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Original article

Chemical composition and biological activities of essential oil and methanol extract of *Scrophularia umbrosa*

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Abstract

Background and objectives: *Scrophularia umbrosa* Dumort is used as a traditional herb in China. In this study, chemical profile, free radical suppression capability, general toxicity and cardiovascular activities of the volatile compounds from *S. umbrosa* were investigated. Moreover, methanol (MeOH) extract of rhizomes were analyzed to purify and identify the constituents. **Methods**: GC/MS was used to identify chemical combination of the volatile oil. Suppression of free radicals of the volatile oil was examined by DPPH method. Also, the essential oil was evaluated for its general toxicity and cardiovascular activity using brine shrimp lethality bioassay and organ bath method, respectively. Preparative HPLC and NMR were applied for investigating the MeOH extract composition. **Results:** Forty one Ingredients were recognized, displaying about 93.08 % of the total volatile oil constituents Ketones (38.49%) with hexahydrofarnesyl acetone (26.18%), phytol (11.86%), palmitic acid (8.92%), β-damascenone (4.1%) and copaene (3.82%) were the main components. The essential oil showed weak free radical scavenging activity (RC_{50}=13.71±0.75 mg/mL). Relatively high levels of toxicity were observed with the essential oil of *S. umbrosa* in comparison with podophyllotoxin. Likewise, the essential oil was able to induced vasorelaxantion in isolated rat aortic rings both in presence and absence of endothelium at a similar rate. An iridoid compounds (sesamoside) was isolated from the MeOH extract of *S. umbrosa.* **Conclusion**: Chemical diversity is probably responsible for various pharmacological activities. However, the essential oil of this plant showed toxicity in preliminary toxicity test; so its toxic effect should be more investigated by various cell lines.

Keywords: brine shrimp, cardiovascular effects, DPPH, *Scrophularia umbrosa*

Introduction

Scrophularia genus belongs to Scrophulariaceae family including about 3000 species and 220 genera [1-3]. *Scrophularia* L. includs approximately 200 species from herbaceous plants, typically known as ‘figwort'. It occurs throughout mountainous regions, forests riversides (e. g. *S. umbrosa*) and seldom in hot deserts [4]. *Scrophularia umbrosa* Dumort with common name “water figwort” is one of the species which is native to Iran [5]. The species of *Scrophularia* are distributed in west, north-west, north, central mountainous region, north-east and

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seldom south of Iran [4]. Many plants of *scrophularia* genus have long been used in Asian countries as a medicinal herb for the cure of diseases; it has been applied for treating various inflammatory conditions like allergy, rheumatic diseases and chronic inflammatory disorders [6-8]. The scientific Genus name, *Scrophularia*, comes from the plant's traditional use as a remedy for scrofula, a tuberculosis infection of the lymph nodes in the neck [9]. Previous pharmacological studies on different species showed multiple biological activities including antitumor, antibacterial, antiprotozoal, hepatoprotective and diuretic properties, as well as treating of gastrointestinal, mental and nervous disorders [10-12]. Some species of this family have been used traditionally to treat eczema, wounds, goiter, ulcers, cancer and fistulae. Some of them were boiled in milk to prepare a poultice which was applied to the abdomen to reduce abdominal pain, whereas their aqueous extracts have been used as a bath to alleviate rheumatic pains [13]. Moreover, various phytochemical studies on *Scrophularia* species have revealed the presence of biologically active phenylethanoids, phenylpropanoids, flavonoids, iridoids, iridoid glycosides and terpenoids [14-17]. In the case of essential oils, despite the several species of *Scrophularia* growing in different parts of Iran, few surveys have been carried out on the chemical composition of their volatile oils (9,18-20). Likewise, to the best of our knowledge, no research has been accomplished on pharmacological activities or chemical profile of *S. umbrosa*; therefore, in this work as part of our in progress studies on Iranian medicinal herbs, we have studied the essential oil of aerial parts and MeOH extract of *S. umbrosa* rhizomes, usually known as water figwort for the first time. Additionally, the free radical scavenging, general toxicity and cardiovascular effects of the essential oil were analyzed.

Experimental

Chemicals, reagents and drugs

Carbachol and DPPH were obtained from Sigma (Germany), phenylephrine from Cintefina (Brazil), KCl and other ingredients of Krebs solution were from Merck (Germany). All solvents used for extraction and fractionation were purchased from Caledon (Canada) and Scharlau (Spain).

Plant material

The aerial parts of *S. umbrosa* were collected from Mishodaghi mountain at E: 45° 47' 24", N: 38° 20' 59" (altitude of 1780 m) in East Azarbaijan province during the flowering period (July, 2012). The identity of the plant was confirmed by anatomical examination in comparison with the herbarium specimens (voucher Nos. Tbz-Fph-762) retained in the Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Essential oil and extract preparation

The air-dried ground aerial parts from *S. umbrosa* (100 g) were subjected to hydrodistillation for 4 h using a Clevenger apparatus. The yielding oil was dried and kept in a refrigerator at 4 °C.

The dried and ground rhizomes of *S. umbrosa* (100 g) were extracted with a Soxhlet apparatus with *n*-hexane, dichloromethane (DCM) and methanol (MeOH), consecutively. Obtained extracts were separately concentrated by rotary evaporator at a maximum temperature of 45 ºC. Two g of the dried MeOH extract (2g) was subjected to solid-phase extraction (SPE) on Sep-Pak (Vac 35 mL, 10 g; C_{18} cartridges, Waters, Ireland) using a step gradient of MeOH: water mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0) as eluent. Solvent of fractions were removed by rotary evaporator at a maximum temperature of 45 °C. In order to purify and isolate phytochemials, the SPE fraction eluted with 10% MeOH was analyzed by reversed-phase preparative HPLC analysis (Knauer, preparative pump 1800, with photodiode array detector (PDA), equipped with a Reprosil 100 C_{18}, 250 mm length, 20 mm i.d, particle size 10 µm, Dr. Maisch column, Germany) using the mobile phase; 0-40 min, linear gradient of 10-30% MeOH in water, 40-42 min, maintained at 30% MeOH in water to isolate compound **1** (1.2

mg, t_R = 40 min). In above prep-HPLC analyses, the flow rate of the mobile phase was 8.0 mL/min and UV λ_{max} 232 nm.

GC/MS and GC-FID analyses

The essential oil was analyzed using a Shimadzu GC/MS-QP5050A gas chromatograph-mass spectrometer (GC/MS) fitted with a fused methyl silicon DB-1 column (60 m×0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.3 mL/min. The column temperature was at 50 °C for 3 min, increased to 260 °C at a rate of 3 °C/min, and finally kept at 260 °C for 5 min. 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 260 °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 alkanes, and computer matching with those listed in the Adams, the Wiley229 and NIST107 mass spectral libraries [21,22]. For quantitation (area %), the GC analyses was also performed on a Shimadzu GC/MS-QP5050A gas chromatograph fitted with a FID detector. The FID detector temperature was 300 ºC. 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.

Extraction and fractionation

The dried and ground rhizomes of *S. umbrosa* (100 g) were extracted with a Soxhlet apparatus with *n*-hexane, dichloromethane (DCM) and MeOH, successively. Two g of the MeOH extract (2×2g) was subjected to solid-phase extraction (SPE) on Sep-Pak 10 g C_{18} cartridges (Waters, Ireland) with a step gradient of MeOH: water mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0) as eluent. All extracts and fractions were separately concentrated using a rotary evaporator at a maximum temperature of 45 °C.

Isolation and identification of compounds

In order to isolate phytochemicals of MeOH in water fractions, they were subjected to reversed phase preparative HPLC (preparative pump 1800, Knauer, Germany), with photodiode array detector (PDA), equipped with a Reprosil 100 C_{18} (250 mm length, 20 mm i.d, particle size 10 µm, Dr. Maisch, Germany) column. The mobile phase consisted of methanol and water. The mobile phase which consisted of 10:90 MeOH - water was applied for 10% sep-pak fraction in 55 min run at flow rate of 8 mL/min and a detector set at 220 nm. The isolated pure compounds were identified by a Bruker Spectrospin 400 MHz NMR-spectrometer. The spectroscopic data of the known ingredients were also compared with the respective published data.

Free radical scavenging activity

DPPH method was used to study the free radical scavenging capacity of the essential oil. DPPH (8 mg) was dissolved in chloroform (100 mL) to obtain a concentration of 80 µg/mL. The essential oil of *S. umbrosa* was dissolved in chloroform to obtain a concentration of 1 mg/mL. Serial dilutions were made to obtain various concentrations of the essential oil and then 5 mL of diluted solutions were mixed with 5 mL DPPH. After the incubation period (30 min) at room temperature, the absorbance was read against a blank at 517 nm with Spectronic Genesys 5 spectrophotometer (USA). The percentage of reduction was drawn against the sample concentration in order to calculate RC_{50} values (essential oil concentration providing 50% loss of DPPH activity). Positive control was Quercetine [23-25].

Brine shrimp lethality assay

To determine the general toxicity of the essential oil, brine shrimp lethality bioassay described by Meyer *et al*. was used with appropriate modifications [26]. The essential oil (0.42

mg/mL) was dissolved in DMSO and diluted with artificial seawater so that the final DMSO concentration did not exceed 1%. Seven distinct concentrations of the essential oils were acquired by serial dilution (0.42, 0.21, 0.10, 0.05, 0.02, 0.01 and 0.006 mg/mL). Afterwards, 10 hatched brine shrimp were moved to test and control tubes containing DMSO and seawater. After 24 h, the number of survivals at each dosage was counted and recorded. LD_{50} values were measured from the best fit line plotted by percentage lethality versus concentration. Podophyllotoxin was used as the positive control.

Cardiovascular effects

Preparation of rat aortic rings

Male Wistar rats (270-300 g) were obtained from the Pasteur Institute of Iran and kept in the central animal laboratory of the Drug Applied Research Center (Tabriz University of Medical Sciences, Tabriz, Iran) in standard polyethylene cages (6 per cage) with a 12 h light- dark cycle and at 21±3 °C. All animal experiments were conducted according to the Declaration of Helsinki in animal research. This study was approved by the ethics committee of Tabriz University of Medical Sciences, Tabriz, Iran (Ethical code: 05/4/8203, 2011/11/05).

After the rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg), the thoracic region was dissected and the thoracic aorta was removed. The acquired segments were immediately transferred to petri dishes containing modified Krebs solution, and the surrounding fat and connective tissues were removed. The trimmed aorta was then cut into 3 to 5 mm rings cautiously to avoid any inadvertent endothelial damage. Two stainless steel triangle hooks were introduced through the lumen of the aorta rings, one fixed to the bottom of the organ bath while the other connected to a force displacement transducer (Letica, Spain). A computer-assisted data acquisition system (PowerLab/4SP; AD Instruments, Australia) recorded the changes in isometric tension during the experiments [27,28]. Modified Krebs-Ringer bicarbonate solution was used as the physiological solution with the following composition: NaCl, 118 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; $MgSO_4.7H_2O$, 2.1 mM; $CaCl_2$. $2H_2O$, 1.25 mM; and glucose, 11 mM. The solution was continuously aerated with carbogene (95% O_2/5% CO_2), and the temperature was held constant at 37 °C. The rings were equilibrated at 2 g resting tension for 45 min, and the bathing solution was changed every 15 min. The intactness of endothelium was confirmed when a remarkable relaxation (more than 40%) occurred in response to carbachol (0.025 mM) in the rings precontracted with phenylephrine (0.1 mM)[29]. To investigate the vascular function, isometric contractions and relaxations were recorded. When uniform responses to consecutive concentrations of KCl were achieved, the main experiment was commenced.

Effects of essential oil of S. umbrosa on the contraction of rat thoracic aortic rings

To evaluate the relaxant effect of the essential oil, it was added in a cumulative manner (2.5-80 µg/mL) during the tonic contraction phase induced by phenylephrin (1 mM), in both endothelium with and endothelium-without aortic rings. The effectiveness of endothelium removal was confirmed by the absence of relaxation induced by carbacol (0.025 mM) in aortic rings precontracted with phenylephrin (0.1 mM).

Statistical analysis

All tests except cardiovascular tests were conducted in duplicate and triplicate and provided as the mean±standard deviations. Cardiovascular tests were explained as mean±standard deviation (SD) of six experiments. Data were analyzed by Microsoft Excel 2007 software. The LD_{50} values were calculated from the linear regression analysis. The percentage lethality was calculated from the mean survival of larvae in oil treated tubes and control.

Results and Discussion

The ground aerial parts of the flowering plant *S. umbrosa* was subjected to hydrodistillation for 4 h using a Clevenger-type apparatus to provide odorous pale yellow oil with a yield of 0.5 %

(v/w). components of the essential oil by comparison with references [9,18-20], has been listed in table 1. This is the first report on the analysis of the essential oil of *S. umbrosa*. A total of 41 compounds, representing about 93.08% of the total essential oil were identified with hexahydrofarnesyl acetone or phytone (26.18%), phytol (11.86%), palmitinic acid (8.92%), β-damascenone (4.1%) and copaene (3.82) as the major constituents (table 1).

The essential oil of this plant, as analyzed in the current study, was clearly rich in ketones, alcohols and acid compounds. According to the previous publications, secondary metabolites such as cucurbitacins, stearic acid derivatives, flavonoids, β- caryophyllene, caryophyllene oxide, 6α-acetoxymanoyl oxide were previously detected in other species of the *Scrophularia* genus [30-33].

A few evaluations conducted on the essential oils of species of this genus and its family showed a significant chemical diversity. However, some compounds such as anethole, anisaldehyde, eugenol, benzaldehyde, eugenol acetate are common in this genus. On the other hand, the presence of aromatic compounds in various genera of the Scrophulariaceae is one of the characteristics of this family [34,35]. Tterpenoids, *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 [36]. For example, 1-octen-3-ol and phytol were found in *S. umbrosa*.

Antioxidant activity of the essential oil was detected by DPPH. This method is based on the ability of antioxidant materials to accept electron or hydrogen to get stable diamagnetic molecule (diphenylpicrylhydrazyl). It was turned out that essential oil of *S. umbrosa* reduced DPPH radicals in a concentration-dependent manner. The lower RC_{50} values demonstrate a stronger ability of the antioxidant substance to scavenge the DPPH radicals while the higher RC_{50} values indicate a lower scavenging activity of the scavengers (table 2).

Oxygen and nitrogen reactive species are essential for detoxification, energy storage, immunological functions and chemical signaling. They also control endogenous enzymes like glutathione peroxidase, superoxide dismutase and catalase. Failure in defense mechanisms is the cause of producing oxygen's free radicals that can disturb vital molecules such as DNA, proteins and lipids. These hurts cause cancer, cardiovascular diseases and other chronic illnesses. Natural antioxidants can guard body versus oxidative harms and decline the risk of various chronic diseases [37]. Medicinal herb's antioxidants such as phenolic compounds represent a large group of antioxidant compounds.

The free-radical-scavenging activity of *S. umbrosa* (RC_{50}= 13.71 ± 0.75 mg/mL) was low maybe due to its low percentage of phenolic compounds. The phenolics quantity of the essential oils of *S. umbrosa* was less than 5% which might elucidate the low potency of this species for free-radical scavenging.

Brine shrimp lethality assay is a convenient method for screening general toxicity of the extracts or pure compounds against brine shrimp larvae, and it can give an indication regarding possible cytotoxicity of the test samples. Relatively high level of toxicity was observed with essential oil of *S. umbrosa* (LD_{50} = 0.19 mg/mL) in comparison with podophylotoxin (table 2).

The vasodilatation effects of essential oil in rat aortic rings with or without endothelium were observed and compared with DMSO-Distilled water. Results have been shown in figures 1 A and B. It is noteworthy that the cardiovascular and cerebrovascular diseases are major causes of death in both developed and developing countries [38]. Many manuscripts have expressed that vascular tone is an important factor for the incidence and progress of the ischemic cardiovascular and cerebrovascular diseases [39,40]. Vasodilators may prevent or treat the vascular obstructive disease through improving blood perfusion of the tissues and organs [41]. Vascular tone and blood pressure are determined by the contractile state of vascular smooth muscle cells within the blood vessel wall, which is regulated by intracellular calcium concentration.

Table 1. Chemical composition of the essential oil from the aerial parts of *Schrophularia umbrosa*

No.	Compounds [a)]	Calc. KI [b)]	Lit. KI[c)]	%	ID [d)]
1	Toluene	756	754	0.86	MS, I_b
2	2-Hexenal (Leaf aldehyde)	835	829	1.08	MS, I_b
3	Z-3-Hexenol (Leaf Alcohol)	845	844	0.66	MS, I_b
5	Benzaldehyde	929	925	0.63	MS, I_b
6	7-Octen-2-one	953	959	0.61	MS, I_S
7	1-Octen-3-ol	965	968	2.95	MS, I_S
8	4-Ethylcyclohexanol	984	1003	0.66	MS, I_S
9	Nonanal	1091	1087	0.62	MS, I_S
10	Pinocarveol	1137	1117	0.93	MS, I_S
11	Decanal	1197	1198	1.24	MS, I_S
12	3-Decen-2-one	1208	1201	0.35	MS, I_S
13	β-Cyclocitral	1214	1221	0.37	MS, I_S
14	E-Cinnamaldehyde	1249	1256	0.85	MS, I_S
15	Pelargonic acid	1265	1260	0.33	MS, I_S
16	p-Vinyl guaiacol	1303	1280	0.53	MS, I_S
17	1-Undecanal	1305	1286	0.79	MS, I_S
18	2-Undecenal	1362	1344	0.31	MS, I_S
19	β-Damascenone	1389	1374	4.10	MS, I_S
20	α-Copaene	1406	1387	3.82	MS, I_S
21	6,10-Dimethyl-2-undecanone	1411	1407	1.03	MS, I_S
22	6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	1425	1420	1.21	MS, I_S
23	Geranyl acetone	1456	1447	1.40	MS, I_S
24	Pentadecane	1493	1500	1.09	MS, I_S
25	(E)-β-Ionone	1498	1481	1.58	MS, I_S
26	γ-Cadinene	1505	1517	0.59	MS, I_S
27	Tridecanal	1521	1497	0.69	MS, I_S
28	α-Muurolene	1530	1506	1.45	MS, I_S
29	(Z)-Calamenene	1548	1531	0.45	MS, I_S
30	δ-Cadinene	1552	1530	1.23	MS, I_S
31	1-Dodecanol, 3,7,11-trimethyl-	1588	1573	0.65	MS, I_S
32	Tetradecanal	1529	1592	0.34	MS, I_S
33	2,6,10-Trimethylpentadecane	1635	1652	0.42	MS, I_S
34	Vinyl octadecyl ether	1698	1689	1.61	MS, I_S
35	Heptadecane	1700	1700	0.68	MS, I_S
36	Myristic acid	1788	1761	2.10	MS, I_S
37	Octadecane	1800	1800	0.58	MS, I_S
38	Hexahydrofarnesyl acetone (phytone)	1885	1862	26.18	MS, I_S
39	Neophytadiene	1890	1883	2.21	MS, I_S
40	Farnesyl acetone	1930	1922	1.99	MS, I_S
41	Methyl palmitate	1932	1914	0.52	MS, I_S
42	Palmitic acid	1956	1960	8.92	MS, I_S
43	1-Octadcanol	2067	2059	0.30	MS, I_S
44	Methyl linolenate	2081	2081	0.26	MS, I_S
45	Phytol	2128	2105	11.86	MS, I_S
46	Palmitaldehyde, diallylacetal	2237	2228	0.89	MS, I_b
47	Tetracosane	2400	2400	1.16	MS, I_b
	Total Identified (%)			93.08	
	Grouped compounds (%)				
	Ketones			38.49	
	Alcohols			18.53	
	Acids			11.36	
	Aldehydes			7.83	
	Hydrocarbons			10.23	
	Others			6.63	

a)Compounds reported in order to their elution from a DB-1 capillary column. b) Kovats index (KI) on DB-1 column, experimentally determined using homologous series of C8-C20 alkanes. c) Literature KI published by Adams [21] and/or listed in the NIST08 mass-spectral library [22]. d) Identification method: MS, based on the comparison of mass spectra with those listed in the Adams, Wiley and NIST08 mass spectral libraries; I_s, Kovats indices based on standard alkanes, I_b, Kovats indices based on bibliography.

Table 2. Antioxidant activity and brine shrimp toxicity of the essential oil from *Scrophularia umbrosa*

Assay	essential oil (mg/mL)	Quercetin/ podophyllotoxin* (mg/mL)
Antioxidant activity (RC_{50})	13.71 ± 0.75	0.005 ± 0.0009
Brine shrimp toxicity (LC_{50})	0.19 ± 0.03	2.7×10^{-3}

*Quercetin and podophyllotoxin were used as positive controls for antioxidant and brine shrimp toxicity assays, respectively

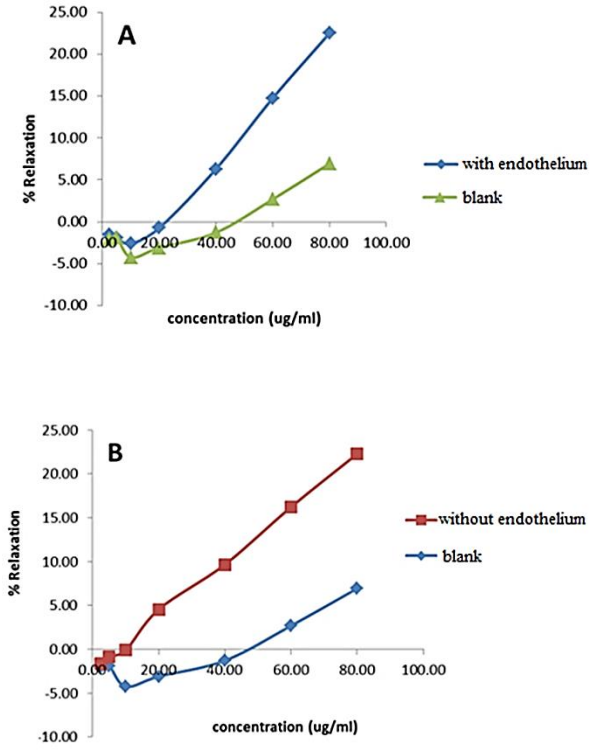

Figure 1. Effect of essential oil of *S. umbrosa* on the contraction induced by phenyl ephrin (1 mM) on rat thoracic aorta rings with endothelium (A) ; without endothelium (B)

The endothelium is shown to have a remarkable role in regulating the vascular smooth muscle tone in duct and stability of vessels by releasing endothelium-derived relaxing factors (EDRF) [42], including nitric oxide (NO), prostacyclin (prostaglandin I2) and an endothelium derived hyperpolarizing factor (EDHF) [43].

In the current study we focused on the vasodilation produced by the essential oil of *S. umbrosa*. Our results demonstrated that the essential oil of *S.umbrosa* was able to induce vasorelaxation in isolated rat aortic rings both in presence and absence of endothelium at a similar rate. It was striking that the results showed that the essential oil induced relaxation was not affected by the removal of the endothelium thus indicated that the vasoactive compounds of essential oil acted directly on the vascular smooth muscle cells (VSMCs). The mechanism of vascular smooth muscle contraction involves different signal transduction pathways, all of which converge to increase calcium levels [44]. Extracellular Ca^{2+} influx, through voltage dependent calcium channel (VDCC) or receptor-operated calcium channel (ROCC), and intracellular Ca^{2+} release results in the increase of intracellular calcium level [44]. It has been recognized that the vasoconstriction of vascular smooth muscle is initiated by an increase in intracellular calcium level. The vasoconstriction is probably achieved by two ways: extracellular Ca^{2+} influx via the voltage-dependent calcium channel evoked by depolarization with high potassium concentration (high K^{+}) or via the receptor-operating calcium channel evoked by NE and release of intracellular Ca^{2+} [45]. Therefore, subsequent studies must be carried out to investigate the mechanism of relation of *S. umbrosa* essential oil.

Reversed-phase preparative HPLC analysis of 10% fraction of MeOH extract of *S. umbrosa* rhizome afforded one iridoid structure, which was identified unequivocally as sesamoside on the extensive ^{1}D H-NMR data analysis (table 3). The spectroscopic data of this compounds was also compared with the respective published data. Sesamoside: ^{1}H- NMR (400MHz, D_2O): δ 5.55 (*d*, 1H, J = 5.62 Hz, H-1), 7.52 (*s*, 1H, H-3), 4.22 (*d*, J=2.07 Hz, 1H, H-6), 3.44 (*d*, J=1.83 Hz, 1H, H-7), 2.47 (*d*, J=5.61 Hz, 1H, H-9), 1.31 (*s*, 3H, H-10), 3.59 (*s*, 3H, H-OMe), 4.64 (Overlapped, 1H, H-1'), 3.54 (*dd*, 1H, H-6'a), 3.75 (*dd*, 1H, J = 11.04/- Hz, H-6'b), 3.15- 3.36 (remaining sugar

protons). Data were in agreement with the published data [46,47].

The results of the present and previous works have shown that the essential oil and methanol extract of *Scrophularia* species are rich in both aromatic and terpenoid compounds in high amounts, which are probably responsible for various pharmacological activities of the species. Pursuant to our results the essential oil of *S.umbrosa* revealed toxicity in preliminary toxicity test; accordingly, its toxic effect should be more investigated by various cell lines. Furthermore, based on the results of our study on rhizome extract, the conclusion can be drawn that fractionation of extracts and running their ^{1}HNMR could be valuable for predicting the natural compounds.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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