulfate and kept with the residue obtained from the solvent [16]. 200 µl of N,O-bis(trimethylsilyl)trifluoroacetamide and pyridine at 60°C for used for salinization of these residues in 2 h. Trimethylsilane (TMS) derivatives were extracted with 0.3 ml of hexane after evaporation under a nitrogen flow in a heat bath at 80°C. After 2 min of centrifugation, the supernatant was withdrawn for injection into the GC-MS [17]. The Gas Chromatography-Mass Spectroscopy (GC-MS) conditions fitted on previous studies and carried on Shimadzu GC-MS-QP5050A fitted with a DB-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness) [17,18]. Compounds of both samples were identified based on computer matching with the NIST NBS54K Library, Kovats indices, and references literatures [19,20]. For compounds quantity determination in both samples, using an Agilent 6890 GC apparatus fitted with a FID detector. For obtaining the good data from GC-FID used the same elution order, detector and column temperatures and other operational conditions.

Broth dilution with shaking (BDS) method

The hydrophobic compounds (n-hexane-ether extract) of *S. amplexicaulis* and *S. oxysepala* tested based on (BDS) method which describe previously [21]. The n-hexane-ether extracts wereadded to 10 ml of Brain Heart Infusion (BHI) broth aliquots (Difco, Detroit, MI, USA) in tubes without any solubilizer or surfactant. An aliquot with 1 × 105 cfu ml^{-1} of culture of *S. aureus* was added to each sample. Cultures were incubated in laboratory condition for 48 h at 37°C with shaking at 40 rpm. By using of turbidiometric method inhibitory activity of each tested extract was monitored. The optical density was determined with a bio photo recorder (TN-1520; Advantec, Tokyo, Japan) at 660 nm (OD660). The minimum inhibitory concentration (MIC) of each sample was determined after 48 h incubation.

Contact toxicity assay

For screening toxicity potential of these plants, the insecticidal test with *Oryzaephilus Mercator* as model was used. All insects were kept in darkness for 3 weeks at 27 ± 2°C. Insects used in this experiment were 1-3 week old. Both extracts were dissolved in a suitable volatile solvent to obtain a concentration of 1, 5, 10 and 15 mg/ml. For control used pure volatile solvents. After the solvent evaporation, 10 adults of *O. mercator* (Silvanidae) were placed in 20 ml glass vials maintained at 27 ± 2°C content and 12 h photo phase. Insect mortality was evaluated after 4, 8, 24 and 48 h of exposure. Responses to treated vial versus control were converted to "percentage of mortality" [22]. All experiments were randomized, with three replicates.

RESULTS AND DISCUSSION

The ground aerial parts of the flowering plants *S. amplexicaulis* and *S. oxysepala* were extracted by the *n*-hexane-ether solvent (1:1, v/v) to give odorous dark yellow amorphous viscous oils. GC-Mass, GC-FID, and their spectrums results were listed in Table 1 and Figure 1a, b. Total volatile identified compounds were 12 and 24 for *S. amplexicaulis* and *S. oxysepala* respectively that were 84.7% and 79.8% of total compounds. *n*-Dotriacontane (15.1%), 1-Docosanol (13.6%) and Eicosane (11.6%) constituted approximately half a percent of the chemical compositions of the *n*-hexane-ether extract of *S. oxysepala*. In the *n*-hexane-ether extract of *S. amplexicaulis* n-Pentatriacontane (32.9%), n-Tetrateracontane (16.8%) and Gamma-Sitostrol (8.7%) were notable (Table 1). Analysis of the *n*-hexane-ether extracts of these plants showed that they were rich in unbranched alkanes and alkanols in *S. amplexicaulis* and unbranched alkanes and fatty acid methyl esters in *S. oxysepala*. This finding show clear correlation to the earlierresearchers that conducted on *Scrophularia* genus and other genus of *Scrophulariaceae* family [23-25]. In some species of *Scrophulariaceae* family have been found various amounts of fatty acids such as γ-Linolenic acid, in one study which conducted on the fatty acid profile of 9 species of *Scrophularia* genus include *S. auriculata*, *S. canina*, *S. grayana*, *S. koraiensis*, *S. lanceolata*, *S. marilandica*, *S. michoniana*, *S. nodosa* and *S. sciophila* indicated that γ-Linolenic acid percentages ranged from 0.00% to 2.17% for *Scrophularia sciophila*. In contrast to γ-Linolenic acid which exists in higher concentration in seeds, stearidonic acid, α-linolenic acid and some of the other monounsaturated C_{20} fatty acids are present in trace amounts in seeds while these compounds occur in a higher percentage in leaves, roots, and stems [26]. In an investigation on constituents of diethyl ether extract of *S. ningpoensis* Hemsl. by GC-MS technique were identified that fatty acids, aliphatic alkanes and aliphatic alcohols consist the major part of lipophilic compounds of this plant. Among the detected fatty acids palmitic acid (25.40%), linoleic acid (10.04%), α-linolenic acid (6.06%), γ-linolenic acid (4.52%) and *cis*-palmitoleic acid (3.94%) were the major constitutions [25]. In similar research about lipid profile of *Diascia* genus floral oils which belong to the *Scrophulariaceae* family, unbranched and (3R)-hydroxy fatty acids with chain length ranging from C_{14} to C_{18} represent the main compounds of the floral oils [27]. Rugutt et al. investigation on another genus of *Scrophulariaceae* family "*Striga"* which shown that fatty acids, aldehydes, and long-chain hydrocarbons consist major components of oils. Among these compounds long-chain alkanes such as n-heptacosane, n-nonacosane and n-pentatricontane were predominant [18]. Investigation on an Ayurvedic traditional remedy, *Bacopa monnieri* (*Scrophulariaceae*) which used as anti-inflammatory herbal therapeutic identified that 9,12-octadecadienonic acid (36.96%), 9,17-octadecadienal (26.65%) and 9-octadecenoic acid (7.79%) consist the major components of the fatty acids meanwhile aliphatic alkanes such as 1-hexadecena (7.74%), 1-tetradecene (6.78%), eicosane (6.57%), 1-octadecene (5.29%), 1-decene (4.60%), E-15-heptadecenal (4.45%) and heptacosane (3.45%) also occur in this plant [28,29].

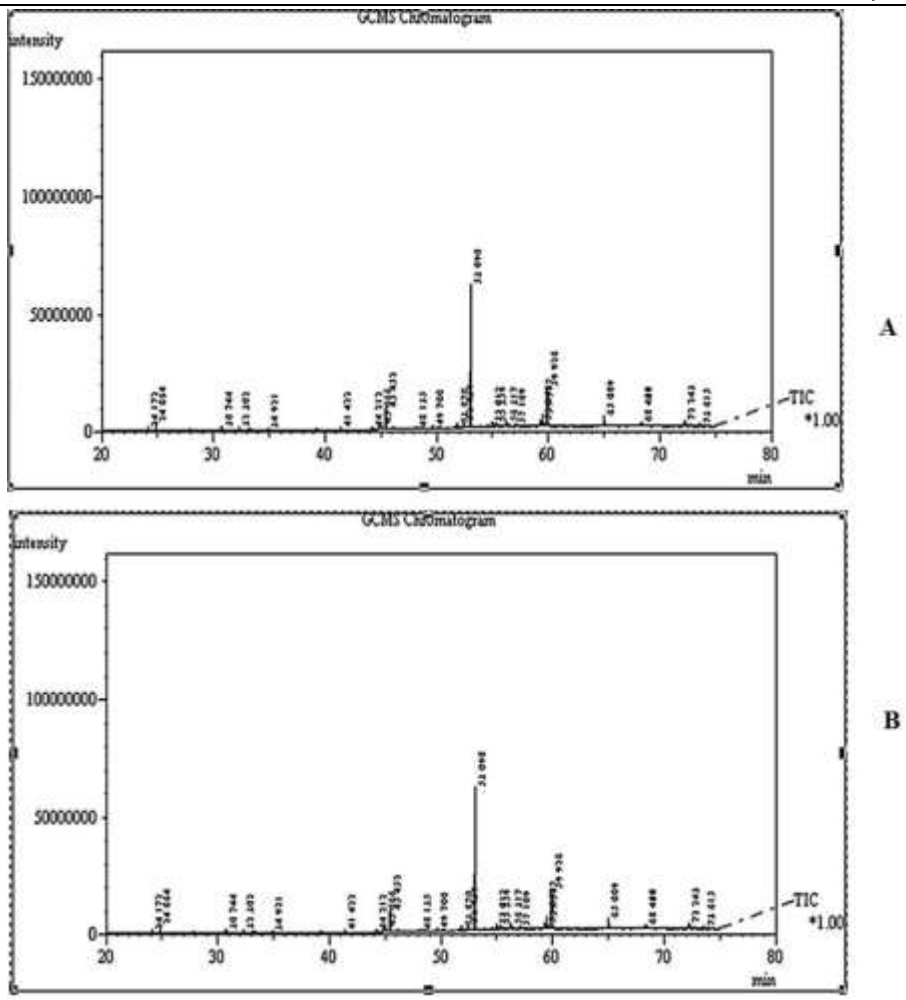

Figure 1(a, b): Chromatogram of GC-MS spectrum of the n-hexane-ether extract of *S. amplexicaulis* and *S. oxysepala*

Table 1: GC-MS and GC-FID data of the components of the n-hexane-ether extract of *S. amplexicaulis* and *S. oxysepala*

S. amplexicaulis			***S. oxysepala***		
Compounds[a]	**RI[b]**	**Percentage in sample (%)[c]**	**Compounds**	**RI[b]**	**Percentage in sample (%)[c]**
Methyl pentadecanoate	1820	1.6	Octadecane	1800	6.4
Hexadecanoic acid methyl ester(Methyl palmitate)	1938	1.2	1-Hexadecanol	1882	1.8
9,12-Octadecadienoic acid methyl ester(Methyl linoleate)	2092	1.1	1-nonadecene	1895	0.4
9,12,15-Octadecatrienoic acid methylester	2096	1.6	Noadecane	1900	8.5
9-Octadecenoic acid (Oleic acid)	2141	3.2	Phytol	1949	1.8
n-Hexacosane	2597	3.9	Eicosane	2000	11.6
n-Octacosane	2800	1.5	10-Methyl eicosane	2074	0.9
Nonacosane	2898	4.2	1-Octadecanol	2082	1.3
Gamma -Sitosterol	3391	8.7	Heneicosane	2100	0.5
n-Pentatriacontane	3505	32.9	1-Docosene	2196	0.3
n-Hexatriacontane	3604	3.1	Docosane	2200	1.9
n-Tetrateracontane	4396	16.8	Totarol	2234	3.4
			Tetramethylheptadecane	2295	1
			Tricosane	2300	3.4
			Tetracosane	2400	1.4
			1-Docosanol	2553	13.6
			1-Tetracosanol	2751	0.4
			n-Octacosane	2800	3.7
			n-Nonacosane	2900	1.8
			Cholesterol	3115	1
			Gamma-Sitostrol	3392	2.4
			n-Pantatriacontane	3500	1.4
			n-Hexatriacontane	3595	0.7
			n-Dotriacontane	3694	15.1
Total		**79.8**	**Total**		**84.7**

[a]Compounds are listed in order of their elution from a DB-5 column; [b]RI=Retention indices as determined on DB-5 column using homologous series of *n-alkanes;*

[c]Percentage obtained by FID peak normalization; Values represent an average of three determinations

S. amplexicaulis and *S. oxysepala* showed the antibacterial activity against *S. aureus* with MIC 45.20 µg/ml respectively (Penicillin as standard, MIC 35 µg/ml). This antibacterial effects previously reported from many lipophilic extracts especially extracts with a high percentage of fatty acid and aliphatic alcohols contents [30,31]. Mechanisms of antibacterial activity of these chemical class of compounds experienced with details in many studies, One of the substantial antibacterial mechanism of aliphatic alcohols is losing of K^+ ions from the bacterial cells, this K^+ ion leakage showed bacterial cell membranes damage [32-34]. Although the antibacterial mechanism of fatty acids it remains unclear but this potential can be attributed to detergency of these compounds. Interfering with electron transport chain capable decline energy production in bacterial cell and caused cell death [35,36]. Although the antibacterial potential of aliphatic alcohols is stronger than fatty acid but studies revealed also synergistic effects of fatty acid and aliphatic alcohols can be improved theantibacterial potential of these compounds. Probably this phenomenon justified suitable antibacterial activity of the lipophilic and oily extract of plants [37]. Resulted data of antibacterial assays showed that *S. oxysepala* extract disclosed better antibacterial potential compared to *S. amplexicaulis* that this out coming from higher aliphatic alcohols content of *S. oxysepala* extract.

Both plants extracts showed mild toxicity in contact toxicity assay model (Table 2) which parallel to the safety of using these traditional remedies. Although based on pervious investigation about insecticidal potential of lipophilic compounds indicated that hydrophobicity of lipophilic compounds caused their accumulation in the cytoplasmic membrane during their metabolism and it alters membrane fluidity, these alterations can be trigged distractive sequences in cytoplasmic membrane, respiratory chain systems and other key cellular reactions [38-40]. Adebowale et al. shown that *Jatropha curcas* L. seed oil which has high percentage of triacyglycerol such as 2-Dioleoyl-3-linoleoyl-rac-glycerol can be reduced the seed beetle *Callosobruchus maculatus* [41]. In other investigation on insecticidal activity of lipophilic compounds has been reported that *Dircapa lustris* compounds include linoleic acid and oleic acid were insecticide against *Aedes aegypti* larvae [42]. Similar activity also observed by two C_{18} acetylenic fatty acids isolated from root of *Ximenia americana* [43]. Other lipophilic compound class such as cyclopropene fatty acids, fatty acid methyl esters, rhamnolipids and alkanolsalso showed suitable insecticidal potential [44-46]. Although these insecticidal potential confirmed in many investigations but probably high lipophilicity of these compounds can be reduced absorption of them during the using by human in traditional treatments [47,48].

Table 2: Contact insecticidal assay of then-hexane-ether extract of *S. amplexicaulis* and *S. oxysepala*

Plant name		**% Mortality[a]**		**Plant name**			**% Mortality[a]**
S. amplexicaulis		***O. mercator***		***S. oxysepala***			***O. mercator***
Dose	**Exposure time (h)**			**Dose**	**Exposure time (h)**		
(mg/ml)	**12**	**24**	**48**	**(mg/ml)**	**12**	**24**	**48**
1	15.7 ± 3.3*	25.3 ± 3.3*	45.3 ± 3.3*	1	0.0 ± 0.0*	0.0 ± 0.0*	3.3 ± 3.3*
5	30.0 ± 3.3*	40.0 ± 3.3*	50.7 ± 0.0*	5	0.0 ± 0.0*	15.7 ± 3.3*	20.3 ± 3.3*
10	45.0 ± 3.3*	51.7 ± 0.0*	61.0± 3.3*	10	21.0 ± 3.3*	30.0 ± 0.0*	50.0 ± 0.0*
15	50.3 ± 0.0*	51.3 ± 0.0*	40.0 ± 0.0*	15	45.3 ± 3.3*	51.3 ± 3.3*	40.0 ± 3.3*

CONCLUSIONS

The hydrophobic compounds of n-hexane-ether extract of *S. amplexicaulis* and *S. oxysepala* investigated in this present study had varying degrees of antibacterial and mild toxicity in contact toxicity assay against *O. mercator*. The difference between observed data of two plant extracts might be attributed to their different chemical compositions and different amounts of them. *S. oxysepala* showed a better antibacterial effect compared to *S. amplexicaulis* because has a highly amount of long chain alcohols. Also, *S. amplexicaulis* and *S. oxysepala* showed similar potencies in toxicity which has been shown that direct correlation between unsaturated compounds and different amounts of them.

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