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Original Article

Extraction and Identification of phytochemicals in Iranian oak (*Quercus brantii* var. *Persica*) Collected in Arghavan Valley, Ilam County by HS-SPME and GC-MS

Mohammad Karimian[1], Reza Najafi[2], Kamkar Jaimand[3], Firoozeh Hatami[3], Naser Abbasi[4]* and Asadollah Jalali Ghalousangh[5]

[1]*Department of Surgery, School of Medicine, Emam Khomeini Hospital, Ilam University of Medical Sciences, Ilam, Iran*
[2]*Department of Pediatrics, School of Medicine, Ilam University of Medical sciences, Ilam, Iran*
[3]*Department of Medicinal Plants & By-products, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, Tehran, Iran*
[4]*Biotechnology and Medical Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran*
[5]*Department of Biology and Vector Control, School of Health, Ilam University of Medical Sciences, Ilam, Iran*

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Abstract

In Iranian traditional medicine *Quercus brantii* var. *Persica* (Jaub. & Spach) Zohary (Oak) is considered warm- and dry-natured. It is used for gastric pain, ulcer, anemia, hemorrhoids and rickets, burns, indigestion, diarrhea, and infection. Oak is readily available and has a variety of medicinal and health effects in both traditional and modern remedies. The main objective of this research is Identification in oak compounds, using Headspace-solid phase microextraction (HS-SPME) and Gas chromatography–mass spectrometry (GC-MS). Oak fruits were collected from Argavan Valley, Ilam, west of Iran, after drying and powdering, chemical compounds were isolated by HS-SPME and identified by using GC-MS method. The results analysis of *Quercus brantii* var. *Persica* revealed the existence of 41 chemical compounds. Major chemicals included β- pinene oxide (8.65%), Tetrahydro- linalyl acetate (8.51%), 2-methoxy –p-cresol (7.65%), 2-methoxy pyrazine (5.08%), 2-acetyl pyridine, 2,3-dimethyl pyrazine (4.42%), Trans- linalool oxide (3.79%), β- pinene(3.66%), Verbenone (3.43%), and Terpin-4-ol (3.27%).

Keywords: *Quercus brantii* var. *Persica*, Ilam, Chemical Compounds, GC-MS

Introduction

Taxonomically, the oaks are in the genus *Quercus* in the family Fagaceae (beech family). The Fagaceae with more than 900 species of trees and shrubs, evergreen and deciduous is one of the most diverse groups, especially in the northern hemisphere. It probably originated in the montane tropics from which its members migrated and diverged into the current area by the late Cretaceous period (about 60 million years ago) [1]. There are about 500 species with more than 45 species of oaks in the world [2,3]. The dominant genus in the Zagros area is oak (*Quercus* spp.). Consequently, these forests are designated as western oak forests (English: "Oak", Persian: "Baloot") of Iran [4-6].

Quercus is one of the native plants of Iran that is widely grown in the forests of Ilam, Fars, Kurdistan, Lorestan and Kohgiluyeh and Boyer-Ahmad. *Quercus brantii* var. *Persica* is the most prevalent among all species [7,8]. Iranian oaks are large trees of 20 meters high with large spherical crown. The leaves of the oak tree are generally uniform and egg-shaped with a serrated margin, with stellate-shaped, dense florets on the leaves and yellow fur on the back. The oak fruit, called acorn,

*Corresponding author: Department of Pharmacology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran
Email Address: ilamfarma@gmail.com

is housed in a cup called gland. This fruit is elongated, quasi-oval and mucronate and is covered in a white velvety and conical shape [9]. They contain a wide range of oils, sugars, amidone, small amounts of quercetin, tannins and pentosan [9]. In all parts of acorn numerous healing properties have been reported, including fruit, leaves, trunk bark, young stem bark, and flowers. The most commonly part of acorn is its fruit [10]. According to Iranian traditional medicine, oak is warm- and dry-natured which is used as food and bread. Brewed oak is used for gastric pain, gastric ulcer, anemia, hemorrhoids, and rickets. Basically oak is effective in the strengthening of body, hemorrhoids, weight loss and diarrhea and is also used as an anti-worm, antidiabetic, analgesic and sedative agent [11]. In the 21st century, which is called Return to Nature and Use of Plants in Treatment, there was a growth expansion of research on medicinal plants, production and supply of new herbal remedies. It is essential to conduct various studies on oak such as physiological, ecological, systematic, phytochemical, etc. Phytochemical studies play a major role to identify constituents for the production of pharmaceutical, hygienic, and perfume products. In addition to the nutritional constituents, oak involves biologically active compounds, including tannins, gallic acid, ellagic acids, and galloil or hexahydroxydiphenoil derivatives, all of which have antioxidant properties [12]. Recent studies have also shown certain effects such as antioxidant [13], microbial [14], burn- and wound-healing [15], antiviral [16], and antifungal [17]; it is used to treat gastric ulcer [18], indigestion or dyspepsia [18,19], candidate vasinosis [20] too. Due to phenolic compounds and tannin, oak can inhibit lipid peroxidation and antioxidant activity [21]. Moreover oak plays a role in regulating blood pressure because of high level of sodium and magnesium [22]. It is also readily available and has a variety of medicinal and health effects in traditional and modern medicine, but also identification compounds can be very important from pharmacological perspective in the treatment of various diseases. To our knowledge, identification of the chemical compounds in *Quercus brantii* var. *Persica* by HS-SPME and GC-MS method is reported for the first time.

Material and Methods

Plant Preparation

In September-October 2019, *Quercus brantii* var. *persica* fruit was collected from Arghavan Valley, Ilam, Ilam province (western Iran). *Quercus brantii* var. *persica* fruit was identified and confirmed using morphological keys of Ilam Province Plant Flora Book at the Biotechnology and Medicinal Research Center of Ilam University of Medical Sciences.

First, *Quercus brantii* var. *persica* fruit was first dried and then powdered with a plant fruit mixer and extraction and analyzed for chemical composition by HS-SPME and GC-MS. The characteristics of *Quercus brantii* var. *persica* fruit used in this study are shown in Table 1.

Methods for Extraction

In this experiment, the essential oil of the Iranian oak fruit extracted by Headspace-solid phase microextraction (HS-SPME) technique, which about 2 grams of dried herb powder packed in the vial and placed in 60-70 °C.

Methods for Identification of Chemical Compounds by HS-SPME and GC-MS

These maximum temperature conditions saturated the vapor content of the substances in Iranian oak fruit essential oil in the upper space of the solid surface. Afterward, the HS-SPME syringe put in the upper part of the container with a lid and the plant substance in the vapor absorbed by the silica phase of the needle. After adequate time and saturation of the silica fiber, the volatile components of the fiber directly placed into the GC-MS device and absorbed by the temperature of the input. Consequently, substances of the fiber reabsorbed and penetrated the GC-MS and recognized [23]. Two grams of each plant extraction used for analysis.

The conditions of instrument were as follows: Gas chromatograph (Agilent6890N) was coupled with Agilent 5973 mass detector; Column: HP - 5 (length 30 m , 0.25 mm (ID) 5 0.25 µm (stationary phase thickness) ؛ Type of injection: Split ;Column temperature application: 50 °C, Holding time 0.00 min and rate 0 °C/min; Temperature 200 °C, Holding time, 0.00 min and rate 5 °C/min and 240 °C; Holding time 0.00 min and 10 oC/min carrier gas: He (99.999%); Injection Type: No Gaps; Library: Wiley 7n; Injection temperature: 250 °C and Flow rate: 0.9 ml/min.

Table 1 Details of the medicinal plant *Quercus brantii* var. *persica* (Jaub. & Spach) Zohary

Region	Herbal family	Scientific name	Persian name	Common name
Ilam city	Fagaceae	*Quercus brantii* var. *persica*	Balout	Oak

Table 2 Identified compounds of *Quercus brantii* var. *Persica* (Jaub. & Spach) Zohary essential oil using HS-SPME (GC-MS) method

No	Compounds name	Retention time	Retention Index	%
1	3Z-hexenal	4.14	855	2.45
2	2-methoxy pyrazine	4.81	897	5.08
3	2,3-dimethyl pyrazine	5.38	920	4.42
4	Tetrahydro citronellene	5.80	937	0.58
5	Cyclohexyl formate	6.50	962	2.48
6	β- pinene	7.07	979	3.66
7	Dehydroxy trans- linalool oxide	7.52	993	2.06
8	3Z-hexenol acetate	7.93	1005	1.11
9	1,4-cineole	8.20	1015	1.80
10	2-acetyl pyrazine	8.40	1021	3.35
11	2-acetyl pyridine	8.85	1034	5.01
12	Lavender lactone	9.03	1040	0.95
13	Isopentyl butanoate	9.69	1058	1.23
14	*Trans*- linalool oxide	10.29	1073	3.79
15	Terpinolene	10.99	1089	0.90
16	Linalool	11.35	1097	0.47
17	1,3,8-p-menthatriene	11.86	1110	1.34
18	2E-heptenyl acetate	11.99	1114	1.25
19	Dehydro Sabina ketone	12.19	1121	2.36
20	Chrysanthenone	12.53	1128	2.64
21	Isopulegol	13.23	1145	2.28
22	Neo- isopulegol	13.39	1148	1.02
23	2E, 6Z-nonadienal	13.67	1155	1.30
24	β- pinene oxide	13.85	1159	8.65
25	1,4-dimethoxy benzene	14.12	1166	1.26
26	Terpin-4-ol	14.71	1177	3.27
27	Lavandulol	14.81	1181	1.96
28	2-methoxy –p-cresol	15.29	1190	7.65
29	Dihydrocitronellol	15.53	1196	1.66
30	Verbenone	15.93	1205	3.43
31	Linalyl formate	16.42	1216	0.60
32	Exo-fenchyl acetate	17.07	1233	1.47
33	Tetrahydro- linalyl acetate	17.16	1234	8.51
34	Pulegone	19.27	1237	0.49
35	Ethyl 2- octynoate	19.40	1284	3.24
36	Isobutyl benzoate	21.39	1329	0.63
37	α-cubebene	22.30	1351	1.31
38	Thymol acetate	22.39	1352	1.27
39	*Cis*-mentholactone	22.97	1367	1.22
40	δ-nonalactone	23.99	1387	0.90
41	Ethyl anthranilate	25.21	1416	0.95

Extraction mode: (HS-SPME); SMPE fibers: 100 µm PDMS thickness (SUPELCO); Sample weight: 0.5g; Extraction temperature: 60oC; Extraction time: 20 min; Ultrasonic time: 10 min (Euronda ultrasound, Italy) and repulse time in Port GC-MS Injector: 3 minutes [24]. The identity of the oil components was established from their GC retention indices, relative to C7- C25 n-alkanes standards mixture, and by comparison of their mass spectra and retention indices with those reported in

the literature [25-27], and by computer matching with the Wiley 5 and NIST mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results

The results of extraction of oak chemical compounds by HS-SPME method and analysis and identification of their compounds by GC-MS are described in Table 1. According to the results of GC-MS method, essential oils of essential oil of oak contain 41 chemical compounds. The results of phytochemical analysis of the essential oil of this plant showed that geranyl acetone (8.65%), heneicosane (8.51%), phenol, 2,6-bis (1,1-dimethylethyl) -4-methyl- (BHT) (7.65%), beta-Myrcene (5.08%), 1-octene (5.01%), dl-limonene (4.42%), tridecane (3.79%), camphane farnesole (3.66%), dihydroactidiniolide (3.43%), and beta-ionone (3.27%) were the major chemical constituents of the plant. Other ingredients of the *Quercus brantii* var. *persica* essential oil are listed in Table 1. Geranyl acetone is a monoterpene ketone. Heneicosane is a direct-chain saturated hydrocarbon of formula C21H44. Butylated hydroxytoluene (BHT) is an organic chemical composed of 4-methylphenol modified with tert-butyl groups at positions 2 and 6. BHT is used in foods, cosmetics and industrial liquids to prevent oxidation and free radical formation. Myrcene or β-myrcene is a natural alkene hydrocarbon that can be *more accurately* classified as a monoterpene. Limonene, (+/-) - is a natural cyclic monoterpene and the main constituent of citrus peel oil and has chemical and anti-tumor activity. Limonene is a colorless hydrocarbon liquid of the cycloterpene class. Tridecane is a straight-chain alkane containing 13 carbon atoms. Camphane or bornane is a compound that is closely related to norborane. Farnesol is an isoprenoid alcohol that is produced as a by-product of the ergosterol biosynthesis pathway. Beta-ionone is a colorless, bright yellow liquid. It is extremely dilute in alcoholic solution and is used in perfumery. Other constituents are alkanes, alkenes, carbohydrates, aldehydes, tannins, sesquiterpenes and monoterpene and so on.

Fig. 1 shows the registered chromatogram of compounds of *Quercus brantii* var. *Persica* fruit essential oil. According to the chromatogram, the plant has 41 peaks, belonging to 41 different chemical compounds.

Discussion

Quercus brantii var. *persica* is a native plant in Iran and there are active ingredients in the essential oil of its fruit such as alkanes, alkenes, carbohydrates, aldehydes, tannins, sesquiterpenes and monoterpenes. These compounds have both medicinal and health and nutritional properties.

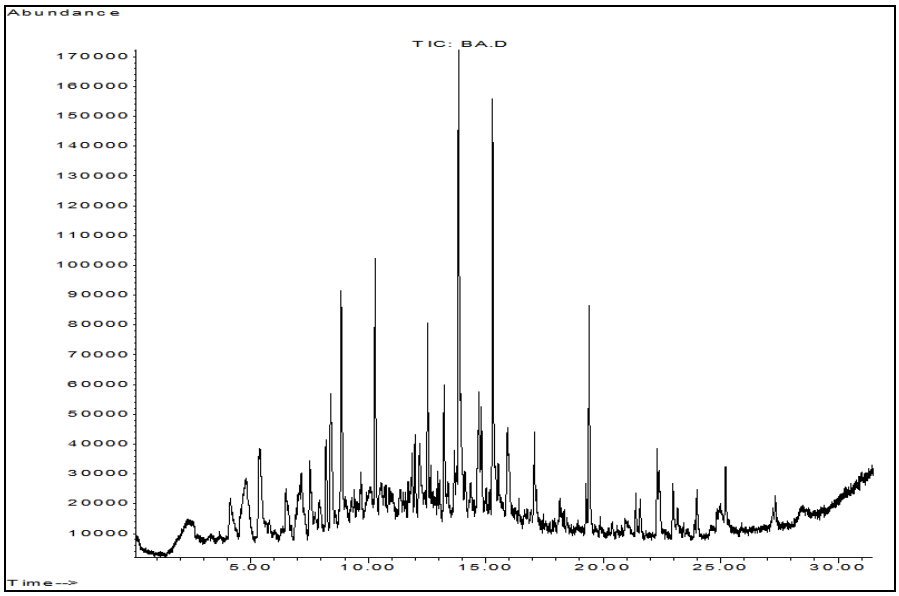

Fig. 1 Chromatogram of *Quercus brantii* var. *Persica* essential oil.

In addition, the lack or low cellular toxicity of *Quercus brantii* var. *persica* extract has been attributed to its flavonoid compound, and particularly tannin [28]. Tannins inhibit their growth by deposition of microbial proteins. Tannins can either make food proteins unavailable to microbes or play the role of enzymes role through the mechanism of iron sequestration, hydrogen bonding and specific dispersal with vital proteins. Tannins can even inhibit their enzyme by inhibiting the reverse transcriptase enzyme in human viruses [29]. Tannins have properties such as the ability to bind albumin, heavy metals and alkaloids. Tannins dissolve in water and become astringent so they can be used to reduce irritation and pain and for inflammation, burns, wounds and swelling [30]. In the oak skin and fruit, there are compounds such as flavonoid, flavobutane, and flobafen. Flavobutans are a mixture of phenols such as alginic acids and pyrogallols. They also contain large amounts of pectin mucilage, quercetin, tanno malic [31]. In addition to nutrients, fruit contains substantial amounts of phenolic compounds and tannin. This study reports for the first time specifically the chemical constituents of *Quercus brantii* var. *persica* essential oil. The main constituents of oak essential oils are alkanes, alkenes, carbohydrates, aldehydes, tannins, sesquiterpenes and monoterpenes, etc. Tannins, oxalates and nitrates are among the preservatives in the plant. This study may be useful to further explore the pharmacological activity of oak due to it can be used for nutritional, health and medicinal purposes.

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