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Research Article

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Chemical composition of *Vitex pseudo-negundo* leavese ssential oil

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ABSTRACT

There is increasing interest in herbal medicine and natural antioxidant products for use as medicines and food additives. The present work was identified the chemical composition compounds of Vitex pseudo-negundo leaves essential oil. This experimental study, which carried out in 2013 in Lorestan Medical Sciences University. Essential oil of Vitex leaves was prepared. The components of Vitex pseudo-negundo leaves essential oil were analyzed with gas chromatography/mass spectrometry (GC/MS). GC/MS data and retention indices for reference essential oil leaves samples were used to identify 30 constituents. These compounds make up a total of 99.00 percent essential oil. 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene; 1,8-Cineole and Phenol, bis(1,1-dimethylethyl are the most of compounds of Vitex pseudo-negundo leaves essential oil. This study showed that essential oil of Vitex pseudo-negundo is a source easily accessible of natural antioxidants such as 1, 8-Cineole and Sabinene and it may be suitable for use in food and pharmaceutical applications.

Keywords: Chemical composition, *Vitex pseudo-negundo*, essential oil

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INTRODUCTION

Oxidative stress caused by an imbalance between oxidants and antioxidants [1]. Therefore, oxidative stress play a key role in the number of diseases such as nephrotoxixity, diabetes, cancer, coronary heart disease, aging and other diseases [2]. Hence the agents that can diminish oxidative stress and balance between oxidants and antioxidants can be beneficial in the treatment of these diseases.

The Vitex plant with local names of the Five-finger and scientific name of vitex pseudo negundo, genus vitex (five finger / pepper) has shrubs with woody bases, 3 meters in height [3]. The species of vitex have high habitat range and is widespread in Western Asia, Syria, Kurdistan, Iran and Afghanistan. In Iran, the species of vitex is widespread in Ahwaz, Fars Province, including Sivand, Kazeron and Firouz Abad, Jahrom [3, 4]. This plant (Five-finger) and other species of this genus are usually growing in the bed of rivers and streams with standing water and some mountainous valleys in the southern Iran [3, 4]. Different species of vitex plant have medicinal uses and are used in the treatment of gynecological diseases [5]. This herb is traditionally used to care and treat many problems of women, such as amenorrhea, dysmenorrhea, corpus luteum insufficiency, hyperprolactinemia, infertility, acne, menopause and breastfeeding disorders [6]. The vitex fruit contains a mixture of iridoids and flavonoids and sex hormone-like compounds [7]. The action mechanism of vitex plant is unclear. The vitex plant may indirectly affect the balance of sex hormones by influencing the hypothalamus and pituitary [8, 9]. Medicinal use of the Five-finger goes back to centuries ago. The first reports of medicinal use are from four centuries BC at which time it had been

used for the treatment of uterine diseases. This is one of the most important herbs in herbal medicine used to treat female hormone diseases, and is considered as a hormone regulator [10]. This plant is widely used in the treatment of breast diseases, menstrual irregularities and uterine bleedings [11-14]. These effects are related to chemical composition of Vitex. According to the useful properties of vitex and its widespread traditional uses and given that chemical composition of vitex leaf (Vitex pseudo-negundo) has not been studied, thus, in this study, the chemical composition of leaf vitex essence (Vitex pseudo-negundo) was identified with using gas chromatography and gas chromatography connected to a mass spectrometer.

EXPERIMENTAL SECTION

Isolation of the essential oil from *vitex pseudo-negundo* leaves:

Vitex pseudo-negundo were prepared July 2013 from cultivated in Khorram Abad (Lorestan province, western Iran). Leaves of the plants were collected during flowering stage and were air-dried at ambient temperature in the shade seperatly. Leaves were hydro -distilled using a Clevenger apparatus for 4 hours, giving yellow oil in 2.287% yield. The oil was dried over anhydrous sodium sulfate and stored at 4ºC. The plant was previously identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI) in Tehran, Iran. A voucher specimen (No. 62316) has been deposited at the TARI Herbarium a voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Gas chromatography/ mass spectrometry

Fid- GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane. 25 m, 0.25 mm i.d., ratio, 1:25, and flame inonization detector. Temperature programme: 60 ºC (2 min) rising to 240 ºC at 4 ºC/min: injector temperature 250 ºC, detector temperature, 260 ºC. GC-MS was performed using a Hewlett-Packard 6859 with a quadrupole detector, on a HP-5 column, operating at 70 eV ionization energy, using the same temperature programme and carrier gas as above. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic to Van Den Dool method [15-17].

Identification of components

The linear retention indices for all the compounds were determined by conjection of the sample with a solution containing the homologous series of C8-C22 n-alkanes.

The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database [17-20].

RESULTS AND DISCUSSION

Chemical composition of Vitex pseudo-negundo leaves essential oil

The yield of the essential oils obtained from Vitex pseudo-negundo leaves was 2.287% (W/W) respectively. Results of the GC-MS analysis of the oils are shown in figure 1 and table 1, where the components are listed in order of their elution from the HP-5MS column. Twelve compounds of Vitex pseudo-negundo leaves were identified (89.41% of the total oils respectively) (Figure 1 and table 1).

The main constituents found in the essential oil Vitex pseudo-negundo leaves were 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (18.2%); 1,8-Cineole (16.7%); Phenol, bis(1,1-dimethylethyl) (16.7%); Veridiflorol (8.32%); dl-Limonene (4.4%); Pyrrolo(3,2,1-JK) Carbazole (4.93%); beta.-Eudesmol (3.11%); beta – Sesquiphellandrene (3.5%); Alpha Terpinol (2.76%); beta – Myrcene (1.37%); 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl(1.41%); Sabinene (5.16%) and dimethyl-6-(4-methyl-3 pentenyl)bicyclo[3.1.1]hept-2-ene (1.31%).

One study on the species of vitex ((V.agnus-castus) collected from Khorasan province, 24 compound were identified, and 1, 8 cineol, alpha-pinene, limonene and Sabinen were introduced as its main components. These compounds are similar to compounds identified in the leaf vitex (Vitex pseudo - negundo), and the results are consistent with the present study [21]. In another study on the essential of Vitex pseudo - negundo species collected from Kermanshah, the compounds of Germacrene D, Bicyclogermacrene D, β- Caryophyllene, α- Eudesmole, Germacrene B and Spathulenol were as reported major components [22]. The 1, 8 cineol and limonene were reported as the main components of the leaf vitex (Vitex agnus-castu) essential oil, which are consistent with the results in this study and similar to the compounds identified [23]. In another study in Turkey, 27 compounds were identified in the composition of the Vitex agnus-castus vitex essence that the compounds of 1, 8 cineol, Sabinen, α- Pinene, *Alpha-Terpineol Acetate* and *Beta-Farnesene* were reported as its major compounds [23]. The two compounds of 1, 8 cineol and Sabinen were similar to the compounds identified in the present study. In another study in Iran on the

essence of flowers, fruit and leaves of vitex (Vitex pseudo - negundo), the compounds of alpha-pinene, limonene and Bicyclogermacrene were introduced as the major constituents [24]. The compounds of alpha-pinene and limonene were similar to the compounds identified in this study. In the essence of Vitex negundo species, 66 compounds were identified that the predominant compounds included β- Caryophyllene, Sabinen and 4-Terniol [25, 26]. The Sabinen compound was similar to the compounds identified in the current study. In another study, the compounds of Aucubin and Casticin were reported as the main components of vitex plant (Vitex agnus-castus), which were not consistent with results and compounds identified in this study [27]. In another study, 85 compounds were reported in the essential oil of Vitex agnus-castus. Its main compounds were compositions β- Caryophyllene, and Beta Selinene and *Beta-Farnesene*. These compounds are not similar to the compounds identified in this study and the results are inconsistent [27]. In a study conducted on the essential oil of vitex (Vitex pseudo - negundo), the major compounds were reported as *Alpha-Terpineol Acetate*, Alpha-Pinene and β- Caryophyllene [21]. Comparing these compounds with the major compounds identified in this study, the results are inconsistent. Another study on the species of vitex (Vitex agnus-castus) showed that the main common ingredients in essence of leaves, flowers and fruit included 1, 8 cineol and Sabinen [28]. Different species of the genus Vitex vary significantly regarding essence content and the type of forming compounds. Obviously, the use of essence varies based on types and the percentages of the components. Studies have shown that some compounds of these plants, such as 1, 2 - cineol have anti-fungal, anti-bacterial, analgesic and antioxidant properties [29]. Viridiflorol has antioxidant effects, while Limonene has analgesic effects [30]. The results of previous studies on different species of vitex and the current study showed antioxidant properties for vitex. Also, some constituents of this plant with high percentage in its essence, such as Cineol, Viridiflorol and Limonene have useful effects [29, 30]. Consequently, performing further studies on its constituents and extracting its effective and important components are required. In addition, using it in the treatment of breast diseases, menstrual irregularities and diseases associated with oxidative stress such as diabetes, cardiovascular disease and cancer in laboratory animals is of great importance. In case of further studies and obtaining optimal results, it can be used in food, pharmaceutical and industrial products.

Table 1. Chemical composition of the *Vitex Pseudo – negundo* leaves essential oil

Number	Compound	Retention Time (min)	Yield%
1	2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene		18.2
2	1,8-Cineole	1031	16.7
3	Sabinene	1020	10.6
4	Phenol, bis(1,1-dimethylethyl)		9.07
5	Veridifloro	1589	8.32
6	Pyrrolo(3,2,1-jk) carbazole		4.93
7	dl-Limonene	1027	4.4
8	beta - Sesquiphellandrene	1523	3.5
9	beta - Eudesmol	1650	3.11
10	Alpha terpineol	1187	2.76
11	dl-Limonene	1027	2.51
12	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)		1.41
13	beta - Myrcene	988	1.37
14	endo-2,6-dimethyl-6-(4-methyl-3-pentenyl)bicyclo[3.1.1]hept-2-ene		1.31
15	Levomenol		1.21
16	Camphene	954	1.16
17	1-(3-Methylbutyl)-2,3,5-trimethylbenzene		1.02
18	beta - Pinene	978	0.91
19	Benzene, 1-methyl-2-(1-methylethyl)		0.89
20	gamma - Terpinene	1059	0.81
21	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetramethyl		0.75
22	beta - Bisabolene	1506	0.74
23	beta - Bisabolene	1506	0.6
24	(3aR,5aS,6R,8aS)-2,3a,6-Trimethyl-3,3a,4,5,5a,6,7,8-octahydrocyclopenta[c]pentalen-3-one		0.59
25	beta - Sesquiphellandrene	1523	0.57
26	4(axial)-n-Propyl-trans-3-oxabicyclo[4.4.0]decane	1013	0.57
27	alpha - Terpinene	1018	0.53
28	2-beta - Pinene	978	0.52
29	delta - terpineol	1191	0.47
30	beta - Selinene	1485	0.47

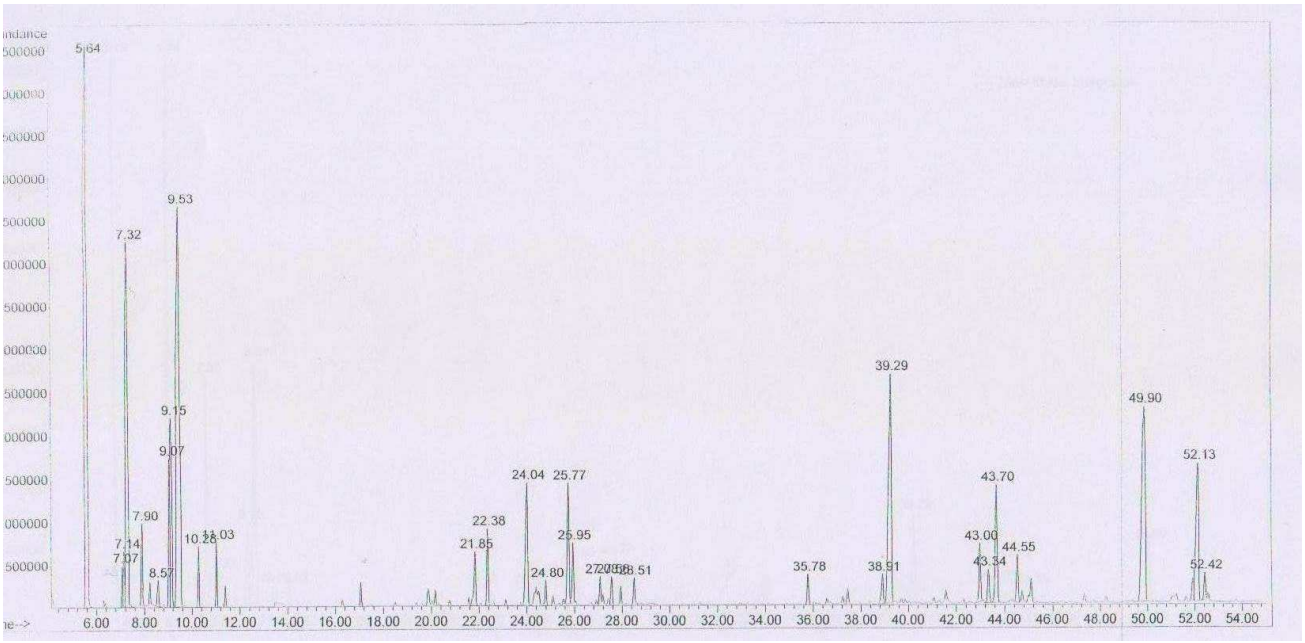

Figure 1. Chromatogram of Vitex negundo leaves by GC-MS

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