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ORIGINAL ARTICLE

Chemical Composition of the Essential Oil, Total Phenolic Content and Antioxidant Activity of *Vitex pseudo-negundo* Seeds Collected from Northeastern Iran

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KEYWORDS

Vitex pseudo-negundo; GC/MS; Hexadecanoic acid; Elemol; Antioxidant activity

ABSTRACT: In this study, the essential oil obtained by hydrodistillation of the seeds of *Vitex pseudo-negundo* (Verbenaceae), growing wild in Sabzevar, Khorasan Razavi Province, Iran, were analyzed by GC and GC/MS. The yield of total volatiles was 0.8% (w/w). Thirty-seven compounds representing 92.4% of the seed oil were identified. The main components of the oil were hexadecanoic acid (8.0%), elemol (7.0%) and α-bisabolol (6.1%). The oil was rich in sesquiterpene hydrocarbons (51.2%). The total flavonoids of different extracts of the plant were found the range 56-195 mg/g, with the maximum amount being in the methanol extract. In addition, the antioxidant activities of the extracts were measured by radical scavenging activity of antioxidants against the free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The antioxidant activities of the extracts of *V. pseudo-negundo* seed were higher than those of the standard synthetic antioxidants, BHT, ascorbic acid and gallic acid.

INTRODUCTION

Vitex pseudo-negundo (Verbenaceae) grows naturally near seasonal rivers in Iran [1, 2]. *V. pseudo-negundo* commonly referred as chaste tree, chaste lamb, tree of chastity and Abraham's balm (English names). The biological activities, e.g., antifungal [3], antioxidant [4, 5], anti- inflammatory [6] and antimicrobial [7] of some species of *Vitex* have been reported.

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Antioxidants have been found in many plant materials and supplements. Because of their natural origin, the antioxidants obtained from plants are claimed to be of greater benefit than those of synthetic ones [8]. Natural antioxidants from plants have fewer side effects than do synthetic antioxidants, some of finding to be genotoxic [9]. Therefore, investigation of the biological activity and chemical composition of medicinal plants as potential sources of natural antioxidants seems meritorious.

The basic aim of this research was to characterize the essential oil of seeds of *V. pseudo-negundo* and to determine the total phenolic content and radical scavenging activity in various extracts of the seeds.

MATERIALS AND METHODS

In this study, the volatile oil prepared by hydrodistillation of *V. pseudo-negundo* seeds from Sabzevar, Khorasan Razavi Province, Iran, was studied by GC and GC/MS. Total phenolic content and radical scavenging capacity were used to determination of antioxidant activity of different extracts of the plant's seed.

Chemicals

Solvents (methanol, chloroform, ethyl acetate) were purchased from Merck (Darmstadt-Germany). Gallic acid and 2, 2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co., St Louis, MO,USA. The Folin-Ciocalteu phenol reagent and butylated hydroxytoluene (BHT) were from FlukaChemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade.

Plant Material

The plant material (Figure 1) was collected in Jun 2013 from Sabzevar in Khorasan Province, Iran (Figure 2). A voucher specimen with assigned number IAUS325 has been deposited in the herbarium of the Research Center of Natural Resources, Sabzevar, Iran. The collected plant material was air-dried in darkness at room temperature (20ºC). Seeds of dried plant were separated from the plant and stored in dark, tightly sealed containers until needed.

Figure 1. *Vitex pseudo-negundo*

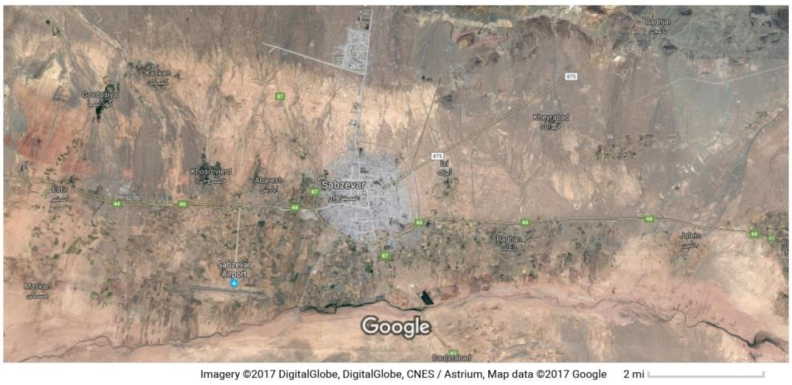

Figure 2. Google map of Sabzevar, northeastern Iran

Essential oil isolation

Air-dried seeds of *V. pseudo-negundo* (100 g) were subjected to hydrodistillation in a Clevenger-type apparatus for three hours to produce oils. The yield of total volatiles was 0.8% (w/w). The oils were dried over anhydrous sodium sulfate and stored in dark and sealed vials at 4°C before analysis.

GC analysis

GC analysis was performed using a Shimadzu GC-9A gas chromatograph, equipped with an 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 (FID) temperatures were 290 °C. Helium was used as carrier gas at a flow rate of 1 mL/min.

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 i.d., 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 very se

lective 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 [10] and stored in an MS library (Wiley 275). The quantification of the components was performed based on GC peak area data obtained from separation on the HP-5MS column.

Preparation of the extracts

The conventional maceration method was used for preparing the extracts. Dried and powdered seed of *V. pseudo-negundo* (50 g) extracted by 400 mL of solvents. This process replicated trice with the same volume of fresh solvent. Three solvents having different polarities (methanol, ethyl acetate, chloroform) were used. An overhead stirrer mixed the materials 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 Phenolic Contents

The total phenolic content of extract was determined spectrophotometrically by Folin-Ciocalteu method according to the procedure reported previously [11] with some modifications. Briefly, 500 µL of extract solution (in methanol, ethyl acetate, chloroform), 1500 µL dis-

tilled water and 500 µL of 1:10 Folin-Ciocalteu reagent were mixed for 1 min. Then 5 min later, 1000 µL of sodium carbonate (5.0%) was added and the mixture was shaken. After two hours in the dark at room temperature, the absorbance at 760 nm was measured using a UV-Visible spectrophotometer, (Unico UV-2100, China). The total phenolic concentration was calculated from gallic acid (GA) calibration curve (5-100 mg/L). The total phenolics content of each extract was expressed as gallic acid equivalents (GAE)/g of extracts averaged from three replicates.

DPPH Radical Scavenging Activity Assay

The ability of the plant extract to scavenge DPPH free radicals was assessed by a standard method [12]. Briefly, 10 µL of the extracts was added to 2.5 mL of a 0.004% solution of DPPH in methanol. Stock solutions of extracts were prepared to have a concentration of 1 mg/mL Dilutions were made to obtain concentrations of 20, 40, 60 and 80µg/mL. Diluted solutions (1 mL each) were mixed with 1 mL of a methanolic solution of DPPH having a concentration of 0.004%. After 30 min 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 µg/mL) were used as reference compounds. The control contained all the reagents except the extract. The percentage of scavenged DPPH was calculated using equation 1. The data reported here are mean values ± standard deviation (n=3).

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

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

RESULTS AND DISCUSSION

Essential oil profile

As a part of on-going work on the chemical analysis of oils obtained from the wild plants of Iran, we decided to re-investigate the oils of this specific plant. Hydrodistilled volatile oils from the crushed dry seeds of *V. pseudo-negundo* (Verbenaceae) from Sabzevar (Iran) were analyzed by GC and GC/MS. The air-dried seed of the plant yielded 0.8% (w/w) oil. The oil was clear and yellowish. The forty-four components identified in the oil of the seed accounted for 92.4% of the compounds. Table 1 lists formulas, percentages, and retention indices of identified compounds in the oil. The main components are hexadecanoic acid (8.0%), elemol (7.0%) and α-bisabolol (6.1%).

GC and GC/MS analysis of the oil revealed several monoterpenoid hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpenoid hydrocarbons (SH), oxygenated sesquiterpenes (OS) and nonterpenoid hydrocarbons (NH).Six monoterpene hydrocarbons (12.0%), seven oxygenated monoterpenes (13.1%), nine sesquiterpene hydrocarbons (22.3%), eleven oxygenated sesquiterpenes (28.9%) and four monoterpene hydrocarbons (16.1%) were detected in this oil. The data lead to a rank order of constituent groups: OS >SH> NH >OM>MH for the seed oil.

Table 1. Constituents of essential oils from seed of *Vitex pseudo-negundo* obtained by hydrodistillation[a]

No.	Compound	Formula	Percentage	RRI[b]	Class
1	**α-Pinene**	$C_{10}H_{16}$	3.3	939	MH[c]
2	β-Pinene	$C_{10}H_{16}$	1.7	979	MH
3	β-Myrcene	$C_{10}H_{16}$	1.5	990	MH
4	p-Cymene	$C_{10}H_{14}$	2.4	1024	MH
1	Limonene	$C_{10}H_{16}$	2.2	1029	MH
2	**1,8-Cineole**	$C_{10}H_{18}O$	3.2	1031	OM[d]
3	Terpinolene	$C_{10}H_{16}$	0.9	1088	MH
4	Linalool	$C_{10}H_{18}O$	2.7	1096	OM
5	Pinocarveol	$C_{10}H_{16}O$	1.6	1139	OM
6	Pinocarvone	$C_{10}H_{14}O$	0.4	1164	OM
7	**Terpinen-4-ol**	$C_{10}H_{18}O$	3.1	1177	OM
8	p-cymen-8-ol	$C_{10}H_{14}O$	0.6	1182	OM
9	bornyl acetate	$C_{12}H_{20}O_2$	1.5	1288	OM
10	**α-copaene**	$C_{15}H_{24}$	4.1	1376	SH[e]
11	β-bourbonene	$C_{15}H_{24}$	0.9	1387	SH
12	β-elemene	$C_{15}H_{24}$	2.7	1390	SH
13	α-cedrene	$C_{15}H_{24}$	1.1	1410	SH
14	β-caryophyllene	$C_{15}H_{24}$	2.5	1417	SH
15	**γ-muurolene**	$C_{15}H_{24}$	3.9	1478	SH
16	α-curcumene	$C_{15}H_{24}$	2.0	1489	SH
17	γ-Cadinene	$C_{15}H_{24}$	0.7	1520	SH
18	**δ-cadinene**	$C_{15}H_{24}$	4.4	1524	SH
19	**elemol**	$C_{15}H_{26}O$	7.0	1548	OS[f]
20	(E)-nerolidol	$C_{15}H_{26}O$	1.1	1561	OS
21	**Spathulenol**	$C_{15}H_{24}O$	4.2	1577	OS
22	Guaiol	$C_{15}H_{26}O$	0.9	1600	OS
23	epi-cedrol	$C_{15}H_{26}O$	0.7	1619	OS
24	α-muurolol	$C_{15}H_{26}O$	2.2	1646	OS
25	selin-11-en-4-α-ol	$C_{15}H_{26}O$	0.9	1658	OS
26	bulnesol	$C_{15}H_{26}O$	1.1	1671	OS
27	**α-bisabolol**	$C_{15}H_{26}O$	6.1	1685	OS
28	Acorenone –B	$C_{15}H_{24}O$	0.9	1690	OS
29	**(E)-nerolidyl acetate**	$C_{17}H_{28}O_2$	3.8	1717	OS
30	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.8	1777	NH[g]
29	**6,10,14-Trimethyl-2-Pentadecanone**	$C_{18}H_{36}O$	4.6	1848	NH
30	dibuthyl phthalate	$C_{16}H_{22}O_4$	1.7	1970	NH
31	**hexadecanoic acid**	$C_{16}H_{32}O_2$	8.0	1992	NH
Total identified		92.4			

[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]Sesquiterpene hydrocarbons, [f]Oxygenated sesquiterpene, [g]Nonterpene hydrocarbons

Content of phenolic compounds

Methanol, ethyl acetate, and chloroform extracts were prepared to examine the total phenolics content and antioxidant activity. The total phenolics contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalents (the standard curve equation: $Y=0.0086x + 0.0175$, $r^2 = 0.999$). The total of phenolic compounds in the extracts examined ranged from 55.8 to 194.5 mg GA/g of dry extract (Table 2). The total of phenolics in extracts of *V. pseudo-negundo* depends on the solvent used for extraction, with the most polar solvent being most effective, which is consistent with the high polarity of most phenols [13].

Table 2. Total content of phenolic compounds in the plant extracts expressed in terms of gallic acid equivalents (mg of GA/g of extract).

Extract	Absorbance	mg of GA/g of extract [1]
Chloroform	1.37	55.8±3.7
Ethyl acetate	1.75	148.7±1.9
Methanol	1.51	194.5±4.2

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

Antioxidant activity

The antioxidant activities of different extracts of the seeds of *V. pseudo-negundo* were determined using a methanolic solution of DPPH. DPPH is a very stable free radical. Unlike *in vitro* generated free radicals, such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by side reactions such as metal ion chelation and enzyme inhibition. "A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm" [14]. This purple color generally fades when antioxidant molecules quench DPPH free radicals [14]. The antioxidant activity of three different extracts from the species *V. pseudo-negundo* is expressed in terms of percentage of inhibition (%) and IC_{50} values (μg/mL) (Table 3). In addition to the plant extracts, the antioxidant activities of three standard compounds--BHT, Gallic acid, and ascorbic acid--were obtained for comparison.

The antioxidant activities of *V. pseudo-negundo* extracts showed values ranging from 8.6% to 72.4%. The most active extract was the methanol extract, which neutralized 50% of free radicals at a concentration of 61.3 μg/mL. A moderate activity was found for ethyl acetate. The chloroform extract had the least activity. In comparison to IC_{50} values of BHT, ascorbic acid, and gallic acid, methanol extract from *V. pseudo-negundo* showed a moderate capacity for neutralization of DPPH radicals. Numerous investigations of plant extracts have revealed the presence of high concentrations of phenols in the extracts obtained using polar solvents. There is a correlation between the concentration of phenolic compounds found in extracts of *V. pseudo-negundo*and their antioxidant activity, with the methanol extract having the highest concentration of total phenols (Table 2) and DPPH scavenging capacity (Table 3).

Table 3. Antioxidant (DPPH scavenging) activity of investigated seed extracts and standard antioxidants presented as percentage of DPPH radicals inhibition and IC_{50} values (µg/mL)

DPPH assay (percentage of inhibition)					
	Extract Concentration				
Solvent	20 ppm	40 ppm	60 ppm	80 ppm	IC_{50}(µg/mL)
Chloroform	9.8±0.7	18.0±0.8	31.8±1.9	42.4±1.4	94.6±6.9
Ethyl acetate	8.6±1.6	26.7±2.2	42.7±2.5	56.1±4.3	75.2±3.3
Methanol	14.0±0.6	23.6±1.0	48.0±1.8	72.4±4.1	61.3±2.7
	Standard antioxidant concentration				
Standard antioxidant	20 ppm	40 ppm	60 ppm	80 ppm	IC_{50}(µg/mL)
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

The chemical composition of the essential oil of the same species can change depending on a variety of conditions, including climate, time of collection, and the ground composition of the sampling area beside of growth stages of plant. In addition, the great value of the species *V. pseudo-negundo*for uses in pharmacy and phytotherapy. This plant is natural sources of antioxidant substances of high importance. The highest concentration of phenolic compounds in the extracts was obtained using the solvent of highest polarity, namely methanol. Further studies of this plant species should be aimed at determining whether the oils and extracts have medicinal value.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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