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Original article

Characterization and Chromatographic Fingerprint Analysis of Traditional Wallflower Oil

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Abstract

Background and objective: Wallflower oil is made from the flowers of *Erysimum cheiri* (L.) Crantz which is a herb rich in cardenolide compounds. Wallflower oil was traditionally indicated for analgesic, anti-inflammatory, hair tonic, and wound healing purposes. In this paper, wallflower oil was prepared based on the method cited in Persian medicine resources. **Methods:** To prepare the oil, 250 g dried flower was soaked in 5000 g distilled water for 20 h. Then, it was boiled for 2 h till half of the water volume evaporated. The obtained decoction was filtered and boiled in 2500 g sesame oil until all the aqueous part evaporated. The quality control tests were performed. **Results:** Acid, peroxide, iodine, and saponification values were determined as 0.72±0.02 (oleic acid%), 7.16±0.10 (meq/kg oil), 104.73±0.71 (g of I_2/100 g oil), and 242.85±0.29 (mg KOH/g oil), respectively. HPTLC analysis revealed the presence of cardenolide compounds in wallflower oil, decoction, maceration, and flower samples. GC-FID results recognized linoleic acid (42.91%), oleic acid (41.22%), and palmitic acid (9.76%) as major fatty acids of wallflower oil. In addition, GC-MS study identified 11 volatile compounds among which, thymol (28.13%), carvacrol (21.63%), and dodecane (11.50%) were recognized as the main components. **Conclusion:** Thymol and carvacrol could be used for evaluation and determination of wallflower oil. On the other hand, presence of cardenolides in wallflower oil and consequent probable cardiac actions should be considered during clinical administrations. This paper recommends further in vitro and in vivo studies as well as clinical trials to evaluate the safety and efficacy of wallflower oil.

Keywords: cardenolides; *Erysimum cheiri*; quality control; traditional pharmacy; wallflower oil

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Introduction

Traditional Persian medicine (TPM), as one of the most popular traditional medicine systems around the world, is a rich source of herbal and natural preparations [1,2]. Herbal oils are a popular class of natural remedies used in TPM since ancient times. Despite the expanded use of theses preparations, there are many medicinal oils with unknown formulations in Persian medicine which reveals the necessity of characterization tests and performing quality controls on this class of remedies. In addition, there is a risk for existence of unsafe compounds in natural formulations, so there is an emerging need for detecting such hazardous compounds.

Wallflower (*Erysimum cheiri* (L.) Crantz) from Brassicaceae family is considered as a medicinal

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plant in TPM. This perennial herb has fragrant flowers with four petals in a cruciform corolla, four oblong sepals, and six stamens [3,4]. Wallflower produces 2 types of toxic chemicals, mustard oils and cardenolides [5]. Phytochemical investigations have reported the presence of bipindogenin, cannogenol, digitoxygenin, strophanthidin, and suzarigenin derivatives as cardenolide compounds in wallflower. Nevertheless, it has been used in various formulations in traditional medicine [6,7]. Wallflower oil, called "Roghan-e-Kheiri" in TPM, has been recommended for anti-inflammatory, analgesic, and hair tonic purposes [3,8]. Also, the cerate made from wallflower oil was frequently indicated for treatment of anal fissure, wounds, and skin fissure [3,7,9,10]. Nowadays, there are some wallflower preparations supplied in traditional herbal markets [8,11]. According to Mosleh et al., a TPM formulation containing wallflower oil could significantly relieve anal fissure in a randomized, controlled clinical trial [7]. Another study indicated the therapeutic potential effects of low-dose wallflower cardenolides on ischemic ulcers and anal fissure tissue [8]. Thus, standardization and dose adjustment of TPM preparations containing wallflower seems to be necessary in order to prevent any probable side effects [3].

By investigating the literature, we found that neither characterization tests nor quality controls of wallflower oil had been studied. Therefore, physicochemical tests along with HPTLC, GC-FID, and GC-MS fingerprints of WO were conducted for the first time in this study.

Materials and Methods

Ethical considerations

The study was approved by Research Ethic Committee of Shiraz University of Medical Sciences (ethical approval ID: IR.SUMS.REC.1395.150).

Chemicals

Digoxin was purchased from Zahravi Pharmaceutical Co. (Iran). Anhydrous sodium sulphate, dichloromethane, 3,5-dinitrobenzoic acid, ethyl acetate, isooctane, isopropanol, lead acetate, methanol, sodium chloride, sodium hydroxide, sodium hydrogen sulphate, potassium hydroxide, and pre-coated silica gel 60 F_{254} aluminum HPTLC plates (size: 20 cm x 10 cm, thickness: 0 .2 mm) were supplied from Merck Company (Germany). Ethanol was purchased from Jahan Alcohol Teb Co. (Iran).

Plant material

Wallflower was collected from Shiraz in April 2018. It was identified as *Erysimum cheiri* (L.) Crantz by an expert botanist and a sample of the herb was deposited at the Herbarium Center of Shiraz School of Pharmacy under the voucher number of 784.

Preparation of wallflower oil

A common method cited in major Persian medicine resources was adopted to prepare wallflower oil [10,12,13]. First, the macerated extract was prepared by soaking 250 g dried flowers of *E. cheiri* in 5000 mL distilled water for 20 h. Then it was boiled for 2 h till half of the water evaporated. Thereafter, the obtained decoction was filtered and subsequently the filtrate was boiled in 2500 mL sesame oil until all the aqueous part evaporated. The ratio of plant to sesame oil was 1:10.

Physicochemical tests

Acid, peroxide, iodine, and saponification values of wallflower oil were determined according to the British Pharmacopeia (BP) method [14].

High performance thin layer chromatography (HPTLC)

HPTLC analysis of wallflower oil was carried out on a CAMAG HPTLC system, equipped with an automatic TLC sampler ATS4, TLC scanner 3, and Win-CATS software. In order to obtain the cardenolide extract of wallflower oil, 30 mL ethanol 50% was mixed with 10 mL lead acetate 10% and added to each sample (including 2 g primary dried flowers as well as the maceration, decoction extracts, and wallflower oil obtained from 2 g of dried flowers). Then they were heated under reflux for 15 min. After cooling and filtering the mixtures, extraction was performed with 15 mL (3 times) of dichloromethane: isopropanol (3:2). The lower phases were combined, dried with anhydrous sodium sulphate, and evaporated to dryness. Finally, the residue was dissolved in one mL of dichlololoromethane: isopropanol (3:2) for chromatography analysis [15]. On the other hand, digoxin was accurately weighed 1 mg), dissolved in dichloromethane: isopropanol (3:2), and made up to a volume of 10 mL. To prepare Kedde reagent, 10 mL ethanolic 3,5-dinitrobenzoic acid 3% was mixed with 10

mL NaOH 2M. Twenty-five µL of each solution was applied in the form of bands with 6 mm width. The solvent system was ethyl acetate: methanol: water (81:11:8) and ascending development process was performed at 25 °C. After the plate was dried and sprayed with Kedde reagent, it was scanned in absorbance mode at 600 nm using the deuterium light source. The scanning speed was adjusted to 20 mm/s and data resolution was observed at 100 m/step.

Gas chromatography-flame ionization detector (GC-FID)

One hundred µg wallflower oil was weighed in a 10 mL test tube and dissolved in 2 mL isooctane. Then 2M potassium hydroxide in methanol (0.1 mL) was added and the mixture was centrifuged. Afterward, the clear supernatant was collected and sodium chloride solution (2 mL) was added. The obtained mixture was vortexed and the clear supernatant was transferred to a vial. After adding sodium hydrogen sulphate (1 g) to the vial, the prepared supernatant was ready for injection (1 µL). The chromatography was performed using a BPX70 column (120 m× 0.25 mm× 0.25 µm) equipped with a flame ionization detector (FID) system. Split injection (split ratio 1:40) was performed with He as the carrier gas at a flow rate of 0.7 mL/min. The oven temperature started from 120 °C to the maximum temperature of 240 °C and the temperature ramp rate was adjusted to 4°C/min which was held at 240 °C for 7 min. Both the injector and detector temperatures were 250 °C. Retention indices of the components were determined relative to the retention times of a series of methylated fatty acids (C_8-C_{24}).

Gas chromatography-mass spectrometry (GC-MS)

Essential oil was obtained from 100 g traditional wallflower oil with 1000 mL water by Clevenger apparatus (4.5 h). The acquired essential oil was used to GC analysis. This procedure was carried out on a GC instrument with Agilent 7000 Series Triple Quadrupole Mass detector, and a fused silica capillary column (Agilent DB1 capillary; 30 m×0.25 mm i.d. and film thickness 0.25 µm). The sample was injected in the split mode with a split ratio of 1:30. The flow rate of He as the carrier gas was 1.2 mL/min. Oven temperature was adjusted at the T_{start} of 70 °C and T_{end} of 280 °C for 4 min at 3 °C/min (total run time 74 min). Also, mass spectrometer was regulated in EI mode (70eV) and 30-600 m/z mass range with 250 °C interface temperature. Finally, identification of the components was done by a comparison of their mass spectra and KI with Adams libraries spectra, Wiley (275), and NIST Database [16,17]. Retention indices of the components were determined relative to the retention times of a series of n-alkanes (C_9-C_{30}).

Results and Discussion

The physicochemical characteristics including acid, peroxide, saponification, and iodine values of wallflower oil were determined (n=3) as 0.72±0.02 (oleic acid%), 7.16±0.10 (meq/kg oil), 242.85±0.29 (mg KOH/g oil), and 104.73±0.71 (g/100 g oil), respectively. According to the results of HPTLC fingerprinting method for cardnenolide profile [15], the spots appeared as violet-red zones in visible light and 366 nm after applying the reagent, the similar patterns of cardenolide compounds were observed in flower, maceration and decoction samples. However, a new cardenolide band, above the R_f range of digoxin, was detected as the major cardenolide compound in wallflower oil (Figure 1). Digoxin was used as a marker of cardenolides in this investigation.

GC-FID profiles of fatty acids are shown in Table 1. The major fatty acids of wallflower oil (fatty acid g/100 g total oil) were identified as linoleic acid (42.91%), oleic acid (41.22%), and palmitic acid (9.76%). According to previous studies, geraniol, nerol, linalool, cheiroline, anis aldehyde, benzyl alcohol, salicylic acid, anthranilic acid, and acetic acid were identified as chemical compounds in the essential oil of fresh flowers [3].

Following determination of an optimized GC method, the essential oil was subjected to GC-MS for analysis of the essential oil. The obtained components were identified by comparing their mass spectra and KI with Adams 2007 libraries spectra, Wiley (275), and NIST database as well as observing Kovats retention index and relevant literatures (Table 3). Eleven components were identified in essential oil composition of traditional wallflower oil. Thymol (28.13%), carvacrol (21.63%), and dodecane (11.50%) were identified as the main components.

According to the results, no similarities were found between the essential oil composition of traditional wallflower oil and the essential oil obtained from the fresh flowers.

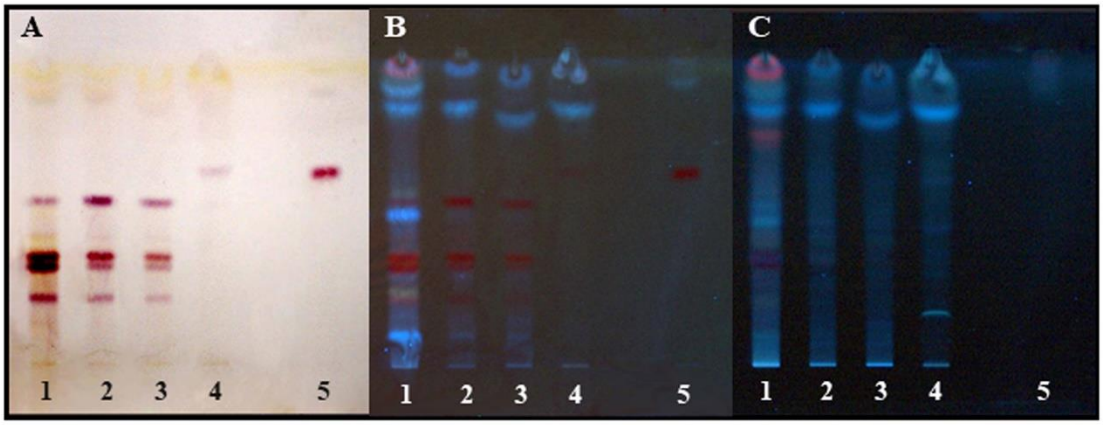

Figure 1. HPTLC pattern of total cardenolide extracts in different wallflower preparations; A: visible range (after applying the reagent); B: 366 nm (after applying the reagent); C: 366 nm (before applying the reagent); 1: flower; 2: maceration extract, 3: decoction extract; 4: oil; 5: digoxin

Table 1. Fatty acid composition of wallflower oil (g/100 g total oil) obtained by GC-FID

Fatty acids	Percentage	Retention Time (min)
Caprylic acid (C8:0)	0.03	10.46
Capric acid (C10:0)	0.02	11.12
Lauric acid (C12:0)	0.03	11.27
Myristic acid (C14:0)	0.02	11.56
Palmitic acid (C16:0)	9.76	12.77
Palmitoleic acid (C16:1)	0.24	13.64
Stearic acid (C18:0)	5.34	14.81
Oleic acid (C18:1)	41.22	15.83
Linoleic acid (C18:2)	42.91	17.56
Linolelaidic acid (C18:2T) + Trans linolenic acid (C18:3T)	0.16	19.92
α-Linolenic acid (C18:3)	0.27	20.36

Table 2. Chemical composition of essential oil obtained from wallflower oil by GC-MS

No.	Components	Percentage	Calculated KI	Reported KI
1	Nonanal	4.50	1087	1081
2	Undecane*	5.34	1103	1100
3	Dodecane*	11.50	1203	1200
4	Unknown	1.67	1210	-
5	2,5-Bornanedione	2.38	1222	1264
6	Unknown	5.40	1227	-
7	Thymol	28.13	1268	1271
8	Carvacrol	21.63	1277	1277
9	Tridecane*	7.38	1301	1300
10	Unknown	1.53	1335	-
11	Unknown	1.28	1365	-
12	Unknown	4.30	1376	-
13	Tetradecane*	3.11	1400	1400
14	α-Bergamotene	tr	1430	1434
15	α-Bulnesene	tr	1480	1490
16	Hexadecane*	1.84	1599	1600

tr = trace (< 1%)

* Similar component was identified in sesame oil base spectra

The increased interest of using traditional medicines around the world reveals the necessity of quality control and standardization of traditional drug preparations [6]. Several traditional Persian medicine (TPM) oil formulations are prepared by boiling aqueous extracts in oily vehicles [10,12]. In the present study, wallflower oil was prepared according to the method described in TPM resources [10,12,13]. Then characterization and quality control were performed for the first time in this study. The results of HPTLC analysis revealed the presence of cardenolide compounds in flower, maceration, decoction, and wallflower oil samples. Despite the similar cardenolide patterns in flower, maceration, and decoction samples, a different fingerprint profile was observed in wallflower oil extract (Figure 1). Both the prolonged heating process and boiling the decoction in an oily vehicle can bring about new chemical reactions as well as thermal degradation of the existing primary compounds. In this context, lipophilic compounds are more likely to be dissolved in the oily base of wallflower oil. Also, the aqueous phase is considered to be trapped in the oily phase during boiling and evaporation processes. Although the heat-sensitive components are

probable to be decomposed by overheating, various amounts of active constituents are supposed to be extracted into the oily vehicles of TPM oils [18,19].

According to GC-FID results, the major fatty acids of wallflower oil were identified as linoleic acid (42.91%), oleic acid (41.22%), and palmitic acid (9.76%). GC-MS profile of essential oil represented thymol (28.13%), carvacrol (21.63%), and dodecane (11.50%) as the main volatile components. Hence, thymol and carvacrol could be used for evaluation and determination of wallflower oil in further investigations.

Conclusion

Thymol and carvacrol as two major components of the essential oil obtained from traditional wallflower oil could be used for evaluation and determination of wallflower oil in further studies. Additionally, due to the cardenolide moiety detected in wallflower oil and the consequent probable cardiac actions, this paper suggests further in vitro and in vivo studies to evaluate the safety and efficacy of wallflower oil.

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Author contributions

Ghazaleh Mosleh participated in conceptualization, methodology, investigations, writing and editing the original draft; Amir Azadi participated in conceptualization, methodology, review, editing the manuscript and supervision of the study; Abdolali Mohagheghzadeh participated in conceptualization, methodology, review and editing the manuscript, and was involved in supervision, and administration of the project.

Declaration of interest

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

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Abbreviations

TPM: traditional Persian medicine; HPTLC: high performance thin layer chromatography; GC-FID: gas chromatography with a flame ionization detector; GC-MS: gas chromatography-mass spectrometry