

Research Article



## Characterization of Terpenoids in the Essential Oil Extracted from the Aerial Parts of *Scrophularia Subaphylla* Growing in Iran

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### Abstract

**Purpose:** The aim of this work was to investigate the volatiles released from aerial parts of *Scrophularia subaphylla* (*Scrophulariaceae*) which is a perennial herb growing in Azarbaijan province in Iran.

**Methods:** A combination of GC-MS and GC-FID were applied for analyzing the chemical compositions of the essential oil extracted by hydro-distillation from the aerial parts of *Scrophularia subaphylla* (*S. subaphylla*).

**Results:** Thirty six compounds, representing 97.32% of total oil were identified. High content of terpenoids (60.02%) were identified in the essential oil with Linalool (22.35%), phytol (15.74%) and geraniol (7.27%) as the most dominant compounds, while other main components were representatives of fatty acids (24.31%), indicated mainly by palmitinic acid (17.29%). DPPH assay was used for assessing the antioxidant properties of compounds. However, no remarkable free radical scavenging activity was observed. Furthermore, Disc diffusion method was applied for evaluating the antimicrobial activity of essential oil vs. gram positive and gram negative bacteria strains. The examined oil showed weak antibacterial effect.

**Conclusion:** Main constituents of *S. subaphylla* were terpenoids. In comparison with other genesis of *Scrophularia*, antioxidant and anti bacterial properties of *S. subaphylla* essential oil were not noticeable.

### Introduction

The genus *Scrophularia* (commonly known as figworts) is one of the largest genera of the *Scrophulariaceae* family which is distributed widely in Asia, North America and central Europe, especially in the Mediterranean region.<sup>1</sup> This genus is represented in Iranian flora by 42 species, of which 19 are endemic.<sup>1,2</sup> *Scrophularia subaphylla* (*S. subaphylla*) is one of the endemic species growing in East Azarbaijan province of Iran. It is a multi-stemmed perennial herb with square stems, woody rhizomes, opposite leaves and open two-lipped flowers.<sup>3</sup> Several investigations on this genus have revealed various pharmacological activities such as wound healing, anti-inflammatory, antibacterial, cardiovascular, diuretic, protozoacidal, fungicidal, cytotoxic and anti-nociceptive.<sup>4-10</sup> Most of these mentioned characteristic were proven to attribute non-volatile components, which were known as secondary metabolites. Iridoids, phenylpropanoids, phenolic acids and flavonoids have been identified as main secondary metabolites.<sup>4-10</sup> Despite the high number of *Scrophularia* species, there are a few studies about the chemical composition of the essential oils of these plants.<sup>11-13</sup> Moreover, to our knowledge,

biological activities of the essential oils from *Scrophularia* genus, especially Iranian endemic taxa, have not yet been exhaustively investigated except two recent reports by Pasdaran et al which indicated antioxidant and antimicrobial properties of essential oils of *S. amplexicaulis* and, insecticidal effects of oil obtained from aerial parts of *S. oxycephala*.<sup>11,12</sup> Therefore, in this work we present for the first time the chemical composition of the essential oil from aerial parts of *S. subaphylla* occurring in East Azarbaijan province as well as its antioxidant and antimicrobial properties.

### Material and Methods

#### Plant material

Flowering aerial parts of *S. subaphylla* was collected during July 2013 from Mishodagh Mountain near the Marand city (Yam) in East Azarbaijan province, Iran. Voucher specimen was identified and retained in the Herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran, under the accession code Tbz-fph 747.

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### Essential oil Isolation

The air-dried aerial parts of plant (100 g) were cut into small pieces and submitted to hydro-distillation for 3 h in a Clevenger-type apparatus using hexane (2 mL) as collector solvent. The pale yellow-colored essential oil was dried over anhydrous sodium sulphate, then the solvent was evaporated and the oil was stored in sealed vials at - 4° before analyses.

### GC-MS analysis

GC-MS analysis was performed on a Shimadzu GCMS QP 5050A gas chromatograph-mass spectrometer fitted with a fused capillary column DB-1 (60 m, 0.25 mm id, film thickness 0.25 µm), with the following temperature program: 3 min at 50°C, then at 3 °C/min to 260°C, held for 5 min, for a total run of 80 min. Injector and transfer line temperatures were 240°C. Helium was utilized as the carrier gas, at a flow rate of 1.3 ml/min. Essential oil were diluted 1:100 in hexane, and the volume injected was 1µL; split ratio, 1:29; acquisition mass range, 30-600 m/z, ion source temperature 270 °C; quadrupole 100 °C; Solvent delay 2 min; scan speed 2000 amu/s and EV voltage 3000 volts. All mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 eV. Qualitative identification of essential oil constituents was based on direct comparison of the Kovats Indices (KI) and MS data with those for the standard compounds as well as using the spectrophotometer database like NIST NBS54K and Wily 229 library along with comparison of their retention indices (RT) and MS fragmentation pattern with the authentic reference compounds that were reported in the Adam'S literature.<sup>14</sup> Also flame ionization detector (FID) which was operated in ionization potential mode at 70 ev, was used for quantification purpose for calculating the relative area percentage (area %) without the use of correction factors.

### Antimicrobial assay

Lyophilized form of two gram positive and gram negative bacteria, *Staphylococcus aureus* ATCC (6538) and *Pseudomonas aeruginosa* ATCC (9027) respectively, were purchased from institute of Pasture, Iran. They were used to evaluate the antibacterial activity of *S. subaphylla* essential oil. Activated bacteria were maintained in suitable agar media at 4°C as stock cultures for further uses. A single colony after transferring in to Muller Hinton Broth media incubated at 37°C for 24h then centrifuged. For providing an optical density equal to 10<sup>8</sup> CFU/ml of bacterial concentration, turbidity was corrected with adding a saline solution. After that, sterilized discs (6 mm diameter, Whatman paper) were impregnated with different concentration of test sample (which were dissolved in 10% aqueous DMSO previously) as well as positive reference (Amikacine). Negative control were prepared from the same solvent (DMSO) employed to dissolve the plant oil. Afterward, petridishes transferred in to the refrigerator to facilitate the diffusion of oil

approximately for 30 min, thereafter, plates were incubated at 37 °C for starting the growth of bacteria. Finally, the diameter of the inhibition zones was considered as the antibacterial activity of the test sample.<sup>15</sup>

### Antioxidant activity

The *in vitro* antioxidant property of the essential oil was evaluated through the modified DPPH assay. 2 ml of 0.08 gr/ml DPPH which was prepared in chloroform was added in to different dilutions of essential oil and negative control (chloroform). Afterwards, when the mixture was homogenous, was kept in room temperature for 30 min to any reaction to occur. The same procedure was repeated for positive control (quercetin). Finally, the absorbance of test specimens was recorded against negative control at 517 nm using UV/Visible Spectrophotometer 160A (USA). Percentage of DPPH reduction was calculated according to the following equation:

$$\% \text{ inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \times 100$$

Also RC<sub>50</sub> was extrapolated from dose-response curve. The experiment was performed in duplicate.<sup>16</sup>

### Results and Discussion

The composition of the essential oil obtained from the aerial parts of *S. subaphylla* is compiled in Table 1, where the components are listed in order to their elution on the DB-1 column.

Hydro-distillation gave an odorous light yellow oil with a yield of 0.05 % W/W, based on the dry mass. The very low yield (0.05%) was consistent with those reported in the researches for other species of the genus *Schrophularia*.<sup>11-13</sup> so it seems that this genus contains oil-poor plants. A total of 36 volatiles were identified, accounting for 97.32% of the total volatiles. The components of essential oil were separated into two classes, which were terpenoids (60.02%) and non-terpenoid (37.30%) compounds. Terpenoids were represented mainly by oxygenated monoterpenes (39.42%), with Linalool (22.35%), geraniol (7.27%) and α-terpineol (5.25%) as the most abundant compounds, while the content of oxygenated sesquiterpenes was rather low (4.86%), represented mainly by nerolidol B (4.21%). Among oxygen containing components, alcohols were the most abundant. Moreover, as shown in Figure 1. The oil was characterized by high amount of diterpenes, notably phytol, which accounted for 15.74% of the oil. Among non-terpenoid compounds, fatty acids constituted the main fraction (20.53%) of this part, with palmitic acid (17.29%) as the major components. This finding previously was confirmed by Mitsuo Miyazawa et al in other species of *Scrophularia*.<sup>13</sup> Apart from the main compounds reported above, 1-Octen-3-ol (2.25%) and hexahydrofarnesyl acetone (3.75%) exceeded a content of 2% of the total oil composition, whilst the remaining compounds (n=27) were present in scant amount, most of them existing at contents lower than 1%.

**Table1.** Chemical constituent of the essential oil from aerial parts of *S. subaphylla*

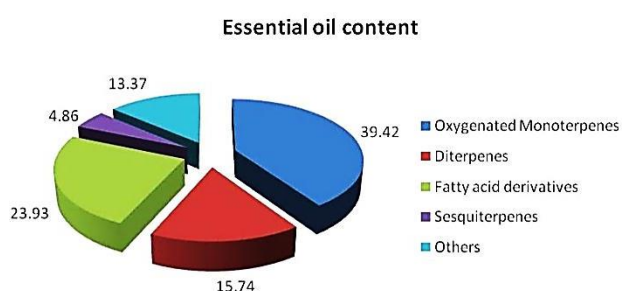
Compounds <sup>a</sup>	K.I	Area %	Identification method
1-Octen-3-ol	963	2.25	GC-MS, I <sub>s</sub>
n-Octan-3-ol	980	0.68	GC-MS, I <sub>s</sub>
n-Nonanal	1083	0.85	GC-MS, I <sub>s</sub>
L-Linalool	1086	<b>22.35</b>	GC-MS, I <sub>s</sub>
α-Terpineol	1174	5.25	GC-MS, I <sub>s</sub>
n-Decaldehyde	1185	0.52	GC-MS, I <sub>s</sub>
4-α-dimethyl-3-Cyclohexene-1-acetaldehyde	1192	0.25	GC-MS, I <sub>s</sub>
Z- geraniol	1211	1.94	GC-MS, I <sub>s</sub>
Geraniol	1236	<b>7.27</b>	GC-MS, I <sub>s</sub>
Z-2-Decenal	1238	0.49	GC-MS, I <sub>s</sub>
Undecanal	1287	0.36	GC-MS, I <sub>s</sub>
E,E-2,4-Decadienal	1290	0.27	GC-MS, I <sub>s</sub>
Heptylidene acetone	1319	1.08	GC-MS, I <sub>s</sub>
E-2-dodecenal	1341	0.2	GC-MS, I <sub>s</sub>
β-Damascenone	1364	1.19	GC-MS, I <sub>s</sub>
Palmitaldehyde	1389	0.5	GC-MS, I <sub>s</sub>
Geranyl acetone	1430	0.56	GC-MS, I <sub>s</sub>
β-Ionone	1466	1.22	GC-MS, I <sub>s</sub>
Nerolidol B (E OR TZ)	1549	4.21	GC-MS, I <sub>s</sub>
α-Bisabolol	1670	0.17	GC-MS, I <sub>s</sub>
Tetradecanal	1696	0.24	GC-MS, I <sub>s</sub>
Nerolidol	1702	0.48	GC-MS, I <sub>s</sub>
Myristic acid	1743	1.02	GC-MS, I <sub>s</sub>
n-Octadecane	1800	0.69	GC-MS, I <sub>b</sub>
Hexahydrofarnesyl acetone	1831	3.75	GC-MS, I <sub>s</sub>
Farnesyl acetone	1895	0.26	GC-MS, I <sub>s</sub>
Methyl eicosanoate	1909	0.37	GC-MS, I <sub>s</sub>
Palmitic acid	1949	<b>17.29</b>	GC-MS, I <sub>s</sub>
Palmitic acid ethyl ester	1978	0.49	GC-MS, I <sub>s</sub>
Heneicosane	2100	0.62	GC-MS, I <sub>b</sub>
Linoleic acid	2109	1.31	GC-MS, I <sub>b</sub>
Oleic Acid	2116	0.91	GC-MS, I <sub>b</sub>
Phytol	2124	<b>15.74</b>	GC-MS, I <sub>b</sub>
Linolenic acid methyl ester	2125	1.68	GC-MS, I <sub>b</sub>
Ethyl linoleate	2139	0.47	GC-MS, I <sub>b</sub>
Hexadecanal diallyl acetal	-	0.39	GC-MS, I <sub>b</sub>
<b>Total identified</b>	-	<b>97.32</b>	-
<b>Non-terpenoid</b>	-	<b>37.30</b>	-
<b>Terpenoids</b>	-	<b>60.02</b>	-

<sup>a</sup> Compounds listed in order of elution from a DB-1 column, <sup>b</sup> Identification Method (I<sub>s</sub> = Kovats retention index according to authentic standard, I<sub>b</sub> = Kovats retention index according to bibliography).

To the best of our knowledge, the current work represents the first study of the essential oil of *S. subaphylla* but there are some reports about chemical composition of other species of *Schrophularia*. The comparison of our results with previous researches illustrates considerable differences in terms of chemical

composition of the oil. In our examined oil, linalool (22.35%), palmitic acid (17.29%) and phytol (15.74%) were identified as the main components whereas according to a report by Pasdaran et al, eugenol (53.8%), eugenol acetate (24.5%) and caryophyllen oxide (6.4%) were the main components of the essential oil of *S.*

*amplexicaulis*.<sup>11</sup> Moreover, the oil from the aerial parts of *S. oxycephala* represented phytol (25.3%), methyl benzyl alcohol (9.3%) and dihydroeugenol (6.7%) as principle components.<sup>12</sup> It is notable that linalool was found at a relatively high level in our study whereas it was not detected in considerable amount in the oil of the other species.<sup>11-13</sup> Conversely, eugenol and its derivatives was absent in our examined oil compared with that of oil composition of other species.<sup>11-13</sup> It is noteworthy to mention that phytol as a diterpenoid compound present in all of the investigated *Scrophularia* species in variable value.<sup>11-13</sup> These findings showed that the genus *Scrophularia* had a remarkable variation in essential oil composition. Furthermore, in the current study was observed weak antibacterial activity of essential oil (Mean Inhibition Zone Diameter  $\pm$  SD (MIZD  $8 \pm 0.3$  mm) in comparison to Amikacin (MIZD  $15 \pm 0.1$  mm). Our finding is in contrary to the previous study which were reported the significant antibacterial activity of *Scrophularia* genesis.<sup>11</sup> Also in the case of *S. subaphylla* was not found remarkable antioxidant activity ( $RC_{50}$   $5.2 \times 10^{-2}$  mg/ml<sup>1</sup>) in comparison to quercetine as a positive control and other species of *Scrophularia* like *S. amplexicaulis* oil.<sup>11</sup> Due to the fact that the phenolic compounds have been known the most likely constituents responsible for the free radical scavenging activity, lack of these sorts of constituents in our examined oil may be led to weak activity in this assay



**Figure 1.** Identified chemical groups from the essential oil of *S. subaphylla*.

### Conclusion

Main constituents of *S. subaphylla* were identified as terpenoides. In comparison with other species of *Scrophularia*, antioxidant and anti-bacterial properties of *S. subaphylla* essential oil were not remarkable.

### Acknowledgments

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### Ethical Issues

Not applicable.

### Conflict of Interest

The authors report no conflicts of interest.

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