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Chemical composition and biological activities of *Scrophularia striata* extracts

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Aim. **The aim of this study was to investigate chemical composition (GC/MS analysis), antioxidant (DPPH) and antibacterial (MIC analysis) activities of three extracts obtained from *Scrophularia striata*.**
Methods. **The extraction of *Scrophularia striata* plant was extracted through ethanolic, hydro alcoholic and aqueous methods. The chemical composition of the three extracts is determined by GC-mass analysis method. DPPH assay was performed for measurement of antioxidant activity. MIC and MBC were determined with micro well dilution assey.**
Results. **GC-MS analysis revealed that ethanolic and aqueous extracts were rich in Bis(2-ethylhexyl) phthalate (25.17%, 27.855% respectively) and hydro alcoholic extract in Oleyl Alcohol (24.81%). All of the extracts had the antibacterial activity. Among all extracts, hydro alcoholic extract indicated the most activity against pathogenic bacteria, and had high antioxidant activities as measured by DPPH scavenging (0.407 mg/mL). The aqueous extract had the lowest amount of total phenolic compounds (88.42±2.55 mg).**
Conclusion. **A strong correlation was observed between the antibacterial, radical scavenging capacity and the phenolic and flavonoid compounds of the extracts. As expected, amounts of the total phenolic and flavonoid components were very high in the hydro alcoholic extract.**

KEY WORDS: Biological processes - Plants, medicinal - Oils, volatile.

Herbal products have been used since ancient times in traditional medicine, involving both eastern and western medical traditions.[1] The extracts and essential oils of many herbs have been shown to exert biological activity *in vitro* and *in vivo*, which justifies research on traditional medicine focused on the characterization of their antimicrobial activity.[2] During the last two decades, the development of drug resistance as well as the appearance of undesirable side-effects of certain antibiotics has led to more investigations for new antimicrobial agents, mainly among plant extracts, to discover new chemical structures which could overcome the so-called disadvantages.[3-6]

The Scrophulariaceae, also known as the figwort family, comprise approximately 5100 species belonging to 268 genera.[7] Species of *Scrophularia* all share square stems, opposite leaves and open two-lipped flowers forming clusters at the end of their stems. The genus is found throughout the Northern Hemisphere, but concentrated in Asia with only a few species in Europe and North America.[8]

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Phytochemically this family is a rich source of iridoid glycosides, especially from the genera *Buddleja*, *Scrophularia* and *Verbascum*.[9-11] Several *Scrophularia* species, such as the *S. striata*, *S. ningpoensis*, *S. deserti*, *S. sambucifolia* and *S. frutescents* have been used by herbal medicine practitioners around the world.[7, 12, 13] Additionally, extracts of different species of the genus *Scrophularia* have been used in traditional medicine to treat a wide variety of diseases, including dermatosis,[12] (antipyretic,[7] inflammation, laryngitis, tonsillitis,[14] tumors and lung cancer [9]).

The therapeutic benefit of medicinal plants is often attributed to their antimicrobial and antioxidant properties.[15, 16] Several studies have tried to evaluate therapeutic profiles of *Scrophularia* species in Iran and some other countries.[17, 18]

This study was conducted firstly to determine chemical composition of *Scrophularia striata* extracts (Aqueous, Hydro alcoholic and ethanolic) by a GC-MS, and secondly to evaluate antioxidant and antimicrobial activities of the extracts.

Materials and methods

Plant material

The *Scrophularia striata* was collected from Ilam (a province in west of Iran) and identified by the department of pharmacognosy, Tehran University, Tehran, Iran.

Preparation of the extracts

The ethanolic extract

The dried and powdered plant materials (100 g) were extracted successively with 500 mL of ethanol (96%) by soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent.[19, 20] The aqueous extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuum at 40 °C using a rotary evaporator (model Zirbus 302®).

The hydro alcoholic extract

The extract was prepared similar to the ethanolic extract preparation method by an ethanol-water solution (50:50).[21]

The aqueous extract

The dried plant (10 g) refluxed with 100 mL of double distilled water and after boiling, kept for 15 minutes at this temperature. The infusion was filtered and concentrated.

All extracts were kept under -20 °C. Before starting the antimicrobial assay, ethanolic and hydroalcoholic extracts were dissolved in DMSO (5%) with a proportion of 10% weight to volume (w/v) which was maximum concentration used in the study. Then, the mixture was sterilized by the filter (Millipore filter, Orange scientific, Gyro disc CA-PC, FD0055-2).

GC-MS analysis of the extracts

Chemical composition of all three extracts was analyzed by gas chromatography. The chromatograph (Agilent 6890 UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 50 °C, temperature ramp 5 °C/min, 240 °C/min to 300 °C (holding for 3 min), and injector temperature at 290 °C. The carrier gas was helium and the split ratio was 0.8 mL^{-1}/min. To confirm the analysis results, all three extracts were also analyzed by gas chromatography–mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent UK) with the same capillary column and analytical conditions. The MS was run in electron-ionization mode with ionization energy of 70 eV.

Antibacterial properties

Bacterial strains

The tested bacteria (gram negative and positive) included *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19118, Salmonella *thyphimurium* ATCC 13311and *Escherichia coli* ATCC 43894. The bacteria were obtained from Department of Food Hygiene and Aquatics, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Micro-well dilution assay

The minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values

were determined by the broth microdilution method.[22] The inoculums of the bacterial strains were prepared from 12 h broth cultures (medium LB, pH: 7.4) and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts were dissolved in 10% DMSO (dimethylsulfoxide) and diluted up to the highest concentration (10%). Serial two-fold dilutions were made in a concentration range from 1.25-10% in sterile test tubes containing nutrient broth (Merck KgaA, Darmstadt, Germany).

The 96-well plates were prepared by dispensing into each well 95 µL of nutrient broth and 5 µL of the inoculums. A 100 µL aliquot from the stock solutions of each extract was added into the first wells. Then, 100 µL from the serial dilutions were transferred into four consecutive wells. The last well containing 195 µL of nutrient broth without extract and 5 µL of the inoculums on each strip was used as the negative control. The plates were mixed on plate shaker at 300 rpm for 20 s and then incubated at 37 °C for 18-24 h.

Microbial growth was determined by absorbance at 600 nm using the EL -800 universal microplate reader (Biotek Instrument Inc, Highland Park, VT, USA) and confirmed by plating 5 µL samples from clear wells on nutrient agar medium. The MIC and MBC were defined as the lowest concentration of the compounds to inhibit the growth of microorganisms and show bactericidal effects on micro-organisms.

Antioxidant activity

DPPH assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH (diphenylpicrylhydrazyl). This spectrophotometer assay (Pharmacia, Uppsala, Sweden) uses stable radical DPPH (Sigma, Aldrich) as a reagent.[23, 24] Aliquots (50 µL) of various concentrations of the extracts were added to 5 mL of methanol (0.004%) of DPPH. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in the following formula:

$$I\,\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} was the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} was the absorbance of the test compound. Extracts concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against essential oil concentration. Synthetic antioxidant reagent i.e. ascorbic acid was used as the positive control.

Determination of total phenolics

Total phenolic constituent of the extracts were performed employing the literature methods,[25, 26] involving Folin-Ciocalteu reagent and gallic acid (both Sigma-Aldrich) as standard. Briefly, an aliquot (0.1) mL of extract solution containing 1 mg of extract was transferred to a volumetric flask, then 46 mL distilled water and 1 mL Folin-Ciocalteu reagent were added, and the flask was shaken thoroughly. After 3 min, 3 mL of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions (0-1000 lg in 0.1 mL). A standard curve was obtained according to the following equation:

Absorbance = 0.0012 × Gallic acid (µg) + 0.0033

Determination of flavonoid content

Total flavonoid content was determined according to the method of Slinkard *et al.*[26] Briefly, 0.5 mL of samples were mixed with 1.5 mL of methanol, 0.1 mL of aluminum chloride (10%), 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water and incubated at 25 °C (30 min). The absorbance of the reaction mixture was recorded at 415 nm. Total flavonoid content was calculated as quercetin equivalents.

Statistical analysis

The results were computed as mean ± standard deviation and were subjected to one-way analysis of variance using SPSS 17.0. All experiments were conducted in triplicate. The statistical significance was determined at $P<0.05$.

Results

Three extracts of *S. striata* were analyzed by GC-MS. The relative concentrations of the identified vol-

TABLE I.—*Aqueous extract constituents of S. stirta identified by GC-MS.*

Compounds	RT (minute)	Composition (%)
ethyl-butanal- 2	10.024	7.56
3-methyl-2-heptanol	10.41	6.45
1-methyldodecylamine	26.905	4.52
1-pentadecanol	30.362	27.60
Phenol, 4-(3,4-dihydro-2,2,4-trimethyl-2H-1-benzopyran-4-yl)	31.371	2.37
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	32.381	3.31
Hexadecanoic acid, methyl ester	33.001	6.55
1,2-Benzenedicarboxylic acid, butyl cyclohexyl este	33.423	4.57
9-Octadecenoic acid (Z)-, methyl ester	33.658	3.29
Octadecanoic acid, methyl ester	35.036	2.076
2,3,6- trichloro benzaldehyde	36.158	3.542
Bis(2-ethylhexyl) phthalate	38.669	27.855
Total		99.693

TABLE II.—*Ethanolic extract constituents of S. stirta identified by GC-MS.*

Compounds	RT (minute)	Composition (%)
tert-butyl hydroperoxide	5.62	4.27
1,3-dimethyl- Benzene	6.71	7.07
2-ethyl-butanal	10.10	6.47
3-methyl-2-heptanol	10.40	5.05
1-methyldodecylamine	26.90	5.05
Oleyl Alcohol	30.36	3.88
1,2- Benzenedicarboxylic acid, bis(2-methylpropyl) ester	32.38	2.35
Hexadecanoic acid, methyl ester	33.00	8.85
n-Hexadecanoic acid	33.35	3.41
1,2- Benzenedicarboxylic acid, butyl cyclohexyl ester	33.42	3.18
9-Octadecenoic acid (Z)-, methyl ester	33.66	3.85
Hexadecanoic acid, ethyl ester	33.73	5.30
Ricinoleic acid	34.14	6.44
Octadecanoic acid, methyl ester	35.03	2.61
2,3,6 –Trichlorobenzaldehyde	36.16	6.63
Bis(2-ethylhexyl) phthalate	38.67	25.17
Total		92.51

TABLE III.—*Hydro-alcoholic extract constituents of S. stirta identified by GC-MS.*

Compounds	RT (minute)	Composition (%)
2-ethyl-butanal	10.028	2.54
Cyclohexene, 1-methyl-4-(1-methylethylidene)	12.955	1.67
Phenol, 2,4-bis(1,1-dimethylethyl)	27.05	3.05
Oleyl Alcohol	30.36	24.81
Phenol, 4-(3,4-dihydro-2,2,4-trimethyl-2H-1-benzopyran-4-yl)-	31.375	1.65
unknown	31.471	3.40
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	32.385	12.41
Hexadecanoic acid, methyl ester	32.999	5.10
1,2--Benzenedicarboxylic acid, butyl cyclohexyl ester	33.421	1.79
9--Octadecenoic acid (Z)-, methyl ester	33.661	1.77
Octadecanoic acid, methyl ester	35.029	1.84
Di-n-octyl phthalate	35.51	21.24
2,3,6-Trichlorobenzaldehyde	36.156	3.72
Bis(2-ethylhexyl) phthalate	38.667	14.91
Total		99.92

atile components (%) and retention time have been presented in Tables I-III. Twelve components representing 99.693% of the aqueous extract *S. striata* were identified, Di-(2-ethylhexyl) phthalate (27.85% of the total extract) were the major compound (Table I). Sixteen components in ethanolic extract of *S. striata* were identified representing 92.51% (Table II). Main constituents of this extract were found as Bis(2-ethylhexyl) phthalate (25.17%) and Hexadecanoic acid, methyl ester (8.85%). Fourteen components representing 99.92% of the total volatiles were identified for hydroalcoholic extract *S. striata* (Table III). The main components were Oleyl alcohol (24.81%) and Di-n-octyl phthalate (21.24%). GC-MS analysis revealed that all extracts from *S. striata* were rich in Bis(2-ethylhexyl) phthalate and Oleyl alcohol.

The antibacterial activity of these extracts was conducted by broth microdilution susceptibility method. All extracts of *S. striata* showed levels antibacterial activity (Table IV). The MIC and MBC values of the all extracts ranged 1.25-10%. Among all extracts, hydro alcoholic extract showed the best activity against pathogenic bacteria, followed by alcoholic and aqueous extracts. The best of antibacterial MIC value was 1.25% obtained with *S. aureus* cultures from alcoholic extract. All extracts were active against *S. aureus*, but the aqueous extract was not effective against *S. typhimurium* and *E. coli*. Aqueous extracts had the lowest antibacterial activity amongst all other extracts.

The results of antioxidant activity assessment showed that among all the solvent extracts of *S. striata*, hydro alcoholic extract had the highest antioxidant activities as measured by DPPH scavenging (IC_{50}=407 µg/mL) while for alcoholic and aqueous extracts this was 455 µg/mL and 412 µg/mL, respectively.

Total phenol and flavonoid content of various extracts of *S. striata* have been shown in Table V. Hydroalcoholic extract had the highest amount of total phenolic compounds (158.33±6.03 mg gallic acid equivalent/g of extract) and total flavonoid content (66.26±4.33 mg quercetin equivalent/g of extract powder).

Discussion

Several studies have indicated that the many species of *Scrophularia* contains substances that possess antimicrobial properties.[3, 7, 12, 13]

The results of antibacterial assay indicated that *S. striata* extracts consist of broad spectrum antimicrobial compounds or pharmacological active metabolic (Table IV).

A study of the chemistry and antibacterial activity of *Scrophularia deserti* led to the isolation of eight compounds. Three out of the eight compounds isolated from the plant exhibited antimicrobial activity.

The unsaturated and hydroxylated fatty acid, 3(f)-hydroxy-octadeca-4(E), 6(Z)-dienoic acid, exerted an anti-*staphylococcal* and anti-*Mycobacterial* activity against all the strains tested with MIC values

TABLE IV.—*Minimal inhibition and bactericidal concentrations (MIC % and MBC %) of the extracts of S. striata.*

Bacteria	Extracts					
	Aqueous		Hydro alcoholic		Alcoholic	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	5	10	1.25	2.5	2.5	5
L. monocytogenes	10	*	2.5	5	2.5	5
S. *typhimurium*	*	*	5	10	5	10
E. coli	*	*	5	10	10	*

TABLE V.—*Phenol and flavonoid contents and antioxidant activities of the extracts of S. striata.*

Extracts	Total phenol contents (mg/g)*	Total flavonoid contents (mg/g)**	DPPH free radical scavenging IC50 (mg/mL)***
Alcoholic	94.90±3.52[a]	52.38±5.11[a]	0.412[a]
Hydro alcoholic	158.33±6.03[b]	66.26±4.33[b]	0.407[a]
Aqueous	88.42±2.55[c]	37.69±3.51[c]	0.455[b]

*mg gallic acid equivalent/g of extract powder, **mg quercetin equivalent/g of extract powder, ***Vitamin C used as control (0.170±0.1 mg mL^{-1}) Means followed by the same letter each column are not significantly different (P<0.05).

ranging from 32 to128 µg/mL.[7] The antibacterial activity of unsaturated fatty acids, such as 3(f)-hydroxy-octadeca-4(E), 6(Z)-dienoic acid, against both *S. aureus* [27, 28] has long been known. However, it has only recently been deciphered that these compounds exert their antibacterial effect by inhibiting an enzyme or enzymes.[29]

Phenolic fractions of *S. frutescens* and *S. sambucifolia* were effective against Gram-positive bacteria, specifically against *Bacillus* sp., The identification of the phenolic acids showed that the Ferulic, isovanillic, p-hydroxycinnamic, p-hydroxybenzoic, syringic, caffeic, gentisic and protocatechuic acids were isolated from *S. frutescens* and ferulic, p-coumaric, vanillic, phydroxybenzoic and yringic acids were isolated from *S. sambucifolia*.[12]

From the phythochemical analysis results in our study, phenolyic compounds and unsaturated fatty acids compounds detected in remarkable value in three extracts of *S. striata* like other species of *Scrophularia* genus. Phenolic compounds have a broad spectrum of biological activities, among which their antimicrobial and antioxidant affects standout.[2, 30, 31] These preliminary results suggest that the antibacterial effect exhibited by *S. striata* extracts can be attributed to the presence of phenolics compounds, therefore these species could be considered as potentially antiseptic agents on bacteriologic infections, especially in processes where Gram-positive bacteria are involved. However, antibacterial activity of all extracts tested was noted weaken or not effective (Table IV) against *E. coli* and *S. typhimurium*. Results obtained in this study show that *S. striata* extracts have selective antimicrobial activity on the basis of the cell-wall differences of bacterial microorganisms (Gram-positive or Gram-negative bacteria) as reported previously.[32, 33]

Antioxidant activity of the various extracts of *S. striata* has been determined by DPPH assay. In all extracts, the antioxidants react with the stable free radical, that is, 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1- diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant and it has been found that known antioxidant such as cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol etc.) reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability.[34]

Radical scavenging activity of the various extracts showed in Table V. It is extremely important to point out that, a strong correlation was observed between the radical scavenging capacity and amount of phenolic and flavonoid compounds of the extracts.

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators.[35] Flavonoids, as one of the most diverse and widespread groups of natural compounds, are also probably the most natural phenolics.[36, 37] These compounds possess a wide spectrum of chemical and biological activities including radical scavenging properties. Shimoi *et al*. (1996) concluded that plant flavonoids which show antioxidant activity *in vitro* also function as antioxidants *in vivo*.[37] A strong relationship between total phenolic content and antioxidant activity in fruits, vegetables, grain products, and plants subject of ethnopharmacological treatments has also been reported.[38]

Data obtained from the total phenolic assay supports the key role of phenolic compounds in free radical scavenging and/or reducing systems. As expected, amount of the total phenolics was very high in hydro alcoholic extract (158.33±6.03 mg gallic acid equivalent/g of extract). It was followed by alcoholic and aqueous extracts (Table V).

It is extremely important to point out that: there is a positive correlation between antioxidant activity potential and amount of phenolic and flavonoid compounds of the extracts. Amount of total flavonoids was also found in the highest value in hydroalcoholic extract (66.26±4.33 mg quercetin equivalent/g of extract powder). Finally, the free radical scavenging activity of all extracts tested in compared with Ascorbic acid was very low (Table V).

Conclusions

These results indicated that the various extracts of S. *striata* could be considered as a natural food preservatives and enhance the human health as natural antioxidant. Complementary investigations of individual compounds are necessary to assess the effectiveness of this extract in food system, perfumes and pharmaceuticals fields.

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