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RESEARCH ARTICLE

Phytochemical screening of two Ilam native plants *Ziziphus nummularia* (Burm.f.) Wight & Arn. and *Ziziphus spina-christi* (Mill.) Georgi using HS-SPME and GC-MS spectroscopy

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ABSTRACT

Essential oils are aromatic compounds widely used in the perfumery, pharmaceutical and food industries. There are several methods to extract essential oils and extracts. These methods include distillation, pressure or scraping, pressing and razor technique, headspace solid-phase micro extraction (HS-SPME), solvent extraction, extraction by bioactive hydrolyzing enzymes, and CO_2 extraction. The aim of this study was to investigate the amounts of active ingredients of essential oils of medicinal plants *Ziziphus nummularia* and *Ziziphus spina-christi* by HS-SPME so that it can more easily move towards production of effective herbal remedies by identifying the main and active ingredients of the plants. The main chemical compound of *Z. spina-christi* essential oil was found to be trans-caryophyllene (17.31%), followed by alpha-pinene (15.50%), beta-caryophyllene (10.86%), and beta-pinene (7.32%). The main compound of *Z. nummularia* essential oil was tetradecane (16.76%), followed by hexadecane (9.35%), dl-limonene (5.75%), cyclohexan-1-ol, 3 meth (5.54%), trans-caryophyllene (5.47%), and beta-myrcene (5.28%). Chemical compounds of *Ziziphus spina-christi* included carbobicyclic or bicycleheptane, sesquiterpenes derived from germacrene, bicyclic monoterpenes, and monoterpenes, while the main compounds of *Z. nummularia* included monoterpenes, aliphatic hydrocarbons, alkane hydrocarbons, primary terpene compounds, and decarbonated alcohol. Identification of chemical and biological constituents of essential oils of medicinal plants is a valuable way to identify medicinal compounds that can be used to treat diseases by combining the traditional effects and the main compounds in the experimental pharmacy studies.

Introduction

Medicinal herbs are used for the treatment of variety of diseases and disorders (1–7). Herbs exert their effect through the active ingredients and antioxidants available (4–10). *Ziziphus* is an important genus of medicinal herbs that is important in traditional medicine. It is a genus of thorny trees and shrubs belonging to the Rhamnaceae family. There are about 3 species of *Ziziphus* that are distributed in tropical and subtropical regions. The fruit of this tree, also called *Ziziphus*, is edible. These trees are abundant in the wild. The trees are found in the tropical, western, and southern coasts of Iran and mainly in Ilam, Khouzestan, Sistan and Balouchestan, Hormozgan, Kerman, Fars and Bushehr provinces. The plants are found scattered and people use its fruit (11). *Ziziphus* species include *Ziziphus abyssinica*, *Z. lotus*, *Z. mauritiana*, *Z. mucronata*, *Z. nummularia*, *Z. spina-christi* and *Z. zizyphus* (12). *Zizyphus spina-christi* belongs to Kingdom: Plantae, Order: Rosales. This plant is a tree of approximately 2 meters height with small, heart-shaped and extended leaves and three prominent veins, with its earrings turning into thorn. An important feature is the secretory system producing mucilage and gum, which contains single

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cells or secretory bags found in the skin and core of the stem in the parenchyma tissue of the vascular bundle around the veins of the leaf and petioles as well as outer part of the fruit (13, 14). In traditional medicine, *Z. spina-christi* is used for wound healing and hair growth and as an antimicrobial, antifungal, laxative, anti-bronchitis, analgesic, immune system-boosting, skin-refreshing, and detoxifying agent, to treat bone fracture, cancer, low back pain, to lose weight, etc. (15–17). The plant contains important medicinal compounds such as alkaloids, flavonoids, glycosides, saponins and essential oils (18, 19). It is found in Iran, Pakistan, Afghanistan, Iraq, Egypt and Israel (20–22). *Z. nummularia* or Ramilak is another species of *Ziziphus* is found in Afghanistan, India, Iran, Lebanon, Pakistan, Zimbabwe, Mauritania, Nigeria and Uganda. *Z. nummularia* leaves have been widely used in traditional medicine for the treatment of cold, skin diseases, pain, diabetes, wounds and also as an anti-inflammatory, antibacterial, antifungal, sedative and hypnotic agent (23–29). Phytochemical analysis revealed the presence of a number of phyto constituents such as flavonoids, tannins, sterols, saponins, pectins, glycosides, alkaloids and tripepnoids. Cyclopeptide alkaloids have been identified as active ingredient of *Ziziphus nummularia* (25–29).

Ziziphus spina-christi and *Z. nummularia* are used as edible fruits, jams, health products for skin and hair and medicinal effect. In traditional and persian medicine of Iran and especially in Ilam province (west of Iran) from fruits and leaves of *Ziziphus spina-christi* and *Z. mauritiana* are widely used as medicinal and hygienic products so, In this study, we tried to understand the effective ingredients of these two plant. Finally, the active ingredients of these herbs will be helpful to produce effective pharmaceutical products.

Fig. 1. *Ziziphus spina-christi*

Fig. 2. *Ziziphus nummularia*

Materials and Methods

Plant preparation

In October–November 2019, two species *Z. spina-christi* (Fig. 1) and *Z. nummularia* (Fig. 2) were procured from Dehloran County (south of Ilam province, west of Iran). The plants were identified and confirmed using morphological keys of Ilam Province Plant Flora at Ilam University of Medical Sciences Biotechnology and Medicinal Research Center. The collected plants were cleaned and dried in the shade in the open air. The dried plants were pulverized by a plant mixer and analyzed by HS-SPME for chemical composition.

Identification of chemical compounds by SPME-HS method

The essential oil of the plant was extracted by HS-SPME technique. About 2 gms of dried plant (from each plant separately) and its powder were placed in standard vial device 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 (Headspace of the (the vial is a part of the HS-SPME equipment). The SPME syringe with a lid on it was then placed in the headspace of the vial and the essential oils in the vial was 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 the essential oils in the fiber in the vial were adsorbed due to the temperature of the input and then the essential oils in HS-SPME device entered into the GC/MS apparatus for identification (30).

HS-SPME method

About 2 gm of each plant extract was used for analysis. The device condition was as follows: Gas chromatograph (Agilent 6890N) was coupled with 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, hold time 0.00 min and rate of -°C/min; temperature 200 °C, hold time, 0.00 min and rate of 5 °C/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: (HSSPME); SMPE fiber: PDMS 100 μm thickness (SUPELCO); sample weight: 0.5 gm; 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 (31).

Results

In this study, essential oils of two plant species *Z. nummularia* and *Z. spina-christi* were extracted by HS-SPME and analyzed by GC-MS. Based on the results, a total of 41 chemical compounds were isolated from *Z. spina-christi* essential oil (Table 1). The results of phytochemical analysis showed that the main chemical compounds of *Z. spina-christi* essential oils was trans-caryophyllene (17.31%), followed by alpha-pinene (15.50%), beta-caryophyllene (10.86%), and beta-pinene (7.32%). The other compounds of the essential oil of *Z. spina-christi* are are listed in Table 1. Based on the results of GC-MS analysis, a total of 39 chemical compounds were also isolated from the species *Z. nummularia*. The main compound of *Z. nummularia* essential oil was tetradecane (16.76%), followed by hexadecane (9.35%), dl-limonene (5.75%), cyclohexan-1-ol, 3 meth (5.54%), trans-caryophyllene (5.47%), and beta-myrcene (5.28%). Supplementary information on other chemical compounds of the essential oil of *Z. nummularia* is given in Table 2.

Table 1. Identified compounds of *Ziziphus spina-christi* essential oils of by HS-SPME (GC-MS) method

No.	Retention time	Compound	%
1	5.942	α-Thujene	1.53
2	6.133	α-Pinene	15.50
3	7.07	Sabinene	2.54
4	7.170	β-Pinene	7.32
5	7.508	β-Myrcene	6.26
6	7.88	1-Phellandrene	0.61
7	8.018	Delta 3-Carene	0.68
8	8.22	4-Carene	0.14
9	8.560	β-Phellandrene	10.86
10	8.735	cis-Ocimene	2.02
11	9.03	trans-.beta.-Ocimene	0.26
12	9.348	ɤ-Terpinene	0.30
13	9.698	trans-Sabinene hydrate	0.65
14	10.242	Terpinolen	0.05
15	10.583	Linalool	0.28
16	11.748	trans-Pinocarveol	0.23
17	12.082	L-Menthone	4.90
18	12.332	Menthofuran	2.43
19	12.737	Menthol	1.28
20	12.99	3-p-Menthanol	0.50
21	13.053	trans-Carane	0.67
22	13.23	A-Terpineol	1.07
23	14.481	Pulegone	0.30
24	14.937	Piperitone	0.21
25	15.898	Carane	4.75
26	16.295	cis-Carane	0.27
27	17.848	α-Muurolene	0.16
28	18.088	α-Copaene	2.46
29	18.338	α- Bourbonene	0.23
30	18.448	β-Cubebene	0.08
31	18.517	β-elemene	0.32
32	19.266	trans-Caryophyllene	17.31
33	19.579	α-Bergamotene	0.64
34	20.121	trans-β-Farnesene	1.70
35	20.814	Germacrene-D	3.84
36	20.957	β-Selinene	0.70
37	21.185	Bicyclogermacrene	4.62
38	21.805	δ-Cadinene	1.15
39	22.207	CIS-α-Bisabolene	0.13
40	23.328	Caryophyllene oxide	0.93
41	23.581	p-Menth-3-en-9-ol	0.10

Ziziphus spina-christi essential oils was trans-caryophyllene (17.31%), followed by alpha-pinene (15.50%), beta-caryophyllene (10.86%), and beta-pinene (7.32%). The other compounds of the essential oil of *Z. spina-christi* are listed in Table 1.

Table 2. Identified compounds in the essential oil of *Ziziphus nummularia* by HS-SPME (GC-MS)

No.	Retention time	Compound	%
1	6.669	α- Pinene	2.82
2	7.846	β-Pinene	1.78
3	8.298	β-Myrcene	5.28
4	9.561	dl-Limonene	5.75
5	12.018	Nonanal	1.40
6	13.761	Menthone	2.20
7	14.533	Menthol	1.23
8	14.63	Pyrazine, 2-methoxy-3-(2-methylpropyl)	3.60
9	15.19	Dodecane	4.87
10	15.487	Decanal	1.24
11	18.459	Menthyl acetate	1.88
12	18.557	Tridecane	1.45
13	20.311	5-Methyltridecane	1.20
14	20.843	2-Methyltetradecane	1.27
15	21.106	Copaene	1.93
16	21.666	Cyclotetradecane	1.44
17	21.774	Tetradecane	16.76
18	22.46	trans-Caryophyllene	5.47
19	22.877	α-Zingiberene	0.27
20	22.98	α-Guaiene	1.26
21	23.415	trans-Geranylacetone	1.33
22	23.557	Pentatriacontane	0.94
23	24.089	α-Amorphene	0.78
24	24.398	β-Ionone	2.73
25	24.615	Pentadecane	1.93
26	24.746	α-Muurolene	0.99
27	25.369	δ-Cadinene	1.85
28	25.832	Methylundecane	1.30
29	25.986	5-Methylpentadecane	1.13
30	26.455	3-Methylpentadecane	0.38
31	27.192	Hexadecane	9.35
32	28.358	Phytane	1.43
33	28.558	Lanol	0.54
34	29.564	Heptadecane	1.52
35	29.656	3-Cyclohexen-1-ol, 3-methyl-	5.54
36	31.37	Octadecane	1.51
37	31.485	Tritetracontane	1.17
38	31.965	Tetrahydrogeranylacetone	0.87
39	33.153	Dibutyl phthalate	1.60

Fig. 3 and 4 comparatively illustrate the chromatograms of the compounds of *Z. spina-christi* and *Z. nummularia* essential oils. Apart from the difference in the percentages of compounds of the two plants', as shown in the chromatogram (No. 3) and Table 1, the plant has 41 chemical compounds in total. The main compounds of *Z. spina-christi* included carbobicyclic or bicycleheptane compounds, sesquiterpene derived from germacrene, monoterpenes as bicyclic monoterpenes. The chromatogram of *Z. nummularia* showed that the plant has a total of 39 chemical compounds of which, the main compounds are monoterpenes, aliphatic hydrocarbons, alkane

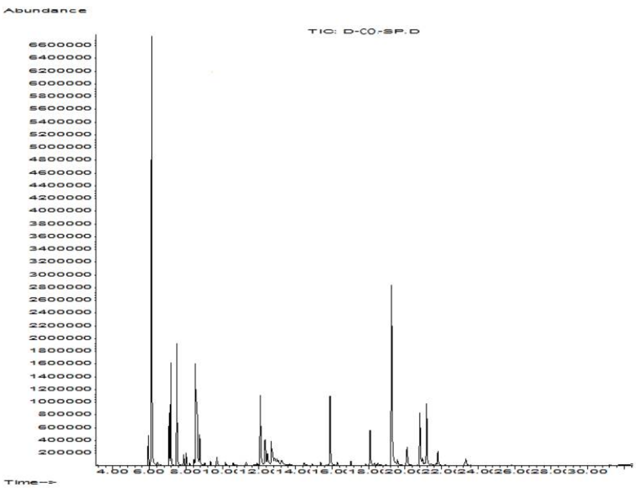

Fig. 3. Chromatogram of the essential oil of *Ziziphus spina-christi*.

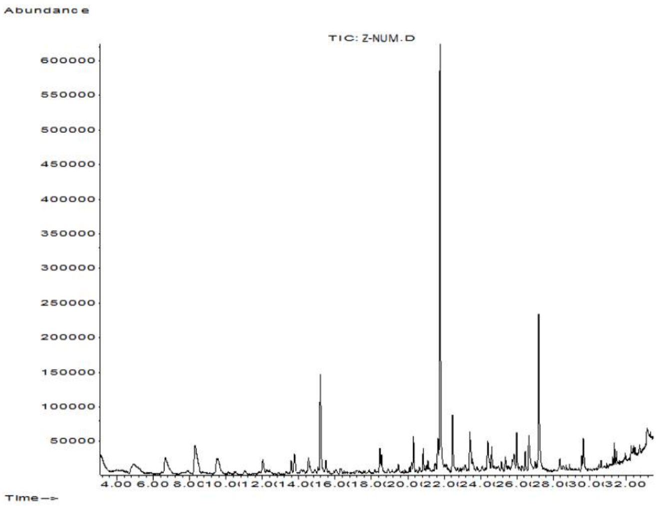

Fig. 4. Chromatogram of the essential oil of *Ziziphus nummularia*.

hydrocarbons, primary terpenes and decarbonated alcohol.

Based on the results of GC-MS analysis, a total of 39 chemical compounds were also isolated from the species *Z. nummularia*. The main compound of *Z. nummularia* essential oil was tetradecane (16.76%), followed by hexadecane (9.35%), dl-limonene (5.75%), cyclohexan-1-ol, 3 meth (5.54%), trans-caryophyllene (5.47%) and beta-myrcene (5.28%). A list of the main constituents along with the chemical formula and the chemical structure of the two plants are outlined in Table 3. View results of the phytochemical analysis show that there are common compounds between the two plants that differ in percentage. The results were reported in Table 4. Different compounds of *Z. spina-christi* are include α-Thujene, Menthofuran, Piperitone, Pulegone, Sabinene and ɤ-Terpinene. Also different compounds of *Z. nummularia* include Decanal, α-Zingiberene, α-Guaiene, α-Amorphene, phytane and Lanol.

Table 3. Structure and chemical composition of the two plants

Z. spina-christi		*Z. nummularia*	
Compound	**Chemical formula**	**Compound**	**Chemical formula**
trans-caryophyllene	$C_{15}H_{24}$	Tetradecane	$C_{14}H_{30}$
alpha-pinene	$C_{10}H_{16}$	Hexadecane	$C_{16}H_{34}$
beta-caryophyllene	$C_{15}H_{24}$	dl-limonene	$C_{10}H_{16}$
beta-pinene	$C_{10}H_{16}$	cyclohexan-1-ol, 3 meth	C_7H_{120}

Table 4. Comparison of differences in the percentages of common constituents between the two medicinal plants

Compound	**Medicinal plants**	
	Z. spina-christi **(%)**	***Z. nummularia*** **(%)**
alpha-pinene	15.50%	2.82%
Beta-pinene	7.32%	1.78%
beta-myrcene	6.26%	5.28%
trans-Caryophyllene	17.31%	5.47%
α-Muurolene	0.16%	0.99%

Discussion

According to the studies (33–35), few phytochemical studies have been conducted on *Ziziphus nummularia* and *Z. spina-christi* (32). One of the important factors in the differences in the chemical composition of a particular species is the growth in different geographical areas and ecological conditions. Despite being part of the plant family Rhamnaceae, These factors have certain differences and similarities with respect to chemical composition and secondary metabolites. In a study, benzaldehyde, phenylacetaldehyde, phenylethylalcohol, benzeneacetonitrile, 2-ethyl hexanoic acid, octanoic acid, 2-methoxy 4- (1-Propanol) -6-acetate phenol, nonanoic acid, decanoic acid, 1-hydroxy 2,4,6-trimethylbenzene and 5-hydroxymethyl-2-furaldehyde were identified from *Z. spina-christi* honey (33). Another study revealed that *Z. spina-christi* seed contained compounds such as 4-hydroxymethyl-1-methyl pyrrolidine-2-carboxylic acid (less polar and major compound) and 4-hydroxy-4-hydroxymethyl-1-methyl pyrrolidine -2-carboxylic acid (34). Chemical compounds of hexane and ethanolic extracts of *Z. nummularia* leaf revealed that hexane extract of the plant included 105 chemical compounds and its ethanolic extract included 56 chemical compounds including palmitic acid, linoleic acid, stearic acid, squalene, stigmasterol campesterol, vitamin E, geranyl linalool isomer, trans-geranylgeran oil, 1-eicosanol, gamma sitosterol, betulin, lupeol and phytol in the n-hexane extract while in addition to palmitic acid, linoleic acid, gamma sitosterol, stigmasterol, phytol, squalene, oleic acid, tricosane, tetradecane, 2-methoxy-4-vinylphenol, ethyl alpha-d-glucopyranoside and behenylbehenate were identified in the extracts of both extract (35). An investigation on the chemical constituents of *Z. nummularia* revealed that 4-hydroxy-cyclohexanone, 2,7-dimethyloctane-3,5-dione, hepta-cosane and 9,12-octadecadienoic acid methyl ester as the main compounds of the plant (36).

Hexadecanoic acid has antimicrobial, anti-allergic, antioxidant and pesticide effects (37–39). Octadecane has antifungal effect (40) and geranyl linalool isomer has antioxidant effects (41), partly explaining the properties of the two studied plants. The HS-SPME (GC-MS) technique helps to identify the essential oil of the plants. Identifying the plant's active ingredients along with documentation of the traditional and ethno-botanical effects of those plants leads us to a better understanding of *Ziziphus nummularia* and *Z. spina-*

christi pharmacological effects to produce natural and herbal remedies that are effective against diseases.

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Authors' contributions

MB, NA and AJ reviewed the literature and prepared the first draft of manuscript; NA, MB, AJ and IS reviewed the literature, helped in preparing first draft of manuscript, checked and corrected the grammar. This study was designed and performed by MB, SSH and NA. All authors read and approved the final report.

Conflict of interests

The authors declare that they have no conflict of interests.

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