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FULL PAPER

Extraction and identification of the components of *Thymbra spicata* L. and *Satureja khuzestanica* Jamzad Oils native to Ilam province by headspace-solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS)

Maryam Shahmohammadi | Mahmood Bahmani |Hori Ghaneialvar* |Naser Abbasi

Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran

Satureja khuzistanica Jamzad (Lamiaceae) and *Thymbra spicata* L. (Lamiaceae) are medicinal plants belonging to the genera of Satureja and Thymbra, having many uses in traditional Iranian medicine. *S. khuzistanica* is used to treat different diseases such as cramps, nausea, and infectious diseases. Medicinal plants, extracts and essential oils are of special importance for pharmacy, health and food industries. *S. khuzistanica* and *T. spicata* plants were collected from Dehloran located in south of Ilam province in western Iran. Then, they were dried and powdered. Headspace-solid phase microextraction (HS-SPME) and Gas Chromatography-mass Spectrometry (GC-MS) techniques were used to obtain extracts of mentioned plants and identify their active compounds. Essential oil from *T. spicata* contained 45 chemical compounds, whose main ones were isothymol (36.24%), gamma-terpinene (17.94%), *O*-cymene (10.92%), trans-caryophyllene (7.81%), alpha-pinene (7.24%), and camphene (3.61%). Also, essential oil from *S. khuzestanica* contained 43 chemical compounds which main ones were carvacrol (71.99%), camphene (2.84%), alpha-pinene (1.63%), 1,8-Cineole (1.51%). carvone (1.44%) and beta-pinene (1.42). Results from present study indicated that antioxidative and antimicrobial compounds such as carvacrol were found in both plants (*S. khuzestanica* and *T. spicata*) from the Laminaceae family. Therefore, essential oils of these plants can find applications as antioxidant, antimicrobial and antiseptic compounds for medicinal and health purposes.

***Corresponding Authors:**
Hori Ghaneialvar
Email: ghaneialvar-h@medilam.ac.ir, ghaneihuri@yahoo.com
Tel.: +989183440794

KEYWORDS

Thymbra spicata L.; *Satureja khuzestanica* Jamzad; Thumol; Carvacrol; essential oils; HS-SPME; GC-MS.

Introduction

Medicinal plants are rich resources used to treat different kinds of diseases [1,2]. They play a significant role in the enhancement of primary public health care worldwide. Different plant parts are being used now as important and cheap medication sources with fewer side effects [3], due to population explosion, an insufficient supply of drugs, side effects of chemical drugs, and drug resistance by infectious pathogens [4]. Natural substances found in plants include primary and secondary metabolites [5,6].

One of the most important strategies for herbal medicine production from medicinal plants is the extraction method [7,8]. Classical methods of extracting effective plant substances, including distillation with water or water vapor and extraction with organic solvent by soaking or using soxhlet extractor, have disadvantages such as loss of volatile compounds, degradation of unsaturated and unstable compounds, low efficiency, long extraction time, and retention of the toxic solvent [8].

Extensive use, high price and sensitivity of essential oils and other natural compounds to extraction conditions have led to the production and expansion of new extraction methods [8,9]. Besides preserving the qualitative properties of natural compounds, these novel methods are faster and cheaper. Before identifying and using essential oils, they must be removed from their base in some way [9]. Several methods have been developed for extracting plant extracts and essential oils, including distillation, pressure/scratching/razor sharpening, High Space Solid Phase Micro-Extraction (HS-SPME), solvent-aided extraction, and extraction by hydrolyzing enzymes or by carbon dioxide [8-11].

S. khuzistanica is widely distributed on mountains in northern, northwestern, northeastern, central, and southwestern regions of Iran, growing also in some other places such as Ilam, Khuzestan, and Lorestan provinces. Belonging to the Laminacease family and genus *Satureja* [12,13], this aromatic plant has a variety of effects, including the treatment of muscle pains, cramps, nausea, and infectious diseases, as well as antimicrobial, antioxidant, hypnotic, and antispasmodic effects. Active ingredients of *Satureja khuzistanica* (the main active ingredients of the essential oil) include such compounds as carvacrol, thymol, p-cymene, beta-caryophyllene, and linalool that have antioxidant properties [14-18].

Being from the genus Thymbra, *T. spicata* L. of the genus *Thymbra* belongs to the family Laminaceae [19]. This aromatic plant is native to Ilam province, being listed as an endangered species on Red Data Book of Iran. In addition to Iran, this plant has a wide range of distribution in the Mediterranean region, including Iraq, Lebanon, Turkey, parts of Eastern Europe, Greece, Italy, Spain and Africa. It is found in semi-arid, hot, and mountainous areas [19-21]. The most important chemical compounds of this plant essential oil include carvacrol, gamma-terpinene, p-cymene, alpha terpinene, thujene, trans-caryophyllene, and beta-myrcene [22]. Based on the results from several studies, essential oil of *S. khuzistanica* have different types and amounts of compounds in essential oil in the full flowering stage in three different habitats located in the western and southwestern provinces of Iran, so does the mean extracted essential oil [23-25].

Considering the effects of essential oil extraction method on the identification of compounds and their percentages to select the best one, the present study used GC-MS method as a completely suitable one to analyze essential oil. We used methods of HS-SPME and GC-MS, which prevents the loss of essential oil volatiles, to identify the constituents of *T. spicata* and *S. khuzistanica*, both of which belong to the Laminaceae family, in the southern region of Ilam (Dehloran). Proper knowledge of the main and effective compounds of these plants can be an effective step in the production of herbal medicines derived from these plants.

Materials and methods

Plant preparation

S. khuzistanica (Herbarium code= 573) (Figure 1) and *T. spicata* (Herbarium code= 347) (Figure 2) plants were collected from Dehloran city located in the south of Ilam province in the west of Iran. These plants were identified and determined using the morphological keys of Ilam Province Plant Flora Book Published by Biotechnology and Medicinal Plants Research Center of Ilam Medical Sciences University. Then, they were dried in fresh open air under shade. Characteristics of two plants are shown in Table 1.

FIGURE 1 Picture of *S. khuzistanica*

FIGURE 2 Picture of *T. spicata*

TABLE 1 Characteristics of *S. khuzistanica* and *T. spicata L.* plants

Scientific name	Plant family	English name	Persian name	Region	Geographic characteristics
***Thymbra spicata* L.**	Laminaceae	Mediterranean thyme	Avishan zoufaei	Dehloran	32° 41' 28" North, 47° 15' 58" East
***Satureja khuzestanica* Jamzad**	Laminaceae	Satureja	Marze khozestani	Dehloran	32° 41' 28" North, 47° 15' 58" East

Essence extraction and compound identification using the HS-SPME and GC-MS methods

Dried plants were powdered using a mixer (350 g Electric Herb Grain Grinder Power Machine Multifunction Mills Hi-Vevor US) and prepared for extraction using HS-SPME method.

Essence extraction by-HS-SPME method

Essential oils of *T. spicata* and *S. khuzistanica* plant were extracted by HS-SPME procedure. Two grams of each dried plant and its powder were placed separately in a standard vial device set at 60 °C. Such optimum thermal conditions could saturate vapor content of substances found in essential oils on the solid surface headspaces (Headspace of the vial is a part of the HS-SPME equipment). The HS-SPME syringe with a lid was then placed on the vial headspace, absorbing essential oils by silica phase in the instrument needle. Once silica fiber was allowed to be saturated sufficiently with volatile components, it was placed directly onto GC-MS input section, in which essential oils were absorbed due to the input temperature; then, essential oils in HS-SPME device were entered by GC-MS apparatus to be identified [9,26].

Identification of chemical compounds by GC-Mass Method

To perform analyses, about 2 gr of each plant was used. The device was under following conditions: Gas chromatograph (Agilent6890N) coupled with Agilent 5973 Mass detector; Column: HP - 5. (30 m long × 0.25 mm (ID)×0.25 μm (stationary phase thickness); Injector type: split/splitless and column temperature program: 50 °C, temperature at 200 °C; hold-time: 0.00 min Hold-time: 0.00 min, with an increase of 5 °C/min; at 240 °C, Hold-time: 0.00 min, with an increase of 10 °C/min. Carrier gas: He (99.999%); Injection type: splitless; Library: Wiley 7n; Injector temperature: 250 °C; flow rate: 0.9 mL/min. Extraction mode: HS-SPME; SMPE fiber: PDMS 100 μm thick (SUPELCO); sample weight: 0.5 g; Extraction temperature: 60oC; Extraction time: 20 min; Sonication time: 10 min (Euronda sonication instrument, Italy) and Desorption time in GC-MS injector port: 3 min [9,26].

Analysis of essential oils

The essential oils were decomposed by the standards of the GC-MS device and the information was taken in Excel and sorted. In the GC-MS device, while the GC device is responsible for the separation of volatile compounds, the mass spectrometer helps to identify each of the isolated components based on the mass property with high accuracy.

Results

Results from analyses carried out on essential oil extracted by using GC-MS method from *T. spicata* showed that essential oil in question contained 45 chemical compounds, whose main ones included Isothymol (36.24%), Gamma-terpinene (17.94%), o-Cymene (10.92%), trans-Caryophyllene (7.81%), Alpha-pinene (7.24%) and Camphene (3.61%) (Table 2).

TABLE 2 Identified compounds of *T. spicata*

Compound	Retention time	Percent
2-hexenal	5.78	0.32
Tricyclene	5.85	0.19
Alpha-thujene	5.95	0.57
Alpha-pinene	6.13	7.24
Gamma-terpinene	6.39	0.02
Camphene	6.49	3.61
Sabinene	7.09	0.13
Beta-pinene	7.17	0.59
1 OCTEN 3 OL	7.33	0.14
beta-myrcene	7.52	1.78
3-Octanol	7.73	0.12
l-Phellandrene	7.89	0.20
Delta.3-carene	8.03	0.73
alpha-terpinene	8.21	1.97
o-cymene	8.49	10.92
dl-Limonene	8.56	2.31
Cis-ocimene	8.76	0.28
Trans-beta-ocimene	9.05	0.28
Gamma-terpinene	9.43	17.94
Cis-sabinenehydrate	9.71	0.53
Alpha-terpinolene	10.17	0.12
Thujene	11.02	0.02
Trimethylcyclopentadiene	11.29	0.03
Camphor	11.83	0.07
Menthone	12.08	0.11
Verbenol	12.40	0.05
Endo-borneol	12.53	0.18
L-menthol	12.72	0.07
Fenchyl acetate	13.87	1.09
Carvacrol-methyl-ether	14.55	0.09
Thymoquinon	14.85	0.78
Bornyl acetate	15.72	0.50
Isothymol	16.66	36.24
Carvacrol	17.99	0.09
Gamma-cadinene	18.18	0.44
Trans-caryophyllene	19.31	7.81
Alpha-bergamotene	19.59	0.24
Aromadendrene	19.76	0.48
Alpha-humulene	20.14	0.34
Germacrene-d	20.82	0.13
Bicyclogermacrene	21.19	0.82
Beta-bisabolene	21.41	0.08
Delta-cadinene	21.80	0.07
Caryophyllene oxide	23.33	0.29
Spinacene	29.18	0.01

Monoterpene=70%
Sesquiterpene=18.23%
Aliphatic compounds=11.46%
Oxygenited terpenes= 0.29

Chromatogram and molecular and chemical structure of the main constituents of *T. spicata* essential oil are shown in Figures 3 and 4.

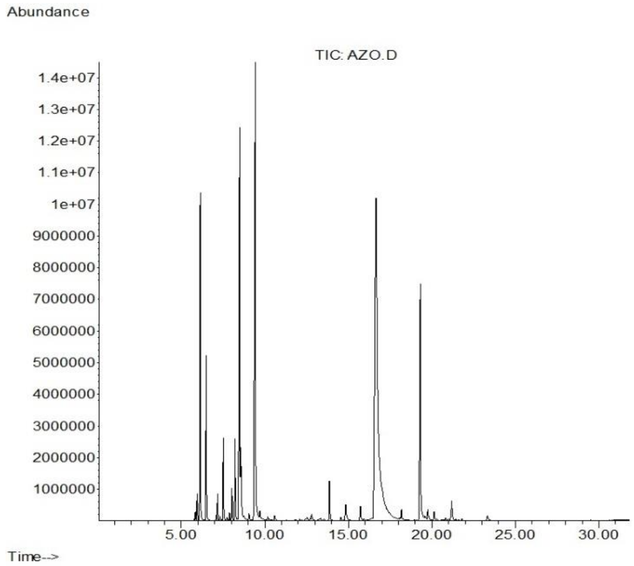

FIGURE 3 Chromatogram of *T. spicata* essential oil

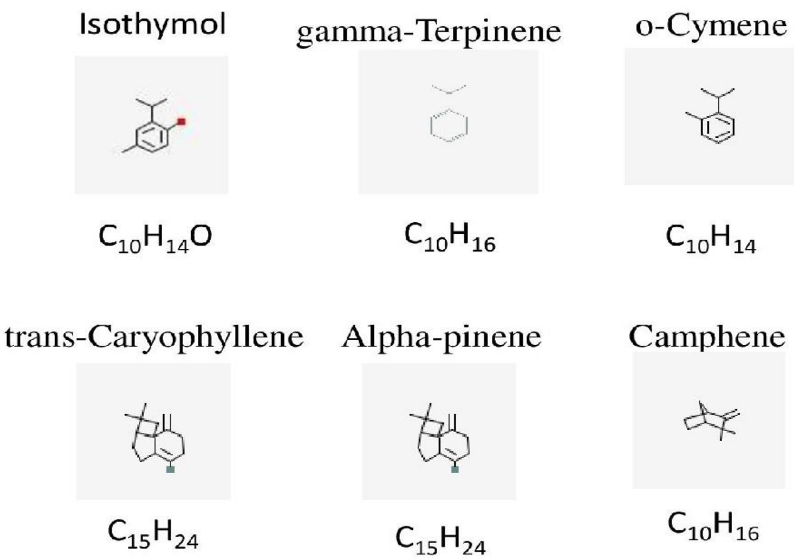

FIGURE 4 Molecular and chemical structures of the main constituents of *T. spicata* essential oil

Also, the analyses showed that *S. Khuzestanica* essential oil contained 43 chemical compounds, whose main ones included Carvacrol (71.99%), Camphene (2.84%), Alpha-pinene (1.63%), 1, 8-Cineole (1.51%), Carvone (1.44%) and Beta-pinene (1.42%) (Table 3).

TABLE 3 Identified compounds of *S. khuzestanica*

Compound	Retention time	Percent
Tricyclene	5.81	0.18
Alpha-pinene	6.10	1.63
Camphene	6.46	2.84
Verbenene	6.6	1.26
Sabinene	7.07	0.05
Beta-pinene	7.16	1.42
1-Octen-3-ol	7.40	0.92
Beta-myrcene	7.50	0.42
l-Phellandrene	7.88	0.05
3-carene	8.01	0.30
Alpha-terpinene	8.21	0.05
P-cymene	8.44	0.78
1,8-cineole	8.77	1.51
Cis-ocimene	8.74	1.39
Trans-beta-ocimene	9.03	0.27
Gamma-terpinene	9.36	0.11
Trans-Sabinene-hydrate	9.72	0.29
Terpinolen	10.16	0.24
Linalool	10.64	1.34
Camphor	11.94	0.77
Isomenthone	12.06	1.14
Menthofuran	12.32	0.98
Borneol	12.71	0.59
4-Terpineol	12.88	0.19
Krypton	13.15	0.26
Eucarvone	13.73	0.22
Isobornyl acetate	14.15	0.76
Cuminic aldehyde	14.58	0.12
Thymol	14.67	0.13
Carvacrol	14.83	71.99
Carvone	15.69	1.44
Eugenol	15.88	1.40
Beta-elemene	18.52	0.31
Alpha-gurjunene	18.95	0.08
Trans-caryophyllene	19.24	1.00
Delta-cadinene	19.92	0.07
Trans-beta-farnesene	20.07	0.87
Germacrene D	20.80	0.62
Beta-ionone	20.91	0.08
Geranyl acetate	21.36	0.26
Alpha-amorphene	25.15	0.83
Caryophyllene oxide	23.35	0.50
Alpha-cadinol	24.74	0.36
Monoterpene=90.90% Sesquiterpene=7.80% Other compounds =0.92%		

Chromatogram and molecular and chemical structure of the main constituents of *S. Khuzestanica* essential oil are shown in Figures 5 and 6.

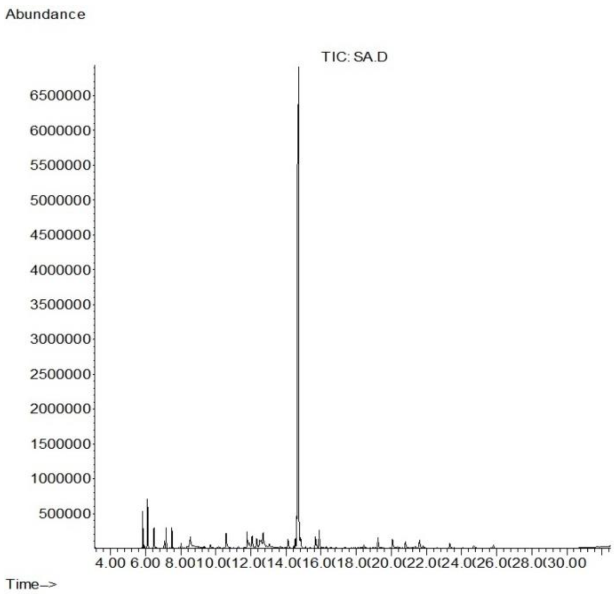

FIGURE 5 Chromatogram of *S. Khuzestanica* essential oil

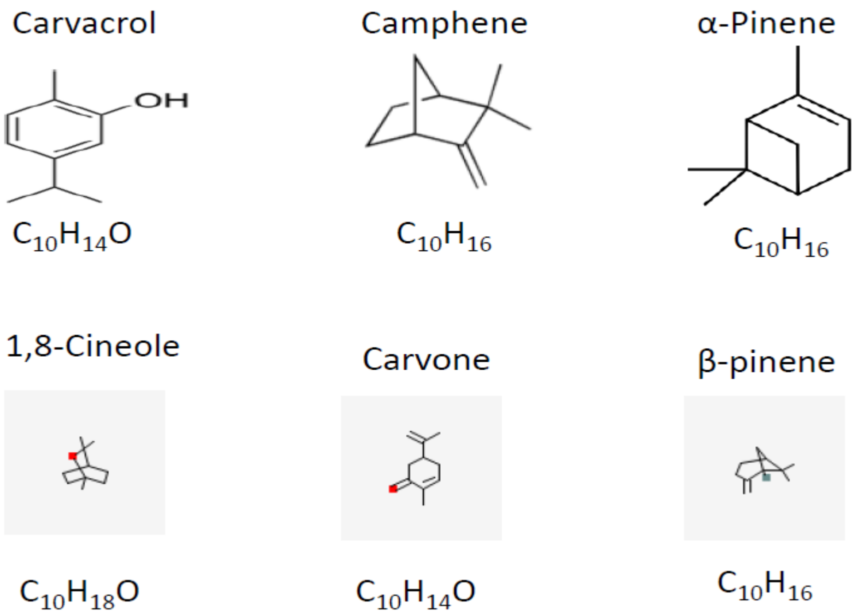

FIGURE 6 Molecular and chemical structures of the main constituents of *S. Khuzestanica* essential oil

Discussion

We reported the chemical compositions of essential oils of *T. spicata* and *S. khuzestanica* native to Ilam province (western Iran). Based on the results, main components of *T. spicata* essential oil included isothymol gamma-terpinene, and o-cymene and main components of *S. khuzestanica* essential oil were beta-thujene, 2-hexenal, trans-caryophyllene and beta-myrcene. Studies have shown that there is a significant difference in the amount of some constituents of *Satureja khuzestanica* essential oil in three habitats, indicating that ecological and climatic conditions of the habitat can affect the amount and type of essential oil compositions [23]. According to one study, which was in agreement with ours, types and amounts of essential oils of *Satureja thymbra* and *Coridothymus capitatu* were strongly influenced by the natural habitat of the plant so that essential oils of plants extracted from both species in dry and low-altitude habitats were rich in carvacrol and those extracted from wet and high-altitude habitats were rich in thymol [24].

In another study carried out in Khuzestan and Lorestan provinces, the main composition of *S. khuzestanica* was carvacrol, with its percentage being 90.9% in extracted essential oil [27]. Again, another study showed that the percentage of carvacrol content in *S. khuzestanica* essential oil in Lorestan province was 97.89% [28]. Saei-Dehkordi and his colleagues found that *S. khuzestanica* essential oil in Khuzestan province (southern Iran) contained 53.8% carvacrol [29] while Yousefzadi and co-workers found 92.87% carvacrol in *S. khuzestanica* in southern Iran [30]. Also, the results of Abbasi's study in Lorestan (southwest of Iran) showed that essential oil of this plant contained 9.88% carvacrol [31]. In Egypt, Abou Baker et al., showed that carvacrol (48.51%) and gamma-terpinene (36.63%) were the main components of *Satureja hortensis* essential oil [32]. *S. khuzestanica* essential oil is an antimicrobial compound because of its high level of carvacrol [30-33]. Based on our results, carvacrol (71.99%) was the main compound of *S. khuzestanica* essential oil in Ilam province (western Iran).

Turkish researchers have identified carvacrol (43.6%), gamma-terpinene (16.69%) and p-cymene (13.97%) as the main components of *T. spicata* essential oil (ubsp, spicata E0). According to studies performed, presence of carvacrol, Gamma-terpinene, p-Cymene, Caryophyllene and Alpha-myrcene gave some antimicrobial property to *T. spicata* L. essential oil [34].

Research indicates that p-cymene alone is not an effective antimicrobial compound, but if combined with carvacrol, it could be effective because of its synergic effect [35, 36]. Climatic conditions and soil in which medicinal plants grow cause the type and amount of their effective compounds to be different. In our study, carvacrol and cymene levels were 0.9% and 17.94%, respectively. In Serhat's study in Turkey, carvacrol and cymene compounds were reported to be 43.6% and 17.97%, respectively. The amount of thyme essential oil in Iran (Ilam) was different from that of collected in Turkey, affecting the medicinal activity of this essential oil. Aromatic medicinal plants such as *S. khuzestanica* and *T. spicata L.* can be used as antimicrobial compounds for medicinal and health purposes because of their active compounds [37,38]. Medicinal plants belonging to the Laminaceae family are rich in phenolic compounds having antimicrobial, herbicidal and pesticidal properties [39-42]. On the other hand, studies show that compounds such as terpenoids, phenolics, nitrogen compounds, and vitamins have antioxidant activities [43,44].

Plants and fruits contain a wide range of plant antioxidants, the most important of which are phenolic acids, polyphenolic compounds, terpenes, and terpenoids such as monoterpene, sesquiterpene, diterpene, triterpene, tetraterpene, and polyterpene [45-49]. Types of triterpene and tetraterpene included carvone, 1,8-cineol, isothymol, caryophyllene, α-pinene, *β*-pinene, etc [45-49]. Isothymol (Carvacrol) is a phenol compound derived from monoterpene cymene, which is added to food as an inhibitor of bacterial growth. In fact, carvacrol is a thymol isomer and smells like thymol. Carvacrol and thymol have various biological and medicinal properties such as antioxidant, antibacterial, antifungal, anti-cancer, anti-inflammatory, hepatosteroid, antispasmodic, and vasodilator. They are small, lipophilic molecules that can easily cross the blood-brain barrier and exert their effects [45]. α - pinene *β*-pinene has anti-inflammatory and possibly antimicrobial effects via PGE1 [46].

Eucalyptol (1,8-cineol) is the ingredient in many brands of cough suppressants and mouthwash; this compound controls excessive airway mucus secretion and asthma by inhibiting anti-inflammatory cytokines [47]. Gamma-terpinene is a compound that has antioxidant properties and is known as a plant metabolite, an essential oil component and a human xenobiotic metabolite [48]. Caryophyllene is one of the chemical compounds with antioxidant properties that are helping to improve cold tolerance at low ambient temperatures [49]. Medicinally, cement is a combination of metabolic and medicinal tones used to prevent coughs and eliminate phlegm, flavoring, and the production of fungicides and pesticides [50]. Antioxidants are compounds that exert their effects by the mechanism of free radical scavenging, inhibition of glycosylation, inhibition of oxidative stress and protection of the body against damage, and injuries caused by oxidative stress [51-53]. *T. Spicata* and *S. Khuzestanica*, are widely used in traditional and modern Iranian medicine. Many licensed herbal medicines have been produced from these plants and their active ingredients, which include analgesics and anti-inflammatory drugs. Despite the valuable medicinal effects of these plants, some side effects have been observed [54-56] that need to be used with a doctor's prescription. For savory, side effects have been observed on some diseases. Savory is contraindicated in people with thyroid problems. People with diabetes should consume safflower in consultation with their doctor. Also, savory consumption should be avoided during pregnancy. Studies show that consumption of thyme species is dangerous for pregnant women and lactating mothers, but over time it has been shown that consumption of this plant increases the risk of miscarriage [57]. People who consume thyme oil are at risk for heart problems such as shortness of breath, seizures, cardiac arrest, and even coma. Thyme plant species have negative effects on thyroid function. In general, excessive consumption causes poisoning [58].

Conclusion

Results of this study indicated the presence of antioxidant and antimicrobial compounds such as carvacrol in both plants of the Laminaceae family (*S. khuzestanica* and *T. spicata L.)*. Therefore, essential oils extracted from mentioned plants will be used as antioxidant, antimicrobial and antiseptic compounds for medicinal and health purposes.

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Authors' contributions

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

Conflict of interests

All authors declare that they have no conflict of interest.

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Orcid:
Maryam Shahmohammadi: *https://orcid.org/0000-0002-2148-8434*
Mahmood Bahmani: *https://orcid.org/0000-0002-9643-9274*
Hori Ghaneialvar: *https://orcid.org/0000-0003-4536-6041*
Naser Abbasi: *https://orcid.org/0000-0003-4457-3997*

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