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RESEARCH COMMUNICATION

Phytoconstituents, extraction and analysis of chemical compounds of *Crataegus pontica* K.Koch fruit using HS-SPME and GC-MS methods

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ABSTRACT

Essential oils were extracted by HS-SPME method from the fruit of the *Crataegus pontica* K.Koch collected from the southern regions of Ilam province. Then, to identify chemical compounds, the essential oil was injected into a chromatograph gas device connected to a mass spectrometer (GC-MC). Of the 50 compounds identified in this essential oil, beta-Thujene (17.21%), alpha-pinene (15.40%), 2-Hexenal (12.42%), trans-Caryophyllene (8.76%), beta-Myrcene (7.89%), 1-Pentadecene (5.89%), Sabinene (4.33%) and trans-beta-Farnesene accounted for %3.50 of the major fruit essential oil compounds of *C. pontica*.

Introduction

Hawthorn (*Crataegus pontica* K. Koch), belonging to the Rosaceae family, is one of the edible fruits. It is a 6–10 meters tall tree with young, hairy, thorny branches, leaves 3 to 7 cm long and 2.5 to 6.5 cm wide and bayonet, with short, fluffy petioles (1). This plant is distributed in Lorestan, Ilam, Chalous, Chaharmahal and Bakhtiari, Kermanshah, Islamabad Gharb, Sarpol-e Zahab, Ardabil, Qom, Hamedan, Khuzestan and Urmia in Iran. The ripe, yellow fruits of the common hawthorn tree are eaten raw in the fall semester and have healing effects (1). The use of hawthorn in the treatment of diseases and medical disorders dates back to ancient times (1). It has been used as one of the useful medicinal plants for the treatment of cardiovascular diseases (2–4). In traditional Iranian medicine, hawthorn is used to treat neurological disorders such as anxiety, insomnia, dizziness, tingling in the ears (tinnitus) and heart failure, angina and aortic oedema, anti-anxiety, improved digestion, anti-hypertension, skin beauty, analgesic, treatment of constipation and diarrhoea (5). Phytochemical studies show that flavonoids and anthocyanins are the most important chemical compounds in hawthorn fruit (6, 7). Quercetin, isocoercistin, vitoxin, hyperoside and chlorogenic acid are other compounds of hawthorn (8, 9). In this phytochemical study, it was tried to extract essential oils and volatile oils from the dried powder of hawthorn fruit by headspace-solid phase microextraction (HS-SPME), and then its chemical compounds were analyzed using GC-MS technique.

Materials and Methods

Method of preparing the plant material

In September of 2019, the hawthorn fruit was collected from the mountainous areas of Maymeh section of Dehloran city in the south of Ilam province (western Iran) [32° 41' 28" North, 47° 15' 58" East]. The plant was identified and confirmed using the flora of Ilam province (Fig. 1). The species was determined at the Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. The collected fruit was cleaned and dried in the laboratory at room temperature. The dried fruit was powdered by a mixer. The plant essence was extracted by HS-SPME method and the chemical composition of the fruit was identified by GC-MS method. The details of hawthorn are specified in Table 1.

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Fig. 1. *Crataegus pontica* K. Koch fruit

Extraction of essential compounds by HS-SPME method

In this experiment, the essential oil of hawthorn fruit was extracted by HS-SPME technique. In the HS-SPME technique, 2 gms of dry powder is placed in the vial, and the vial temperature rises between 60–70 °C. These temperature conditions are optimal so that the vapours of the substances in the essential oil are saturated in the space above the solid surface. The SPME syringe is then placed in the upper part of the container with the lid closed, and the material in the plant vapour is absorbed by the silica phase in the needle of the device (10, 11).

Table 1. Details of *C. pontica*

Scientific name	Common name	Persian name	Plant family	Collection area	Geographical coordinates
Crataegus pontica K.Koch	Hawthorn	Zalzalak	*Rosaceae*	Dehloran, Ilam province	32° 41' 28" North, 47° 15' 58" East

Identification of essential plant compounds by GC-MS method

After extracting the essential oil, silica fibre from the volatile compounds, the fibre is placed directly in the inlet of the GC-MS device, and due to the temperature of the input part, the materials in the fibre are absorbed and enter the GC-MS device (10). About 2 (g) of each plant powder was used to identify essential substances. The condition of the device was as follows: The gas chromatograph (Agilent6890N) was accompanied by the mass detector Agilent 5973. Column: HP – 5 (30 m long 0.25 mm (ID) 5 0.25 µm) fixed phase thickness); Type of injection: Split / gap and column temperature programme: 50 °C, holding time 0.00 min and rate -oC/min; temperature 200 °C, holding time 0.00 minutes and rate 5 °C/min and temperature 240 °C, holding time 0.00 minutes and rate 10 °C/min Carrier gas: helium (99.999%); Type injection: no gap; Library: Willy 7n; injector temperature: 250 °C and flow rate: 0.9 ml per minute. Extraction mode: (HSSPME); SMPE fibers: PDMS 100 micrometer thickness (SUPELCO); Sample weight: 0.5 g; Extraction temperature: 60 °C; Extraction time: 20 minutes; Ultrasound time: 10 minutes (Eurovond ultrasound) Tools, Italy and time discharge in the port GC-MS injector: 3 minutes (11).

Results

Crataegus pontica fruit extract was extracted by HS-SPME method, and GC-MS method was analyzed. The results of the phytochemical analysis of hawthorn fruit essential oil are given in Table 2. Essential oil of *Crataegus pontica* fruit contain 50 chemical compounds. Accordingly, the major fruit essential oil compounds of this plant are α-bisabolol oxide A (19.07%), α-Pinene (15.50%), β-*Bisabolene* (12.56%), spathulenol (9.23%), β-Farnesene (8.95%), α-Bisabolol (4.72%), caryophyllene oxide (4.46%), trans-Farnesole (3.75%) and dl-limonene (3.47%). Other ingredients of hawthorn essential oil are listed in Table 2.

Discussion

Production, exploitation and processing of herbal medicinal products are increasing due to the long history in traditional medicine and herbal medicine, as well as to avoid or reduce the adverse effects of the increasing use of chemical drugs. In particular, identifying the compounds of medicinal plants to produce natural medicines is more necessary. Recognition of medicinal plants and their medicinal or hygienic and industrial use in their compounds have long been considered by many researchers. So far, no study has been done on the chemical composition of *Crataegus pontica* fruit by HS-SPME and GC-MS method, which can be a new scientific document for reporting chemical compounds. Therefore, in this study, hawthorn fruit extract was extracted by HS-SPME method and by GC-MS method was identified. According to the results, hawthorn fruit has 50 chemical compounds in this essential oil. Essential oils are a complex compound of fat and volatile compounds that are prone to oxidation and degradation. There is only one study on the physicochemical properties of *C. pontica* fruit and that is to measure the phenolic and flavonoid content of *C. pontica* (12) while we have identified and reported every chemical composition of hawthorn fruit essential oil. According to one study, the total phenols, total flavonoid content, and antioxidant activity of *C. pontica* were in the range of 21.19–69.12 mg gallic acid equivalent (GAE)/g dry weight (dw), 2.44–6.08 mg quercetin equivalent (QUE)/g dw and 0.32–1.84 mmol Fe++/g dw, respectively (12). Hyperoside (0.87–2.94 mg/g dw), chlorogenic acid (0.06–1.16 mg/g dw), and

Table 2. Chemical composition of hawthorn fruit

No.	Retention time	Compound	Percentage of compound(s) (%)
1	4.64	2-Hexenal	12.42
2	6.22	ALPHA-PINENE	15.40
3	7.24	Sabinene	4.33
4	7.61	beta.-Myrcene	7.89
5	7.96	l-Phellandrene	0.73
6	8.33	ALPHA. TERPINENE	0.10
7	8.67	beta-Thujene	17.21
8	9.10	3-Carene	0.28
9	9.42	gamma-Terpinene	0.17
10	9.76	cis-sabinene hydrate	0.24
11	10.21	ALPHA.-TERPINOLENE	0.08
12	10.64	Linalool	0.13
13	11.36	Alloocimene	0.51
14	12.56	2-cyclononine-1-ol	0.11
15	13.27	alpha.-Terpineol	0.25
16	14.04	1-Hexadecene	0.32
17	15.17	1-Pentadecene	5.89
18	16.34	Thymol	0.07
19	16.44	3,4-Diethylphenol	0.04
20	16.79	Camphene	0.19
21	17.06	Bicycloelemene	1.34
22	18.12	Copaene	2.34
23	18.36	Beta- Burbonene	0.23
24	18.46	beta-Cubebene	0.19
25	18.54	Beta-elemene	0.34
26	18.98	alpha-Gurjunene	0.30
27	19.12	Isoedene	0.14
28	19.34	trans-Caryophyllene	8.76
29	19.51	Gamma-Cadinene	0.60
30	19.60	alpha.-Bergamotene	1.03
31	19.79	Zingiberene	0.17
32	20.15	trans-beta-Farnesene	3.50
33	20.38	Calarene	0.30
34	20.58	Epizonzrene	0.17
35	20.71	Beta-Himachalene	0.30
36	20.86	GERMACRENE-D	3.44
37	21.10	Gamma-Cadinene	0.44
38	21.21	bicyclogermacrene	1.88
39	21.43	beta.-Bisabolene	0.58
40	21.63	Alpha Amorphene	0.65
41	21.84	delta-Cadinene	2.67
42	22.22	CIS-ALPHA-BISABOLENE	0.60
43	22.59	sesquisabinene hydrate	0.12
44	23.28	Spathulenol	0.22
45	23.33	Caryophyllene oxide	0.23
46	23.45	Hexadecane	0.10
47	23.58	gamma-Gurjunene	0.09
48	25.09	Aromadendrene	0.11
49	25.41	Valeranone	0.19
50	30.51	Bis(2-ethylhexyl) phthalate	2.61

The recorded chromatogram of the essential oils of the hawthorn fruit oil is shown in Fig. 2. This extract has 50 peaks, corresponding to 50 chemical compounds specified in Table 1. Table 2 also shows the molecular formula and major chemical structure of the main constituents of hawthorn fruit essential oil.

isoquercetin (0.24–1.59 mg/g dw) were found to be the most abundant phenolic compounds in the extracts of hawthorn fruits (12).

Essential oils are aromatic oil liquids derived from plant materials such as flowers, seeds, leaves, tree bark, wood, fruit, roots and buds (13). The results of the phytochemical analysis of *Crataegus oxyacantha* essential oil showed that the plant contains twenty-five compounds, Longifolenaldehyde, β-Selinene and the main eugenol composition is slightly combined (14).

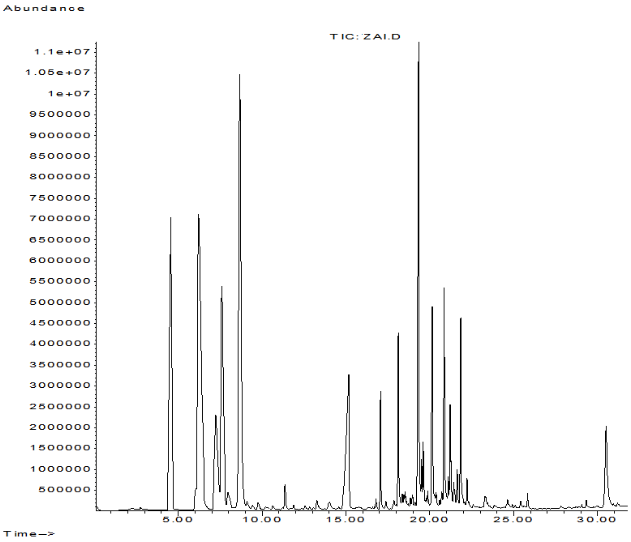

Fig. 2. Chromatogram related to the essential oil of *Crataegus pontica* fruit.

The molecular formula and major chemical structure of the main compounds of *Crataegus pontica* fruit essential oil are shown in Fig. 3-9.

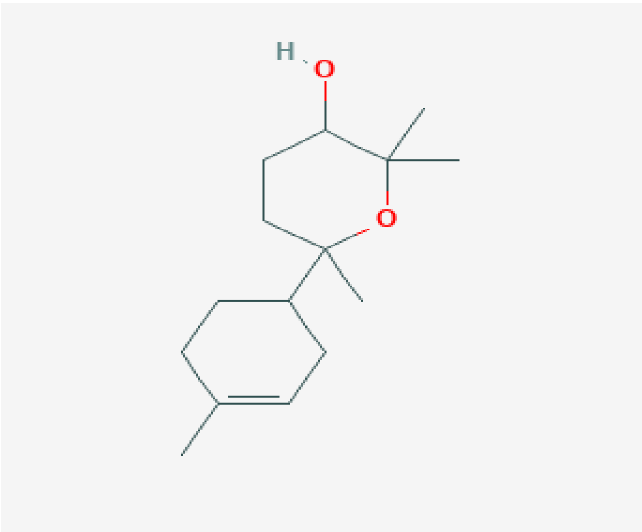

Fig. 3. alpha-Bisabolol oxide A

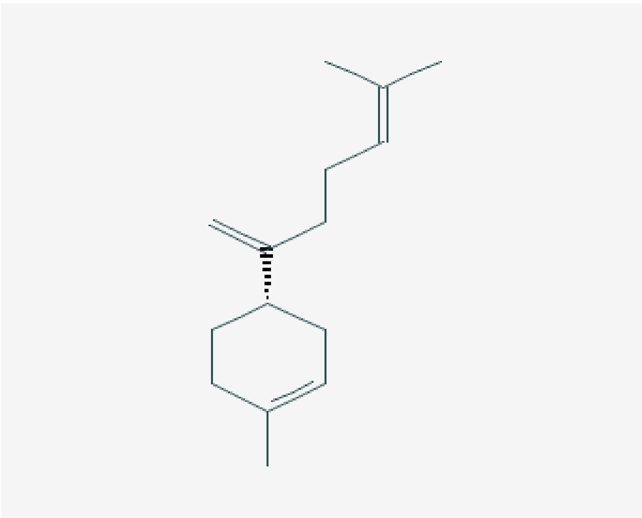

Fig. 4. Beta-Bisabolene

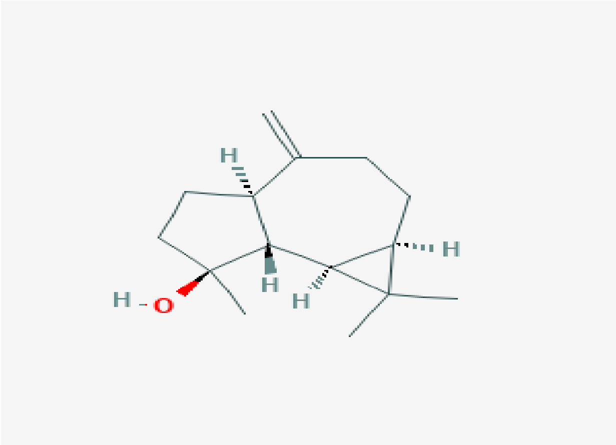

Fig. 5. Spathulenol

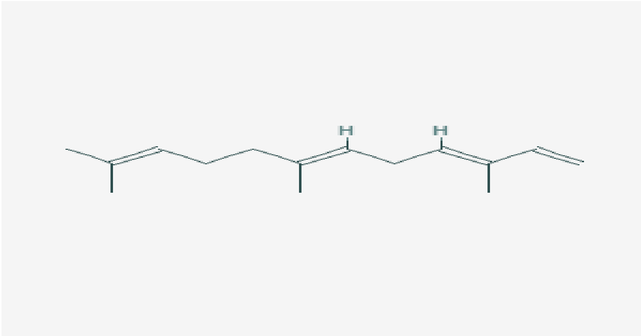

Fig. 6. Farnesene

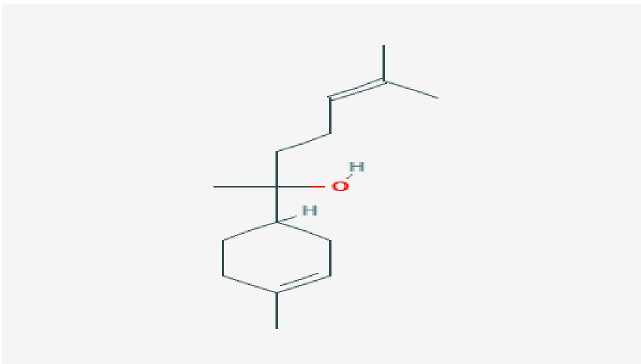

Fig. 7. alpha-Bisabolol

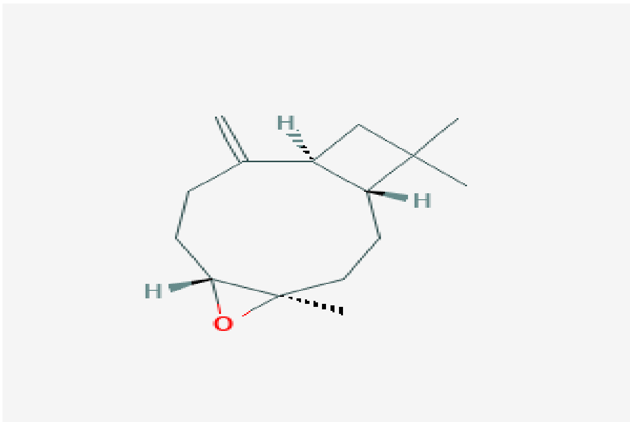

Fig. 8. Caryophyllene oxide

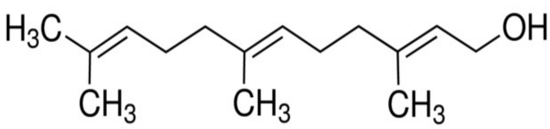

Fig. 9. trans-Farnesole

The results of a study showed that *C. pinnatifida* extract had compounds of 4 monoterpene glycosides, pinnatifidanosides A-D; phenolic glycoside, pinnatifidanoside E; byzantionoside B, (3S, 5R, 6R, 7E, 9R) -3.6-epoxy-7- megastigmen-5, 9-diol-9-O-β-D-glcp, (65,7Z, 9R) - roseoside , icariside B6, linalool oxide β-D-glc, shanyenoside A, dihydrocharcone-2'-β-D-glc, eriodectyol, vitexin, 2 " -O-rhamnosyl vitexin (15). The results of a study shown that *C. pinnatifida* extract had Norhawthornoids A, B compounds; sesquiterpenoids shnyegenin B, shnyeside B, (3S, 5R, 6R, 7E, 9S) - megastiman-7-ene-3,5,6,9-tetrol, euodionosides D, (6R, 9R) -3-oxo-α- ionol-9-O-β-D-glucopyranoside, (6S, 7E, 9R) -6,9-dihydroxy-4,7-megastiymadien-3-one-9-O- [β-D-xylopyranosy-β-D - glucopyranoside], linarionosides A, B, C; 3,9-dihydroxy-5-megastigmen-3-O- [β-D-xylopyranosy-β-Dglucopyranoside], pinnatifidanosides C, F, G. (16). The results of a study showed that *C. azarolus* var. *aronia* contains azarolic acid compounds, 4 known phenolic compounds; 4 is known as triterpene acids (17).

The results of a study showed that *C. monogyna* extract had 3 hydroxycinnamic and 1 hydroxybenzoic acid compounds, 6 glucosylated flavonols and 2 flavones, 2 cyanidin glycosides; (picatechin, a dimer B2, two trimers, C1 and C2) (18). The results of a study revealed that *C. pycnoloba* extract had a composition of 4 dibenzofurans discovered compound 6-hydroxy-2,3,4-trimethoxydibenzofuran; ursolic aldehyde (19). As can be seen from different studies, different species of hawthorn have different compounds. In this study, *C. pontica* essential oil was studied for the first time, and the most important composition of *C. pontica* fruit essential oil included beta-Thujene. Beta-Thujene is a natural organic compound of the monoterpene class that is found in the essential oils of a variety of plants and helps to flavour some plants. Thujene has antibacterial and antifungal properties and can be used for antimicrobial purposes. Studies have shown that medicinal plants and natural products have beneficial effects on human health due to the active ingredients and their medicinal and antioxidant compounds and have a therapeutic effect on various organs and various disorders and diseases of the body (20-29).

Conclusion

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 beta-Thujene, alpha-pinene, 2-Hexenal and trans-Caryophyllene and the highest concentration of this compound among them. These compounds can be used as antioxidant compounds after further testing, so identifying the active ingredients of each herb is a useful way to produce effective drugs. In the future.

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Authors' contributions

NB, AGH and MT reviewed the literature and prepared the first draft of manuscript; PSH helped in preparing first draft of manuscript, verified and corrected. All authors read and approved the final report.

Conflict of interests

All authors declare that no conflict of interest exists.

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