Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2022.574.581

Research Article
Comparative Evaluation of Chemical Carbon Acid Extract of the Ordinary Harmala (*Peganum harmala*) in Central Asia Region

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Abstract

Background and Objective: Numerous researches were conducted on the chemical composition of harmala (*Péganum hármala*), but there are no available comparative studies with a focus on methods and ecology of plants. The chemical composition of the thick carbonic acid extract was studied, based on the ordinary harmala. The objective of the study was to observe the ordinary harmala grown in the central Asia region, which was obtained by the maceration extraction method. **Materials and Methods:** For this purpose, the chemical composition of the object was determined using a physicochemical method, Gas Chromatography/Mass Spectrometry (GC/MS). This definition is supplemented by a comparative analysis of extracts (alcoholic and carbon dioxide, respectively sample 1 and sample 2) ordinary harmala by their chemical composition. **Results:** With the above extraction parameter from 2600 g of medicinal plant raw materials, a thick carbon dioxide extract with a total weight of 7.2 g was obtained (samples 1: Alcoholic and sample 2: Carbon dioxide). The yield of the extract is 0.27%. According to the results of carbon dioxide extraction, it was revealed that the most optimal extraction parameter is the extraction parameter of sample 1, due to the high extract yield, 5.1 g (0.19%). **Conclusion:** Based on the results obtained, prove that carbon dioxide extract is the most optimal for the further development of a medicinal product based on an extract of ordinary harmala with anticholinesterase therapeutic effect.

Key words: Alkaloids, carbon dioxide dense extract, harmala, *Péganum hármala*, anticholinesterase action

Citation: Shynykul, Z., 2022. Comparative evaluation of chemical carbon acid extract of the ordinary harmala (*Peganum harmala*) in Central Asia Region. Asian J. Plant Sci., 21: 574-581.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Known in Iran as "Espand" and in North America as "Harmel" and "African Rue", in the USA as "Mexican Rue" or "Turkish Rue", "Arruda of Syria"[1]. In central Asia, ordinary harmala (Péganum hármala) has a completely different name, like "Adyraspan" and mainly grows in the Southern and Southeastern, as well as in small quantities in the Southwestern regions of the Republic of Kazakhstan. The annual stock of ordinary harmala in the Republic of Kazakhstan is approximately 9 tons[2].

Also, studies by Moloudizargari *et al.*[1] confirm the effectiveness of various pharmacological and therapeutic effects of harmala and its alkaloids, especially harmine and also harmaline. Analytical evaluations of the chemical composition of the plant, show that the most important constituents of the plant are beta-carboline alkaloids, such as harmaline, harmalol and harmine. Harmine is the most studied among these naturally occurring alkaloids[1].

A wide range of therapeutic specifies and a large-scale distribution area is the main feature of this medicinal plant. For the same features, the plant is included in several arid floras that spread in the desert, semi-desert areas with high temperatures and dryness[1,2].

The populations of ordinary harmala (*Péganum hármala*) in particular are found in areas with low salt content[3].

An amazing plant of Central Asian origin today can be found in Australia and the southwestern and northern regions of Africa, such as Algeria, Egypt, Libya, Morocco and Tunisia[4]. Another interesting fact is about the unusual type of distribution of the medicinal herb, ordinary harmala (*Péganum hármala*) says that its distribution area is expanding with growth in European countries, in the same way as in Asian countries in the southeastern regions, namely Italy (Apulia and Sardinia)[5].

According to scientific research materials, it became known that the main constituents among phytochemical compounds in the composition of ordinary harmala are alkaloids (more than 10%), anthraquinones and flavonoids[6].

One of the important studies on the chemical composition of ordinary harmala is the work of researchers from Georgia. The results of the study indicate that studies of l-peganin for specific pharmacological activity revealed that the alkaloid, similar to standard peganin, is a pharmacologically active compound and has an anticholinesterase effect. The anticholinesterase activity of d, l-peganin was established in acute experiments on rabbits, in chronic experiments on rats on isolated organs[7].

This was explained by the 14% content of essential oils in the composition of ordinary harmala (*Péganum hármala*), the iodine number of which is 133-135[8]. Also according to Hajji *et al.*[9], *P. hármala* is a source of vegetable oil, the content of which in seeds was at a the considerable level when the extract was analyzed using gas chromatography (GC) and thin-layer chromatography.

Sharifi-Rad *et al.*[10] found that *P. harmala* seeds contain considerable level of oleic (9-octadecenoic acid), linoleic (9,12-octadecadienoic acid), stearic (octadecanoic acid) and palmitic (hexadecanoic acid) acid.

What is characteristic, according to Seilkhan *et al.*[11], signs of resistance to various conditions are ring-shaped sclerenchyma in stems and roots, as well as the presence of two-sided columnar mesophyll in leaves.

In all the above data, the object of the study is a liquid alcoholic extract based on ordinary harmala (*Péganum hármala*), which were obtained by the maceration extraction method.

Classical extraction is a process of processing raw materials with any kind of solvent. In this case, part of the substances with the greatest affinity for the solvent passes into it and a mixture of the solvent and the target component of the extract dissolved in it is obtained. But the solvent used often cannot be completely removed from the obtained extract; in addition, the feedstock undergoes some changes due to the use of chemical solvents, which casts doubt on the "naturalness" of such extracts[12]. The yield of biologically active substances from the feedstock is directly related to the extractant itself and the condition of the extraction process.

So, ordinary harmala or "burial ground" (*Peganum harmala*) is a perennial herb of the Peganaceae family (Engl.) Tiegh. ex Takht., "20-80 cm high, with a strong specific smell"[13,14].

The characteristics and chemical compounds of *P. harmala* have been studied all over the world, quinazoline, beta-carboline alkaloids-peganine/vasicine, desoxypeganine/desoxypeganine (desoxyvasicine/desoxyvasicine), peganine glycoside/peganine glycoside[15].

Totally 105 compounds were identified, the main components of which are oxygenated monoterpene oxygenated sesquiterpenes, alkaloids of quinazoline[16,17].

Peganum harmala used in the preparation of drinks for religious rituals with an effect on the central nervous system as an inhibitor of the enzyme monoamine oxidase, the action of which is due to the presence of harmonic-alkaloids of the β-carboline group and harmaline and harmine are often found in the seeds of *P. harmala*[18].

According to research by Iranshahy *et al.*[19] *P. harmala* EtOHB phytochemical analysis demonstrates the presence of alkaloids, saponins, coumarins, anthraquinones, anthocyanins, anthracene derivatives, monoterpene, sesquiterpenes, diterpenes, triterpenes and tannins (condensed and hydrolyzed). The result is negative for the presence of lignans, naphthoquinones and xanthenes.

A chemical study of the chloroform extract of ripe fruits and flowers of *P. harmala* revealed three alkaloids in ripe fruits and two alkaloids in the flower and leaves of this plant. The alkaloids found in ripe fruits were harmine, peganine and harmaline. Two alkaloids, harmine and peganin, were found in the *P. harmala* flower, while harmaline was found only in ripe fruits[19].

Based on the studies of Shahverdi *et al.*[20], the most common compound found in dichloromethane extract is harmine.

To study the chemical composition of the carbon dioxide extract of ordinary harmala (*Péganum hármala*), the purpose of this work was a comparative study on the composition of harmala grown in Central Asia (Kazakhstan).

MATERIALS AND METHODS

Study area: The present study was conducted during 2020 in the Kazakhstan region, with the assistance of Al Farabi Kazakh National University Laboratories. Samples were selected from different regions of Kazakhstan.

Obtaining a liquid alcoholic extract: A liquid extract of ordinary harmala (*Péganum hármala*) was obtained by the percolation method developed by Gholamhossainian *et al.*[21].

The 55 g of dry, pre-dried grass of ordinary harmala (*Péganum hármala*) was crushed with a universal feed grinder of the KDU-2 brand. The degree of fineness was checked according to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, volume I, through a sieve No. Ethyl alcohol, 76%, 300 mL was chosen as the extractant.

Obtaining a thick carbon dioxide extract of 2600 g of medicinal plant raw material was measured in an ACS System Electronic Scale-150. Then it was crushed and crushed by a universal feed crusher of the KDU-2 brand and the degree of crushing was checked by sifting through a sieve No. 1 (d = 1 mm).

One of the following important technological processes in the preparation of the extractant is carried out directly in the most complex extraction apparatus UUPE-5. Through the condenser from a standard carbon dioxide cylinder with a capacity of 25 L, liquid carbon dioxide GOST 8050-85 is condensed and pumped into the storage tank. The crushed raw materials are placed in the extraction tank and the extractor is hermetically sealed. The system of valves of the installation is opened and carbon dioxide is supplied to the extractor. Flowing through the plant material in it, it washes away the lipophilic component of the plant cell and in the form of a miscella, enters the evaporator. In the evaporation chamber, the process of separating the miscella into extract and carbon dioxide takes place. The released carbon dioxide is pumped again, through the condenser, into the storage tank.

The extraction process is hermetically sealed and takes place within 2-4 hrs, the solvent of the lipophilic part of the cells is food carbon dioxide.

After the expiration of the extraction period, the process is stopped by blocking the circulation of carbon dioxide by a valve system. The 100% extract concentrate accumulated in the evaporator is discharged from the evaporator into a receiving vessel.

The residual gas from the extractor is pumped back into the storage tank. The extractor is unlocked and the used raw materials are replaced with fresh ones.

The main technological parameters of the process:

- Working temperature: t = 21 °C
- Working pressure: 51 atm
- Full cycle of obtaining a thick extract: 9 hrs

Methods for the analysis of the phytochemical composition of extracts of ordinary harmala (*Péganum hármala*)

Analysis methods: Gas chromatography with mass spectrometric detection (Agilent 6890N/5973N) given in Table 1.

Table 1: Analysis conditions of ordinary harmala plant

Sample volume	5 µL
Sample injection temperature (splitless)	260°C
Separation process	Using a chromatographic capillary column DB-WAXetr with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm
Carrier gas velocity He (helium)	1 mL min^{-1}
Chromatographic temperature	From 80°C (exposure 0 min) to 150°C
Heating rate	10°C min^{-1}
Detection mode	SCAN m/z 34-750
Registration and processing of the results	Agilent MSD ChemStation software (version 1701EA)
Interpretation of the obtained mass spectra	Libraries Wiley 7th edition and NIST'02

RESULTS AND DISCUSSION

Obtaining a liquid alcoholic extract: Percolation process lasting 24 hrs a liquid extract of dark emerald (green) colour with a specific pungent odour was obtained. With the extraction parameter from 2600 g of medicinal plant raw materials, a thick carbon dioxide extract with a total weight of 7.2 g was obtained (samples No. 1 and 2). The yield of the extract is 0.27% in Table 2. The pressure was 51 and 45 and extraction temperature was 22 and 19°C, respectively for samples 1 and 2, in process, due to these regulated measures, 5.1 and 2.1% extracts were harvested from samples 1 and 2, respectively.

According to the results of carbon dioxide extraction, it was revealed that the most optimal extraction parameter is the extraction parameter of sample No. 1 (Table 1 and 2) due to the high extract yield, 5.1 g (0.19%).

Table 3 shows chromatographic analysis of the chemical composition of an alcoholic extract of ordinary harmala (*Péganum hármala*), as a routine method for essential plant analysis. This method was also well described in Abd-ElGawad *et al.*[22] for analysis of essential plants specially harmala and the related result was similar with the present profile.

In Table 3, twenty chemical compounds were isolated and identified from the alcoholic extract of ordinary harmala (*Peganum harmala*) According to the results, the most compounds of ordinary harmala seed extract include Bis(2-ethylhexyl) phthalate, Nonacosane9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl), [2R-[2R*(4R*,8R*)], vitamin E, Campesterol, Stigmasterol with more than 1% concentration are detailed in Table 1. Among these components, amounts of 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester and 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]- were above 3%, presented as major components of analysis.

Bis (2-Ethylhexyl) phthalate is mostly common in plastic related components, whereas, it has organic nature and is found in some plants such as harmala and it's considered a toxic component[23,24]. It is visible in low amount in harmala originated from central Asia, according to present finding (Table 3). The abundant fatty acid in the present analysis was hexadecanoic acid with a concentration of 14.9% and it was lower than the report of Moussa and Almaghrabi[3] in a variety of Saudi Arabia. Also, Octadecadienoic acid was detected in

Table 2: Extraction process at different technological parameters

Samples	Extraction weight (g)	Working pressure (atm)	Extraction temperature (°C)	Extraction time (hrs)	Extract yield (g) (%)
1	2600	51	22	9	5.1 (0.19)
2	2600	45	19	9	2.1 (0.08)
∑					7.2 (0.27)

Table 3: Chromatographic analysis of an alcoholic extract of ordinary harmala (*Péganum hármala*)

Retention time (min)	Compounds	Identification probability (%)	Percentage (%)
16.1	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	91	0.9
19.8	Cedrol	87	0.7
21.9	2-Pentadecanone, 6,10,14-trimethyl-	85	0.4
23.8	n-Hexadecanoic acid	87	14.9
26.0	Ethyl Oleate	89	3.2
26.1	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	88	13.2
26.2	9-Octadecenoic acid, (E)-	74	8.3
26.3	9,12-Octadecadienoic acid (Z,Z)-	92	24.2
26.3	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	86	3.6
26.5	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	90	8.9
27.2	Deoxyvasicinone	76	5.8
28.7	4,8,12,16-Tetramethylheptadecan-4-olide	81	0.8
30.1	Butyl 9.cis.,11.trans.-octadecadienoate	75	0.9
30.8	Bis(2-ethylhexyl) phthalate	92	1.2
32.3	Nonacosane	83	1.2
32.6	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	84	3.2
34.5	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	90	3.7
36.4	Vitamin E	89	1.4
38.4	Campesterol	76	1.8
38.8	Stigmasterol	77	1.4

Table 4: Chromatographic analysis of the CO_2 extract of ordinary harmala (*Péganum hármala*)

Retention time (min)	Compounds	Probability of identification (%)	Percentage (%)
25.0	Trans-p-Dimethylamino cinnamonitrile	75	20.3
25.2	Phytol	70	4.4
26.1	Vasicine	78	33.6
27.2	Deoxyvasicinone	72	3.4
28.2	Vasicinone	80	6.3
30.5	Harmine	95	31.9

Table 5: Comparative analysis of phytochemical composition of extracts of ordinary harmala (*Peganum harmala*)

	Main phytochemicals in the extracts of ordinary harmala (*Péganum hármala*)			
	Alkaloids and their derivatives		Terpenoid and diterpene alcohols	
Samples	Title	Percentage (%)	Title	Percentage (%)
Liquid alcohol extract	Deoxyvasicinone	5.8	Cedrol	0.7
Thick carbon dioxide extract	Deoxyvasicinone	3.4	Phytol	4.4
	Vasicinone	6.3		
	Harmine	31.9		
	Vasicine	33.6		

Table 6: Pharmacological activity of phytochemical compounds according to the results of the comparative analysis in the composition of extracts of ordinary harmala (*Peganum harmala*)

BAS	Sample	BAS group	Pharmacological activity
Vasicine	Thick carbon dioxide extract	Quinazoline alkaloids	Anticholinesterase, bronchodilatory, expectorant action
Harmine	Thick carbon dioxide extract	Alkaloids derived from β-carboline	Reversible monoamine oxidase inhibitor and central nervous system stimulant
Vasicinone	Thick carbon dioxide extract	Quinazoline alkaloids	Bronchodilator, expectorant action
Deoxyvasicinone	Both samples	Quinazoline alkaloids	Bronchodilator, expectorant action
Cedrol	Liquid alcoholic extract	Terpenoid alcohol, sesquiterpene series	Antiseptic, anti-inflammatory, antispasmodic and tonic, diuretic, astringent
Phytol	Thick carbon dioxide extract	Diterpene alcohols, monounsaturated	Anti-inflammatory, healing, sunscreen, firming the skin, soothing, rejuvenating

concentration 24.2%, which was somewhat in higher amount when compared with the report of Moussa and Almaghrabi[25], (13.8%). In addition, present findings related to these two major fatty acids of harmala, was completely in accordion with a systematic analysis of Shahrajabian *et al.*[25], which showed these components are major fatty acids of harmala plant originated from the Middle East region and West of China. Also, 9,12,15-Octadecatrienoic acid was detected in the concentration of 8.9% (Table 3) which Shahrajabian *et al.*[25] reported as major fatty acid of harmala. Deoxyvasicinone component which is presented in 5.8% in this analysis (Table 3), can be found in low level whereas it is newly considered as medicinal components for herbal therapy[26].

In the chromatographic analysis of the CO_2 extract of ordinary harmala in Table 4, the percentage and identification probability of phenolic components of harmala plant was identified. According to Table 4, Harmine with 31.9% was the major component in the CO_2 extract of ordinary harmala (*Péganum hármala)* with the greatest probability of identification.

As shown in Fig. 1, with the peaks of the chemical composition of CO_2 extract of harmala (*Peganum harmala*), 20 compounds were identified and most of the harmala seed compounds (Table 3) belong to Stigmasterol the time of 38.8 min and as presented in Table 3 and 4, which detected chemical components are listed base on time and percentage. Stigmasterol was in concentration of 1.4% and this variety was somewhat less than the report of Amariz *et al.*[18] with gas chromatography method.

The gas chromatogram shows the high currency of analyzed data in Table 3 and 4, based on findings of Amariz *et al.*[18] and Moussa and Almaghrabi[3], gas chromatography is useful and accurate method for detection of fatty acids of harmala plant extract.

Phytochemical composition of extracts of ordinary harmala (*Peganum harmala*) in "Alkaloids and their derivatives" and "Terpenoid and diterpene alcohols" are presented in Table 5. Phytol is the major component (4.4%) in the alcoholic part (Terpenoid and diterpene alcohols) and Vasicine (33.6 %) is the major component of the alkaloid part (Alkaloids and their derivatives).

In Table 6, the pharmacological activity of phytochemical compounds has been presented based on comparisons between two samples liquid alcoholic extract and thick carbon dioxide extract.

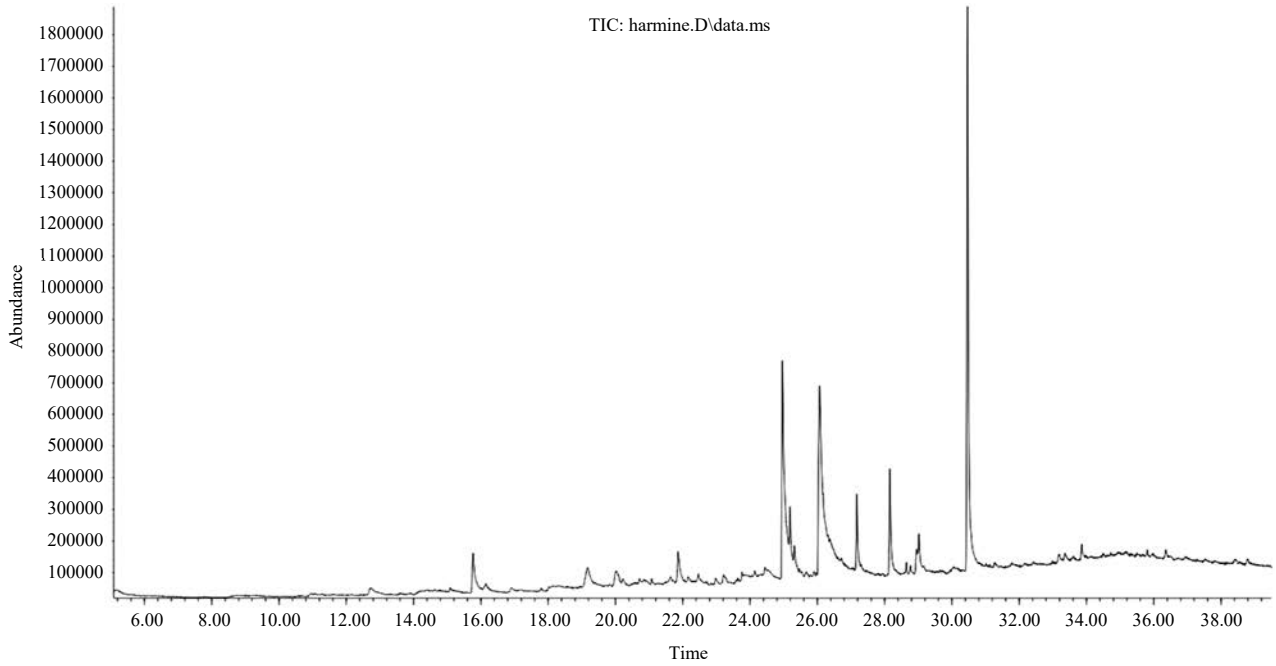

Fig. 1: Chromatogram of the analysis of CO_2 extract of ordinary harmala (*Peganum harmala*)

Also, pharmacological usages and specifies of each component are described. In both samples (liquid alcoholic and thick carbon dioxide-extracts) quinazoline alkaloids were presented as detected major pharmacological components.

In general, these main results (Table 3-6 and Fig. 1) are consistent with the study, showing that, in terms of its phytochemical composition, dense carbon dioxide extract is most effective as a valuable product for further development of pharmaceuticals with various therapeutic effects. These results are in agreement with Abbas *et al.*[26], who showed the efficiency of these components as anti-inflammatory and antioxidant agents. In Roselló-Soto *et al.*[27] suggested that optimum conditions for extracting the highest amount of flavonoids from harmala have occurred with 66.79°C and 32.41% ethanol density, also the ratio of solvent to plant, was 27.81 mg g^{-1} and the extraction time was 76.31 min. In the present study, the extraction with a similar percentage of the extract was considerable less than Roselló-Soto *et al.*[27]. In Table 3, the related value for the time was 30.5 min and the percentage was 31.9. This means extraction with CO_2 method is a very efficient method for extraction of phenolic components from harmala plant.

Present analysis (Table 4-6) show potential therapeutic components of harmala including phytopreparations with anticholinesterase action, which currently have a high level of demand among the population (Table 6). Present findings, which are presented in Table 6 also reported in Vietnam-variety of Harmala by Nguyen *et al.*[28].

The anticholinesterase effect is manifested by the alkaloid vasicine (*L-peganine*), as previously mentioned in the studies of the authors Doskaliyev *et al.*[29].

In a comparative analysis (Table 1) of two completely different extracts based on the same raw material, ordinary harmala (*Peganum harmala*), it can be noted that the yield of the largest amount of biologically active substances is observed in a thick carbon dioxide extract. The present observation was in agreement with Nasseh *et al.*[30], who assessed ion absorption in carbon synthesis from Harmala. Present finding related to the largest amount of biologically active substances (Table 1 and 2), also in agreement with Shao *et al.*[31] great concentration of active phenols could inhibit the growth of some bio-organism and seeding of some plants.

Based on current attempts for optimization of excretion methods for harmala, which reviewed by Asghar and Abooali[32]. The same finding can be a rationale for optimizing the extraction process, as a completive finding. In addition, the findings of the present study are related to the CO_2 extraction method efficacy is applied method as a green technique for nanoparticles preparation of bioactive components of harmala.

CONCLUSION

Based on the obtained results, present research proves that carbon dioxide extract is the most optimal for the further development of a medicinal product based on an extract of ordinary harmala (*Peganum harmala*) with an anticholinesterase therapeutic effect. In addition, this method is most efficient for the detection of bioactive components of local herbs for possible use in pharmacology and herbal therapy. In addition, this variety of harmala (Kazakhstan) has similar fatty acids components of harmala plant originated from Middle East region and West of China, which studied in kinds of literature.

SIGNIFICANCE STATEMENT

The present study showed an effective method for analysis of chemical compositions of Harmala plant, based on plant origin. Carbon dioxide extract is the most optimal for the further development of a medicinal product based on an extract of ordinary harmala with anticholinesterase therapeutic effect.

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