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Original Article

Extraction and Phytochemical Analysis of *Anthemis susiana* Nábělek Using Headspace-solid Phase (HS-SPME) and Gas Chromatography–mass Spectrometry (GC-MS)

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Abstract

Chamomile has several variants, such as *Anthemis Susiana* Nábělek, *Matricaria chamomilla* L., *Chamomilla recutita* (L.) Rauschert, *Matricaria suaveolens* Koch and etc. Medicinal herbs, extracts and essential oils are of particular importance in the pharmaceutical, cosmetic and food industries. The phytoanalysis of these compounds is of particular importance. In April 2019, *Anthemis Susiana* Nábělek samples were collected from the Thermal Springs area of Dehloran, south of Ilam province, western Iran. The plant was dried and then pulverized. The essential oil was extracted from the plant using HS-SPME and analyzed using GC-MS. GC-MS results showed that *Anthemis susiana* Nábělek contains 63 chemical compounds. The main chemical compounds included alpha-bisabolol oxide A (19.07%), alpha-pinene (15.50%), beta-bisabolene (12.56%), spathulenol (9.23%), beta-farnesen (8.95%), alpha-bisabolol (4.72%), caryophyllene oxide (4.46%), trans-farnesol (3.75%), and dl-limonene (3.47%).

Keywords: Alpha-bisabolol oxide A, Alpha-pinene, Chamomile, Essential Oils, GC-MS, Microextraction

Introduction

Chamomile is a generic name used for different variants of the Asteraceae family. Asteraceae which commonly referred to as the aster, daisy, composite, or sunflower family, is a very large and widespread family of flowering plants. This family has several species, including *Anthemis Susiana* Nábělek, *Matricaria chamomilla*, *Chamomilla recutita, Matricaria suaveolens* and etc. In traditional medicine, chamomile is used as an analgesic, anti-inflammatory, sedative, antispasmodic, digestive, anti-gastrointestinal spasm [1,2], stomach boosting [1-3], appetizing, anti-flatulence, carminative, antimicrobial [2-4], anti-gout and anti-anxiety agent [4-6] and also to beautify and refresh the skin [3, 4] , treat liver and spleen swelling [7,8], anxiety diarrhea, and migraines, boost stomach, and prevent gastric ulcer [9, 10]. Shushi chamomile is scientifically called *Anthemis Susiana* Nábělek. This one-year-old herbaceous plant has a stem 5 to 15 cm high, is ascending, branching, and sometimes branchless, slightly thick. The plant leaves have a rectangular-elliptic shape of 15 to 25 mm long and 5 to 10 mm wide with partly linear and pointed sections. Inflorescence tail is 1.5–5 mm long and slightly thick. All its flowers are fertile, tabs flowers and white flowers are 8–10 mm long, and tuberose flowers are yellow [11]. Chamomile species is a medicinal plant that is often referred to as the star of medicinal species. Studies have shown that medicinal plants have beneficial health effects for humans due to their active ingredients and medicinal and antioxidant compounds [12-15]. The essential oils of *Anthemis Susiana* Nábělek are a mixture of more than 120 different compounds. These compounds are mainly composed of monoterpene, sesquiterpene and oxygenated derivatives such as alcohols, esters, aldehydes and aliphatic ketones. The beneficial pharmacological, cosmetic, health and nutritional effects of the plant have been proven through genuine traditional medicine and its traditional and pharmacologically modern applications. Chamomile has a domestic and international consumer

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market that is increasing on a daily basis. In order to identify the most effective compounds in the essential oil of this plant, which the compounds has a great use in traditional Iranian medicine. The chemical constituents of essential oils of chamomile species have been studied but no chemical constituents of chamomile have been reported so far. From a historical point of view, in traditional medicine and herbal medicine, plants are very important and extensive research has been done to find herbal products and medicines since ancient times, but the important point is that a small percentage of plant species as a source Medicinal compounds have been used. Since chamomile has long been used and used medicinally, chamomile is also a species that has not been studied, so the study of its chemical composition is of particular importance. In this phytochemical study, the chemical constituents of essential oils of chamomile were investigated and analyzed for the first time by Headspace-solid phase microextraction and Gas chromatography–mass spectrometry.

Material and Methods

Plant Preparation

In April 2019, *Anthemis Susiana* Náběleksamples were collected from the thermal springs area of Dehloran, south of Ilam province, western Iran. The sample was taken from an altitude of about 300 meters above sea level. Figure of chamomile collection area in Dehloran city. Dehloran Waterfall Area is 213 meters above sea level. The plant was identified and confirmed using morphological keys of Ilam Province Plant Flora Book at Ilam University of Medical Sciences Biotechnology and Medicinal Research Center. The collected plants were cleaned and shadow-dried in the open air. The dried plant was pulverized by a plant mixer and analyzed by HS-SPME for chemical composition analysis. The characteristics of the medicinal plant *Anthemis Susiana* Nábělek used in this study are shown in Table 1.

Identification of Chemical Compounds by HS-SPME

In this experiment, the essential oil of the plant was extracted by HS-SPME technique. In this technique, about 2 grams of dried plant and its powder were placed in the vial and the vial temperature was set at 60-70 °C. These optimum temperature conditions will saturate the vapor content of the substances in the plant essential oil in the headspace of the solid surface. The SPME syringe with a lid on it was then placed in the headspace of the container and the material in the vapor waz absorbed by the silica phase in the instrument needle. After the silica fiber was allowed to sufficiently saturate with volatile components, the fiber was directly placed into the GC/MS input section and materials present in the fiber were absorbed due to the temperature of the input and then entered into the GC/MS apparatus for identification [16].

Fig. 1 Chamomile collection area in Dehloran city

HS-SPME Method

2 g of each plant extract was used for analysis. The device condition was as follows: Gas chromatograph (Agilent6890N) was coupled to Agilent 5973 Mass detector; Column: HP - 5. (30 m length × 0.25 mm (ID) × 0.25 µm (stationary phase thickness); Injector type: split/splitless and column temperature program: 50 °C, temperature 200 °C, hold time, 0.00 min and rate of 5oC/min and temperature 240 °C, hold time 0.00 min and rate of 10 °C/min. Carrier gas: He (99.999%); Injection type: splitless; Library: Wiley 7n; Injector temperature: 250 °C and flow rate: 0.9 mL/min. Extraction mode: (HS-SPME); SMPE fiber: PDMS 100 µm thickness (SUPELCO); sample weight: 2 g; extraction temperature: 60 °C; extraction time: 20 min; sonication time: 10 min (Euronda sonication instrument, Italy) and desorption time in GC-MS injector port: 3 min [17].

Table 1 Specifications of *Anthemis Susiana* Nábělek

Plant	Scientific name	Herbal family	Location	Geographical coordinates
Shushi chamomile	*Anthemis susiana* Nabelek	Asteraceae	Dehloran	32° 41' 28" North, 47° 15' 58" East

Table 2 Identified compounds of essential oils by HS-SPME (GC-MS)

No.	RI	Compound	%
1	5.16	α-Pinene	2.92
2	5.43	Camphene	0.00
3	5.91	β-Pinene	0.19
4	6.10	β-Myrcene	0.07
5	6.23	p-Cymene	0.18
6	6.39	α- Phellandrene	0.16
7	6.48	δ.3-Carene	0.02
8	6.60	α -Terpinene	0.01
9	6.82	dl-Limonene	3.47
10	7.36	β-Phellandrene	0.01
11	9.51	endo-Borneol	0.04
12	9.69	4-Terpineol	0.07
13	9.90	Adamantane, 1-methyl-	0.23
14	10.05	Myrtenal	0.12
15	10.31	Terpinene-3-ol	0.07
16	10.57	*Trans*-Carveol	0.07
17	11.92	Nerol	0.26
18	12.05	benzyl nonanoate	0.19
19	12.74	Carvone	1.09
20	14.21	Mesitylaldehyde	0.64
21	14.28	Benzaldehyde, 2,4,6-trimethyl-	0.58
22	14.34	Mesitaldehyde	0.44
23	14.47	β-Bourbonene	0.45
24	14.62	Tetradecane	0.49
25	14.80	β-Ethylnaphthalene	0.11
26	15.41	trans-Caryophyllene	0.78
27	15.81	β-Farnesene	8.95
28	15.97	Aromadendrene	0.72
29	16.26	α -Bergamotene	1.27
30	16.50	α -Amorphene	0.37
31	17.10	α-Curcumen	0.20
32	17.34	Epi-ligulyl oxide	1.05
33	17.62	δ.-Selinene	0.28
34	17.81	β.-Bisabolene	12.56
35	18.30	δ.-Cadinene	0.34
36	18.53	Spathulenol	9.23
37	18.91	o-Cymene	2.39
38	19.61	Longifolenaldehyde	0.23
39	20.16	Caryophyllene oxide	4.46
40	20.47	N-Acetyltyramine	0.98
41	20.89	Santalol	1.12
42	21.03	Bisabolene epoxide	0.75
43	21.67	Valerenol	0.24
44	22.00	Ledene	0.26
45	22.28	Aromadendrene oxide	1.13
46	22.56	γ.-Gurjunene	0.43
47	22.81	β-Selinene	0.73
48	23.29	α-Farnesene	1.81
49	23.71	(+)-.β.-Atlantone	0.78
50	23.88	α-Bisabolol oxide B	2.76
51	24.59	α-Bisabolene oxide A	0.85
52	25.13	Trans-Farnesol	3.75
53	25.31	Bergamotol	0.38
54	25.65	Tetradecanoic acid	1.44
55	26.13	α-Bisabolol	4.72
56	26.45	Sinularene	0.33
57	27.62	α -Bisabolol oxide A,	19.07
58	28.28	Isoaromadendrene epoxide	1.12
59	28.95	Nonadecane	0.44
60	30.86	Oplopenone	0.16
61	31.66	Eicosane	0.15
62	33.83	Phytol	0.11
63	34.06	Heptacosane	1.77

RI: Retention time

Fig. 2 illustrates the recorded chromatogram of the *Anthemis susiana* Nábělek essential oil composition. This plant has 63 peaks that belong to 63 different chemical compounds of the plant (Fig. 1).

Results

In this study, HS-SPME method was used to extract *A. susiana* essential oil and the essential oil analyzed by GC-MS method. According to the results of the GC-MS method shown in Table 1, essential oils obtained from *A. susiana* essence contain a total of 63 chemical compounds. The results of phytochemical analysis of essential oil of this plant showed that its major chemical constituents included alpha-bisabolol oxide A (19.07%), alpha-pinene (15.50%), beta-bisabolene (12.56%), spathulenol (9.23%), beta-farnesene (8.95%). %), Alpha-Bisabolol (4.72%), caryophyllene oxide (4.46%), trans-farnesole (3.75%) and dl-Limonene (3.47%). Other compounds of the essential oils of *A. susiana* essential oil are listed in Table 1.

Discussion

So far no study has been done on the chemical composition of *Anthemis Susiana* Nábělek. This study reports for the first time the chemical constituents of *A. susiana* essential oil. According to the results, the major compound of essential oil of this plant is alpha-bisabolol oxide A (19.7%).The study of Jaymand (2001) showed that the main compounds of essential oil of chamomile (*Mathricaria chamomilla* L.) included sesquiterpene compounds such as (-)-α-biabolol (56.86%) trans-trans-farnesol (15.64%), cis-β-farnesene (7.12%), guaiazulene (4.24%), and chamazulene (2.18%), accounting for 86.04% of the total essential oil [18]. The study of Ayogi *et al*. (2010) showed that GC-MS analysis of *Matricaria chamomilla* L. showed the presence of compounds such as cis-anethol (51.72%), cis-beta-ocimene (8.32%), methyl-eugenol (8.06%), limonene (4.94%) and linalool (4.41%) in Tarragon, essential oil, and E-beta-farnesene (24.19%), guaiazulene (10.57%), alpha-bisabolol oxide A (10.21%), alpha-farnesene (8.7%) and alpha-bisabolol (7.27%) in the essential oil of *Matricaria chamomilla* L. [19]. The results of one study revealed the main constituents of *Anthemis coelopoda* Boiss.

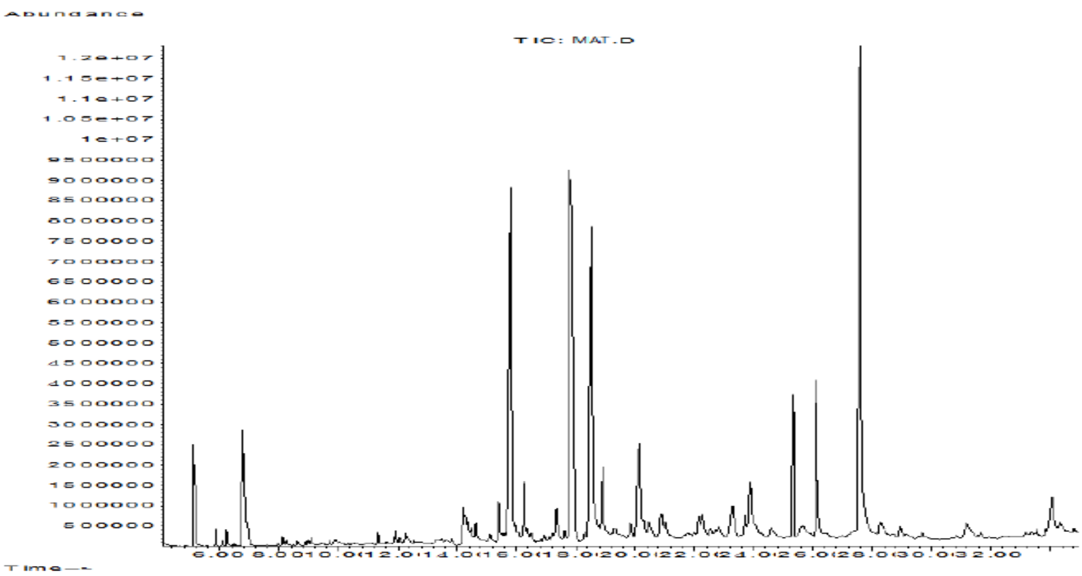

Fig. 2 Chromatogram of the essential oil of *Anthemis Susiana* Nabelek

The flower oil contained cis-chrysanthenyl acetate (27.3%), hexylbutanate (16%) and myrcene (7%), while its leaf oil contained isobornyl formate (30.6%), trans-ethyl chrysanthemate (15%) and p-mentha-1.5-diene-8-ol (13.4%) [20].The main use of chamomile is related to the pharmaceutical and cosmetic industries [21, 22]. Due to containing certain active ingredients such as kamazolene, alpha-bisabolol, and alpha-bisabolol-A [22-24], chamomile essential oil has many therapeutic properties including anti-microbial, anti-inflammatory, anti-spasm and antiseptic properties and is used for the treatment of gastrointestinal diseases [6]. The plant is effective in relaxing the nervous system and reducing seizures and is also used for preparing compresses and naturally making hair shiny [25]. One study showed that 1-alpha bisabolol oxide amounts in different parts of Iran including Kerman, Isfahan, Shiraz, Tehran, Baba Meydan, Nourabad (Fars province), Gachsaran, Larestan and Behbahan are 22, 9-7, 11, 10-9, 65-58, 58-55, 55-42, 40-42, and 28-29%, respectively. Besides that, the amounts of 3-alpha bisabolol oxide A in Kerman, Isfahan, Shiraz, Tehran, Baba Meydan, Nourabad (Fars province), Gachsaran, Larestan and Behbahan have been reported to be 47-43, 53-54, 33, 55-54, 9-8, 16-15, 32-30, 28-14, and 12%, respectively [26]. The results of studies in Egypt, Estonia and Slovakia showed that the concentrations of alpha bisabolol oxide in chamomile (*Matricaria recutita*) were 9.28% and 15.80% and those of bisabolol oxide A in Egypt, Slovenia and Slovakia were 49.51%, 37% and 39.90%, respectively [27, 28]. In Würzburg, Germany and Riva del Garda, Italy, 1,8-cineole was found to be the main compound and alpha-pinene and camphor were reported as being the second and third main compounds in Germany and Italy; chrysanthenyl acetate and camphor were not found in the essential oil [29, 30]. Different species of chamomile have different active ingredients. Substances such as 3-alpha-bisabolol oxide A, cis-anethol, kamazolene, alpha-bisabolol and alpha-bisabolol-A are the main constituents of other chamomile species, while in our study it was determined that alpha-bisabolol oxide A is the main compound of Susiana chamomile. It seems that the marked fluctuations in the type and amount of compounds of essential oils of these plants are due to ecological differences such as latitude, longitude, elevation, temperature, humidity, climate and soil, and different climatic and edaphic conditions affect metabolic pathways and the biosynthesis of active ingredients in these plants, and therefore a variety of secondary metabolites are biosynthesized under different environmental conditions.

In general, it can be said that the essential oil of this plant is influenced by various environmental and intrinsic factors both in quantity and in its constituents the main compound being alpha-bisabolol oxide A and the highest concentration of this compound among them. These compounds can be used as antioxidant compounds after further testing.

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Authors' contributions

MB reviewed the literature and prepared the first draft of manuscript; NA, MB, RA and IS reviewed the literature, helped in preparing first draft of manuscript, checked and corrected the grammar. All authors read and approved the final report.

Conflict of Interests

All authors declare that no conflict of interest exist.

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