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ORIGINAL ARTICLE

Sclerorhachis Platyrachis (Boiss.) Podlech Ex Rech. F.: an Indigenous Medicinal Plant from Northeastern Iran; Essential Oil Composition, Total Flavonoid Content and Antioxidant Activity

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KEYWORDS

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ABSTRACT: In this study, the essential oil obtained by hydrodistillation of the aerial parts of *Sclerorhachis platyrachis* (Boiss.) Podlech ex Rech. f. (Compositae), growing wild in Sabzevar, Khorasan Razavi Province (Iran), was analyzed by GC and GC/MS. The total yield of volatiles was 0.38% (w/w). Sixty- three compounds representing 89.2% of the aerial parts oil were identified. The main components of the oil were β-pinene (17.5%) and γ-terpinene (15.4%). The oil was rich in monoterpenoids, and among them, monoterpene hydrocarbons (48.7%) predominated over oxygenated monoterpenes (11.8%). The total flavonoid content of different extracts of the plant was in the range 52.4-172.3 mg/g, with the maximum amount being in the methanol extract. The antioxidant activities of the extracts were also measured based on radical scavenging activity of antioxidants using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The results showed that IC_{50} values of extracts of *S. platyrachis* are higher than for the standard synthetic antioxidants, BHT, ascorbic acid and gallic acid.

INTRODUCTION

Approximately eight thousand species of plants can be found in Iran, of which about two thousand have medicinal and/or aromatic properties [1]. According to the Flora Iranica, 130 plant families growing in Iran have members with pharmaceutical and aromatic properties [2]. These 130 families include 700 genera and 2250 species. From a

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comprehensive survey of medicinal plants and traditional medicines in Iran, one can realize that due to the lack of both communication in the past and a common scientific language, many pharmaceutical plants were recognized and used by particular traditional physicians residing in specific regions, but were not used in other regions [1]. In our current study, a native aromatic and medicinal plant from Asteraceae family was chosen found only in Khorasan Province of Iran. This plant is *Sclerorhachis platyrachis* (Boiss.) Podlech ex Rech. f. which residents of villages near Sabzevar use to relieve menstrual pain.

The genus *Sclerorhachis,* includes two endemic species, *S. platyrachis and S. leptoclada*, found in Khorasan Province, northeastern Iran [2, 3]. In recent years, there have been three reports on chemical components of *S. platyrachis* [4-6]. These include a study of the composition of the essential oil from aerial parts of *S. platyrachis* (and of *Anthemis talyshensis*) by Aghajani et al. [4] and the identification of a sesquiterpenoid [5] and a eudesmane-type terpenoid [6].

The basic aims of the present research were to carry out a detailed analysis of the essential oil from the aerial parts of *S. platyrachis*, to determine the total content of flavonoids in various extracts of of *S. platyrachis* and of other phenolic substances as well as to examine the antioxidant activity of plant extracts.

MATERIALS AND METHODS

Chemicals

Solvents (methanol, chloroform, ethyl acetate), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent and butylated hydroxytoluene (BHT) were purchased from Merck (Darmstadt, Germany) products.

Plant Material

The plant material was collected on June 2012 in northern Sabzevar in Khorasan Province, Iran, at an altitude of 1530 meters. A voucher specimen has been deposited in the herbarium of the Research Center of Natural Resources, Sabzevar, Iran. The plant material was dried in a dark room at ambient temperature. Dried plant were crushed and stored in clean containers.

Essential oil isolation

Air-dried aerial parts of *S. platyrachis* (100 g) were subjected to hydrodistillation in a Clevenger-type apparatus for three hours to produce oils. The total yield of volatiles was 0.38% (w/w). The oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C before analysis.

GC analysis

GC analysis was performed using a Shimadzu GC-9A gas chromatograph, equipped with a HP-5MS fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 50 °C for five minutes and then programmed to 250 °C at a rate of 3 °C/min. The injector and detector temperatures were 290 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s.

GC/MS analysis

GC/MS analysis was carried out on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.32 µm). The oven temperature was programmed from 60 to 220°C at 6 °C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The chromatograph was coupled to a Hewlett-Packard 5973 mass selective detector with an ionization voltage of 70 eV.

Qualitative and quantitative analyses

Constituents of the volatile oils were identified by comparison of their retention indices relative to C9-C21 n-alkanes and of their mass spectral fragmentation patterns with those reported in the literature [7] and stored in a MS library (Wiley 275). The quantification of the components was performed based on their GC peak areas from the HP-5MS column separation.

Extraction of phenolic and antioxidant materials

A conventional maceration method was used for preparing the extracts. Dried and powdered aerial parts of *S. platyrachis* (50 g) extracted by 400 mL portions of various solvents. The extractions were carried out three times with each solvent, using new batches of *S. platyrachis* each time. Solvents having different polarities (methanol, ethyl acetate, chloroform) were used. Mixing was accomplished using an overhead stirrer for 24 h. All the mixtures were filtered through Whatman paper No. 41. The solvents were removed below 40°C using a rotary evaporator (Heidolph, Germany) and stored at 4 °C for further use.

Determination of Total Phenolics Content

The total phenolics content of extract was determined by Folin-Ciocalteu reagent using a modification of the procedure of Singleton et al. [8]. Briefly, 0.5 mL of the methanol, ethyl acetate, or chloroform) extract, 1.5 mL distilled water and 0.5 mL of 1:10 Folin-Ciocalteu reagent were shaken vigorously. Then 5 minutes later, 1.0 mL of sodium carbonate (5.0%) was added. The mixture was shaken, and after standing for two hours in the dark at room temperature, its absorbance at 760 nm was measured using a UV-Visible spectrophotometer, (Unico UV-2100, China). The total phenolics concentration was calculated by comparison with a gallic acid (GA) calibration curve (5-100 mg/L). Total phenolics content was expressed in terms of gallic acid equivalents (GAE)/g for the average of three replicates.

DPPH Radical Scavenging Activity Assay

Antioxidant activity was assessed based on the ability of plant extracts to scavenge DPPH free radicals, by the standard method Mensor et al. [9]. Briefly, the stock solution of extracts was prepared at concentration of 1000 ppm. Then diluted solution of 20, 40, 60 and 80 ppm were prepared. The volume of 2.5 mL of each solutions and one mL of a 0.004% solution of DPPH in methanol were mixed. After standing for half hours in the dark at room temperature (23 °C), the absorbance was recorded at 517 nm. Ascorbic acid, gallic acid and BHT (20, 40, 60, 80 ppm) were used as positive references.

Control samples contained all the reagents and pure solvents rather than the extracts. Percentage of scavenged DPPH was calculated using equation 1. The data are presented as mean values ± standard deviation (n = 3).

DDPH scavenging activity = 100 × (Ac – As)/Ac, (1)

Ac and As represents the absorbance of the control and of the sample resectively. IC_{50} values denote the concentration of sample required to decrease the absorbance at 517 nm by 50%.

RESULTS AND DISCUSSION

As a part of our on-going study on the chemical analysis of oils obtained from the wild plants of Iran, we decided to investigate the oils of this specific plant. Hydrodistilled volatile oils from the crushed dry aerial parts of *S. platyrachis* (Boiss.) Podlech ex Rech. f. (Compositae) from Sabzevar (Iran) was studied by GC and GC/MS. The crushed and dried aerial parts of the plant yielded 0.38% (w/w) oil. The oil was clear and yellowish. Sixty-three components, representing 89.2% of the total constituents, were quantified and recognized in the oil from the aerial parts of *S. platyrachis*. Table 1 lists formulas, percentages, and retention indices of the identified compounds in the oil. As can be seen, the main components are β-pinene (17.5%) and γ-terpinene (15.4%). Other considerable components were p-cymene (4.9%), hexadecanoic acid (4.9%), limonene (4.4%), camphor (3.9%) and E-β-ocimene (3.4%).

GC and GC/MS analysis of the oil from the aerial parts of *S. platyrachis* revealed several monoterpenoid hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpenoid hydrocarbons (SH), oxygenated sesquiterpenes (OS), nonterpenoid hydrocarbons (NH) and one oxygenated diterpene (OD). Eleven monoterpene hydrocarbons (48.7%), twelve oxygenated monoterpene (11.8%), fifteen sesquiterpene hydrocarbons (9.9%), five oxygenated sesquiterpene (6.0%), nineteen nonterpene hydrocarbons (12.4%) and one oxygenated diterpene (0.4%) were detected in this oil. These data lead to a rank order of constituent groups: MH>NH>OM>SH>OS>OD for the oil of the aerial parts. The main components in this oil were β-Pinene (17.5%) and γ-terpinene (15.4%). These results are in contrast with those of Aghajani et al. [4], who found the major components to be α-pinene (31.2%), camphor (24.8%) and β-pinene (14.7%), and very little γ-terpinene (0.3%). In the earlier study, the oil consisted mainly of monoterpenes and small fraction of sesquiterpenes (0.6%), with monoterpene hydrocarbons (56.5%) predominating over oxygenated monoterpenes (28.2%).

It is evident from these data that there are significant differences in the results of the two studies for the aerial parts of *S. platyrachis*. The differences may be related to environmental conditions such as climate, altitude, collection time, and ground composition of the sampling area.

Table 1. Constituents of the essential oils from aerial parts of *Sclerorhachis platyrachis* obtained by hydrodistillation [a]

No.	Compound	Molecular formula	Class	RRI[b]	Percentage
1	α-Thujene	$C_{10}H_{16}$	MH[c]	930	0.1
2	α-Pinene	$C_{10}H_{16}$	MH	939	1.7
3	Camphene	$C_{10}H_{16}$	MH	954	0.4
4	β-Pinene	$C_{10}H_{16}$	MH	979	17.5
5	Myrcene	$C_{10}H_{16}$	MH	990	0.4
6	α-Terpinene	$C_{10}H_{16}$	MH	1014	0.3
7	p-Cymene	$C_{10}H_{14}$	MH	1024	4.9
8	Limonene	$C_{10}H_{16}$	MH	1029	4.4
9	E-β-Ocimene	$C_{10}H_{16}$	MH	1050	3.4
10	γ-Terpinene	$C_{10}H_{16}$	MH	1059	15.4
11	cis-Sabinene hydrate	$C_{10}H_{18}O$	OM[d]	1070	0.2
12	Terpinolene	$C_{10}H_{16}$	MH	1088	0.2
13	Pentyl butyrate	$C_9H_{18}O_2$	NH[e]	1091	0.4
14	Linalool	$C_{10}H_{18}O$	OM	1096	2.1
15	2-Methyl buthyl 2-methyl butanoate	$C_{10}H_{20}O_2$	NH	1103	0.5
16	cis-p-Mentha-2-en-1-ol	$C_{10}H_{18}O$	OM	1122	0.1
17	trans-Pinocarveol	$C_{10}H_{16}O$	OM	1139	0.1
18	Camphor	$C_{10}H_{16}O$	OM	1146	3.9
19	n-Hexyl isobutyrate	$C_{10}H_{20}O_2$	NH	1151	0.5
20	n-Amyl isovalerate	$C_{10}H_{20}O_2$	NH	1155	0.4
21	Pinocarvone	$C_{10}H_{14}O$	OM	1164	0.1
22	Borneol	$C_{10}H_{18}O$	OM	1167	0.4
23	Lavandulol	$C_{10}H_{18}O$	OM	1170	0.2
24	Terpinen-4-ol	$C_{10}H_{18}O$	OM	1188	1.7
25	α- Terpineol	$C_{10}H_{18}O$	OM	1191	2.0
26	Hexyl 2-methyl butyrate	$C_{11}H_{22}O_2$	NH	1241	0.6
27	Hexyl isovalerate	$C_{11}H_{22}O_2$	NH	1246	0.4
28	Thymol	$C_{10}H_{14}O$	OM	1299	0.9
29	α-Cubebene	$C_{15}H_{24}$	SH[f]	1345	1.5
30	β-Bourbonene	$C_{15}H_{24}$	SH	1387	0.6
31	cis-Jasmone	$C_{11}H_{16}O$	OM	1392	0.1
32	Methyl eugenol	$C_{11}H_{14}O_2$	NH	1403	0.3
33	β-Caryophyllene	$C_{15}H_{24}$	SH	1417	1.2
34	β-Copaene	$C_{15}H_{24}$	SH	1430	0.2
35	Aromadendrene	$C_{15}H_{24}$	SH	1439	0.3
36	α-Humulene	$C_{15}H_{24}$	SH	1452	0.1
37	(E)-β-Farnesene	$C_{15}H_{24}$	SH	1454	0.4
38	Alloaromadendrene	$C_{15}H_{24}$	SH	1458	0.3
39	Germacrene D	$C_{15}H_{24}$	SH	1484	1.3
40	β-Selinene	$C_{15}H_{24}$	SH	1489	0.3
41	Bicyclogermacrene	$C_{15}H_{24}$	SH	1500	0.7
42	α-Muurolene	$C_{15}H_{24}$	SH	1504	0.2

Table 1. Continued

43	γ-Cadinene	$C_{15}H_{24}$	SH	1513	1.7
44	δ-Cadinene	$C_{15}H_{24}$	SH	1522	1.0
45	Cadine-1,4-diene	$C_{15}H_{24}$	SH	1533	0.1
46	Dodecanoic acid	$C_{12}H_{24}O_2$	NH	1568	0.2
47	Spathulenol	$C_{15}H_{24}O$	OS[g]	1577	2.4
48	Caryophyllene oxide	$C_{15}H_{24}O$	OS	1582	0.7
49	β-Eudesmol	$C_{15}H_{26}O$	OS	1649	0.3
50	α-Cadinol	$C_{15}H_{26}O$	OS	1652	1.8
51	α-Bisabalol	$C_{15}H_{26}O$	OS	1685	0.8
52	Heptadecane	$C_{17}H_{36}$	NH	1700	0.4
53	Tetradecanoic acid	$C_{14}H_{28}O_2$	NH	1768	0.9
54	Octadecane	$C_{18}H_{38}$	NH	1800	0.1
55	6,10,14-Pentadecanone	$C_{18}H_{36}O$	NH	1827	0.9
56	Pentadecanoic acid	$C_{15}H_{30}O_2$	NH	1865	0.1
57	Dibutyl phthalate	$C_{16}H_{22}O_4$	NH	1965	0.8
58	Hexadecanoic acid	$C_{16}H_{32}O_2$	NH	1972	4.9
59	Eicosane	$C_{20}H_{42}$	NH	2000	0.1
60	1-Octadecanol	$C_{18}H_{38}O$	NH	2081	0.7
61	Phytol	$C_{20}H_{40}O$	OD[h]	2111	0.4
62	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	NH	2136	0.1
63	Tricosane	$C_{23}H_{48}$	NH	2300	0.1
	Total identified				89.2

[a] The compounds have been arranged according to their retention indices on an HP-5 MS capillary column, [b] Kovatz retention indices given in the literature [c] Monoterpene hydrocarbons, [d] Oxygenated monoterpene, [e] Nonterpene hydrocarbons, [f] Sesquiterpene hydrocarbons, [g] Oxygenated sesquiterpene, [h] Oxygenated diterpene

Total content of phenolic substances

Methanol, ethyl acetate and chloroform extracts were prepared in order to determine the total phenolics content and antioxidant activity. The total amount of phenolic substances was assessed using the Folin-Ciocalteu reagent and is expressed in terms of gallic acid (GA) equivalents (the standard curve equation: $Y=0.0086x + .0175$, $r^2 = 0.998$). The values obtained are expressed as mg of GA/g of dried extract (Table 2). These values ranged from 52.4 to 172.3 mg. As anticipated, the amount of phenolic substances extracted from *S. platyrachis* depends on the polarity of solvent used for extraction, with more phenolic material being extracted with the more polar solvent (e.g., methnol) [10].

Table 2. Total phenolic contents in the extracts as gallic acid equivalents (mg of GA/g of dried extract).

Extract	Absorbance	mg of GA/g of dried extract [1]
Chloroform	0.66	70.9±1.9
Ethyl acetate	1.50	52.4±0.5
Methanol	0.52	172.3±5.9

[1] Each value is the average of three analyses ± standard deviation.

Antioxidant activity

The antioxidant activity of the different extracts of *S. platyrachis* was determined using a solution of DPPH reagent in methanol. A fresh solution of DPPH has a dark purple color that its maximum absorption occurs at 517 nm.

This purple color changes into a colorless product, resulting in a decrease in absorbance [11]. The antioxidant activity of three different extracts of *S. platyrachis* is expressed in terms of percentage of inhibition of oxidation and IC_{50} values (µg/mL) (Table 3). A parallel evaluation was made to examining antioxidant activity of the plant extracts and three standard compounds. The standard substances were BHT, gallic acid and ascorbic acid examination of antioxidant activities of extracts of *S. platyrachis* showed values ranging from 4.6% to 53.5%. The methanol extract was the most effective in neutralizing DPPH radicals, with an IC_{50} of 71.1 µg/mL. The chloroform extract had a moderate activity. The least effective extract for inhibiting DPPH radicals was the ethyl acetate extract. The methanol extract, however, was not as effective as BHT, ascorbic acid or gallic acid in scavenging of DPPH free radicals.

Furthermore, the extracts that had the highest antioxidant activity (Table 3) also had the highest concentration of phenols (Table 2). There is a logical correlation between the values of both antioxidant assays of *S. platyrachis*.

Table 3. Antioxidant (DPPH scavenging) activity of plant extracts and synthetic antioxidants as percent of inhibition of DPPH radicals and IC_{50} values (µg/mL).

Extract	20 ppm	40 ppm	60 ppm	80 ppm	IC_{50}
Chloroform	4.6±1.9	21.65±2.2	32.1±1.6	42.2±1.2	92.3±3.6
Ethyl acetate	4.7±1.1	15.6±2.9	28.0±1.1	36.5±2.0	103.4±4.6
Methanol	11.7±1.3	30.7±2.2	43.3±1.7	53.5±2.5	71.1±1.6
Synthetic antioxidant	20 ppm	40 ppm	60 ppm	80 ppm	IC50
BHT	76.0±4.8	93.8±0.8	94.6±1.1	96.7±0.9	14.9±0.9
Ascorbic acid	34.2±5.2	51.8±2.4	67.2±0.6	85.5±1.7	40.3±1.1
Gallic acid	82.8±1.6	91.0±1.9	91.5±2.6	92.6±0.9	7.9±1.2

CONCLUSIONS

This study showed considerable amounts of β-pinene (17.5%) and γ-terpinene (15.4%), results that differ from a previous study on the same species [4]. These results suggest that the chemical composition of the essential oil of the same species can change depending on a variety of conditions, possibly climate, time of collection, and the ground composition of the sampling area besides the stage of plant growth. In addition, the results of our study suggest that *S. platyrachis* may be of use in pharmacy and phytotherapy. From these results, it may be concluded that this plant is a natural source of antioxidant substances. The highest concentration of phenolic compounds was obtained using the solvent of highest polarity, specifically, methanol. It is proposed that the high content of phenolic compounds correlates with the high antioxidant activity of the methanol extract. Further studies of this plant species should be directed toward *in vivo* studies of its medicinally active components.

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REFERENCES

1. Mozaffarian V., 2013. Identification of Medicinal and Aromatic Plants of Iran. Farhang Moaser Publisher, Tehran, 1st edit.
2. Rechinger K.H., 1986. *Sclerorhachis*, in: Flora Iranica, Compositae No. 158. Edits., K.H. Rechinger and I.C. Hedge, P 47, Akademische Druck and Verlagsanstalt, Graz, Austria.
3. Mozaffarian V., 1996. A Dictionary of Iranian plant names. Farhang Moaser Publischer, Tehran, 1st Edit., p 491.
4. Aghajani Z., Masoudi S., Rustaiyan A., 2005. Volatile oils of *Anthemis talyshensis* A. Fedor and Sclerorhachis platyrachis (Boiss.) Podlech ex Rech. f. from Iran. J Essent Oil Res. 17 (4), 355-357.

5. Kheyrabadi R., Habibi Z., Ng S.W., 2011. 2-{(1S,2S,4aR,8R,8aR)-8-Hydroxy-4a,8-dimethyl-1-[(2E)-2-methylbut-2-enoyloxy]perhydronaphthalen-2-yl}acrylic acid from *Sclerorhachis platyrachis*, Acta Crystallographica Section E, Structure Reports Online, 67, Part 10: pp. o2699.

6. Habibi Z., Kheyrabadi R., Ghasemi S., Yousefi M., Ng S.W., 2012. A new sesquiterpenoid from *Sclerorhachis platyrachis* (Boiss.) Podlech ex Rech. f. Phytochemistry Letters. 5(4): 705-707.

7. Adams R.P., 2007. Identification of Essential oil Components by Gas Chromatography/Mass Spectrometry. Allured Pub. Corp., Carol Stream, IL., USA.

8. Singleton V.L., Orthofer R., Lamuela-Raventos R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Meth Enzymol. 299, 152–178.

9. Mensor L.I, Menezes F.S., Leitao G.G., Reis A.S., Dos Santos T., Coube C.S., Leitao S.G., 2001. Screening of Brazilian plants extracts for antioxidants activity by use of DPPH free radical method. Phytother Res. 15, 127-130.

10. Mohsen M.S., Ammar S.M.A., 2008. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem. 112, 595-598.

11. Amarowicz R., Pegg B.R., Rahimi-Moghaddam P., Bar B., Weil J.A., 2003. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 84, 551-562.