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Research Article

TOWARD OBTAINING OPTIMAL PHARMACEUTICAL STANDARDIZATION OF JOVARISH-E-KAMMUNI

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ABSTRACT
Jovarish-e-kammuni is a poly herbal medicine with carminative and digestive properties used for treatment of dyspepsia and obesity. In this study we have tried to run the Pharmacopoeia standards for Jovarish-e-kammuni. According to the method described in traditional manuscripts, we prepared 2 batches of formulation and then evaluated DNA bar coding, quality control factors, physicochemical factors. DNA bar coding indicated that internal transcribed spacer region of our herbal ingredients completely matched the sequences existed in the Gene bank. Additional analysis of the drug indicated that other physicochemical parameters were within acceptable range. The parameters measured in this study are presented a standard criterion for Jovarish-e-kammuni.

Keywords: *Obtain Optimal, Pharmaceutical Standardization, Jovarish-e-kammuni*

INTRODUCTION
Jovarish-e-kammuni is a poly herbal medicine with carminative and digestive properties used for treatment of dyspepsia and obesity (Aghili and Qarabadin-e-kabi, 2013). Jovarish consists of 6 ingredients (Table 1). Despite its extensive usage in traditional medicine, there is few scientific sop prepared (Aghili and Qarabadin-e-kabi, 2014). In order to obtain optimal pharmaceutical standardization, herbal medicines need to meet several quantitative criteria obtained through Standardization process. These factors could be of different classes, from chemical determination to bar coding of DNA. In this study, scientific validation of this drug is performed through applying physicochemical analysis, thin layer chromatography, analysis of microbial load, and estimation of aflatoxins, heavy metals and pesticide residue. Furthermore, DNA bar coding was used for standardization of ingredients and Distinguish adulterations

MATERIALS AND METHODS
The raw components of formulation were collected. Voucher specimens of each plant which are deposited in central herbarium of Tehran University are as follows.

Table 1: Ingredients of formulation

No	Common name	Scientific name	Voucher NO	Part used	Amount
1	caraway	Bunium persicum (Boiss)B.Fedtsh	PMP-647	Fruit	15 gr
2	Ginger	Zingiber officinale Rose.	PMP-224	Rhizome	6 gr
3	RUE	Ruta graveolens L.	PMP-406	Leaf	6 gr
4	Black peper	Piper nigrum L.	PMP-646	fruit	4.5 gr
5	Sodium borate	-	-	-	1 gr
6	Honey	-	-	-	90 gr

Bunium persicum (Boiss)B.Fedtsh (PMP-647), *Zingiber officinale Rose* (PMP-224), *Ruta graveolens L.* (PMP-406), *Piper nigrum L.(* PMP-646)

The formulation was prepared in two laboratory scale batches. Complete ingredients including herbs used if the formula listed in Table 1.

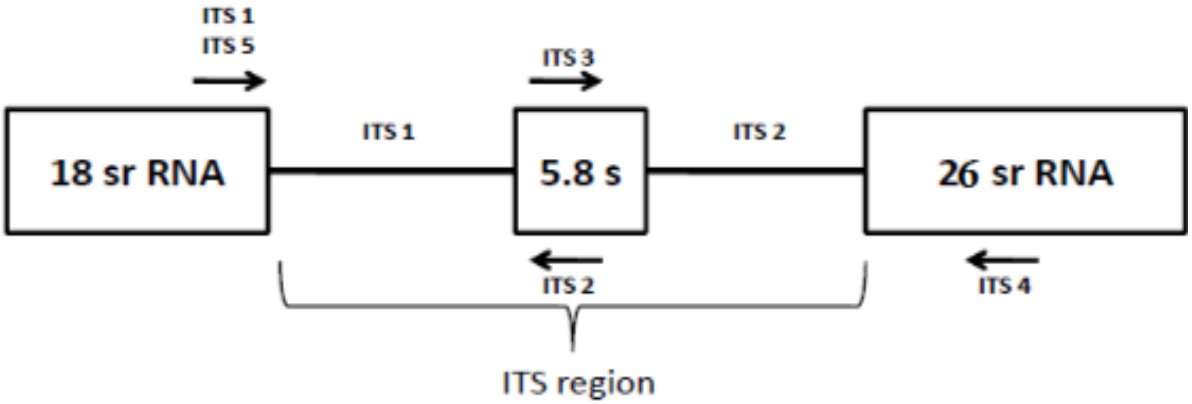
Method of Preparation

All the components were taken of standard quality clean, dried and made powder of herbal ingredient and sieved (mesh 40).

The powder of component 1-5 were mixed then added honey and mixed well and after that packed in tightly closed container (Aghili and Qarabadin-e-kabi, 2014).

Powder DNA Bar Coding

In DNA bar coding, a short sequence from a standard region of the genome is used to distinguish unknown species (Ref.4). Different regions of nuclear and chloroplast DNA can be used as DNA barcode. One of the most used nuclear DNA sequences is internal transcribed spacer (ITS) that includes ITS1+5.8 S+ITS2 regions of nuclear DNA (Figure 1).



As we used powdered plant material of four species in our Jovarish-e-Kammuni, we tend to introduce a DNA barcode to easily and accurately distinguish its components. ITS region can easily be amplified using universal primers.

DNA Extraction, Amplification, and Sequencing

Total DNA was separately extracted for each of four components. In addition, we successfully tested the probability of DNA extraction from large particles existed in the powder. In order to prevent contamination, the particles were washed carefully prior to extraction.

Genomic DNA was isolated from approximately 15 mg of each sample following the CTAB protocol of Doyle and Doyle (1987) using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) and the manufacturer's protocol. Polymerase chain reaction (PCR) amplification of the ITS region was carried out in a Labcycler Thermal Cycler using approximately 40 ng genomic DNA as a template in a 25 µL reaction mixture containing 2.5 µL 10 × buffer, 1.25 mmol/L MgCl2, 0.1 mmol/L each dNTP, 0.5 µmol/L each primer, 1% Tween, and 1.0 U Taq DNA polymerase. The entire ribosomal ITS region (ITS1 + 5.8s + ITS2) was amplified using primer pairs AB 101 (forward, 5'- ACG AAT TCA TGG TCC GGT GAA GTG TTC G - 3') and AB 102 (reverse, 5' - TAG AAT TCC CCG GTT CGC TCG CCG TTA C - 3') PCR protocol for primer pair AB101 - AB102 followed a pretreatment of 5 min at 95° C, 35 cycles of 30 sec at 95° C, 30 sec at 50° C, and 1 min 30 sec at 72° C, and a final extension of 7 min at 72° C. The PCR products were checked in a 1 % agarose gel, stained with Gel Red and visualized under UV light. The PCR products were purified and were directly sequenced using an Illumine sequencer.

Forward and reverse sequences were visually compared and edited using Sequencer 4.1.4 (Gene Codes, Ann Arbor, MI). Then we searched the Gene bank for the most similar sequences in the Gene bank using BLAST (Basic Local Alignment Search Tool) at NCBI

Physico-chemical Analysis

All samples were analyzed for total ash, acid insoluble ash, loss of drying, bulk density, Fructose to glucose ratio test and PH.

Thin Layer Chromatography

Preparation of extract for TLC

1g of each batch were soaked in chloroform and ethanol then refluxed for 10 min and filtered. Then filtrates were concentrated on water bath up to 3 ml.

Condensed extracts utilized on percolated silica gel 60 F 254 TLC plate and for solvent system used toluene: ethyl acetate 9:1 and 6:4.After plates were dried, observing the color spots at UV 254 nm and 366 nm (Wagner *et al*., 1984).

Quality Control

Microbial evaluation and measurement of heavy metals were performed according to the WHO methods. Aflatoxins and pesticide residues measurement were performed by standard methods **(**Anonymous, 1998 and 2000).

RESULTS AND DISCUSSION

Results

Jovarish-e-kammuni is semisolid, dark brown with effective smell and sweet-bitter taste.

DNA Bar Coding Result

Extraction of DNA from plant tissues was possible from either separated or mixed components. In our Jovarish-e-Kammuni, leaf particles of *Ruta graveolensL.* were green, fruit particles of w *Bunium persicum (Boiss)B.Fedtsh* are dark-brown with distinctive yellow ribs, fruit particles of *Piper nigrumL.* (Endocarp) were white, and rhizome particles of *Zingiber officinaleRose.* were yellow (Figure 2).

Our results showed that ITS region of our material completely match the sequences already existed in the Gene bank. For example our DNA sequence of *Bunium persicum (Boiss)B.Fedtsh* was 100 % identical to Gene bank accessions DQ435230 (ITS1) and DQ435269 (ITS2). We observed a little variation in the BLAST results for our *Ruta graveolensL.* Our sample was 99% identical to Gene bank accession FJ434146.1 comparing to the other Gene bank accessions of *Ruta graveolensL.* in the Gene bank, FJ434146.1 with a 4 % threshold can considered as the DNA barcode for *Ruta graveolensL* (96-100% identical). Our DNA sequence of *Piper nigrumL* was 100 % identical to Gene bank accessionsEF060077.1. in the Gene bank, KC 582874 with a 5 % threshold can considered as the DNA barcode for *Zingiber officinaleRose* (95-100% identical).

TLC Analysis Result

Both ethanol and chloroform extract determined specific spot in 254 and 366 nm. The R_f values are presented in Table 2.

Table 2: R_f value

R_f value of chloroform extract		**R_f value of ethanolic extract**	
254 nm	366 nm	254 nm	366 nm
0,81 pink	0.66 green	0.75 pink	0.62 yellowish green
0.73 light pink	0.61 dark pink	0.62 light pink	0.59 dark pink
0.5 blue	0.58 light violet	o.12 light blue	0.54 light violet
o.1 blue	0.5 orange		0.47 orange

Table 2-1: Solvent system, Toluene:Ethyl acetate (9:1)

R_f value of chloroform extract		**R_f value of ethanolic extract**	
254 nm	366 nm	254 nm	366 nm
0.72 blue	0.93 dark pink	0.86 blue	0.96 orange
0.70 blue	0.84 blue	0.78 dark blue	0.88 brown-orange
0.59 dark blue	0.78 light blue	0.65 blue	0.84 fluorescent blue
0.54 lightblue	0.4 blue	0.52 light blue	0.8 dark pink
0.49 light blue	0.34 blue	0.43 light blue	0.74 green
			0.34 blue

Table -2-2 solvent system , Toluene:Ethyl acetate(6:4)

Chemical Analysis Result

Physico chemical factors are determined in Table3

Table 3: Physicochemical limiting factor

Total ash(%w/w)	2.4
Acid insoluble ash(%w/w)	0.48
PH	4.2
Moisture (%w/w)	18
Fructose to Glucose ratio (%w/w)	0.85
Bulk density	1.7

Quality Control Analysis

The estimatin of other parameters such as heavy metals test, microbial load, aflatoxins and pesticide residue are given in Table 4,5,6,7

Table 4: Heavy metals test

Heavy metal	**Results**	**WHO & FDA limits**
Arsenic	0.014	10 ppm
Cadmium	ND	0.30 ppm
Lead	ND	10 ppm
Mercury	ND	10 ppm

Table 5: Microbial test

total bacterial count	$1.8 * 10^5$
total fungal test	none
entrobacteriaceae	absent
salmonella	absent
staphylococcus aureus	absent

Table 6: Aflatoxins

B1	none
B2	none
G1	none
G2	none

Table 7: Pesticide residue analysis

Diazinone	ND	endrin-keton	ND	triallate	ND	ioxanyl octonoate	ND
Metribuzine	ND	azinphos-ethyl	ND	parathion-ethyl	ND	dialifos	ND
Fenitrothion	ND	dieldrin	ND	heptachlor	ND	coumaphos	ND
chlorthal-dimethyl	ND	methoxychlor	ND	aldrin	ND	deltamethrin	ND
Ethin	ND	pyrozophos	ND	parathion-ethyl	ND	carbophention	ND
bromopropylate	ND	trifluralin	ND	chlorthion	ND		
Fenpropathrin	ND	triadimefon	ND	bromophos-methyl	ND		
Tetradifon	ND	pethoxamide	ND	heptachlor-epoxide	ND		
Phosalone	ND	butachlor	ND	chlordan total	ND		
cypermethrin total	ND	hexaconazole	ND	methidation	ND		
fenvalerate total	ND	imazalil	ND	bromophos-ethyl	ND		
Famoxadon	ND	oxadiazon	ND	endosulfan total	ND		
HCH total	ND	nitrofen	ND	endrine	ND		
Propetamphos	ND	diclofop methyl	ND	DDT	ND		

Conclusion

Jovarish-e-kammuni is frequently used in the treatment of dyspepsia. Therefore, using a standard drug is important. According to this study DNA bar coding is used for raw plant materials. By this method we distinguished species and results showed ITS region of materials completely matched the sequences in the Gene bank.

Then other analysis for quality control and physicochemical parameter estimated.

Based on the above result, this study is a step toward standardization of Jovarish-e-kammuni.

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This article based on PhD thesis. The authors declare that there is no conflict of interest.

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