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PHYTO, PHYSICOCHEMICAL SCREENING OF *BHUVANESHVAR VATI*

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ABSTRACT

Ayurveda is one of the traditional medicinal systems of India. Ayurvedic formulations are amalgamation of various herbs used to cure various lifestyle diseases. Standardization of drug means confirmation of its identity and determination of its quality and purity by using various tests for phytochemical and physicochemical analysis. The main objective of this study was to screen *"Bhuvaneshvar Vati"* for ots phytochemical and physicochemical properties which can play a key role in its standardization. *Bhuvaneshvar Vati* is an ancient ayurvedic preparation used in the treatment of diarrhoea (Atisara). The formulation was prepared as per the guidelines mentioned in Ayurvedic Pharmacopoeia of India. The powder characteristics, test for phytochemical constituents and biochemical tests were performed on formulation. In addition to these tests, the tests like hardness, friability, organoleptic characteristics & TLC was also performed. Instrumental analysis was done by using HPLC & HPTLC. The results obtained were useful for standardization of ASU drugs.

KEYWORDS: *Bhuvaneshvar Vati*, Standardization, Formulation, Ayurveda.

INTRODUCTION

"Ayurvedic Drug" is a form of alternative medicine that is the traditional system of medicine of India and seeks to treat and integrate body, mind, and spirit using a comprehensive holistic approach especially by emphasizing diet, herbal remedies, exercise, meditation, breathing, and physical therapy. Many ayurvedic drugs have been found far superior to their allopathic counterparts. Ayurveda is completely natural and it believes that to follow the nature is the only way to achieve the complete wellness. As far as the preparation used in Ayurvĕdic system of medicine, a drug formulation or design may not be a problem, because many formulations are well documented in classical texts. But, there is confusion with respect to standards to be followed while preparing a formulation as well as basic parameters to assess the quality of the finished product.

Standardisation is the process of implementing and developing technical standards Standardization can help to maximize compatibility, interoperability, safety, repeatability, or quality. Standardisation of ASU dug is maintaining the same physico-chemical properties and quality through out the preparation to get identical therapeutic efficacy in same batch.The current research on ayurvedic formulation Bhuvneshvar Vati aims to analyze its phyto and physicochemical properties which will paly a key role in setting standards for its quality production.Bhuvaneshvar Vati is a herbal formulation used extensively as treatment of Diarrhoea, Dysentry, Ulcerative colitis. It Balances vatta and pitta.

Bhuvaneshvar Vati is prepared from five herbs Amlaki (*Embilica officinalis*), Haritaki (*Terminalia chebula*) and Bibhitaki (*Terminalia bellerica).* Yawani (*Trachyspermum ammi),* Bilva (*Aegle marmelos)* and exciepients Saidhava (Rock Salt), Grihadhuma(Activated Charcoal).

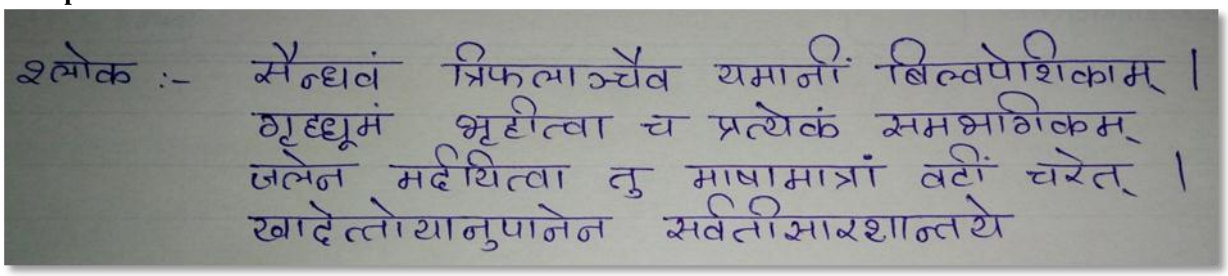
In order to assess the quality of inhouse formulation,it was prepared at laboratory scale as per pharmacopoeial standards and it was subjected to various quality control tests.

MATERIALS AND METHODS

1. Raw Materials, Chemicals and Reagents

Plant Raw materials used for the preparation of Bhuvaneshvar Vati were procured Ayurvedic Proprietory Medicines Shop (Mumbai) with the knowledge of Ayurvedic physician. The materials were dried in an oven preset at 45°C, powdered, sieved through an 85-mesh (BSS) sieve and stored in air tight containers.The Gallic Acid standard was procured from Himedia and Assigned purity: 98%.

2. Preparation of Bhuvaneshvar Vati



- Mix the coarsely fine powders of all ingredients in sufficient amount of water to make firm dough.
- Accurately weigh 2gm dough and roll out the vatis.
- Heat at 110° C in an oven.

Table 1: Formulation composition.

Sr.No.	Ayurvedic Name	Botanical / Scientific identity	Quantity
1.	Amlaki	*Embilica officinalis*	10gm coarse powder each
2.	Haritaki	*Terminalia chebula*	
3.	Bibhitaki	*Terminalia bellirica*	
4.	Yawani	*Trachyspermum ammi*	
5.	Bilva	*Aegle marmelos*	
6.	Saidhava	Rock Salt	
7	Grihadhuma	Activated Charcoal	

3. Quality Evaluation of Bhuvaneshvar Vati

- **Organoleptic evaluation**

The formulation was studied for its preliminary characters like colour, texture, odour and taste.

- **Preliminary Phytochemical and Biochemical Evaluation**

Phytochemical screening of some major secondary metabolites (Flavonoids, Tannins, Alkaloides, Glycosides, Terpenoids, Steroids, Phlobatannin, Phenolic Compounds and Saponins) and Biochemical for Carbohydrates, Proteins and Fats in Bhuvaneshvar Vati was carried out by performing preliminary colour based tests.

- **Physical Evaluation**

The prepared formulation was subjected for physical studies Friability, Hardness, LOD and Ash Value.

- **Chromatographic Evaluation**

Preparation of Standard

Gallic Acid standard was prepared in methanol with initial concentration of 1000 ppm. Further dilution of 100 ppm was prepared using mobile phases.

Preparation of Sample

All the raw materials and prepared formulation powders were dissolved in Methanol and kept overnight.Next day al the solutions were filtered through whattman filter paper to obtain clear extracts.

- **High Perfiormance Thin Layer Chromatography(HPTLC) Fingerprinting**

10 µl of the filtered solution of formulation extract and standard was applied on the TLC plate as per condtions mentioned in table 1a followed by development, derivatizing with vanillin sulphuric acid agent and scanning at 513 nm.

Table 1a: Chromatographic Conditions for HPTLC.

Stationary Phase	HPTLC plates silica gel 60 F 254
Plate size	10.0x10.0 cm
Mobile Phase	Ethyl Acetate : Methanol : water (40.48 : 5.46 : 4.04)
Saturation Time	20 min.
Standard Used	100 ppm Gallic Acid
Spot Volume	10 µl
Band Length	8.0mm
Solvent Front	80mm
Wavelength and Lamp	366nm & Mercury lamp
Sample Applicator	CAMAG Linomat 5
Sample Detection	CAMAG Visualizer : 200480
Number of Tracks	7

- **High Performance Liquid Chromatography (HPLC) evaluation.**

HPLC was also performed to find out the Gallic acid content in prepared formulation as per conditions mentioned in table 1b.

Table 1b: Chromatographic Conditions for HPLC.

Mobile phase	**Acetonitrile : water (20:80) [pH 3 by ortho phosphoric acid]**
Stationary Phase	C_{18} (4.6 × 250 mm, 5 µm).
Flow rate	1 ml/min
Injection volume	20 µl
Detection	UV at 272nm

RESULTS AND DISCUSSION

As a part of standardization, inhouse formulation Bhuvaneshvar Vati was tested for the relevant phyto and physico-chemical parameters. The formulation was found to be black colored, spherical in shape, characteristic bitter odor with no specific taste(table 2). Physicochemical parameters and Physical properties such as total ash values, loss on drying, Friability and Hardness were determined and the values are presented in (table 3). Qualitative tests for Phytochemical evaluation helped to understand the presence of various therapeutically active constituents in Bhuvaneshvar Vati and it was found to be having important phytoconstituents like Glycosides,alkaloids,Saponins and steroids (table 4). These Chemical constituents could have pharmacological action on their own or in conjugation with other constituents in terms of efficacy, which possibly help the body to fight with ailment Biochemical tests showed presence of Carbohydrates (table 5).These Phytochemical and Biochemical tests are important to obtain preliminary information on the quality. According to Mohan et al. different chemical compounds detected in whole plant extracts could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

The prepared formulation was also assesed by hyphenated techniques like HPTLC and HPLC for presence of marker compound Gallic acid. HPTLC fingerprinting data clearly indicates that ga,llic acid is present in all the raw materials and formulation and this can be used to perform stability studies of this formulation(Fig 1). HPLC analysis data was also aligned with data obtained by HPTLC and formulation was found to be having marker compound gallic acid with significant quantity (Fig 2).

Table 2: Organoleptic Characters.

Sr. No.	**Characters**	**Bhuvaneshvar Vati**
1	Colour	black
2	Taste	tasteless
3	Texture	spherical
4	Odour	bitter

Table 3: Physicochemical evaluation

Sr. No.	**Parameters**	**Bhuvaneshvar Vati**
1	Friability	0.15 %
2	Hardness	18.2 kg
3	LOD	6.98 %
4	Total Ash	12 %

Table 4: Phytochemical Evaluation.

Sr No.	**Tests**	**Observation**	**Results**
1	Tannin: 1ml Aq. Extract + 0.1% $FeCl_3$ dropwise	Brownish green or Blue black colour	-
2	Alkaloids: 1ml Alc. Extract + 1ml conc. HCl + Hager's Reagent	Yellow ppt	+
3	Glycosides: 1ml extract + 0.5ml Glacial Acetic acid + few drops of Dil. $FeCl_3$ till colourless + 1ml Dil. H_2SO_4	Brown Ring	+
4	Flavonoids: 1ml extract+ 1ml Dil. ammonia solution + Conc. H_2SO_4	Yellow colour disappear	-
5	Steroids: 1ml extract + 1ml chloroform + Conc H_2SO_4	Red colour after stand	+
6.	Phlobatannin: 0.5ml aq. Extract+ Boil with 1ml 1% HCl	Ppt present	-
7.	Phenolic Compounds: 1ml extract + dropwise $FeCl_3$	Violet colourppt	-
8.	Saponin: 1ml extract + Few drops of olive oil+ Shake vigorously	Froth	+
9.	Terpenoids: 1ml extract +0.5ml $CHCl_3$+ 1ml Conc. H_2SO_4	Yellow colour	-

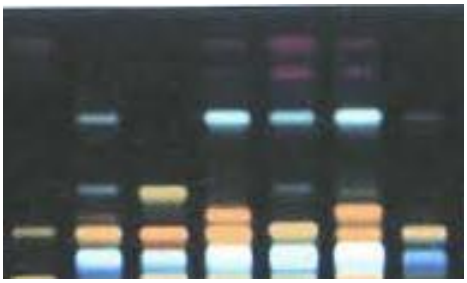
Key : + positive, - Negative

Table 5: Biochemical Evaluation.

Sr no.	Tests	Observation	Results
1.	Carbohydrate: 1ml extract + 1ml Fehling A + 1ml Fehling B	Blue Colour	+
2.	Proteins: 1ml extract + 1ml 4% NaOH + few drops 1% $CuSO_4$	Violet or pink colour	-
3.	Fats and Fixed oils: 1ml extract + 1ml KOH + 2drops of phenolphthalein + heat for 15mins on water bath	Formation of froth and neutralisation of alkali	-
4	Starch: 1ml extract + iodine	Blue colour	-

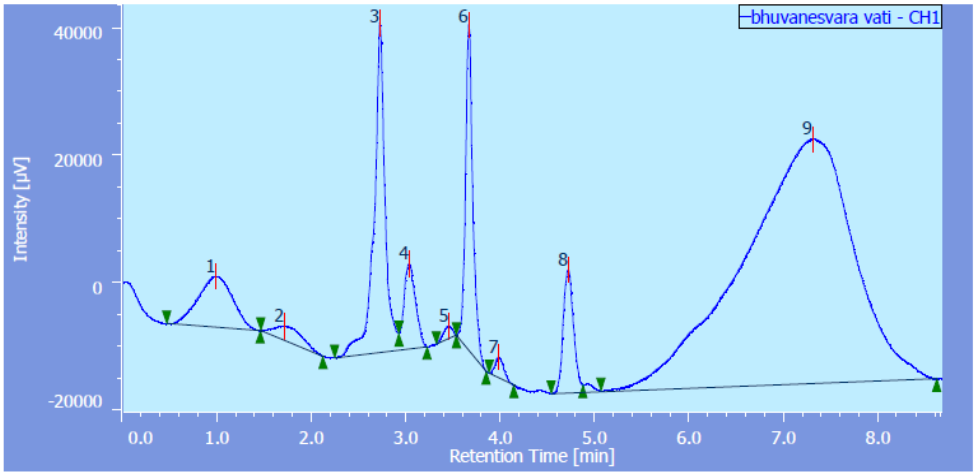
Fig 1 : HPTLC fingerprint

solvent front : 6.5 cm



Track No.	Sample Name
1	Gallic Acid
2	Haritaki
3	Amalaki
4	Bibhitaki
5	Yawani
6	Formulation
7	Bilva

Fig 2 : HPLC Analysis



#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor
1	Unknown	1	0.992	202592	7981	4.648	4.223	N/A	33	1.119	0.937
2	Unknown	1	1.717	49128	2214	1.127	1.172	N/A	127	2.615	1.279
3	Unknown	1	2.725	422376	51602	9.690	27.303	N/A	4468	1.563	N/A
4	Unknown	1	3.033	115563	13357	2.651	7.068	N/A	2725	1.994	N/A
5	Unknown	1	3.458	13290	1940	0.305	1.027	N/A	5029	1.275	0.788
6	gallic acid	1	3.667	275679	51103	6.324	27.039	N/A	12225	2.044	1.202
7	Unknown	1	3.983	21190	3199	0.486	1.693	N/A	8009	3.887	1.209
8	Unknown	1	4.717	147003	19354	3.372	10.240	N/A	8874	2.296	N/A
9	Unknown	1	7.308	3112159	38244	71.396	20.236	N/A	201	N/A	0.803

CONCLUSION

Quality control parameters are of key importance if traditional medicines are to be given credibility as modern medicine has.In order to have consisitancy and uniformity in the production of these medicines on large scale, there is a need to set a standard protocol for preparation and for assesment of quality, efficacy. Ayurvedic classical preparation, Bhuvaneshvar Vati has been screened for its phytochemical and physicochemical properties using the various modern scientific quality parameters. The results obtained can be used as reference while setting the pharmacopoeial standards for Bhuvaneshvar Vati to ensure the quality of the medicine.

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