TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Integr Med, Volume 6, Issue 3, Summer 2021

Original Research

Extraction and Identification of Chemical Compounds of *Peganum harmala* L. Seed Essential Oil by HS-SPME and GC-MS Methods

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Received: 8 Apr 2021 **Revised:** 12 Jun 2021 **Accepted:** 13 Jun 2021

Abstract

Peganum harmala is a plant that belongs to the family Zygophyllaceae. In traditional medicine, this plant has therapeutic effects such as repelling intestinal worms, increasing sexual potency, increasing milk secretion, anti-rheumatism, regularizing, anti-parasitic, hypnotic, diaphoretic, anti-cancer and analgesic. The aim of this study was to identify the chemical composition of *P. harmala* seed essential oil. *P. harmala* seed essential oil was extracted by Head Space-SPME [HS-SPME] method and its chemical compounds were identified by GC-MS method. Totally, 41 chemical compounds were identified in *P. harmala* seed essential oil. According to the results, the most compounds of *P. harmala* L. seed essential oil includes 2,3-dimethyl benzofuran (28.32%), cis-linalool oxide (7.46%), [2E]- decenal (6.57%), 4aα,7β,7aα-nepetalactone (6.49%), 3-oxo-p-menth-1-en-7-al (6.36%) and trans- β- terpineol (5.86%), respectively.

Keywords: Medicinal plant; Essential oil; *Peganum harmala* L.; 2,3-Dimethyl benzofuran; Head-space solid-phase microextraction (HS-SPME); Gas chromatography mass spectrometry (GC-MS)

Introduction

Esfand or Harmel medicinal plant with the scientific name of *Peganum harmala* L., is a plant that grows in the Mediterranean regions of North Africa, Asia, Turkey, Syria, Iran, India and Spain. *P. harmala* is stable and hairless and belongs to the family Zygophyllaceae [1]. Morphologically, *P. harmala* L. as bright green leaves full of water with narrow, long and irregular divisions. *P. harmala* stem is zigzag, woody and contains several branches. Also, its fruit is in the form of a capsule of three houses and in the shape of a sphere. Each capsule house contains a large number of seeds. Its seeds are angular, dark brown, with a distinct odor [1]. *P. harmala* is wide-

Citation: Asadzadeh R, Abbasi N, Bahmani M. **Extraction and Identification of Chemical Compounds of *Peganum harmala* L. Seed Essential Oil by HS-SPME and GC-MS Methods.** Trad Integr Med 2021; 6(3): 229-235.

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ly used in traditional medicine and is used to repel intestinal worms, increase sexual potency, increase milk secretion, treat rheumatism, analgesic, laxative, antiparasitic, and stomach pain reliever [2-4]. Other effects of *P. harmala* include its anti-cancer, hypnotic, diaphoretic, anti-cancer properties [3,4]. Alkaloids derived from this plant include harmine, harmalin and harmalol [5]. *P. harmala* seeds are rich in lipids, proteins, carbohydrates, minerals, alkaloids and amino acids. *P. harmala* seed fatty acids include palmitic acid, linolenic acid, stearic acid, linoleic acid, etc. [6]. HS-SPME method refer to methods in which the volume of the extraction phase is much less than the sample size and therefore due to the low capacity of the extraction phase, the extraction is not complete and only a small fraction of the analyte is transferred into the extraction phase and in some cases, after extraction, the concentration of the species in the sample solution is equal to its initial concentration. So, in fact, extraction is of equilibrium type. A method that removes essential oils under temperature and pressure conditions [7-9]. GC-MS analysis of P. harmala in Algeria showed the presence of harmaline (48.009%), harmine (38.440%), tetrahydroharmine (8.513%), tetrahydroharman (0.061%) and 6-methoxytetrahydro-1-norharmanone (0.057%) were present in the it's seed extract but so far, the active ingredients of the essential oil of this plant have not been done by HS-SPME method [10].

In this phytochemical study, essential oils and volatile extracts were extracted from dried Peganum harmala seed powder by HS-SPME method and its chemical compositions were investigated using GC-MS technique.

Materials and Methods

Preparation of medicinal plant and its identification

In this study, *Peganum harmala* (Figure 1) was collected in May 2020 from Dehloran city with geographical coordinates of 45 degrees and 30 minutes to 48 degrees and 4 minutes east longitude and between 32 degrees and 7 minutes to 33 degrees and 20 minutes north latitude located in the south Ilam province located in western Iran. The shoots were dried for 72 hours at room temperature. Dried plant seeds were powdered using a mixer and prepared for testing.

Figure 1. *Peganum harmala* medicinal plant

Extraction of chemical compounds of Peganum harmala plant by HS-SPME method

In this study, the essential oil of *P. harmala* seed was extracted by HS-SPME technique. In the

HS-SPME technique, about 2 grams of dried plant powder is placed in a vial and the vial temperature rises between 60 and 70 oC (Extraction time: 20 min). This temperature condition is in the optimal state, causing the extraction of vapors of substances in plant essential oil in the space above the solid surface in a saturated form. The SPME syringe was then placed in the upper space of the container with the lid closed and the material in the plant vapors was absorbed by the silica phase in the needle of the device. After sufficient time and saturation of silica fiber, the volatile compounds of the fiber are placed directly in the input part of the GC-MS device and due to the temperature of the input part, the material in the fiber was desorbed and entered to the GC/MS device and identified [11].

GC-MS device conditions

The device condition was as follows:

The gas chromatograph (Agilent 6890N) was coupled to the Agilent 5973 bulk detector. Column: HP-5. (30 m length, 0.25 mm (ID), 0.25 µm fixed phase thickness). Type of injection: split / gap and column temperature program: 50 °C, holding time 0.00 min and -oC/min rate; 200 °C temperature, holding time, 0.00 min and 5 oC/min and 240 min temperature, holding time 0.00 min and 10 oC/min Carrier gas: He (99.999%); Injection type: no gap; Library: Willey 7n; Injector temperature: 250 °C and flow rate: 0.9 ml per minute. Extraction mode: (HSSPME); SMPE fibers: PDMS thickness 100 micrometers (SUPELCO); Sample weight: 0.5 g; Extraction temperature: 60 oC; Extraction time: 20 minutes; Ultrasound time: 10 minutes (Euronda ultrasound instrument, Italy) and disposal time in GC-MS injector port: 3 minutes [8,9].

Results

In this study, the essential oils and volatiles of *Peganum harmala* seeds were extracted by solid phase micro-extraction in the upper space (HS-SPME) and its chemical compounds were analyzed by GC-MS technique. The results of phytochemical analysis using HS-SPME technique showed that 41 chemical compounds were isolated and identified from *Peganum harmala* seed. According to the results, the most compounds of *Peganum harmala* seed essential oil include 2,3-dimethyl benzofuran (28.32%), cis-linalool oxide (7.46%), [2E]-decenal (6.57%), 4aα,7β,7aα-nepetalactone (6.49%), 3-oxo-p-menth-1-en-7-al (6.36%) and *trans*-β-terpineol (5.86), respectively. The results of the percentage of other chemical compounds are detailed in Table 1.

Table 1: Chemical compounds of *Peganum harmala* seed essential oil

No.	RT	R.I.	Compound	%C	Molecular formula
1	6.246	6.246	Camphene	0.03	$C_{10}H_{16}$
2	6.371	6.371	2-ethoxy thiazole	0.14	C_5H_7NOS
3	6.56	6.56	5-methyl furfural	3.45	$C_5H_4O_2$

4	6.983	6.983	1-octen-3-one	3.72	$C_8H_{14}O$
5	7.703	7.703	ethyl hexanoate	4.08	$C_8H_{16}O_2$
6	7.794	7.794	Meta-mentha-1[7],8-diene	1.57	$C_{10}H_{16}$
7	8.023	8.023	Dehydroxy cis-linalool oxide	0.20	$C_8H_{16}O$
8	8.217	8.217	1,4-cineole	0.64	$C_{10}H_{18}O$
9	8.629	8.629	Limonene	0.09	$C_{10}H_{16}$
10	8.806	8.806	2-methyl hexanoic acid	0.05	$C_7H_{14}O_2$
11	9.029	9.029	5-methyl hexanoic acid	0.06	$C_7H_{14}O_2$
12	9.515	9.515	[3Z]-octen-1-al	5.38	$C_{10}H_{18}O$
13	10.063	10.063	[2E]-octen-1-ol	0.52	$C_8H_{16}O$
14	10.418	10.418	Benzyl formate	0.15	$HCO_2CH_2C_6H_5$
15	10.835	10.835	Tetramethyl pyrazine	0.62	$C_8H_{12}N_2$
16	11.944	11.944	1-octen-3-yl acetate	0.71	$C_{10}H_{18}O_2$
17	13.384	13.384	γ-heptalactone	0.17	$C_7H_{12}O_2$
18	14.036	14.036	Trans- β- terpineol	5.86	$C_{10}H_{18}O$
19	14.52	14.52	Cis-linalool oxide	7.46	$C_{10}H_{18}O_2$
20	16.673	16.673	2,3-dimethyl benzofuran	28.32	$C_{10}H_{10}O$
21	17.219	17.219	[3Z]- hexenyl 3-methyl butanoate	0.17	$C_{11}H_{20}O_2$
22	17.813	17.813	Isoamyl hexanoate	0.36	$C_{11}H_{22}O_2$
23	18.162	18.162	Linalool acetate	2.25	$C_{12}H_{20}O_2$
24	18.453	18.453	[2E]- decenal	6.57	$C_{10}H_{18}O$
25	19.854	19.854	Cis-2-tert-butyl cyclohexa-nol acetate	0.31	$C_{12}H_{22}O_2$
26	20.814	20.814	4-hydroxy cryptone	1.04	$C_9H_{14}O_2$
27	21.1	21.1	Cis-2,3- pinanediol	1.24	$C_{10}H_{18}O_2$
28	21.391	21.391	Isobutyl benzoate	0.23	$C_{11}H_{14}O_2$
29	21.545	21.545	3-oxo-p-menth-1-en-7-al	6.36	$C_{10}H_{14}O_2$
30	22.454	22.454	α- longipinene	2.85	$C_{15}H_{24}$
31	22.729	22.729	4aα,7α,7aα-nepetalactone	0.18	$C_{10}H_{14}O_2$
32	23.46	23.46	Linalool isobutanoate	0.75	$C_{14}H_{24}O_2$
33	23.734	23.734	Geranyl acetate	0.20	$C_{12}H_{20}O_2$

34	24.1	24.1	Isobutyl phenylacetate	0.13	$C_{12}H_{16}O_2$
35	24.249	24.249	4aα,7β,7aα-nepetalactone	6.49	$C_{10}H_{14}O_2$
36	24.397	24.397	Ethyl decanoate	1.31	$C_{12}H_{24}O_2$
37	24.54	24.54	Cyperene	0.39	$C_{15}H_{24}$
38	24.746	24.746	Methyl eugenol	0.80	$C_{11}H_{14}O_2$
39	24.918	24.918	cycloseychellene	1.27	$C_{15}H_{24}$
40	25.363	25.363	[E]- caryophyllene	3.61	$C_{15}H_{24}$
41	25.695	25.695	Methyl undecanoate	0.24	$C_{12}H_{24}O_2$

RT: Retention time; %C: % Compound

Chromatogram (Figure 2) shows the peaks of chemical composition of *Peganum harmala* seed essential oil. As shown in the chromatogram 41 chemical compounds were identified for *Peganum harmala* seeds and most of the *Peganum harmala* seed essential oil compounds belong to 2,3-dimethyl benzofuran.

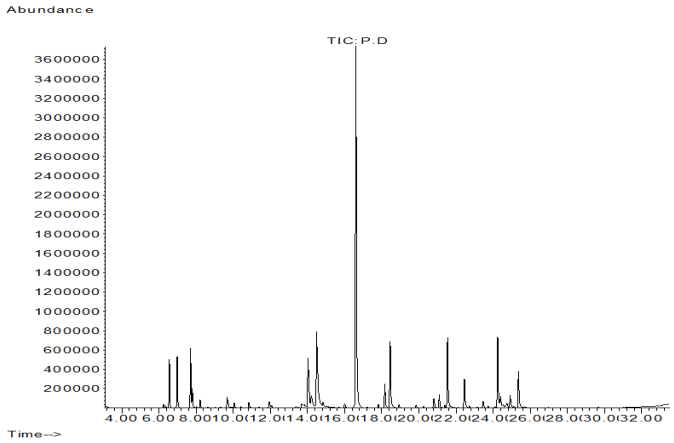

Figure 2. Chromatogram related to *Peganum harmala* seed essential oil

Discussion

So far, few studies have been conducted on the essential compounds of *Peganum harmala*, including studies in Algeria, Egypt, Libya, Morocco and Tunisia. Studies show that the most common constituents of *Peganum harmala* in Algeria are Eugenol (17.5%), Thymol (7%), epi Cadinol (5.3%), respectively [11]. In another study in Egypt, most compounds of *Peganum harmala* essential oil include Eugenol [12] and n-Tetradecanol (12.3%) [11]. In Libya, Eugenol with 17.5 % and n-Tetradecanol with 11.3 % are the most chemical compounds of *Peganum harmala* essential oil [10]. In Morocco, the combination of Eugenol with 13.2% and n-Tetradecanol (11.1%) is the highest chemical composition of the essential oil of this plant [11]. In Tunisia, Eugenol with 69.2% and Eugenol acetate with

9% are the most chemical compounds of this plant essential oil [11]. In our study, *Peganum harmala* seed essential oil contained the main constituents include 2,3-dimethyl benzofuran (28.32%), Cis-linalool oxide (7.46%), [2E]-decenal (6.57%), 4aα,7β,7aα-nepetalactone (6.49%), 3-oxo-p-menth-1-en-7-al (6.36%) and Trans- β- terpineol (5.86%). The results of our study differ from those of Libya, Morocco, Tunisia and Algeria. Because the main ingredient identified in previous studies was Eugenol, while the main ingredient identified in our study was 2,3-dimethyl benzofuran. One of the most important reasons for the differences in the chemical composition of *Peganum harmala* seeds in different regions is due to climatic conditions and soil conditions in each region. One of the main reasons for choosing HS-SPMAE method is more accuracy and less sample size for extracting essential chemical compounds.

Peganum harmala include of peganin and isopeganin, vesicine and vesicine are other compounds identified in P. harmala [12-15]. Studies show that beta-carotenes make up more than 60% in P. harmala seeds [16-19]. The substance of 2,3-dimethyl benzofuran with the chemical formula C10H10O is one of the phenolic compounds. 2,3-Dimethylbenzofuran, also known as benzofurans and it is a phenolic tasting compound. This compound includes spices, extracts, colorings, flavors, etc added to food for human consumption [20]. Phytochemical research has shown that medicinal plants with the use of standalone medicinal and therapeutic effects and their beneficial effects through their active ingredients [21]. Different varieties of P. harmala plant have different active ingredients such as eugenol and pulegone in the event that the main constituent of harmal essential oil in our study was 2,3-dimethylbenzofuran. It seems that the extreme fluctuations in the type and amount of compounds in the essential oils of these plants are due to ecological differences such as; Longitude, altitude, temperature, humidity, climate and soil and different climatic and edaphic conditions, metabolic pathways and biosynthesis of active substances in these plants affect and as a result, various secondary metabolites under different environmental conditions. 2,3-dimethyl benzofuran in P. harmala can be used as a natural medicine.

Authors' contributions

All of the authors reviewed and contributed to data collection and preparation of the manuscript. The first draft was prepared by all the authors. All authors read the final version and confirmed for the publication.

Conflicts of Interest

The authors declared no competing interests.

Ethical considerations

Ethical issues [including plagiarism, data fabrication, double publication and etc.] have been completely ob¬served by thee author.

Funding/Support

This study received no funding or grant.

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