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Standardization of an Ayurvedic Formulation: Bhuvnesvara Vati

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ABSTRACT

Bhuvnesvara Vati (BV) is a well known formulation used in Ayurveda for a wide range of disorders. It is having different ingredients which are having a wide range of uses in health maintenance. Information on the qualitative and quantitative parameters of Bhuvnesvara vati (BV) to guarantee the quality and the safety of the product to the consumer is less; many of these parameters were vary according to the method of preparations. The quality control of Bhuvnesvara Vati which will assist the regulatory authorities, scientific organizations and manufacturer in developing standards. With this aim, In the recent study, an attempt has been made to develop standardization methods of Bhuvnesvara Vati (BV). A comparative study has been made between in-house preparation and the two marketed formulations. These all formulations were standardized on the basis of organoleptic properties, physical characteristics and physico-chemical properties. The set parameters were found to be sufficient to evaluate the vati and can be used as reference standards for the said formulation which will be part of the quality assurance for these formulations.

Keywords: Bhuvnesvara Vati (BV), Ayurvedic formulation, Standardization, physico-chemical parameters.

INTRODUCTION

Knowledge of herbs has been handed down from generation to generation for thousands of years [1]. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness, and providing leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [2]. According to WHO more than 80% of world's population relies on traditional herbal medicine for their primary health care [3]. India, with its mega diversity and knowledge rich ancient traditional systems of medicine, viz. ayurveda, siddha, unani, amchi and local health traditions, provides a strong base for the utilization of large number of plants in general health care and alleviation of common ailments of the people [4]. Although the herbal medicines have been enjoying renaissance, one of the impediments in the acceptance of these formulations is the lack of standardization and quality control profiles. Due to the complex nature and inherent variability of the chemical constituents of plant based drugs, it is difficult to establish quality control parameters [5]. However, with the increasing demands for these phyto-pharmaceuticals, their standardization is also becoming mandatory. Standardized products provide more security and increase the level of trust that people have in herbal drugs [6]. The present study was, therefore, undertaken to develop standardization parameters for Bhuvnesvara Vati (BV). A comparative study has been made between in-house preparation and the two marketed formulations. These formulations were standardized on the basis of organoleptic properties, physical characteristics and physico-chemical properties.

MATERIALS AND METHODS

Physico-chemical studies like total ash, acid insoluble ash, water & alcohol soluble extract, loss on drying at 100-105^{0}C and successive extractive values by cold maceration method were carried out as per the WHO guidelines [7], Preliminary phytochemical tests were performed as per the standard methods [8].

Plant material

The crude drugs used in preparation were purchased from local market and were identified morphologically and microscopically and compared with standard pharmacopoeial monograph.

Preparation of formulations

Three sample batches of Bhuvnesvara Vati were prepared as per the procedure mentioned in Ayurvedic formulary of India and were named as BV-I, BV-II, BV-III. The same procedure was performed for each batch of Bhuvnesvara vati, Two Marketed formulations named M-I and M-II were purchased from local pharmacy.

Standardization parameters

The various standardization parameters studied were Organoleptic properties, Physical Characteristics, Loss on drying, determination of foreign matter, determination of extractive values, Physico-chemical investigations, Preliminary Phytochemical analysis.

Organoleptic Evaluation

The evaluation of organoleptic characters [9] of the samples was based on the method described by Wallis. Organoleptic evaluation refers to evaluation of the formulation and raw material by color, odor, taste and texture etc.

Table 1 Organoleptic properties of Bhuvnesvara vati and its raw material

S. No.	Name		Colour	Odour	*Taste*
1	*Emblica officinalis*		Dark drown	Characteristic	*Sour and astringent*
2	*Terminalia chebula*		Light brown	Characteristic	*Bitter*
3	*Terminalia belerica*		Light brown	Bitter	*Astringent*
4	*Trichyspermum ammi*		Grayish brown	Pungent	*bit bitter and pungent*
5	*Aegle marmelus*		Pale red	Aromatic	*Astringent and mucilagenous*
6	Bhuvnesvara vati	BV-I	Dark Brown	Slight Bitter	*Salinity and Astringent*
		BV-II	Dark Brown	Slight Bitter	*Salinity and Astringent*
		BV-III	Dark Brown	Slight Bitter	*Salinity and Astringent*
		M-I	Dark Brown	Slight Bitter	*Salinity and Astringent*
		M-II	*Dark Brown*	*Slight Bitter*	*Salinity and Astringent*

Determination of physical characteristics of raw materials:

The raw materials of BV were subjected to evaluation of physical properties which were determined in form of bulk density [10], tap density [10], angle of repose [10], hausner ratio [11] and carrs index [11]. The term bulk density is defined as the mass of a powder divided by the bulk volume. Tap density is defined as the mass of a powder divided by the Tapped volume. Hausner Ratio is related to interparticle friction and as such can be used to predict the powder flow properties. The Carr index is a method of measuring the powder flow from bulk density.

Table 2 Physical characteristics of raw materials of Bhuvnesvara vati

S.no	Name	Tap density	Bulk density	Angle of repose	Hausner ratio	Carr's index
1	*Emblica officinalis*	0.5067	0.4143	35.01	1.223	22.37
2	*Terminalia chebula*	0.5076	0.4067	37.25	1.248	24.82
3	*Terminalia belerica*	0.5138	0.4042	36.02	1.271	27.13
4	*Trachyspermum ammi*	0.5420	0.4346	36.55	1.247	24.75
5	*Aegle marmelus*	0.5232	0.4152	37.48	1.269	26.93

Physico-chemical investigation

Determination of Total ash [7]

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. For its detection, About 4gm of the ground air-dried material is accurately weighed and placed in a previously ignited and tarred silica crucible. The material is spread in an even layer and ignited by gradually increasing the heat to a temperature of 500-600°C until it is white, indicating the absence of carbon. The material is cooled in a desiccator and weighed. The content of total ash is calculated in mg per gm of air-dried material.

Acid-insoluble ash [7]

To the crucible containing the total ash, 25 ml of hydrochloric acid is added, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass is rinsed with 5 ml of hot water and this liquid is added to the crucible. The insoluble matter is collected on an ashless filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter is transferred to the original crucible, dried on a hot-plate and ignited to constant weight. The residue is allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The content of acid-insoluble ash is calculated in mg per gm of air-dried material.

Table 3 Percentage ash value of Bhuvnesvara vati and its raw materials

S.no.	Name		Total ash* (% w/w ± S.D., n=6)	Acid insoluble ash* (% w/w ± S.D., n=6)
1	*Emblica officinalis*		4.629 ± 0.389	1.204 ± 0.023
2	*Terminalia belerica*		8.132 ±0.785	0.821 ± 0.012
3	*Terminalia chebula*		3.891 ±0.423	0.423 ± 0.008
4	*Trichyspermum ammi*		4.289±0.356	0.621 ± 0.021
5	*Aegle marmelus*		6.246±0.965	0.636 ± 0.052
6	Bhuvnesvara vati	BV-I	25.831 ± 0.376	0.589 ± 0.065
		BV-II	25.826 ± 0.651	0.595 ± 0.054
		BV-III	25.826 ± 0.965	0.599 ± 0.043
		M-I	32.852 ± 0.368	0.863 ± 0.046
		M-II	33.832 ± 0.468	0.852 ± 0.056

Determination of extractive values [7]

The percent extractive values were determined in various solvents ranging from non polar semi polar to polar behavior. The extractive values are recorded in petroleum ether, chloroform, alcohol and water with a view to study the distribution of various constituents of Bhuvnesvara vati (BV-I, BV-II and BV-III), Marketed formulation (M-I and M-II) and its raw materials *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Trichyspermum ammi* and *Aegle marmelus*. Accurately weighed 4.0 gm of coarsely powdered air-dried material is placed in a glass-stoppered conical flask and macerated with 100 ml of the solvent for 6 hours, shaking frequently, and then allowed to stand for 18 hours. The mixture is filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate is transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. The residue is dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The findings were recorded in terms of percentage extractive values in table 5.7.

Table 4 Extractive values (%w/w ±S.D., n=6) Bhuvnesvara vati and its raw materials

S.no.	Name		Water soluble	Alcohol soluble	Chloroform soluble	Petroleum ether soluble
1	*Emblica officinalis*		40.334±1.067	25.25± 1.422	3.25 ± 0.144	0.924±0.073
2	*Terminalia belerica*		28.127±1.788	19.75±1.248	7.542±0.972	1.026±0.884
3	*Terminalia chebula*		38.756±2.102	20.21±1.667	4.578±0.549	1.329±0.069
4	*Trichyspermum ammi*		13.532±0.938	2.538±0.369	1.203±0.974	0.532±0.236
5	*Aegle marmelus*		55.128±0.723	7.128±0.435	2.014±0.736	0.892±0.845
6	Bhuvnesvara vati	BV-I	40.745±0.328	25.912±1.212	3.361±0.268	0.957±0.347
		BV-II	40.232±1.089	25.456±0.653	3.386±0.547	0.921±0.232
		BV-III	39.979±1.129	25.236±0.581	3.364±0.612	0.969±0.721
		M-I	42.679±1.246	23.258±0.256	2.258±0.856	1.001±0.128
		M-II	39.212±0.325	25.163±1.456	2.962±0.425	0.998±0.358

Determination of loss on drying [7]

Accurately weighed 5g of the air-dried material, is placed in a previously dried and tared flat weighing bottle. The sample is dried in an oven at 100-105°C until two consecutive weighing do not differ by more than 5mg.

Table 5 Percent loss on drying of Bhuvnesvara vati and its raw material

S.no.	Name		%LOD	±S.D. (n=6)
1	*Emblica officinalis*		6.40	0.612
2	*Terminalia chebula*		7.62	0.474
3	*Terminalia belerica*		4.60	0.319
4	*Trichyspermum ammi*		3.20	0.729
5	*Aegle marmelus*		6.49	0.436
6	Bhuvnesvara vati	BV-I	5.661	0.342
		BV-II	5.663	0.129
		BV-III	5.667	0.369
		M-I	5.98	0.362
		M-II	6.04	0.456

Determination of foreign matter [7]

Accurately weighed (250g) sample of plant materials are spread in a thin layer and foreign matter is sorted by using a magnifying lens (6x or 10x). The remainder of the sample is sifted through a No. 250 sieve; dust is regarded as mineral admixture. The portions of this sorted foreign matter are weighed and the content of each group is calculated in grams per 100g of air-dried sample.

Table: 6 Percent foreign matters in raw materials of Bhuvnesvara vati

S.no.	Name		%Foreign Matter	±S.D. (n=6)
1	*Emblica officinalis*		1.21	0.032
2	*Terminalia chebula*		1.52	0.084
3	*Terminalia belerica*		1.16	0.082
4	*Trichyspermum ammi*		2.06	0.365
5	*Aegle marmelus*		0.221	0.312
6	Bhuvnesvara vati	BV-I	NIL	NIL
		BV-II	NIL	NIL
		BV-III	NIL	NIL
		M-I	NIL	NIL
		M-II	NIL	NIL

Qualitative phytochemical studies [8]

To detect the presence of various phytoconstituents in formulations as well as in raw materials phytochemical investigation is performed. The tests are performed on alcohol, water, chloroform and petroleum ether extract. Qualitative phytochemical analysis was done for Bhuvnesvara Vati (BV), two marketed formulations and all the raw ingredients of formulation using the procedures of Kokate (1994). Alkaloids, carbohydrates, tannins and phenols, fixed oils and fats, saponins and gums and mucilage's were qualitatively analyzed.

Table 7 Qualitative chemical analysis of various extract of Bhuvnesvara vati and its raw materials

S. No.	Chemical Test	Extract	BV-I	BV-II	BV-III	M-I	M-II	EM	TB	TC	TA	AM
1.	Alkaloids	Alcohol	+	+	+	+	+	-	-	-	-	+
		Water	+	+	+	+	+	-	-	-	-	+
		Chloroform	+	+	+	+	+	-	-	-	-	+
		Pet. ether	-	-	-	-	-	-	-	-	-	-
2.	Carbohydrate and Glycoside	Alcohol	+	+	+	+	+	+	+	+	+	+
		Water	+	+	+	+	+	+	+	+	+	+
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	-	-	-	-	-	-	-	-	-	-
3.	Tannins and phenols	Alcohol	+	+	+	+	+	+	+	+	+	+
		Water	+	+	+	+	+	+	+	+	+	+
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	-	-	-	-	-	-	-	-	-	-
4.	Gums and Mucilage's	Alcohol	-	-	-	-	-	-	-	-	-	-
		Water	+	+	+	+	+	+	+	+	+	+
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	-	-	-	-	-	-	-	-	-	-
5.	Fixed oils and fats	Alcohol	-	-	-	-	-	-	-	-	-	-
		Water	-	-	-	-	-	-	-	-	-	-
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	+	+	+	+	+	+	+	+	-	-
6.	Saponins	Alcohol	+	+	+	+	+	+	+	+	+	-
		Water	+	+	+	+	+	+	+	+	+	-
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	-	-	-	-	-	-	-	-	-	-
7.	Protein and free amino acid	Alcohol	+	+	+	+	+	+	+	+	+	+
		Water	+	+	+	+	+	+	+	+	+	+
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	-	-	-	-	-	-	-	-	-	-
8.	Volatile oil	By hydro distillation method	-	-	-	-	-	-	-	-	+	-
			-	-	-	-	-	-	-	-	+	-
			-	-	-	-	-	-	-	-	+	-
			-	-	-	-	-	-	-	-	+	-
9.	Phytosterol	Alcohol	+	+	+	+	+	+	+	+	-	-
		Water	-	-	-	-	-	-	-	-	-	-
		Chloroform	-	-	-	-	-	-	-	-	-	-
		Pet. ether	+	+	+	+	+	+	+	+	-	-

*- : Absent, + : present, **EM:** Emblica officinalis,**TB:** Terminalia belerica,**TC:** Terminalia chebula,**TA**: Trichyspermum ammi **AM**: Aegle marmelus*

RESULTS AND DISCUSSION

Three batches of Bhuvnesvara vati designated as BV-I, BV-II and BV-III were prepared in laboratory using method described in Ayurvedic Formulary of India. Two marketed formulations named M-I and M-II was purchased from local pharmacy store.

Results of organoleptic properties reveal that the Bhuvnesvara vati is dark brown in colour with salinity and astringent taste having slight bitter odour. The results were reproducible with each batch and hence these results may be considered as standard for further reference. The results for the marketed formulations (M-I and M-II) and Laboratory formulation are found comparable (table-1).

Crude powdered drugs possess some physical properties which are determined in form of tap density, bulk density, angle of repose, Hausner ratio and Carr's index. Among the raw materials *Emblica officinalis* and *Terminalia chebula, Trichyspermum ammi* showed good flow properties while a little poor flow was observed in case of *Terminalia belerica* and *Aegle marmelous.* This is further confirmed by high value of Hausner's ratio and Carr's index (Table 2).

The total ash value indicates both physiological ash, and non-physiological ash. The total ash value for *Emblica officinalis, Terminalia belerica, Terminalia chebula, Trichyspermum ammi* and *Aegle marmelous* was 4.629± 0.389 %, 8.132 ±0.785 % and 3.891±0.423 %, 4.289±0.356 and 6.246±0.965 respectively. The total ash value for BV-I, BV-II and BV-III are 25.831±0.376 %, 25.826±0.651%, and 25.826±0.965 respectively while in M-I and M-II it is 32.852±0.368 % and 33.832±0.468%. This shows that M-I and M-II contain higher amounts of inorganic components. The yield of acid insoluble ash for *Emblica officinalis, Terminalia belerica, Terminalia chebula, Trichyspermum ammi* and *Aegle marmelous* was 1.204±0.023, 0.821±0.012, 0.423±0.008, 0.621±0.021 and 0.636± 0.052 respectively which indicates the amount of silica present, especially as sand and siliceous earth. The acid insoluble ash for BV-I, BV-II and BV-III are 0.589±0.065 %, 0.595±0.054 % and 0.599± 0.043 % respectively and for M-I and M-II 0.863± 0.046 % and 0.852± 0.056 % (Table 3).

Extractive values for raw material *Emblica officinalis, Terminalia belerica, Terminalia chebula, Trichyspermum ammi* and *Aegle marmelus* in water are 40.334±1.067%, 28.127±1.788%, 38.756±2.102%, 13.532±0.938% and 55.128±0.723 respectively, in alcohol 25.254±2.422%, 19.753±1.248%, 20.212±1.667%, 2.538±0.369 and 7.128±0.435 respectively, in chloroform 3.25±0.144%, 7.542±0.972%, 4.578±0.549%, 1.203±0.974 and 2.014±0.736 respectively and in petroleum ether 0.924±0.073%, 1.026±0.884%, 1.329± 0.069, 0.532±0.236 and 0.892±0.845 respectively. The extractive values for the three batches of Bhuvnesvara vati BV-I, BV-II and BV-III are 40.745± 0.328%, 40.232± 1.089%, and 39.979±1.129% in water, 25.912±1.212%, 25.456±0.653%, 25.236±0.581% in alcohol, 3.361±0.268%, 3.386±0.547%, 3.364±0.612% in chloroform and 0.957±0.347%, 0.921±0.232%, 0.969±0.721% in petroleum ether respectively. While in M-I and M-II the values are 42.679±1.246 % and 39.212±0 .325% in water, 23.258±0.256 % and 25.163±1.456% in alcohol, 2.258±0.856% and 2.962±0.425% in chloroform and 1.001±0.128% and 0.998±0.358% in petroleum ether respectively. Highest extractive values are obtained in water and alcohol (Table 4). This indicates high proportion of tannins, carbohydrates and glycoside etc in the formulations as well as raw material.

Loss on drying or heating to constant weight is determined for crude drugs and finished formulation. The percent loss on drying was found to be 6.40±0.612 for *Emblica officinalis*, 4.60±0.319 for *Terminalia belerica*, 7.62±0.474 for *Terminalia chebula*, 3.20±0.729 for *Trachyspermum ammi* and 6.49 ± 0.436 for *Aegle marmelus.* The percent loss on drying for BV-I, BV-II and BV-III are 5.661±0.342, 5.663±0.129 and 5.667± 0.369, while it is 5.98±0.362 for M-I and 6.04±0.456 for M-II (Table 5).

The percent of foreign matter was found to be 1.21±0.032 for *Emblica officinalis*, 1.16±0.082 for *Terminalia belerica*, 1.52±0.084 *Terminalia chebula*, 2.06±0.365 for *Trichyspermum ammi* and 0.221±0.312 for *Aegle marmelous.* Laboratory formulations of Bhuvnesvara vati are prepared after removal of foreign matter but there is possibility that this may be present in M-I and M-II, because it was obtained in finished form (Table 6).

To detect the presence of various phytoconstituents in formulations as well as in raw materials phytochemical investigations were performed (Table 7). The tests were performed on alcohol, water, chloroform and petroleum ether extract. The raw materials and formulations were found to have phenolics (tannins), carbohydrates, glycosides, phytosterols, fixed oil and fats, saponins and proteins, alkaloid. Marketed formulations are found to contain the same constituents.

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