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Original article

Preparation of *Saussurea costus* Traditional Oil and Investigation of Different Parameters for Standardization

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Abstract

Background and objective: Medicinal oils are one of the most common and special dosage forms in oral and topical therapies of Persian medicine (PM). The oil of *Saussurea costus* (bitter qust) root is prominent topical oil with different applications in PM. In this study, the oil of bitter qust was prepared according to ancient Persian medical texts. **Methods:** To prepare traditional qust oil, 100 g of the root was soaked in 600 mL aqueous ethanol 25% overnight. The supernatant was then filtered and boiled in 800 g sesame oil until all water was evaporated. The essential oil of the root and volatile components of its traditional oil were extracted using hydro-distillation method in a Clevenger-type apparatus and were analyzed by gas chromatography-mass spectrometry (GC-MS) method. Total phenolics, flavonoids, tannins and polysaccharides were determined by spectrophotometric methods to evaluate the chemical parameters of traditional bitter qust oil. **Results:** The content of volatile compounds in both investigated samples was determined (0.5% and 0.1% (v/w), respectively). Dehydrocostus lactone and 1, 3-cyclooctadiene were two similar main compounds in the both analyzed samples. Total phenolics (788.290±0.61 mg/L gallic acid equivalent (GAE)), flavonoids (303.2±2.52 mg/L catechin equivalent (CE)), tannins (23.97±0.52 mg/L GAE) and polysaccharides (9.240±0.13 mg/L dextrose equivalent (DE)) contents were determined. **Conclusion:** According to the obtained data, dehydrocostus lactone could be used for determination and evaluation of traditional bitter qust oil.

Keywords: bitter qust oil; costus root; Persian medicine; *Saussurea costus*; traditional oil

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Introduction

Oils are one of the oldest dosage forms in ancient medical systems such as Persian medicine (PM) [1,2]. Persian medicine is a prominent, popular and historical medicinal system similar to other various traditional systems of medicine like Chinese medicine, Ayurveda and Homeopathy [3]. PM encompasses two fundamental parts disease prevention in the first step and treatment of different disorders at the next stage [4]. Medicinal herbs are mainly recruited in PM to treat diseases [5] while oils are one of the most common preparations [6]. Various topical and systematic applications of herbal oils have been introduced in PM medical texts [5,7]. Traditional

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oils are called "Adhan" (singular form: “Dohn”) in pharmaceutical books of PM and their therapeutic usage and preparation procedures have been explained in detail [8].

More than thirty species of plants have been introduced in PM to prepare herbal oils. These drugs were administered in various diseases especially arthritis, sciatica and muscle aches [9].

Traditional oils are divided into two categories; those which are taken directly from the oily parts of the plants (sesame seed, olive fruits) and the others obtained from extraction of non-oily parts of the plants in oil vehicle that causes trapping of hydrophobic and hydrophilic agents in the vehicle [1,2,10,11]. The mentioned oils are both categorized as fixed oil. *Saussurea costus* (Falc.) Lipsch. (bitter qust) oil is an example of the second mentioned category that has different therapeutic applications in the traditional medicine of Iran and other countries such as India and China [12].

Saussurea costus, is one of the main species of the genus *Sausssurea* (Asteraceae family) [13]. The reported active ingredients of this well-known medicinal plant are mainly terpenes, while different amounts of flavonoids, anthraquinones, alkaloids, tannins and inulin were reported in the previous studies from the plant [14-16]. Sesquiterpene lactones, such as custonolide and dehydrocostus lactone are the major components of *S. costus* with several pharmacologic effects including anti-inflammatory, antiulcer, anti-cancer and hepatoprotective activities which were demonstrated in the various experiments [14].

The present study has aimed to prepare traditional bitter qust oil (TQO) based on traditional methods of Persian medicine and determine some active ingredients of the oil to conduct the oil standardization. Further, chemical composition of essential oil of the plants root was analyzed, since relatively non-polar constituents of the essential oil could trap in the fixed oil during preparation of TQO. As it has been mentioned before [1,2,11], other hydrophilic compounds like phenol, tannins, flavonoids and polysaccharides may be extracted in traditional oils, therefore these compounds were detected in the TQO.

Material and Methods

Plant material

Roots of *S. costus* were purchased from herbal market (Tehran, Iran, 2016) and identified at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran with the voucher number of PMP-240.

Chemicals

All chemical substances were of analytical grade and obtained from Merck Company (Germany).

Preparation of TQO

To prepare TQO, 100 g of plant's root coarse powder was soaked overnight in 600 mL aqueous ethanol 25%. The supernatant was then filtered using whatman filter paper (No.1, Sigma-Aldrich, Germany) and boiled in 800 g sesame oil (Omid Company, Iran) until all water was evaporated [5,6].

Extraction and analysis of essential oils

The oil from the powdered roots of the plant (100 g) and TQO (100 mL) were extracted using hydro-distillation method in a Clevenger type apparatus for 4 h at room temperature. The obtained volatile fractions were separately collected and dried using sodium sulphate anhydrous [17].

Gas chromatography-mass spectrometry (GC-MS)

The volatile compounds were analyzed using an Agilent Technologies Gas chromatography device connected to Mass system (Agilent, USA) with DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 50 ºC for 5 min and raised to 280 °C at a rate of 10°C/min. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were 280 ºC. 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.

Methanol extraction of the TQO

The methanol extraction of TQO was needed for further experiments. TQO (10 mL) was mixed with methanol (10 mL) and after complete mixing, the methanol phase was separated by decantation funnel, the procedure was repeated three times to optimize extraction. The obtained methanol phase was then centrifuged for 8 min at

4000 rpm (Megafuge 1.0, Germany) to remove oil droplets and the remained methanol was removed using rotary evaporator (Heidolph, Germany). The extraction yield was 1.1% and the concentration of 1 mg/mL in methanol was used for analysis.

Quantification of total flavonoids content

Determination of total flavonoids was based on complex creation by the AlCl3 in a methanol medium [18]. For the measurement, sodium nitrate (0.15 mL, 5% in water) and $AlCl_3$ (0.15 mL, 10% in methanol) were added to 1 mL of the methanol extract of TQO and standard solutions of catechin (50, 75, 125, 150 µg/mL). Sodium hydroxide 4% (2 mL) was then added to the experimental system after 6 min and the volume reached to 5 mL with distilled water. Eventually, passing 15 min, the absorbance was measured at 510 nm by UV-visible spectrophotometer (Camag, Korea).

Quantification of total polysaccharide content

Inulin is a primary storage polysaccharide in the roots of Asteraceae members and some of monocotyledons [16]. *Saussurea costus* is one of the sources of inulin therefore, polysaccharide determination is a useful test for the TQO. The procedure was done according to a previous study [19]. The methanol extract of TQO (1 mL) was mixed with phenol 4% (0.5 mL) and sulfuric acid 96% (2.5 mL) that breaks all the glycoside linkages. After 5 min, absorbance of colored aromatic complex that achieved between phenol and the carbohydrate was measured at 490 nm using UV-visible spectrophotometer and was compared to absorbance of dextrose (repeated unit in the structure of inulin) standard solutions (0.1, 1, 10, 100 µg/mL).

Quantification of total phenolics and tannins contents

Determination of total phenols and tannins was performed using Folin-Ciocalteu reagent [20]. In the first step, 0.1 mL of the methanol extract of TQO and gallic acid standard solutions (50-200 µg/mL) were mixed with distilled water (0.4 mL), Folin-Ciocalteu (0.25 mL) and sodium bicarbonate 20% (1.25 mL); after 40 min the absorbance of the sample was measured at 725 nm. In the second step, the methanol extract of TQO (1 mL) was vortexed with powdered polyvinyl poly pyrrolidone (100 mg), and distilled water (1 mL). The mixture was refrigerated for 15 min at 4 °C. Then it was centrifuged for 10 min at 3000 rpm to precipitate tannins. The supernatant (0.2 mL) was mixed with distilled water (0.3 mL), Folin-Ciocalteu (0.25 mL), sodium bicarbonate (1.25 mL) and the absorbance of sample was measured at 725 nm after 40 min. The total tannins content was estimated with subtracting the first and second absorption values.

Statistical analysis

All tests were performed in triplicate and the data were reported as mean±standard deviation (SD).

Results and Discussion

The TQO of the *S. costus* root was successfully prepared from aqueous ethanol (25%) extract of the plant regarding the Persian medicine instructions. According to the Persian traditional text, by evaporation of the aqueous phase, the constituents of the extract would be trapped in the sesame oil (vehicle) [1]. The prepared TQO was subjected to essential oil extraction which yielded 0.1% v/w volatile oil. The essential oil of *S. costus* roots was subsequently extracted to yield bright yellow color oil (0.5% v/w).

The data from GC-MS conduced to the identification of more than 90% of the oils chemical compounds. The essential oil of the root contained thirty-one compounds of which, ten chemicals were also identified in the TQO (table 1). The predominant compounds in the root oil of the plant were dehydrocostus lactone (17.73%) and 1, 3-cyclooctadiene (16.10%). The main constituents of the TQO were thymol (14.44%), 1, 3-cyclooctadiene (14.34%) and dehydrocostus lactone (6.77%). There were other compounds identified just in TQO which were derivatives of fatty acids and probably relevant to the sesame oil (47.9%). Although, both investigated oils contained monoterpenes and sesquiterpenes, the essential oil of bitter qust root (65.57%) was much more reach of sesquiterpenes comparing to the TQO (12.94%); however, percentage of monoterpenes in TQO (14.88%) was higher than the oil of root plant (4.28%). The other class of compounds identified in the oils was hydrocarbons, 20.30% and 15.26% in root oil and TQO, respectively.

Table1. Chemical composition of essential oils of *Saussurea costus* root and traditional qust oil (TQO)

Identified compounds	KI_1	KI_2	KIr	% in root oil	% in TQO
Thymol	1256	1249	1290	1.07	14.44
Carvacrol	1274	-	1298	0.70	-
beta-Elemen	1354	1345	1382	5.90	2.79
trans-Caryophyllene	1402	-	1409	4.37	-
alpha-Ionone	1417	1390	1426	1.67	0.44
trans-α-Bergamotene	1425	-	1434	0.53	-
alpha-Humulene	1433	-	1449	0.45	-
Geranylacetone	1447	-	1453	0.54	-
beta-Selinene	1478	1449	1481	3.15	1.81
alpha-Curcumene	1481	-	1485	2.93	-
alpha-Selinene	1482	1459	1494	1.43	0.82
Cetene	1487	1464	-	0.38	0.55
cis-gamma-Bisabolene	1494	-	1515	0.48	-
Elemol	1509	1525	1547	2.70	0.75
gamma-Eudesmol	1595	-	1630	1.01	-
beta-Eudesmol	1622	-	1649	1.14	-
alpha-Eudesmol	1630	-	1652	1.44	-
Elema-1,3,11(13)-trien-12-ol	1648	-	-	11.56	-
7-Tetradecyne	1658	-	-	1.61	-
1,3-Cyclooctadiene	1665	1658	-	16.10	14.34
Cyclododecene, 12-methyl-1-(1-propynyl)	1682	-	-	0.36	-
(3E,5E,8Z)-3,7,11-Trimethyl-1,3,5,8,10-dodecapentanene	1702	-	-	0.17	-
Z-alpha-trans-Bergamotol	1711	-	1693	0.30	-
(+)gamma-Costol	1753	-	-	1.18	-
Valerenol	1775	-	1699	5.28	-
(-)alpha-Costol	1783		-	3.74	-
2(3H)-Benzofuranone	1859	2006	-	1.41	0.37
Germacra-1(10),4,11(13)-trien	1918	-	-	0.21	-
(-)Isodiospyrin	1961	-	-	0.27	-
Dehydrocostus lactone	2028	2051	-	17.73	6.77
Costunolide	-	-	-	0.34	-
Monoterpenes	-	-	-	4.28	14.88
Sesquiterpenes	-	-	-	65.57	12.94
Hydrocarbons	-	-	-	20.30	15.26
Other compounds	-	-	-	-	47.90
Total				90.15	90.98

KI_1: Kovats index of essential oil constituents of *Saussurea costus* root, KI_2: Kovats index of volatile constituent of traditional qust oil, KI_r: Kovats index reported in databases

For determination of flavonoids in TQO, the standard curve was plotted with different concentrations of catechin ($y=0.002x+0.0227$, $R^2=0.9975$) and total flavonoids content of TQO was calculated 303.2±2.52 mg CE/L.

The polysaccharide content of the TQO was estimated 9.240±0.13 mg DE/L based on plotting the standard curve with different concentrations of dextrose ($y=0.0122x+0.0231$, $R^2=0.9994$). Determination of total phenolic and tannin contents was done in two steps. The standard curve was plotted with different concentrations of gallic acid ($y=0.0074x-0.0756$, $R^2=0.9795$) and the amount of phenolics and tannins were calculated as 788.290±0.61 and 23.97±0.52 mg GAE/L, respectively.

One of the special truths about application of medicinal plants in treatment of diseases is the presence of many different active components with various pharmacological effects that can demonstrate synergistic effects, decreasing side effects or other surprising actions. *Saussurea costus* is a historical herbs that is well known from 2500 years ago and has been used in various ancient systems of medicine [13]. The roots and root oil of *S. costus* have been sold out as an important drug in the international herbal markets [14]. It has been prescribed as neuroprotective, anticonvulsant, anti-cancer, anti-ulcer, anti-arthritic, hepatoprotective, anti-viral, anti-inflammatory herb and for treatment of cough and cold in old systems of medicines such as India, China and Iran [14]. Terpenes, phenols, flavonoids, tannins and inulin, that are present in the *S. costus* root oil, demonstrate some pharmacological activities such as anti-

inflammatory, analgesic, anti-cancer, hepatoprotective, anti-ulcer, anti-bacterial and anti-fungal effects which is conformed to its traditional usage [15].

The plant has reputation in the treatment of various diseases related to muscular and neural organs [7], which are current complications and challenges in the societies. The presented dosage form of *S. costus* for muscular and neural disorders is in oil form which has been prepared according to traditional manuscripts [8].

According to the previous studies, the main components in the essential oil of *S. costus* root were sesquiterpenes [21], which were also identified in TQO and the obtained root oil. Sesquiterpene lactones are colorless, bitter, relatively stable and lypophilic constituents that are common in Asteraceae family but also occur in some other flowering plants such as Umbelliferae, Magnoliaceae, Lauraceae, Winteraceae, Illiciaceae, Aristolochiaceae, Menispermaceae, Curtiariaceae and Acanthaceae [22]. These compounds have reputation as antitumor and anti-inflammatory agents in vitro and in vivo [14,23]. Dehydrocastus lactone and costunolide are two sesquiterpenes introduced in other studies as major active constituents of *S. costus* essential oil [13,24]. Anti-tumor, anti-inflammatory, imunomodulatory, hepatoprotective, anti-ulcer and anti-viral activities have been confirmed for dehydrocastus lactone and costunolide [13,14].

A portion of essential oil and hydrophobic constituents of *S. costus* root were imported in the prepared oil, certainly. The hydrophilic compounds consistent to the previous reports [2,10,11] were also trapped in the sesame oil through the Persian medicine procedure for preparation of TQO, because in this process, the aqueous extract of the plant enters in an oily vehicle and the water is then eliminatedg from the system. The hydrophilic and hydrophobic contents of TQO were successfully determined in the present experiment. The qualitative tests on the traditional oil aren't practicable and repeatable; so a methanol extract of the oil was prepared. We suggested that dehydrocostus lactone could be a suitable option for identification and qualification of the bitter qust preparations such as TQO. Although its amount is little in the prepared oil, specification of this chemical constituent in Asteraceae family and *S. costus* and its important pharmacological activities are distinguishing options for the selection.

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Author contributions

Sahar Bagheri, Nastaran Ebadi, Zahra Taghipour and Azadeh Manayi contributed to the design and implementation of the research to the analysis of the results and to the writing of the manuscript. Tayebeh Toliyat and Mehran Mirabzadeh Ardakani 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.

References

[1] Hamedi A, Zarshenas MM, Sohrabpour M, Zargaran A. Herbal medicinal oils in traditional Persian medicine. *Pharm Biol*. 2013; 51(9): 1208-1218.

[2] Zargaran A, Faridi P, Daneshamouz S, Borhani-Haghighi A, Azadi A, Hashempur MH. Renovation and standardization of a historical pharmaceutical formulation from Persian medicine: chamomile oil. *Trad Integr Med*. 2016; 1(3): 108-114.

[3] Zarshenas MM, Zargaran A, Blaschke M. Convenient, traditional and alternative therapies for cardiovascular disorders. *Curr Pharm Des*. 2017; 23(7): 1112-1118.

[4] Nikaein F, Zargaran A, Mehdizadeh A. Rhazes' concepts and manuscripts on nutrition in treatment and health care. *Anc Sci Life*. 2012; 31(4): 160-163.

[5] Aghili Shirazi S. Makhzan Al-adviyah (the Storehouse of medicaments). Tehran: Tehran University of Medical Sciences, 2009.

[6] Zargaran A, Borhani-Haghighi A, Faridi P, Daneshamouz S, Kordafshari G, Mohagheghzadeh A. Potential effect and mechanism of action of topical chamomile (*Matricaria chammomila* L.) oil on migraine headache: a medical hypothesis. *Med Hypotheses*. 2014; 83(5): 566-569.

[7] Avicenna. The canon of medicine. Beirut: Alaalami Beirut Library Press, 2005.

[8] Aghili Shirazi S. Qarabadin-e-kabir. Tehran: Mahmoudi Press, 1970.
[9] Mikaili P, Shayegh J, Sarahroodi S, Sharifi M. Pharmacological properties of herbal oil extracts used in Iranian traditional medicine. *Adv Environ Biol*. 2012; 6(1): 153-158.
[10] Abolhassanzadeh Z, Aflaki E, Yousefi G, Mohagheghzadeh A. Randomized clinical trial of peganum oil for knee osteoarthritis. *J Evid Based Complement Altern Med*. 2015; 20(2): 126-131.
[11] Zarshenas MM, Mahmoodian R, Moein M. Colorimetric determination of flavonoids in *Citrus medica* peel traditional medicinal oil. *Int J Pharmacogn Phytochem Res*. 2015; 7(4): 696-700.
[12] Wei H, Yan LH, Feng WH, Ma GX, Peng Y, Wang ZM, Xiao PG. Research progress on active ingredients and pharmacologic properties of *saussurea lappa. Curr Opin Complement Alternat Med.* 2014; 1(1): 1-7.
[13] Zahara K, Tabassum S, Sabir S, Arshad M, Qureshi R, Amjad MS, Chaudhari SK. A review of therapeutic potential of *Saussurea lappa* an endangered plant from Himalaya. *Asian Pac J Trop Med*. 2014; 7(S1): 60-69.
[14] Pandey MM, Rastogi S, Rawat AK. *Saussurea costus*: botanical, chemical and pharmacological review of an ayurvedic medicinal plant. *J Ethnopharmacol*. 2007; 110(3): 379-390.
[15] Khan MA, Alam A, Husain S, Ahmed S, Nazamuddin M, Ahmed Z. Qust (*Saussurea lappa* Clarke) - a potent herb of Unani medicine: A review. *Int J Curr Pharm Res*. 2013; 5(4): 1-4.
[16] Upton R, Graff A, Jolliffe G, Länger R, Williamson E. American herbal pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines. Boca Raton: CRC Press, 2016.
[17] Manayi A, Saeidnia S, Shekarchi M, Hadjiakhoondi A, Shams Ardekani M, Khanavi M. Comparative study of the essential oil and hydrolate composition of *Lythrum salicaria* L. obtained by hydro-distillation and microwave distillation methods. *Res J Pharmacogn*. 2014; 1(2): 33-38.
[18] Manayi A, Khanavi M, Saeidnia S, Azizi E, Mahmoodpour MR, Vafi F, Malmir M, Siavashi F, Hadjiakhoondi A. Biological activity and microscopic characterization of *Lythrum salicari*a L. *Daru J Pharm Sci*. 2013; 21(1): 61.
[19] Vazirian M, Dianat S, Manayi A, Ziari R, Mousazadeh A, Habibi E, Saeidnia S, Amanzadeh Y. Anti-inflammatory effect, total polysaccharide, total phenolics content and antioxidant activity of the aqueous extract of three basidiomycetes. *Res J Pharmacogn*. 2014; 1(1): 15-21.
[20] Saeidnia S, Nikan M, Mirnezami T, Gohari AR, Manayi A. Micromorphological characterizations and phytochemicals contents of some *Phlomis* species from Iran. *Int J Pharm Pharm Sci*. 2015; 8(1): 157-161.
[21] Liu ZL, He Q, Chu SS, Wang CF, Du SS, Deng ZW. Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res*. 2012; 110(6): 2125-2130.
[22] Picman AK. Biological activities of sesquiterpene lactones. *Biochem Syst Ecol*. 1986; 14(3): 255-281.
[23] Rao Vadaparthi PR, Kumar K, Sarma VU, Hussain QA, Babu KS. Estimation of Costunolide and dehydrocostus lactone in *Saussurea lappa* and its polyherbal formulations followed by their stability studies using HPLC-DAD. *Pharmacogn Mag*. 2015; 11(41): 180-190.
[24] De Kraker JW, Franssen MC, de Groot A, Shibata T, Bouwmeester HJ. Germacrenes from fresh costus roots. *Phytochemistry*. 2001; 58(3): 481-487.

Abbreviations

PM: Persian medicine; TQO: Traditional bitter qust oil; GAE: Gallic acid equivalent; CE: Catechin equivalent; DE: Dextrose equivalen