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STANDARDIZATION OF 'BHUNIMBADI CHURNA' - AN AYURVEDIC POLYHERBAL FORMULATION

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ABSTRACT: Standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value. The present work is an attempt to standardize 'Bhunimbadi Churna', an Ayurvedic polyherbal formulation, used in fever and diabetics etc. The various parameters performed included organoleptic characteristics, physico-chemical parameter, physical characteristics of Churna**,** preliminary phytochemical, Heavy metal and Microbial analysis and high performance thin layer chromatography (HPTLC) analysis. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standard formulation of great efficacy.

INTRODUCTION: 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.

India having a rich heritage of traditional medicine constituting with its different components like *Ayurveda, Siddha* and *Unani*. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will helps not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare.

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In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases.

Standardization of herbal formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles [1, 2].

In the present research work, an attempt was made to standardize " Bhunimbadi Churna " an Ayurvedic polyherbal formulation made up of nine herbs (**Table 1**) used in the treatment of is a polyherbal ayurvedic medicine used as a fever, jaundice, anemia and Antidiabetic activity.

However, the work deals with the details of following latest standardization guidelines involving Good Manufacturing Practices (GMP) for preparation of Ayurvedic medicines. Standardization guidelines to be followed for herbal products provided by international bodies like World Health Organization (WHO), Ayurvedic pharmacopeia of India (API), European

Agency for the evaluation of Medicinal Products (EMEA) and United States Pharmacopeia (USP) have also been considered.

MATERIALS AND METHODS:

Plant material: The crude drugs used in preparation of Bhunimbadi Churna were collected from local market of Vadodara and identified by Dr. M. S. Jangid, Department of Botany, Modasa, Gujarat. Bhunimbadi Churna was prepared, as per the procedure mentioned in Ayurvedic text "Brhat Nighantu Ratnakar"(Ayurved Sar Samgrah), published by 'Shree, Baidyanath Ayurved bhavan Ltd, Zhansi (U.P), 1985: 596. All plant parts were then dried in shade, powdered and passed through sieve no. 85 # and lastly packed in a well closed container to protect them from moisture. Composition of Bhunimbadi churna was given in **Table 1**.

TABLE 1: COMPOSITION OF BHUNIMBADI CHURNA

Plant Name In Sanskrit	Botanical Source	Part Used	Quantity
Chirayata	*Swertia chirata*	Whole plant	4%
Indrajav	*Holarrhena antidysenterica*	Seed	4%
Sunthi	*Zingiber officinale*	Rhizome	4%
Marica	*Piper nigrum*	Fruit	4%
Pippali	*Piper longum*	Fruit	4%
Nagarmoth	*Cyperus rotundus*	Rhizome	4%
Katuki	*Picrorrhiza kurroa*	Rhizome	4%
Chitrak	*Plumbago zeylanica*	Root	8%
Kada chhal	*Holarrhena antidysenterica*	Stem bark	64%

Standardization Parameters: The various standardization parameters studied were botanical parameters, physico-chemical investigations, pH analysis, preliminary phytochemical analysis, determination of physical characteristics of powder formulation, Heavy metal analysis, Microbial analysis and HPTLC fingerprint.

Botanical parameters [3, 4]:

Macroscopic characters: The Organoleptic evaluation refers to evaluation of the formulation by colour, odour and taste **(Table 2)**.

TABLE 2: BOTANICAL PARAMETERS OF BHUNIMBADI CHURNA

Formulations	Appearance	Colour	Taste	Odour
Bhunimbadi Churna	Moderately fine powder	Greyish yellow	Bitter	Characteristic

Microscopic characters: For microscopical study, properly washed plant material was cut in to desirable size. A few mg of powder was washed with plain water, treated with iodine and potassium iodide, drop of glycerine was added and mounted **(Figure 1)**.

Physico-chemical investigations [5]: Physico-chemical investigations of formulations were carried out were the determination of Moisture content, extractive values and ash values **(Table 3)**.

Determination of pH: 10% solution of Polyherbal formulation was prepared in distilled water and pH was determined using pH meter Orion digital pH meter **(Table 3)**.

TABLE 3: PHYSICO-CHEMICAL PARAMETERS OF BHUNIMBADI CHURNA

Parameters	Bhunimbadi Churna
	Physicochemical Parameters
Moisture content	8.11±0.13%
pH	5.35±0.11%w/w
Extractive value	
Water soluble	21.62±0.18%w/w
Alcohol soluble	12.42±0.13%w/w
Ash value	
Total ash	5.26±0.02%w/w
Water soluble ash	1.68±0.06%w/w
Acid insoluble ash	0.49±0.01%w/w

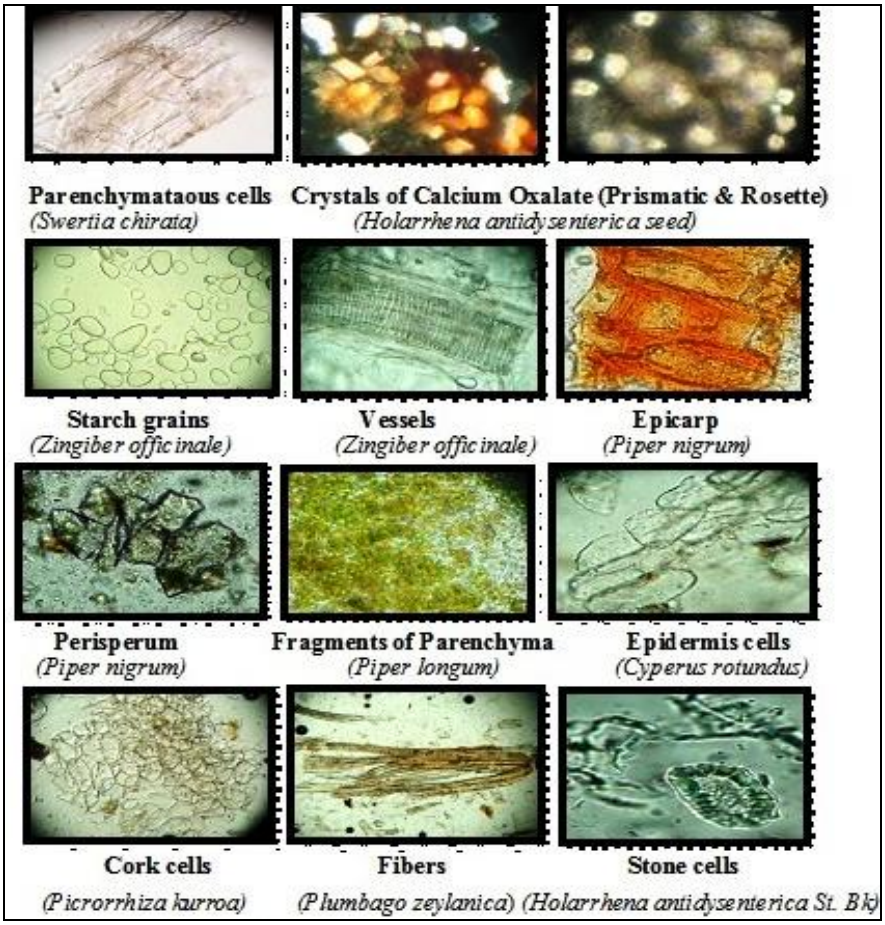

FIGURE 1: POWDER MICROSCOPY OF BHUNIMABADI CHURNA

Determination of Physical Characteristics of Powder Formulation [6]: Physical characteristics like bulk density, tap density, Hausner's ratio, and Carr's index were determined for different formulations the term bulk density refers to packing of particles or granules.

The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device that has a specially cut rotating can. 100 grams of weighed formulation powder was taken and carefully added to cylinder with the aid of a funnel.

The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, it gives the value of tapped density. Hausner's ratio is related to inter particle friction and as such can be used to predict the powder flow properties. Carr's index is a method of measuring the powder flow from bulk density **(Table 4)**.

TABLE 4: PHYSICAL CHARACTERSTICS OF BHUNIMBADI CHURNA

Parameters	'Bhunimbadi Churna'
	Physicochemical Characteristics
Bulk density	0.3692 gm/cm^3
Tapped density	0.5641 gm/cm^3
Hausner's ratio	1.528
Carr's index	34.55
Angle of repose	46.17

Preliminary Phytochemical Analysis [7, 8]**:** Preliminary qualitative phytochemical analysis of all the extracts was carried out on Water extract by employing standard conventional protocols (**Table 5**).

TABLE 5: PHYTOCHEMICAL TESTS FOR BHUNIMBADI CHURNA

Test	Bhunimbadi Churna
Alkaloids	+
Glycoside	+
Flavonoids	+
Tannins	+
Steroids and Triterpenoids	+
Phenolic	+
Carbohydrates	+
Proteins and Amino acids	-

Note: + indicates presence − indicates absence

Heavy Metal Analysis [9, 10]:

Preparation of Samples by Acid Digestion Method: Accurately weighed 2 g of sample was taken in Kjeldahl flask. Acid mixture of HNO3: $HClO_4$ (4:1) was added in the flask and heated continuously till the solution is colourless. The sample was then transferred in a 25 ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared as per the protocol in the manual and the calibration curve was developed for each of them.

Detection: Then samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic Absorbance Spectrophotometer (AAS) 6300 (by SHIMADZU) **(Table 6)**.

TABLE 6: HEAVY METAL ANALYSIS OF BHUNIMBADI CHURNA

Heavy Metal	Limit	Bhunimbadi Churna
Lead (Pb)	10 ppm	9.40 ppm
Cadmium (Cd)	0.3ppm	0.3 ppm
Arsenic (As)	10 ppm	2.15 ppm
Mercury (Hg)	1 ppm	Absent

Microbial Analysis [11]: Microbial analysis was carried for determination of microbial contamination as per procedures of Ayurvedic pharmacopoeia of India and WHO Guideline. The test included total bacterial count, total yeast and mould count, *Escherichia coli* and *Salmonella typhi* **(Table 7)**.

TABLE 7: MICROBIAL ANALYSIS OF BHUNIMBADI CHURNA

Microbial Analysis	Limit	Bhunimbadi Churna
Total aerobic viable count	10^5 /gm	410 cfu/gm
Total yeast and mould	10^3 /gm	Absent
Escherichia coli	Absent	Absent
Salmonella sps	Absent	Absent

HPTLC Analysis [12, 13]**:** The HPTLC finger print profile of Methanolic extract of Bhunimbadi Churna was taken on aluminium plate coated with silica gel 60 $F_{254}$of 0.2 mm thickness (E. Merck) as adsorbent and employing CAMAG Linomat IV applicator. The mobile phase used was Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.5 v/v). The plate was dried and visualised under UV 254 nm and 366 nm. The plate was spray with Anisaldehyde-Sulphuric acid reagent and heated at 105 °C till the spots appeared.

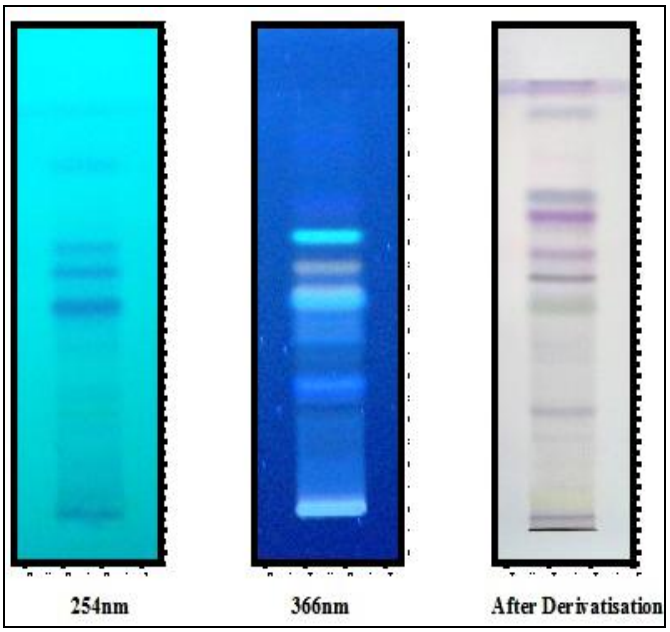

FIGURE 2: HPTLC OF BHUNIMBADI CHURNA

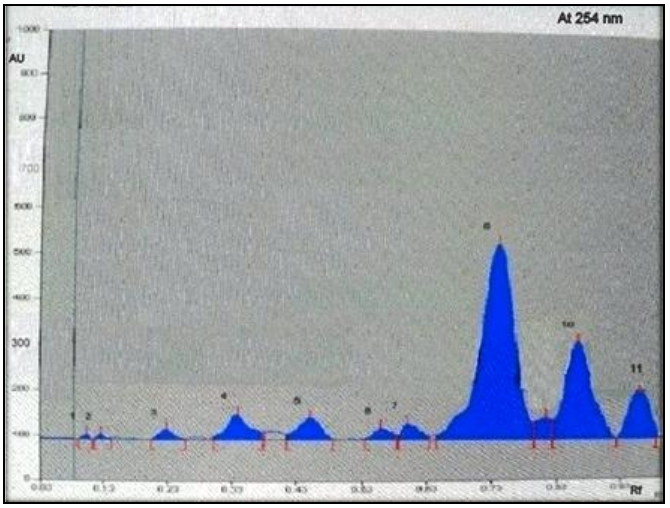

FIGURE 3: HPTLC FINGER PRINTING CHROMATOGRAM OF BHUNIMBADI CHURNA

RESULTS: As a part of standardization procedure, Churna was tested for relevant physical and chemical parameters, and also subjected to microbial screening through quality control measures. Botanical parameters revealed that Churna was Greyish yellow, moderately fine powder, odour- Characteristic, taste- Bitter **(Table 2)** and Microscopic characters showed the characters like orange coloured Parenchymatous cells (*Swertia chirata)*, rosette and prismatic crystals of calcium oxalate *(Holarrhena antidysenterica*), Starch grains and vessels with

spiral thickening up to 85µ in length (*Zingiber officinale*), Epicarp and Perisperm cells (*Piper nigrum*), Fragments of Parenchyma (*Piper longum*), Epidermis cells *(Cyperus rotundus)*, Cork cells (*Picrorrhiza kurroa*), fibers (*Plumbago zeylanica*), Stone cells (5-7µ) (*Holarrhena antidysenterica St. Bk*) **(**Figure 1).

Results of quantitative analysis for Loss on drying at 105º C, pH, Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractives and Water soluble extractive were calculated and results were shown (Table 3). Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Percent weight loss on drying or moisture content was found to be 8.11% w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth.

Physical properties of churna like Bulk density, Tapped density, Hausner ratio and Carr's index indicates very poor flow ability and Angle of repose indicates Poor-must agitate, vibrate flow property (Table 4). Phytochemical analysis revealed the presence alkaloid, glycoside, flavanoid, Steroids & Triterpenoids, tannins and Carbohydrate and absence of protein and amino acid (Table 5). Heavy metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations.

However, heavy metals have been associated with various adverse effects including status hepatotoxicity, epilepticus, fatal infant encephalopathy, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs. Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals (Table 6).

For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained (Table 7) revealed the absence of these microorganisms thereby confirming the non-toxic nature of the formulations.

HPTLC finger printing profile of Bhunimbadi Churna were developed in Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.5 v/v) solvent system. Under 254 nm, it showed 4 spots with R_f value of 0.56, 0.62 , 0.73, 0.85 (all blue colour); under 366nm it showed 5 spots with R_f value of 0.25 (Purple), 0.56 (Dark green), 0.62 (Purple), 0.73 (Black). After derivatization with the Anisaldehyde-Sulphuric acid reagent it showed 11 spots with R_f value: 0.05(Dark purple), 0.09(Yellow), 0.19(Light purple), 0.25(Purple), 0.45(Green), 0.56(Dark green), 0.62(Purple), 0.68(Dark purple), 0.73(Black), 0.82 (Light pink), 0.95 (Black) (Figure 2).

DISCUSSION: Bhunimbadi Churna is a safe polyherbal formulation containing many phytoconstituents. The churna shows very poor flow poor flowability. The churna was evaluated based on different physical and chemical evaluation parameters. The Bhunimbadi Churna is free from any toxic material. The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards.

CONCLUSION: Ayurvedic Bhunimbadi Churna has been standardized by intervention of modern scientific quality control measures in the traditional preparation described in classical texts. Pharmacognostic characters established for the raw materials could be employed as Q.C. standards for evaluating its identity and can be used for routine analysis. Purity and potency of the materials and formulations following the procedure given could be performed in QC/QA laboratory of pharmaceutical house.

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REFERENCES:

1. Ekka NR, Nmedo KP and Samal PK: Standardization strategies for Herbal drugs. Research Journal Pharm. and Tech 2008; 1: 310-312.
2. WHO: Quality Control Methods for Medicinal Plant Materials. AITBS Publishers, Delhi 2002: 65–67.
3. The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare Department

of Indian System of Medicine and Homoeopathy, New Delhi, First Edition 2010; Part-I, Vol-1-4.

4. Sudha Revathy S, Rathinamala R, Murugesan M: Authentication methods for drugs used in Ayurveda, Siddha and Unani Systems of medicine: an overview. International Journal of Pharmaceutical Sciences and Research 2012; Vol. 3(8): 2352-2361.
5. Panchal Mayank**,** Patel D: Phytochemical Screening and Standardization of Formulation "Renolith" For Renal Stone. International Journal of Pharmaceutical Research and Bio-Science 2013; Volume 2(2):99-111.
6. Tekeshwar Kumar, Chandrashekar K.S., Tripathi D.K.: Standardization of 'Gokshuradi Churna'- An Ayurvedic polyherbal formulation. Journal of Chemical and Pharmaceutical Research 2011; 3(3):742-749.
7. Bhargava1 S, Dhabhai K: *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. Journal of Chemical and Pharmaceutical Research 2012; 4(1): 360-364.
8. Ravichandra VD: Pharmacognostical and Phytochemical investigation on leaves of *Ficus microcarpa* Linn. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(2): 137-140.
9. Munish Garg and Jaspreet Singh: Quantitative estimation of Heavy metals and trace elements in marketed Ayurvedic churna preparations in India. International Journal of Pharmaceutical Sciences and Research 2012; Vol. 3(5): 1331-1336
10. Naithani V, Pathak N, Chaudhary M: Evaluation of Heavy metals in two major ingredients of ampucare. International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(2): 137-141.
11. Manoj Kumar Pandey, GN: Standardization of Yakrit plihantak churna: An Ayurvedic polyherbal formulation. International Journal of Pharmaceutical Sciences and Drug Research 2012; Vol. 3(1): 171-176.
12. Eike Reich: HPTLC for the identification and quality control of medicinal plants. CAMAG – Laboratory.
13. Ajay Kr Meena, Rao MM, Panda P: Standardisation of Ayurvedic polyherbal formulation, Pancasama Churna. International Journal of Pharmacognosy and Phytochemical Research 2010; 2(1):11-14.

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