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Standardization and Stability Study of *Jawarish-e-Bisbasa*, A Unani Formulation

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ABSTRACT : Traditional medicines are effective but the Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Department of AYUSH (a Government Body) has given preliminary guidelines for standardizing these conventional formulations, for uniformity of batches in production of herbal formulation and it is necessary to develop methods for evaluation. The present work is an attempt to prepare and to standardize *Jawarish-e-Bisbasa*, a unani formulation, used in carminative in abdominal distension and also in piles. The various parameters performed both for raw materials and finished products included organoleptic characteristics, physicochemical, microscopical, HPTLC analysis, quantitative and qualitative analysis of biological molecules, sterility and antimicrobial activity as well as stability studies. The phytochemical constituents found to be present in the raw material used for the preparation of *Jawarish-e-Bisbasa* possibly facilitate the desirable therapeutic efficacy of the standardized medicinal formulation as a whole, and also could help in knowing the underlying mechanism of pharmacological action. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standards.

Keywords : *Jawarish-e-Bisbasa*, Unani formulation, Standardization, Stability study.

INTRODUCTION

In recent years, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics (Sagar *et al.*, 2005). There are around 6000 herbal manufacturers in India. More than 4000 units are producing Ayurveda medicines. Due to lack of infrastructures, skilled manpower reliable methods and stringent regulatory laws most of these manufacturers produce their product on very tentative basis (Patel *et al.*, 2006).

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs (Patel *et al.*, 2006*b*). "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations (Bhutani, 2003). "Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration (Kokate *et al.*, 2005).

The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy (Lohar, 2009; Anonymous, 1999).

Standardization of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in case of polyherbal drugs (Patel *et al.*, 2006b).

The main problem in poly herbal formulation is that the presence of each ingredient has to be established (Bhutani, 2003). The microscopic Character of each ingredient are very difficult to identify and also some time overlapping with the character of other ingredient. The paper presents development of methods for the evaluation and standardization of *Jawarish-e-Bisbasa*, a Unani formulation which is used as carminative in abdominal distension and also in piles. It consist of *Myristica fragrans*, *Syzygium aromaticum*, *Piper nigrum*, Amomum subulatum as major ingredients.

MATERIALS AND METHODS

For preparation the ingredient were purchased from the local raw materials traders and used as a control. All analytical specification was carried out in triplicate in a sample of *Jawarish-e-Bisbasa* according to the prescribed methods in AYUSH and WHO guidelines. Organoleptic characters and particle size of samples were recorded.

Preparation of *Jawarish-e-Bisbasa :* *Jawarish-e-Bisbasa* was prepared according to the method described in National Formulary of Unani Medicine (Anon., 1993). The powdered ingredients were weighed according to the amount mentioned (Table 1). Initially 125 ml of pure water and 125 g pre-weighed sugar was added in a stainless steel vessel and heated over low flame. When all the sugar dissolved 125 g weighed honey was added. The contents in the vessel were continuously stirred till the consistency of one tar was observed. When the Qiwam (base) is prepared of required consistency, heating was stopped and it was allowed to cool. Each ingredient was added one after the other and mixed uniformly so that the homogeneity of the formulation is maintained.

Table 1: Ingredients of *Jawarish-e-Bisbasa.*

S. No.	*Unani name*	*Botanical name*	*Part used*	*Quantity*
1.	Bisbasa	*Myristica fragrans*	Seed covering	5 grams
2.	Taj-Qualmi	*Cinnamomum tamala*	Bark	5 grams
3.	Heel-e-Khurd	*Eletearia cardamomum*	Seeds	5 grams
4.	Zanjabeel	*Zingiber officinale*	Rhizome	5 grams
5.	Filfil Daraz	*Piper longum*	Fruit	5 grams
6.	Darchini	*Cinnamomum zeylanicum*	Bark	5 grams
7.	Asaroon	*Asarum europaeum*	Root	5 grams
8.	Qaranfal	*Syzygium aromaticum*	Fruit	7.5 grams
9.	Filfil Siyah	*Piper nigrum*	Fruit	10 grams
10.	Heel-e-Kalan	*Amomum subulatum*	Seeds	25 grams

Storage and preservation : *Jawarish-e-Bisbasa* was preserved in dried, airtight, fungus free clean glass or china clay container. During preservation if Jawarish becomes dry it can be brought to normal by consistency by addition of purified honey or Qiwam made up of sugar.

Experimental procedure : ***Physico-chemical analysis:*** Total Ash value, acid insoluble Ash, water soluble extractive value, ethanol soluble extractive value, fixed oil content, loss on drying were determined (Iyengar, 1995; Trease and Evans Wc., 1989).

Phyto-chemical Analysis: Preliminary tests were carried out on methanolic extract for the presence / absence of phyto-constituents like alkaloids, carbohydrates, flavanoids, glycosides, saponins, sterols, terpenes and tannins (Sazada *et al.*, 2009).

Microscopic Analysis: The microscopic Character of each ingredient and final product were carried out (Anonymous, 1992). Permanent slides were prepared and stained with Safronin (1%) + Glycerin (Selvakumar *et al.*, 2010).

HPTLC Profile

For HPTLC profiling, 1 gm of sample was extracted with 10 ml of methanol in a reflux condenser for 1 hr, filtered and concentrated. The chromatograph was performed by spotting extracted samples on pre-coated silica gel aluminum plate 60F-254 (10 cm × 10 cm with 250 μm thickness) using Camag Linomat 5 sample applicator and a 100 μl Hamilton syringe. The sample, in the form length 8 mm were applied as a band 8mm from bottom, 12 mm from left margin of plate and 10 mm apart from each band, at a constant application rate of 150 nl/s using nitrogen aspirators.

Plates were developed using a mobile phase (consisting of Toluene: Ethyl acetate: Methanol (7 : 2 : 1 v/v), for comparing finished product with raw material. For stability studies, Toluene: Ethyl Acetate: GAA (7.5 : 3 : 0.2) was used as the mobile phase.

Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 15 min at room temperature. The length of chromatogram run was 7.5 cm. 10ml of mobile phase was used for development, which required 10minutes to develop. Subsequent to the development, TLC plate was dried with help of air dryer. Densitometric scanning was performed on Camag TLC scanner III in the operated by win CATS planer chromatography version 1.4.3. The source of radiation utilized was Mercuric, Deuterium lamp. The slit dimension setting of length 6.00mm and width 0.45 mm, and scanning rate of 20mm/s was employed for plate. The scanning was performed at 266 nm and 366 nm (Eike, 2006; Sazada *et al.*, 2009).

Microbial Screening: For the finished product microbial analysis was done. (Gopala *et al.*, 2008).

Standard plate count: This method was followed in order to enumerate the total aerobic count in a sample (Gopala *et al.*, 2008).

Antimicrobial test: Formulation was checked for its antimicrobial activity against *Klebsiella pneumonia, Proteus vulgaris, Salmonella typhae, Staphylococcus aureus, Escherichia coli* by Agar diffusion method (Gopala *et al.*, 2008).

Stability studies: Comparison of the finished product (formulation) stored at room temperature for second, third & fourth month was carried out by conducting tests for the parameters resin content, eugenol content, using HPTLC technique (Gopala *et al.*, 2008).

RESULTS

Botanical parameters revealed that brownish yellow in color, with pleasant odor, sweet taste. Results of quantitative analysis for Total ash, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, Lipid content, Volatile Oil content, Particle size (80-100 mesh) and Loss on drying at 105 °C were calculated (Table 2). Phytochemical analysis showed the presence of alkaloids, Glycosides, Tannin/ phenols, carbohydrate and fixed oils (Table 3). Microscopic analysis of sample showed the presence of identifying diagnostic characters, which are not overlapping. It shows Pieces of pitted vessels, fibers, epithelial cells, cork cells etc. (Fig. 1).

Table 2: Physicochemical analysis of *Jawarish-e-Bisbasa.*

S.. No.	*Parameters*	
1	Description	
	Colour	Brownish
	Taste	Sweet
	Odour	Aromatic
	Nature	Semi solid
2	Loss on drying at 105 Degree C	80 %
3	Total Ash	9 %
4	Acid soluble Ash	0.4 %
5	Water soluble Extractive	49 %
6	Alcohol soluble extractive	17 %
7	Lipid content	7 %
8	Volatile oil content	0.5 %

In HPTLC procedure, the comparison of finished product with raw material, observed under Visible light and UV light showed distinctive characteristic patterns (Fig. 2). Rf values were calculated (Table 4). In Stability study of the finished product, the resin content, eugenol content were checked for four months. The results obtained were found to be within acceptable ranges / values and were nearly constant over the tested interval of time of about four months.

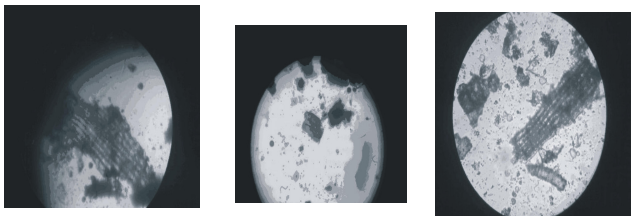
Table 3: Phytocochemical analysis of *Jawarish-e-Bisbasa.*

S. No.	*Chemical constituents*	*Jawarish-e-Bisbasa*
1.	Alkaloid	+
2.	Carbohydrate	+
3.	Steroids	–
4.	Reducing Sugar	+
5.	Glycoside	+
6.	Tannins/ Phenols	+
7.	Fixed oil	+
8.	Protein	+

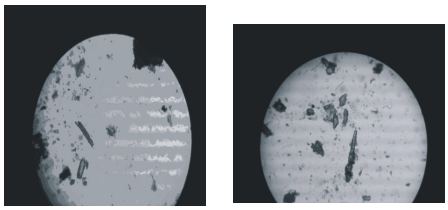
Table 4: Rf Value for HPTLC Analysis.

Jawarish-e-Bisbasa (2½ months)	247
Jawarish-e-Bisbasa (3 months)	550
Jawarish-e-Bisbasa (3½ months)	816
Eugenol 250 ppm	330
Jawarish-e-Bisbasa (4 months)	1014

The results obtained were found to be within acceptable ranges/values and were nearly constant over the tested interval of time of about four months.

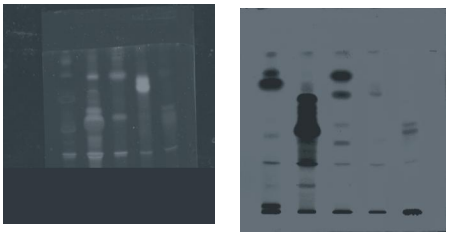


Epithelial cells Pericarp Xylem



Pitted Xylem Prism

Fig. 1. Microscopy of *Jawarish-e-bisbasa*.



Fluorescence at 366 nm Ultraviolet at 266 nm

Fig. 2. Detection under Visible and UV light.

Key: Track1: Qaranfal; Track 2 : Filfil Siyah; Track 3: Darchini; Track 4: Zanjabeel; Track 5: *Jawarish-e-Bisbasa* (Formulation)

For the finished product, microbial analysis was done. Pathogens *Klebsiella pneumonia*, *Proteus vulgaris*, *Salmonella typhae*, were found to be inhibited by formulation. Total aerobic count was done and bacteria, fungi and coliforms were found to be within limits.

DISCUSSION

The standardization of *Jawarish-e-Bisbasa* has become possible by considering various scientific parameters concerning the quality protocol, keeping intact procedures following Unani system of medical knowledge. As a part of quality control analysis, for the parameters-ash content, acid insoluble ash have been found within the standard ranges. But, for the parameters-moisture content, pH, soluble extractives, there are no standard ranges available. The value obtained for each of these parameters was found to be consistent for four months. So, the preparation method and value obtained for above parameters could be considered for laying down new pharmacopoeial standards while preparing *Jawarish-e-Bisbasa* according to traditional methods.

To check expected consistency as part of standardization of *Jawarish-e-Bisbasa*, TLC records were obtained up to four months. The occurrence of same number of spots in TLC plates confirms the consistency of the finished product *Jawarish-e-Bisbasa.* Such a stipulation for obtaining TLC (including the number of spots and corresponding Rf values) could be considered and laid down as part of standardization guidelines in the preparation of *Jawarish-e-Bisbasa*. The fact that the finished product was found to be stable over up to 4 months is more than indicative that the medicine continues to retain its therapeutic value. It has become possible to identify the various phytochemical constituents has also been delineated. The results obtained could be used as a pharmacopoeial standard for the preparation of *Jawarish-e-Bisbasa* for getting optimal efficacy of the medicine.

CONCLUSION

After analysis of sample of *Jawarish-e-Bisbasa* by different parameters such as total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, lipid content, volatile oil content, particle size (80-100 mesh) and loss on drying at 105° C, microscopic analysis, phytochemical analysis, HPTLC chromatogram, microbial screening and stability studies showed reproducible fingerprints between batches. So it can be concluded that these parameters can be used for the evaluation of *Jawarish-e-Bisbasa*. The same protocol may be applied for as a regular quality control and standardization for polyherbal formulations.

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