Original Article

TIPS Trends in Pharmaceutical Sciences 2021: 7(4): 259-268.

Volatile composition analysis and quantitative determination of specific markers in a traditional preparation, *Jawārish-e-Komooni*

Saeedeh Aghajani[1,2], Sanaz Jafari[2], Mohammad M. Zarshenas[1,2,3*], Zohreh Abolhassanzadeh[4]

[1]Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Departmentvof Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Epilepsy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Abstract

Control and standardization process of herbal products is a critical point in traditional medicine. Many herbal formulations that are being used today are not standardized and also there is no noticeable control over them. Out of all the different pharmaceutical dosage forms mentioned in Traditional Persian Medicine literature, *Jawarish-e-Komooni* is an effective formulation due to its positive effect on Gastrointestinal disorders. This formulation includes *Zingiber officinale*, *Bunium persicum*, *Piper nigrum*, and also honey. At this time there have been no noticeable and proven control and standardization or any pharmacognosy studies on this formulation. In this study, *Jawarish-e-Komooni* was prepared according to *Qarābadin-e-Salehi*, one of the most practical Traditional Persian Medicine literature. Then, by using gas chromatography/mass spectroscopy (GC/MS) and HPTLC, the containing of the formulation were assessed. Also for content determination, using Gas chromatography/ flame ionization detector (GC/FID), two of the main ingredients were determined. The HPTLC results showed piperine and gamma-terpinene as the main components of the formulation. In the standardizing process, piperine and gamma-terpinene were respectively proved to be 0.22% and 0.97% of the whole preparation. Also by calculating the standard deviation of the content determination process, we could reach the point where RSD was less than 10%, which is proof of the validity of our method. As mentioned before, standardization is a critical process for all the traditional preparations and it could help us gain repetitive drug responses elsewhere.

Keywords: *Jawarish-e-Komooni*, standardization, Traditional Persian Medicine, Gas Choromatography, HPTLC.

Please cite this article as: Aghajani S, Jafari S, Zarshenas MM, Abolhassanzadeh Z. Volatile composition analysis and quantitative determination of specific markers in a traditional preparation, *Jawārish-e-Komooni*. Trends in Pharmaceutical Sciences. 2021;7(4):259-268.doi: 10.30476/TIPS.2021.91274.1098

1. Introduction

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history. From the global perspective, there is a shift towards the use of the medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent. Though herbal products have become increasingly popular globally, one of the impediments in their acceptance is the lack of a standard quality control profile. The quality of

Corresponding Author: Mohammad M. Zarshenas, Departmentvof Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: zarm@sums.ac.ir

Received: 08/07/2021; Accepted: 01/12/2021

herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish a quality control parameter, though the modern analytical technique is expected to help in circumventing this problem (1). Medical and pharmaceutical manuscripts authored by medieval Persian practitioners present not only an accumulation of traditional medical systems but also contain a collection of ingenious studies that provide valuable information in the field of medicinal herb formulation (2). *Jawārish* is a semisolid traditional formulation which has been described in a series of historical Persian pharmaceutical manuscripts, namely "*Qarābadin*" (pharmacopeia), which consists of medical texts on drug compounds, formulas, and indications (4). The name "*Jawārish*" is derived from the Persian word "*govāresh*" meaning digestion in this language. As the name "*Jawārish*" implies, this combination is used to treat digestive problems (3). *Jawārish-e-Komooni* is one of the dosage forms that has been mentioned in the *Qarābadin-e-Salehi*. This semi-solid traditional formulation included 4 parts (*Zingiber officinale*, *Bunium persicum*, *Piper nigrum*, and also honey). *Zingiber officinale* Roscoe., commonly known as Ginger and belongs to the *Zingiberaceae* family, is cultivated in China, India, and Jamaica. Ginger has traditionally been used as an important spice in food all over the world (5). The rhizome has been administered as antiemetic, anti flatulence, antioxidant, pain-relieving, antibactrial, and antiviral (6). Ginger contains various active constituents, such as phenolic and terpene compounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols and the terpene component in Ginger are β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene (7). *Bunium persicum* (Boiss.) *B. fedtsch*. from Apiaceae family is completely native to Iran. It is traditionally used as anti-diarrhea, anti flatulence, digestive, for asthma and losing weight (5). The γ-terpinene was identified as the major component of the *B. persicum* essential oil. The other main components of this oil were found to be cuminaldehyde (15.5%), ρ-cymene (6.7%), and limonene (5.9%) (8). *Piper nigrum* L. belongs to the *Piperaceae* family, is endemic to southwestern India, Southeast Asia, Brazil, and West Africa. Vietnam and India are the largest producers of Piper nigrum. This plant has traditionally been a widely used spice around the world. In traditional medicine it is used as anti flatulence, appetizing, local anti-inflammation, and disinfectant (5).

A compound with the formulation of one or more plant components can be identified and standardized by using pharmacognosy studies and advanced instrumental methods. The amount of one or more active ingredients in the formulation can be determined to standardize the therapeutic effects and match the pharmacognosy properties. In this regard, the current study was conducted to introduce this semisolid dosage form as well as parallelly determine concerning two major volatile constituents via Gas Chromatography/ Flame Ionization Detector (GC/FID) and High-Performance Thin-Layer Chromatography (HPTLC) techniques.

2. Materail and Methods

2.1. Plants microscopic characterization

To perform this step, first, some fruits of black cumin and black pepper, as well as ginger rhizomes, were powdered separately in a mortar. The powders were then passed through a sieve with mesh 40. In the following, 5 g of each powdered was added to 5 ml of 60% chloral hydrate solution in a test tube. After warming by the heat of the flame, they were poured separately on the watch glass, then some of the powder was placed on a slide and observed under a triangular light microscope. Furthermore, each powder was poured directly onto the watch glass and mixed with a few drops of water, and observed under a microscope.

2.2. Plant material and Jawārish-e-Komooni preparation

As mentioned by the *Qarabadin_e_salehi* book, the *Jawārish-e-komooni* is a semi-solid formulation made with the following ingredients in the constitution as shown in Table 1. All ingredients of this formulation were purchased from a popular medicinal plant market in Shiraz and brought to the Department of Phytopharmaceu-

Table 1. Gredients of the *Jawarish-e-Komooni* formulation.

common name	Scientific name	Voucher number	quantity
Ginger	*Zingiber officinale* Roscoe	PM962	200g
Black pepper	*Piper nigrum*	PM862	200g
Black cumin	*Bunium persicum* (Boiss.) *B. fedtsch*	PM1008	400g
Salt	_	_	100g
Honey	_	_	1800g

ticals (Traditional Pharmacy), Shiraz School of Pharmacy for authentication and specification of a voucher number.

According to *Qarabadin_e-salehi* instruction, 1 kg of *B. persicum* was added to 3 kg of vinegar and after 24 hours the soaked *B. persicum* was placed on a filter paper to dry. All plants were ground via electric miller and sieved through 30 British mesh, separately. Finally, 200 g of *Z. officinale* and *P. nigrum*, 400 g of *B. persicum* and 100 g of salt were mixed together (final weight of the product was 900 g). Then, the honey with 2 times amount of powder was added to the finished product and mixed well.

2.3. Hydro-distillation of the samples

The *Jawarish-e-komooni* as well as each plant were seperately hydro-distilled in a Clevenger apparatus for 4 hours. For this purpose, 300 g of *Jawarish* powder and 50 g of each plant powder were weighed and soaked in a certain amount of distilled water for 24 hours, then poured into the Clevenger apparatus. After finishing the extraction process, the yield of essential oils were calculated on a dry weight basis. Then, the whole of essential oil was poured in the test tubes with screwed caps. The essential oils after drying over anhydrous sodium sulfate, were stored in the refrigerator at -20 °C until GC/MS analysis.

2.4. GC/MS analysis

GC/MS analysis was performed via an Agilent GC-MSD system (model 7890A). HP-5MS capillary column (phenyl methyl siloxane, L × I.D. 30 m × 0.25 mm, with 0.25µm film thickness) was used with helium as a carrier gas at 1 mL/min flow rate. GC oven temperature was scheduled from 60 (0 min) to 220 °C (heating rate of 5 °C/min) and then kept fixed for 10 min at 220°C. The mass spectrometer (Agilent technologies 5975 C) employed at 70 eV. The mass range was documented from 30 to 600 m/z and injected temperature was adjusted at 280 °C.

In order to identify the essential oil components, the Kovats indices were calculated via using retention times of synchronically injected normal alkanes (C9-C24) as well as their mass spectra with Willey (nl7) and Adams libraries spectra (9).

2.5. GC/FID analysis

GC/FID analysis was performed via an Agilent GC-FID system (model 7890A) supplied with a HP-5 column (phenyl-methyl siloxane, L×I.D. 25 m×0.32 mm, with 0.52-µm film thickness) and flame ionization detector (FID). Nitrogen (5^{th} grade), as a carrier gas, was used at 1 mL/min flow rate. The column temperature was programmed from 60 (0 min) to 250 °C at the heating rate of 5 °C/min and then kept stable for 10 min at 250 °C. The injector and detector were modified at 270 °C and 300 °C, respectively. The standard solution of thymol (the main bioactive component in the essential oil) as a reference compound was prepared in methanol (with 99-101% purity). The calibration curve is needed for the quantification of the specific marker. In order to plot the calibration curve, dilutions of gamma terpinene(0.85, 1.7, 8.5, 17, 42.5 mg/ml) prepared by methanol and about 1µg of each one sample injected to GC/FID for three times. Also, the essential oil yielded from *Jawarish* was prepared (0.02 mg/ml) and injected to GC/FID three times a day in three days to determine the difference of inter-day, intraday and relative standard deviation. The limit of detection (LOD) and limit of quantification (QOD) was determined.

2.6. Preparation of methanol extract from Jawarish-e-Komooni

To prepare the extact, 50 g *B. persicum* ,

Table 2. Essential oil extraction.

Common name	Weight (g)	Essential oil (ml)	Yield (%)
Jawarish_e_komooni	300 g	2	0.7
Ginger	50 g	1	2
Black cumin	50 g	1.2	2.3
Black pepper	50 g	0.9	1.7

25 g *Z. officinale* and 25 g *P. nigrum* which powdered in mortar were mixed and 200 g honey was added to the mixture. Then, 300 ml of methanol was added to the mixture and sonicated in sonicator for 20 minutes. Next, the mixture was filtrated with filter paper and the rest was sonicated for 20 minutes with 300 ml of methanol. This step was repeated for one time more. The extracts which obtained from 3 steps were mixed and condensed in a rotary machine.

2.7. High-performance thin-layer chromatography (HPTLC) analysis

HPLC analysis was performed using the CAMAG TLC system equipped with ATS 4 (automatic TLC sample 4) and an automatic developing chamber (ADC2). The stationary phase was a silica gel plate 60F254 (10×10 cm, Merck, Germany) and Toluene: Ethyl acetate: methanol (70:25:5 v/v) the mobile phase was. A stock solution containing 2 mg/ml piperine and decreasing serial dilutions from the stock solution in the range of 1, .5, 0.25 and 0.125 was prepared in methanol to set the calibration curve. Also, 2 mg of *Jawarish* extract was diluted via 1 ml dichloromethane. Then, four samples (2, 1, 0.5, 0.25 and 0.125 mg/ml) were prepared and each one was injected three times.

Finally, the chromatographic spots were visualized first with ultraviolet lamps emitting at 254 and 366 nm and then anisaldehyde-sulfuric acid reagent. All chemicals and solvents were analytical grade from Merck (Germany) or Sigma Aldrich (USA).

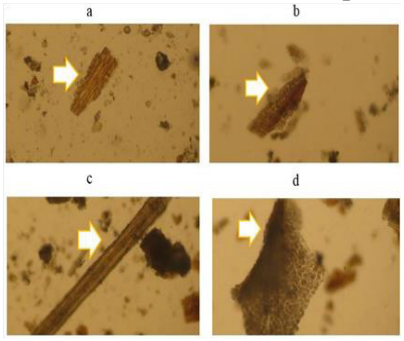

Figure 1. Microscopial charactristics of black cumin by using hydrated chloral (a) Endocarp (b) Epicarp (c) Sclereid of mesocarp (d) Cells of inner fruit with duct.

3. Results and discussion

3.1. Essential oils and Yield determination

The amount of dried samples, essential oil and yield are shown in Table 2.

3.2. Microscopic characterization

In order to facilitate the anatomical description, in most plants, the fruit wall is called pricarp and the pericarp is often divided into three regions called exocarp (epicarp), mesocarp and endocarp (10). Microscopial charactristics of black cumin are presented in Figures 1 and 2. Microscopial charactristics of Black pepper are shown in Figures 3 and 4. Microscopial charactristics of Ginger are depicted in Figure 5.

3.3. GC/MS Essential oil analysis

About 1 µl of dehydrated Jawarish, black pepper, black cumin and Ginger samples without honey were injected into GC/MS device and their components were analyzed. Table 3 and Figures 6-9 are shown the chemical composition and the GC/MS chromatogram of *Jawarish-e-Komooni*, black pepper, black cumin and Ginger essential oils, respectively. According to GC/MS analysis, gamma terpinene is the main volatile composition in the *Jawarish-e-Komooni*.

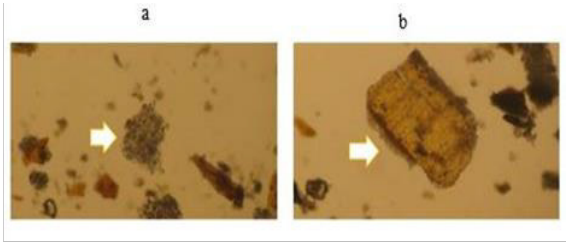

Figure 2. Microscopial charactristics of black cumin by using water(a) Exocarp (b) Starch.

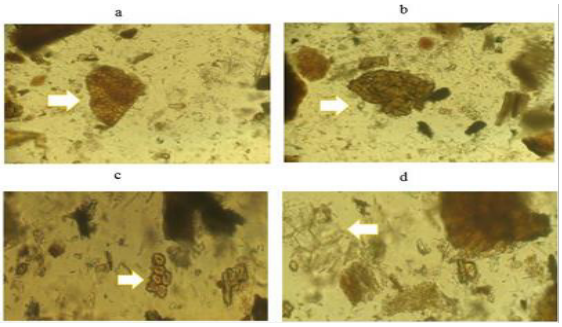

Figure 3. Microscopial charactristics of black pepper by using hydrated chloral (a) Exocarp (b) Sclereid of endocarp (c) Stone cells (d) Pitted cells of mesocarp.

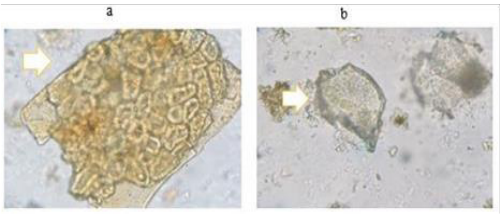

Figure 4. Microscopial charactristics of black pepper by using water (a) Exocarp (b) Starch.

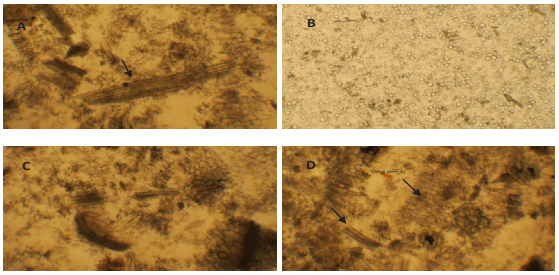

Figure 5. Microscopial charactristics of Ginger by using hydrated chloral (a) xylem with sclerenchyma fiber (b) starch particles (c) parenchyma tissue (d) xylem and parenchyma tissue.

3.4. Quantification of gamma terpinene as a major compound by GC/FID

GC/FID method was applied to determine the exact amount of gamma terpinene in *Jawarish-e-Komooni* formulation. For this purpose, the standard curve of gamma terpinene was plotted and the related equation was calculated according to different concentrations of the standard (Figure 10). Also, table 4 represented the mean±SD area and RSD for every 5 standard concentrations of gamma terpinene, separately. The linearity of the protocol was confirmed by R2 (R2=0.99). The intra-day and inter-day variation was calculated and showed in table 5. Finally, Table 6 represented the exact amount of gamma terpinene in *Jawarish-e-*

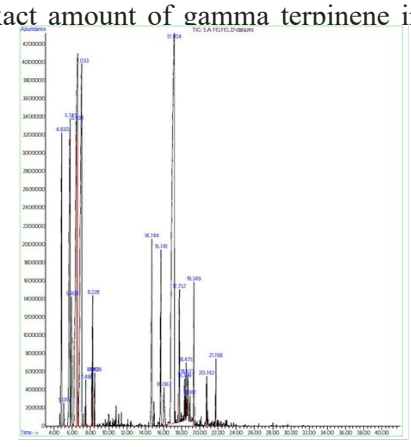

Figure 6. GC-MS chromatogram of black pepper essential oil.

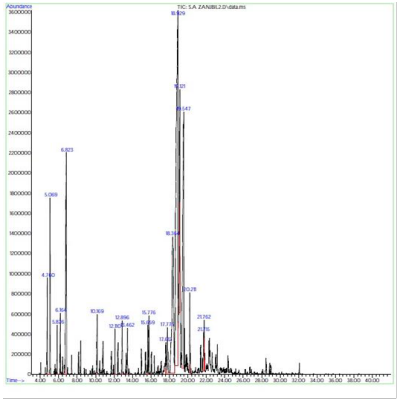

Figure 7. GC-MS chromatogram of Ginger essential oil.

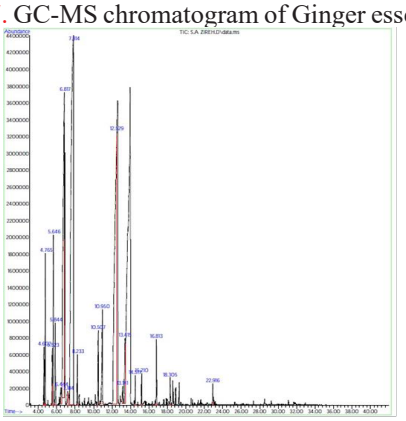

Figure 8. GC-MS chromatogram of black cumin essential oil

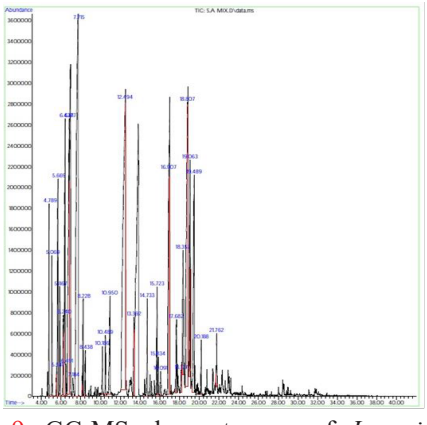

Figure 9. GC-MS chromatogram of *Jawarish_e_komooni* essential oil.

Table 3. Chemical composition of the *Jawarish-e-Komooni* and each plants Essential oils.

component	Jawarish-e-komooni	Black cumin	Black pepper	Ginger	KICAL	MS_K1	Reference
α -Thujene	-	0.71	-	-	923.00	923.00	(11)
α-Pinene	2.06	2.07	5.58	1.38	935.15	938.00	(11)
Camphene	1.33	-	0.30	3.32	951.20	952.00	(12, 13)
Sabinene	0.42	1.17	-	-	974.88	974.00	(11, 12)
β-Pinene	3.34	3.20	9.62	-	979.16	980.00	(12, 13)
β-Myrcene	1.53	1.46	3.48	0.74	991.12	991.00	(11-13)
delta.3-Carene	5.97	-	12.74	-	1014.93	1011.00	(12)
α-Terpinene	0.36	0.60	-	-	1018.72	1018.00	(12)
o-Cymene	9.03	16.63	-	-	1027.16	1029.00	(14)
Limonene	-	-	18.06	-	1030.21	1031.00	(11)
α-Phellanderene	-	-	-	0.98	1007.844	1032.00	(11)
β -Ocimene	0.40	0.59	-	-	1046.14	1050.00	(12, 13)
β-Phellanderene	-	-	-	9.64	1051.00	1053.00	(11)
γ-Terpinene	17.50	36.69	0.39	-	1064.30	1062.00	(11, 12)
α-Terpinolene	0.91	0.63	2.40	-	1090.98	1088.00	(12)
Linalool	0.52	-	0.61	-	1101.80	1101.00	(11)
Borneol	0.55	-	-	1.41	1169.13	1172.00	(12)
4- Terpineol	0.73	1.58	-	-	1178.10	1177.00	(14)
Cuminaldehyde	16.97	26.47	-	-	1221.81	1223.00	(15)
Chrysanthenone	-	2.29	-	-	1223.90	1225.00	(16)
Neral	-	-	-	1.03	1230.11	1231.00	(11)
Ugenol	-	0.50	-	-	1240.80	1243.00	(12)
Geranial	-	-	-	1.32	1257.78	1258.00	(16)
2-Undecanone	-	-	-	1.07	1292.70	1291.00	(17)
delta-Elemene	-	-	4.09	-	1336.22	1337.00	(17)
α-Copaene	1.31	-	3.54	0.95	1385.15	1391.00	(11)
Geranylacetate	0.46	-	-	1.22	1389.13	1392.00	(11)
trans-Caryophyllene	4.79	-	29.76	-	1420.98	1418.00	(13)
α-Humullene	1.22	-	2.29	-	1463.52	1454.00	(18)
trans- β -Farnesene	-	-	-	1.00	1456.76	1459.00	(12)
δ-Curcumene	-	-	-	1.53	1469.58	1478.00	(19)
Germacrene-D	-	0.56	0.56	-	1487.60	1480.00	(19)
α-Curcumene	2.45	-	-	12.51	1480.78	1483.00	(11)
β-Selinene	-	0.64	0.64	-	1493.85	1485.00	(12)
α-Selinene	-	0.60	0.60	-	1502.25	1494.00	(12)
Levomenol	-	-	-	-	1510.11	1507.00	(20)
Zingiberene	9.77	-	-	36.69	1508.68	1508.00	(12)
β-Bisabolene	-	-	-	7.27	1507.51	1509.00	(11)
delta-Cadinene	-	-	1.97	-	1530.26	1530.00	(12)
β-Sesquiphellandrene	5.45	-	-	14.31	1536.23	1543.00	(13)
Germacrene-B	0.65	-	-	1.77	1567.22	1567.00	(11)
Caryophyllene-oxide	-	-	0.71	-	1590.84	1581.00	(12)
% Identification	87.72	95.53	97.34	98.14	-	-	-

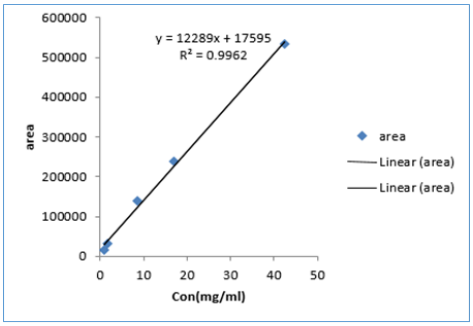

Figure 10. standard curve of gamma terpinene.

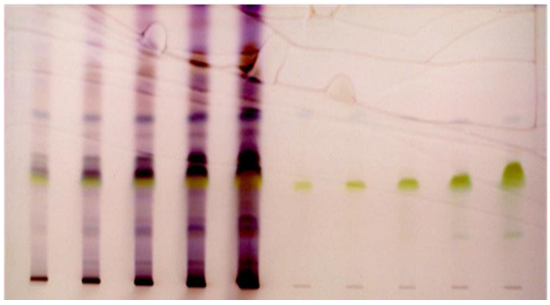

Figure 11. The HPTLC plate at visible light, after derivatization.

Table 4. The Mean±SD area and RSD for standard concentrations of gamma terpinene.

concentration (mg/ml)	Sample number	Area	Mean±SD	RSD%
0.85	1	12564	12203.30±1146.30	9.39
	2	10920		
	3	13126		
1.7	1	34645	31823.30±2449.64	7.70
	2	30241		
	3	30584		
8.5	1	147534	144034.00±4656.01	3.23
	2	145819		
	3	138750		
17	1	280561	262131.00±22438.20	8.60
	2	268687		
	3	237145		
42.5	1	494772	510085.00±20018.47	3.90
	2	532713		
	3	502671		

RSD: relative standard deviation

Table 5. Inter-day and intra-day variations.

Days	AREA			Mean±SD(intra-day)	RSD%(intra-day)	RSD%(inter-day)
	A1	A2	A3			
1	154413	154880	147534	152275.70±4113.04	2.71	
2	159115	162315	145819	155749.83±8747.91	5.62	5.6
3	144034	140119	138750	140967.72±2742.32	1.94	

Table 6. Determined exact amount of gamma terpinene in *Jawarish-e-Komooni*.

No.	Area	Concentration (mg/ml)	Mean±SD	RSD%	LOD (mg/ml)	LOQ (mg/ml)
1	30872.51	1.08	0.97±0.07	7.21	0.26	0.85
2	28887.82	0.92				
3	28883.51	0.92				

Komooni formulation.

3.5. Quantification of piperine by HPTLC method

The presence and the amount of piperine in the jawarish extract, has been detected via HPTLC method. Figure 11 has exhibited the HPTLC plate at visible light, after submerging in the tank.

In order to calculate the exact concentration of the piperine, the area under the curve of different concentrations of the standard (piperine), were calculated. The results have been shown in table 7. Subsequently, a calibration curve was plotted (Figure 12). Centered on the calibration curve, the linearity of the protocol was confirmed by R2 (0.96).

Then, the area under the curve of different concentrations of *Jawarish* extract was calculated (Table 8). Eventually, after considering the

Table 7. The area under the curve of different concentrations of the standard (piperine).

concentration(mg/ml)	Sample number	Area	Mean±SD	RSD%
2	1 2 3		5213.76±15.00	0.29
1	1 2 3		2950.06±42.48	1.44
0.5	1 2 3		1205.56±6.58	0.55
0.25	1 2 3		785.56±22.06	2.81
0.125	1 2 3		332.53±20018.47	3.61

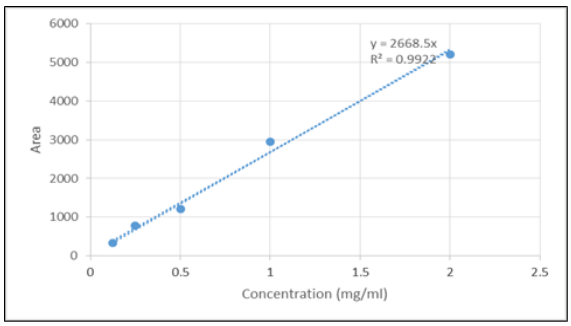

Figure 12. The calibration curve of piperine.

dilution factor and extract percentage, the piperine concentration was determined as 68 mg per 100 g *Jawarish*.

4. Conclusion

Traditional Medicine is the oldest form of health care in the world and is used in the prevention, and treatment of physical and mental illnesses. Different societies historically developed various useful healing methods to combat a variety of health- and life-threatening diseases (21, 22).

Herbal therapy in Iran also dates back to a long time ago and a number of writings regarding this issue are left by great physicians e.g. Avicenna and Rhazes. Nowadays, in spite of remarkable advancement in modern medicine, the use of herbal remedis is increasing worldwide as a complementary and alternative medicince (23).

The development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of marker/bioactive compounds and other major constituents, is a major challenge to scientists. Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs (24). GI disorders are frequent in the general population. Herbal medicine has an important role in treatment of GI disorder. There is a growing appeal for the prescribe and use of herbal medicaments for these disorders. *Jawarish* formulations are the set of herbal compound drugs that have been prescribed for improving GI problems (25). *Jawarish-e-komooni* as a kind of Jawarish, includes *Zingiber officinale*, *Bunium persicum*, *Piper nigrum* and also honey. This formulation is one of the most important and widely used jawarish in traditional Persian medicine, which has widely prescribed by traditional physicians in Iran. Till now, there have been no noticeable and proven control and standardization or any pharmacognosy studies on this formulation. The current study aimed to introduce some specific and

Table 8. The area under the curve of different concentrations of *Jawarish* extract.

No.	AREA	Conc. (mg/ml)	Mean ± SD	RSD%
1	4233.20	1.59	4677.03±387.42	8.28
2	4850.40	1.81		
3	4947.50	1.85		

reliable parameters to standardize *Jawarish-e-Komooni* as a semi-solid herbal medicine in treatment of GI diseases.

In this study, the compound drug form of *Jawarish-e-komooni* was prepared according to the reliable instruction from *Qarabadin-e-salehi* book. The botanical properties of each components were evaluated and identification steps were performed. Then, essential oils of each plants and also jawarish were extracted via Clevenger apparatus. Besides, *Jawarish* extraction was perormed using methanol. According to GC/MS analysis gamma terpinene is the main component from yielded essential oil. Furthermore, standardization based on gamma terpinene and piperine was done via GC-FID and HPTLC method respectively.

Acknowledgments

Authors of this manuscript wish to express their thanks and appreciation to Shiraz University of Medical Sciences, Shiraz, Iran (95-01-70-13363).

Conflict of Interest

All authors declared that they have no conflict of interest

References

1. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines-A review. *Int J Biodiver. Conserv.* 2012 Mar 20;4(3):101-12.
2. Hamedi A, Zarshenas MM, Sohrabpour M, Zargaran A. Herbal medicinal oils in traditional Persian medicine. *Pharm Biol.* 2013 Sep;51(9):1208-18. doi: 10.3109/13880209.2013.777462. Epub 2013 Jun 7. PMID: 23746335.
3. Mahmoudpour Z, Shirafkan H, Mojahedi M, Gorji N, Mozaffarpur SA. Digesters in traditional Persian medicine. *Caspian J Intern Med*. 2018 Winter;9(1):1-6. doi: 10.22088/cjim.9.1.1. PMID: 29387312; PMCID: PMC5771353.
4. Heravi MG. (1765). Qarabadin-e-Salehi. Tehran, Iran: Dar-ol-khalafeh(Litograph in Persian).
5. Iranian Herbal Pharmacopoeia Commission. Iranian Herbal Pharmacopoeia. Ministry of Health, Treatment and Medical Education, Tehran, 2003.
6. Chrubasik S, Pittler MH, Roufogalis BD. Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine*. 2005 Sep;12(9):684-701. doi: 10.1016/j.phymed.2004.07.009. PMID: 16194058.
7. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB. Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). *Foods*. 2019 May 30;8(6):185. doi: 10.3390/foods8060185. PMID: 31151279; PMCID: PMC6616534.
8. Sharififar F, Yassa N, Mozaffarian V. Bioactivity of major components from the seeds of Bunium persicum (Boiss.) Fedtch. *Pak J Pharm Sci*. 2010 Jul;23(3):300-4. PMID: 20566444.
9. Samani SM, Moein M, Petramfar P, Zarshenas MM. Development and Assessment of a Gas Chromatographic Based Method for the Quantification of Thymol from Cream Based Formulation. *Curr Drug Discov Technol*. 2016;13(2):77-83. doi: 10.2174/1570163813666160519111145. PMID: 27193568.
10. Ryding O. Pericarp structure and phylogeny of theLamiaceae-Verbenaceae-complex. *Plant Syst Evol*. 1995 Mar 1;198(1-2):101-41.
11. Högnadóttir A, Rouseff RL. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *J Chromatogr A*. 2003 May 23;998(1-2):201-11. doi: 10.1016/s0021-9673(03)00524-7. PMID: 12862384.
12. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. *Carol Stream*. 1995;16:65-120..
13. Creed MC, Milgram NW. Amyloid-modifying therapies for Alzheimer's disease: therapeutic progress and its implications. *Age (Dordr).* 2010 Sep;32(3):365-84. doi: 10.1007/s11357-010-9142-z. Epub 2010 Apr 20. PMID: 20640545; PMCID: PMC2926857.
14. Binder RG, Flath RA, MonTR. Volatile components of bittermelon. *J Agric Food Chem.* 1989;37(2):418-20.
15. Tongnuanchan P, Benjakul S. Essential oils: extraction, bioactivities, and their uses for food preservation. *J Food Sci.* 2014 Jul;79(7):R1231-49. doi: 10.1111/1750-3841.12492. Epub 2014 Jun 2. PMID: 24888440.
16. Danin A, Ravid U, Umano K, Shibamoto

T. Essential oil composition of Origanum ramonense Danin leaves from Israel. *J Essent Oil Res.* 1997;9(4):411-17.
17. Chen J, Ho CT. Volatile compounds generated in serine-monosaccharide model systems. *J Agric Food Chem.* 1998;46(4):1518-1522.
18. Mondello L, ZappiaG, Cotroneo A, Bonaccorsi I, Chowdhury JU, Yusuf M, et al. Studies on the essential oil-bearing plants of Bangladesh. Part VIII. Composition of some Ocimum oils O. basilicum L. var. purpurascens; O. sanctum L. green; O. sanctum L. purple; O. americanum L., citral type; O. americanum L., Camphor Type. *Flavour Fragr J.* 2002;17(5):335-340.
19. Flamini G, Luigi Cioni P, Morelli I, Celik S, Suleyman Gokturk R, Unal O. Essential oil of Stachys aleurites from Turkey. *Biochem Syst Ecol*. 2005;33(1):61-6.
20. Lee N-H, Lee S-M, Lee T-M, Chung N, Lee H-S. GC-MS Analyses of the Essential Oils Obtained from Pinaceae Leaves in Korea. *J Essent Oil Bear Plant*. 2015;18(3):538-42.
21. Abdullahi AA. Trends and challenges of traditional medicine in Africa. *Afr J Tradit Complement Altern Med*. 2011;8(5 Suppl):115-23. doi: 10.4314/ajtcam.v8i5S.5. Epub 2011 Jul 3. PMID: 22754064; PMCID: PMC3252714.
22. World Health Organisation. General Guidelines for Methodologies of Research and Evaluation of Traditional Medicine; World Health Organisation: Geneva, Switzerland, 2000.
23. Mikaili P, Shayegh J, Asghari MH, Sarahroodi S, Sharifi M. Currently used traditional phytomedicines with hot nature in Iran. *Ann Biol Res*. 2011;2(5):56-68.
24. Choudhary N, Sekhon BS. An overview of advances in the standardization of herbal drugs. *J Pharm Educ Res.* 2011 Dec 1;2(2):55.
25. Jafari S, Jalali A, Abolhassanzadeh Z,Farboodniay Jahromi MA, Zarshenas MM. Gas and high performance thin layer chromatographic based-determinationmethod forquantification of thymol in semisolidtraditional dosage forms. *Biointerface Res Appl Chem.* 2020;10 (2):4982-4987.