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Original article

Determination of Scientific Name of Bitter "Qust": an Important Controversial Plant Source in the Iranian Medicinal Plants Market for Neurological Complications

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Abstract

Background and objectives: Traditional medicine could provide a hopeful area of research to mitigate the suffering of patients. "Qust" is one of the medicinal plants that are mentioned in Persian Medicine (PM) for treatment of neurological diseases**.** There is diversity within the scientific name of "Qust" in different references. Some have introduced *Saussurea costus* (Falc.) Lipsch. (Asteraceae), while others have presented *Costus speciosus* (J. Koenig) Sm. (Costaceae) as "Qust". Since "Qust" is not endemic in Iran, there is difficulty to access to the whole plant for its identification. Hence, this study has aimed to identify available bitter "Qust" which is composed of roots of the plant in the Iranian market. **Methods:** Macroscopic characters and microscopic properties of powders and transverse sections of specimens with essential oil analysis of the Indian and one of the Iran herbal market samples using chromatography-mass spectrometry (GC-MS) were investigated for identification of bitter "Qust". **Results:** Microscopic evaluation showed presence of secretory cavities and their specific size, narrow radial rows of conducting tissue alternating with broad medullary rays in the secondary phloem and xylem, presence of inulin, absence of starch and calcium oxalate crystals in the bitter "Qust" particles. Further, positive response was observed to *S. costus* identifying test. In the analysis of essential oils, active components of *S. costus*, such as dehydrocostus lactone, were identified in the examined essential oils. **Conclusion:** According to the results, it could be concluded that bitter "Qust" in Iran herbal market most probably is *S. costus*.

Keywords: *Costus speciosus*; Persian medicine; Qust, *Saussurea costus*; scientific name

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Introduction

Persian medicine (PM) is a complete medicinal system with a plenary viewpoint of the human being. Disease prevention and treatment of different disorders are the two main principles of this system. The first step in the treatment of diseases is correction of the lifestyle, especially dietary regimes. Pharmacotherapy is the second step and medicinal plants are the most important agents used in this segment of the therapy [1]. Nowadays we have some problems with identification and determination of the scientific name of medicinal plants mentioned in ancient

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medical texts. These problems include alternatives, adulterations, and wrong or incomplete descriptions of medicinal plants that have been entered in some texts of ancient books due to the lack of accurate identification of the plants and trans-multiple translations over the years [2]. Therefore, it is necessary to recognize medicinal plants of ancient medical books and determine their scientific names.

"Qust" is one of the valuable medicinal plants that has been used in some traditional medical systems like Persian and Indian medicine [3,4]. "Qust" is a special plant because it can be applied in treatment and control of neurological diseases, one of the associated problems with today's medicine [5]. Complications of the neurological disorders involve patients in all ages around the world. Traditional medicine is a hopeful way of research for cure or relief the problems of these patients. Treatment of headache, convulsion, tremor, paralysis, amnesia, joint pain, gout, swelling of the spleen and amenorrhea are part of the medical benefits of the plant [3,4].

One of the main problems is the diversity within the scientific name of "Qust" in different references. Some of the references introduce *Saussurea costus* (Falc.) Lipsch. from Asteraceae family as "Qust" and others introduce it as *Costus speciosus* (J. Koenig) Sm. from Costaceae family [6,7]. Crêpe ginger is the English name and "Keukand", "Keu", "Kust" (Hindi), "Pushkarmula" (Marathi), "Kustha" (Sanskrit) and "Kostam" (Tamil) are other common names of *C. speciosus* [8]. *Saussurea costus* is commonly known as costus in English and "Kut", "Kur", "Postkhai", "Sepuddy", "Kot", "Kushta", "Kostum", "Kustam", "Kushta", "Koshta" and "Kuth" in India [6].

Saussurea costus is one of the most important species in the genus *Saussurea* DC. with multiple therapeutic applications in ancient systems of medicine, such as Unani, Ayurveda and Siddha. The plant is native to India, Pakistan and China, and grows in the Himalaya region [9]. Its major components are sesquiterpene lactones, such as custonolide and dehydrocostus lactone.

Costus speciosus is a medicinal and ornamental plant distributed in the moist areas of the Indo-Malayan region and the foothills of the Himalayas in India [10] It is a commercial source of diosgenin [11]; also, Sesquiterpenoids like costunolide and eremanthin have been isolated from hexane extract of *C. speciosus* [12].

Since, the whole plant of bitter "Qust" is not accessible in Iran and several roots with similar external appearance are presented in markets, evaluation of macroscopic and microscopic characteristics of the samples could be helpful in recognition of herbal resources. The present study has aimed to identify the scientific name of bitter "Qust" which has been used for treatment of various neuromuscular diseases in PM.

Material and Methods

Plant material

Four dried root specimens of bitter "Qust" (Sample 1-4) were purchased from various major suppliers of medicinal plant markets in Tehran, Iran during summer 2016. The bitter "Qust" of Iran market is imported from India and Pakistan. A high quality standard specimen of bitter "Qust" was palso urchased from India (Delhi medicinal plant market) for comparison with specimens of Iran market and to reach judgment about their different or similar characteristics.

Macroscopic and microscopic analysis

The organoleptic characteristics of "Qust", including shape, size, colour, taste and odour were determined in macroscopic investigation [13]. In the next step, a part of dried roots was powdered for microscopic analysis and identification tests and another part was sectioned for completion of microscopic analysis and size determination of secretory cavities. All macroscopic and microscopic features of samples were compared with Indian sample and monograph of *S. costus* in references [14, 15].

To investigate microscopic properties, one g of each sample was separately prepared based on the explained process [16]. Aqueous glycerine was applied for conservation of treated specimens. A Carl Zeiss Standard 14 Laboratory Microscope (Germany) and a digital camera were used to take photomicrographs.

To prepare transverse sections of "Qust", dried roots of all samples were macerated separately in distilled water, ethanol 70% and glycerine for 24 h; afterwards, the soaked roots were boiled with distilled water for 3 h and sectioned using a blade.

Identification and inulin test

An identification test of *S. costus* was introduced in the monograph of Japanese pharmacopeia. For the procedure, 0.5 g of pulverized sample was warmed with 10 mL of ethanol 95% for 1 min, and subsequently cooled and filtrated. One mL of the filtrate was shaked with 0.5 mL of hydrochloric acid. Formation of purple colour demonstrated that the sample was the root of *S. costus* [14].

Inulin is a storage polysaccharide in the roots of some monocotyledons and members of the Asteraceae family such as *S. costus* [15] which is a new source of inulin [6]. For microchemical test of inulin, a drop of thymol 15% and concentrated sulphuric acid were added to freehand sections of the roots. A bright red colour served as evidence of presence of inulin in the plant [17].

Extraction and analysis of essential oil

The essential oils of two "Qust" specimens, one from Iran market with the most similarity in macroscopic and microscopic characteristics to those of Indian sample, and the Indian sample were extracted and analysed. The powders of roots (100 g) were subjected to a Clevenger apparatus for 6 h at room temperature. The obtained essential oils were separately stored in dark sealed vials in the refrigerator prior to analysis by GC-MS.

Chemical ingredients of the essential oils were determined by an Agilent Technologies Gas chromatography device connected to Mass system with DB-5 fused silica column (30 m×0.25 mm; 0.25 µm film thicknesses). The oven temperature was held at 50 ºC for 5 min, then programmed at 10 ºC/min to 280 ºC and held for 5 min. The flow of helium gas was 1 mL/min and the injector and detector temperatures were 280 ºC. One µL of diluted essential oil in hexane was injected into the system. Ion source temperature was 150 °C and scan mass range of m/z was 50-550. The compounds were identified by comparison of their mass spectra with the Wiley libraries and retention indices with those reported in the literature [18-20].

Results and Discussion

Macroscopic features of four bitter "Qust" specimens of Iran herbal market were compared with both Indian "Qust" and monograph of *S. costus* root (figure 1). Roots of Indian "Qust" were firm, solid, non-fragile, curved with wrinkled skin and radicles. They had 6 to 14 cm length and 0.5 to 2 cm diameter, grey to brown crust and yellow inner part. Their taste and odour were bitter and spicy. The tested samples were largely similar to Indian sample. They were firm, solid, non-fragile, curved with wrinkled skin, too, and contained radicles in sample 4 and no radicles in Samples 1, 2 and 3. Their size varied between 4.5 to 7.3 cm in length and 1.3 to 3.5 cm in diameter. They were grey or brown in outer part and had a lighter inner part. All samples had a range of spicy and bitter taste with spicy odour, which was stronger in samples 1 and 2, and was similar to lanolin (wool wax) in sample 4. These macroscopic features of samples conformed to the monograph of *Saussurea* root [14].

Images of investigated samples were compared with images of Indian sample and monograph of *S. costus* in American herbal pharmacopeia (AHP) [15]. The transverse section of *S. costus* root represented conducting tissue in narrow radial rows with broad medullary rays in the secondary phloem and xylem, in which secretory cavities were scattered and a white wavy line in the middle showed the vascular cambium [14]. This specific overview of root and secretory cavities were clearly seen in the transverse section of the Indian sample. Also, all samples of the Iranian herbal market (1- 4) showed this schematic image of root and secretory cavities. Samples 3 and 4 were more similar to *S. costus* monograph in AHP and Indian sample (figure 2).

Sample 4 was the most similar one to the section of *S. costus* in AHP and Indian sample because of narrow radial rows of conducting tissue and secretory cavities arranged in central and peripheral part of the section, therefore, it was selected for GC-MS analysis.

Figure 1. Morphological characteristics of "Qust"; four Iran herbal market's samples (1- 4) and Indian sample (5)

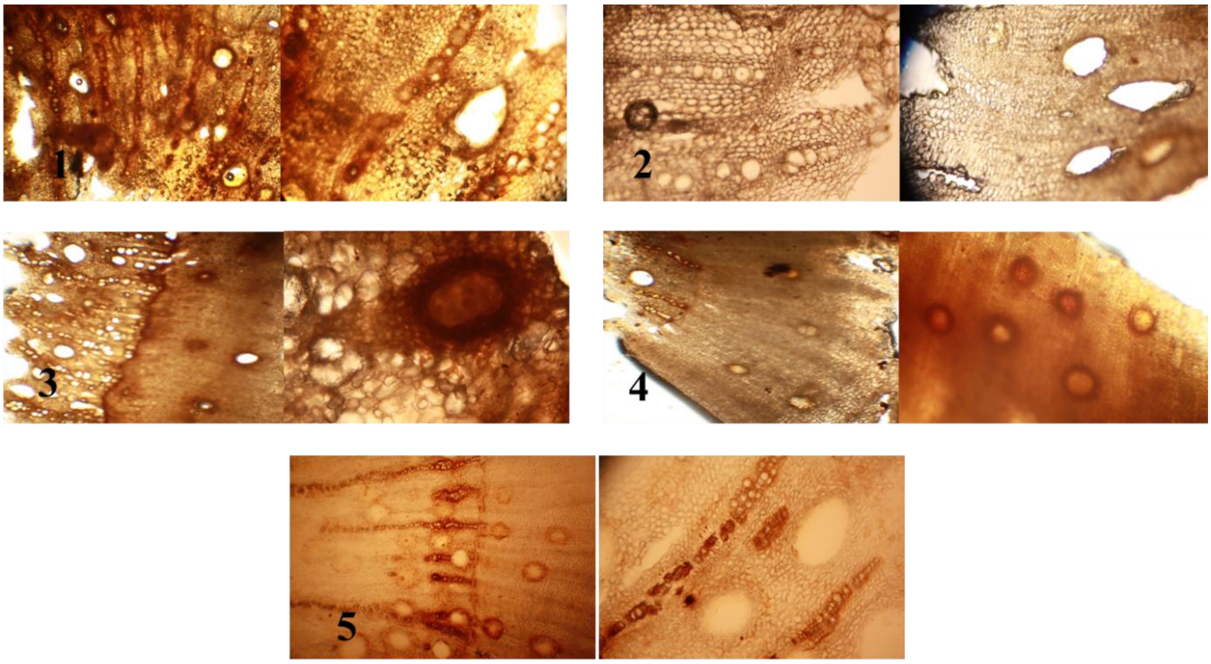

Figure 2. Transverse sections of four bitter "Qust" samples in Iran market (1- 4) and Indian sample (5) showing secretory cavities and conducting tissue in narrow radial rows with broad medullary rays in the secondary phloem and xylem

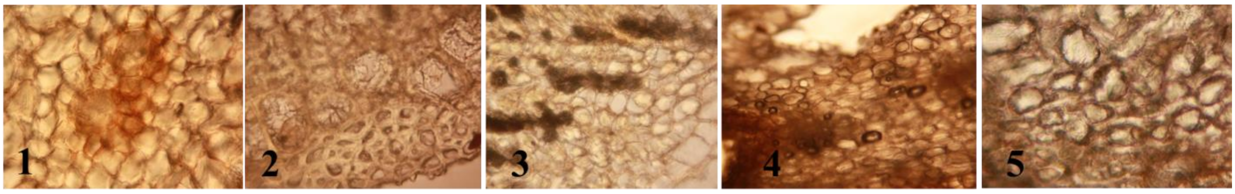

Figure 3. Inulin in four samples of Iran herbal market (1- 4) and Indian sample (5)

Inulin was identified in the transverse section of the Indian samples and all specimens of Iran herbal market as well as in section of *S. costus* as described in AHP monograph (figure 3).

The size of secretory cavities is one of the noteworthy recognition factors in some medicinal plants and their adulterants.

In *S. costus*, the size of the secretory cavities is up to 400 µm diameter, whereas in *Arctium lappa* L. (Asteraceae), an adulterant of *S. costus*, the diameter is 40 µm [15].

In the samples of Iranian herbal market and Indian sample, the diameter of these cavities was measured between 140 and 380 µm. Hence, the measured sizes of the secretory cavities in tested samples were in the range of those in *S. costus*.

Nonexistence of starch granules as well as their structures are useful diagnostic characteristics in identification of "Qust". Root of *C. speciosus* is rich in starch, whereas inulin is the primary storage substance in the roots of members of Asteraceae family, such as *S. costus*, while starch granules were not seen in the plant [15,21]. Iodine test showed that none of the Indian sample and 4 specimens of Iran market contained starch and, therefore, had no relationship with *C. speciosus*.

Crystals of calcium oxalate are another distinctive microscopic difference between *S. costus* and *C. speciosus*. They are common in the ground parenchyma cells of *C. speciosus* rhizomes, while are absent in *S. costus* [8]. No calcium oxalate crystal was found in microscopic investigation of Indian sample and four samples of the Iranian market's powders before and after sulphuric acid test.

Lipids, volatile oils and resins will stain orange-red with Sudan solution. Thus, this chemical test has been accomplished as a confirmatory test since *S. costus* contains essential oils and resins [22]. Contrary to lipids and fixed oils, volatile oils and resins are soluble in alcohol [15].

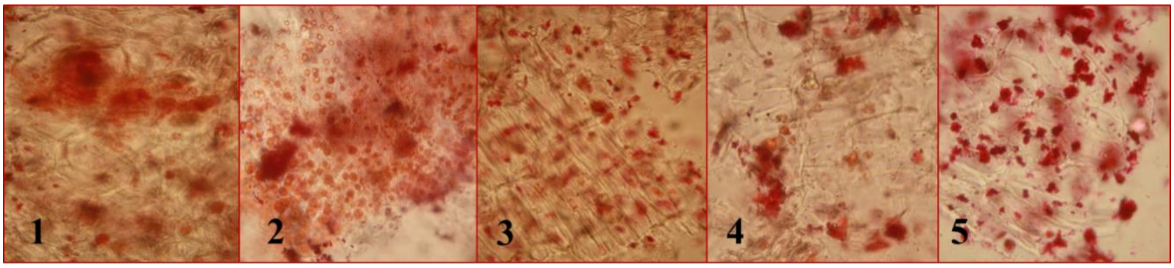

Figure 4. Volatile oils and resins in powder of four Iranian market samples (1- 4) and Indian sample (5) after adding Sudan solution

The Indian sample and all tested specimens showed orange-red colour with Sudan solution. The colour was lost after adding ethanol; therefore, the tested samples probably contained volatile oils and resins (figure 4).

Microscopic analysis of powder of investigated samples revealed presence of various herbaceous cells and vessels, since they are not particular characteristics for the plants, they were not helpful to deduce.

The Indian sample and all the specimens of “Qust” showed purple colour in the identification test of *S. costus*, so, according to Japanese pharmacopeia, they can be *S. costus* [14]. The bright red colour was seen in the inulin test of Indian sample and each one of Iranian market’s specimens confirming that all of them contain inulin [17].

The Indian sample and the most similar sample to Indian sample (No. 4) were selected for GC-MS analysis of essential oils; they respectively yielded 0.37% and 0.5% bright yellow essential oils. Thirty-tree compounds (90.47%) were identified in the essential oil of the Indian sample and thirty-one components (90.40%) in the essential oil of Iranian market specimen. Retention time values of these compounds began from 14.48 and 14.50 min, related to thymol, and they were terminated at 29.55 min related to dehydrocostus lactone and 30.89 min for costunolide in Indian and investigated sample, respectively. 1,3-Cyclooctadiene (19.27%), elema-1,3,11(13)-trien-12-ol (10.03%), beta-elemen (8.84%) and dehydrocostus lactone (8.41%) were major components of the Indian sample. Similarly, the major components of the Iranian market specimen (sample 4) were dehydrocostus lactone (17.73%), 1, 3-cyclooctadiene (16.10%) and elema-1, 3, 11(13)-trien-12-ol (11.56%) (table 1).

“Qust” is one of the special medicinal plants with neuromuscular effects [3-5]. The nonexistence of the exact morphological description of the plant in ancient medical books, long medical history, replacement of the plant with other medicinal plants by the local people, various similar species, many different common names and variations in botanical origins result in no confidence about the scientific name of some medicinal herbs like “Qust” [23]. Similar to Iran medicinal plant market, some kinds of “Qust” consisting of bitter, Indian or black “Qust” and sweet, Arabic or white “Qust” are introduced under “Qust” monographs in traditional medical text of Persian medicine [4,5].

Different species of genus *Costus* are introduced as “Qust” in various references and *C. speciosus* is the most important one [8]. *Costus speciosus* is a member of the Costaceae family and order of Zingiberales from monocotyledons and so has many morphological differences with *S. costus* related to the Asteraceae family and eudicotyledons plants [7,9]. The application of roots as medicinal parts in both of these plants and no access to the whole plant means that there is no clear separation between market’s specimens of these two medicinal herbs.

Common microscopic features of transverse section, absence of starch and calcium oxalate that are present in rhizomes of *C. speciosus* and also positive response to inulin and identification test of *S. costus* led to assume that bitter “Qust” can be *S. costus*. For complete conclusion, essential oils of the best quality specimen of Iran market and Indian sample were compared. Chemical constituents and retention time of both investigated samples were similar to each other with same major components of dehydrocostus lactone, 1, 3-cyclooctadiene and elema-1,3,11(13)-trien-12-ol. Costunolide was identified in the market specimen, though it was not seen in Indian sample.

Table 1. GC-MS analysis of the Indian "Qust" (1) and one of the Iran herbal market "Qust" (No. 4) (2) essential oils

No	Component	KI_1	KI_2	KI_r	Relative content$_1$ (%)	Relative content$_2$ (%)
1	Thymol	1255	1256	1290	3.23	1.07
2	Carvacrol	-	1274	1298	-	0.70
3	beta-Elemen	1354	1354	1382	8.84	5.90
4	trans-Caryophyllene	1403	1402	1409	5.34	4.37
5	alpha-Ionone	1417	1417	1426	2.33	1.67
6	trans- alpha -Bergamotene	1425	1425	1434	0.60	0.53
7	alpha-Caryophyllene	1433	1433	1449	0.64	0.45
8	Geranylacetone	1447	1447	1453	0.89	0.54
9	beta-Selinene	1478	1478	1481	3.23	3.15
10	alpha-Curcumene	1481	1481	1485	2.33	2.93
11	alpha-Selinene	1482	1482	1494	1.08	1.43
12	Zingiberene	1486	-	1495	0.43	-
13	Cetene	1487	1487	-	0.44	0.38
14	cis- gamma-Bisabolene	1494	1494	1515	0.47	0.48
15	beta-Sesquiphellandrene	1499	-	1543	0.27	-
16	Elemol	1509	1509	1547	1.81	2.70
17	gamma-Eudesmol	1595	1595	1630	0.31	1.01
18	beta-Eudesmol	1622	1622	1649	0.63	1.14
19	alpha-Eudesmol	1632	1630	1652	1.08	1.44
20	Elema-1,3,11(13)-trien-12-ol	1649	1648	-	10.03	11.56
21	Bicyclo[10.1.0]tridec-1-ene	1658	1658	-	2.03	1.61
22	1,3-Cyclooctadiene	1667	1665	-	19.27	16.10
23	Cyclododecene, 12-methyl-1-(1-propynyl)	1682	1682	-	0.52	0.36
24	(Z)-cis- alpha-Bergamotene	1686	1685	-	1.19	0.52
25	alpha-nor-Cholest-16-en-ol	1702	1702	-	0.85	0.17
26	(Z)- alpha-trans Bergamotol	1711	1711	1693	1.03	0.30
27	caryophylla-3,8(13)-dien-5 alpha-ol	1719	-	-	0.68	-
28	(+)-gamma Costol	1753	1753	-	1.43	1.18
29	Alloaromadendrene oxide-(2)	1767	-	-	0.32	-
30	Valerenol	1776	1775	1699	6.46	5.28
31	(-)-alpha-Costol	1783	1783	-	4.15	3.74
32	Germacra-1(10),4,11(13)-trien	1849	1859	-	0.16	1.41
33	2(3H)-Benzofuranone	1920	1918	-	0.50	1.41
34	Dehydrocostus lactone	1958	1961	-	8.41	17.73
35	Costus lactone (costunolide)	-	2028	-	-	0.34
	Unknown	-	-		9.02	8.40
	Total identified	-	-		90.98	91.60

KI_1: Kovats index of essential oil constituents of Indian "Qust"; KI_2: Kovats index of volatile constituent of Iran herbal market "Qust"; KI_r: Kovats index reported in databases

However, germacra-1(10), 4, 11(13)-trien, an intermediate compound in costunolide biosynthesis, was identified in Indian sample [24]. Further, the compositions of essential oil of bitter "Qust" were similar to some scientific reports about chemical constituents of *S. costus*. In a recent Chinese study, dehydrocostus lactone and costunolide were reported as the major components of *S. costus* essential oil [22].

A part of chemical constituents identified in bitter "Qust" essential oil in this study, such as α-selinene, β-selinene, α-costol, elemol, α-ionone, β-elemene, α-trans-bergamotene, α-costol, γ-costol, elema-1,3, 11 (13)-trien-12-ol, γ-costal and curcumene were reported as essential oil's components of *S. costus* in the previous studies, [25,26].

Content of some essential oil of *S. costus* look different in different studies that may be probably related to differences in harvesting time, climatic and seasonal conditions, growing locations, and environmental factors like relative humidity, temperature, photoperiod and irradiance along with diversity in storage duration of medicinal plants [9,22]. It seems that differences between the yields of essential oils and contents of dehydrocostus lactone and costunolide in two investigated samples were due to differences in the age of their respective plants; the considerable likeness of the two samples' essential oil components and their retention times were indicative of their probable similar growing place.

Some identical pharmacologic effects like anti-inflammatory, antimicrobial, hepatoprotective, hypolipidaemic, hypoglycaemic, spasmolytic, analgesic and antioxidant activities have been proven in *S. costus* and *C. speciosus* and this similarity can be a reason for application and replacement of these herbs instead of each other [6,7,12,23,27]. Sesquiterpene lactones such as costunolide are common in the Asteraceae family and *S. costus*, but have been separated from some other medicinal plants like *C. speciosus* too [28]; so, it is probable that these active components are responsible for similar pharmacologic effects in *S. costus* and *C. speciosus*.

According to the macroscopic and microscopic properties with chemical tests and identification of dehydrocostus lactone and other specific constituents in the essential oil of both samples (Indian specimen and Iran herbal market sample), it could be proposed that the Iranian herbal market’s specimen is similar to the Indian sample of India with scientific name of *S. costus*.

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Author contributions
Nastaran Ebadi, Sahar Bagheri, Azadeh Manayi and Zahra Niktabe contributed to the design and performance of the research and to the writing of the manuscript. Mehran Mirabzadeh Ardakani, Tayebeh Toliyat, Sima Sadrai and Malihe Tabarrai supervised the project.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

PM: Persian medicine; AHP: American herbal pharmacopoeia