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Development of Evaluation Parameters for Balchaturbhadra Churna & Comparision with Market Formulation

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Subject: Pharmacognosy

Abstract

Most of the traditional systems of medicine are effective but they lack of standardization. So, there is a need to develop a standardization technique. Central Council of Research in *Ayurveda* and *Siddha* has given preliminary guidelines for standardizing these conventional formulations. For the uniformity in production of herbal formulations it is necessary to develop methods for evaluation. In the paper, attempt has been made to evaluate *Balchaturbhadra Churna* , an *Ayurveda* formulation. One sample was procured from manufacturer and was subjected to compare with inhouse preparation sample (Made as per *Bhaisajyaratnavali Book)* by performing physicochemical screening, phytochemical screening, microscopic characterization and fluorescence analysis. It was observed that market samples matched exactly with that of authentic standards of inhouse formulation after performing the standardization.

Key words: *Balchaturbhadra Churna, Ayurvedic drugs, Standardization.*

Introduction

Balchaturbhadra churna is the old age formulation the most commonly employed in Ayurvedic paediatric practice.It is very safe and effective remedy to overcome routine digestive complaints of children like diarrhoea, griping pain etc.It improves digestion hence help to promote healthy physical growth and boost immunity to protect child from minor infections.[1] Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principles. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, its therapeutic effect according to different batches of collection.By considering the increasing demand of Ayurvedic formulations, proper documentation regarding their standardization is more important to assure the quality, purity, safety and efficacy.[2]

Objective

Lack of in-process quality control techniques makes it difficult to maintain the consistency and quality of Ayurvedic formulations. Therefore, there is an immediate need to develop standard protocol for the uniform manufacturing of Ayurvedic formulations. Standardization is a burning topic in Ayurvedic drug industry today, and remains lacking in documentation, validation, and determination of marker compounds. So the standardization of the market formulation is very useful and guarantee the quality and the safety of the product to the consumer would be given. Therefore, establishing quality and standard parameters for the Ayurvedic formulation is important.So, the aim of this work is to development of evaluation parameters for Balchaturbhadra churna & comparision with market formulation. [3]

EXPERIMENTAL

Material

All chemicals and solvents used were of analytical grade. Balchaturbhadra churna contains the crude drugs *(1) Aconitum Heterophyllum* wall. (Fam. Ranunculaceae) (2) *Pistacia Integerrima* (Fam.Anacardiaceae) (3) *Piper Longum* Linn. (Fam. Piperaceae) (4) *Cyperus rotendus* (Fam. Cyperaceae) [2]. All these plant crude drugs required for the preparation of the standard formulation of Balchaturbhadra churna were collected from the local market, vapi. in the month of november, 2010. One market formulation of Balchaturbhadra churna was collected from the Shree Narnarayan,Ayurvedic Pharmacy, Ahmedabad, Gujarat.

Table 1:Composition of market Balchaturbhadra Churna

Name and address of the manufacturer	Ingredients	Quantity (in gm)
Shree Narnarayan, Ayurvedic Pharmacy, Ahmedabad,Gujarat. Mfg Date : Nov 10 Batch no : 120	*Aconitum heterophyllum wall*	12.5
	Piper longum Linn	12.5
	Cyperus rotundus Linn	12.5
	Pistacia integerrima Stew	12.5

[Table 2 Composition of market Balchaturbhadra Churna

Name of the ingredient	Part of the plant used in formulation	Quantity in parts
Aconitum heterophyllum wall.	Roots	1
Piper longum Linn.	Fruits	1
Cyperus rotundus Linn.	Rhizomes	1
Pistacia integerrima Stew	Galls	1
Total	-	4

METHODS

In house formulation of Balchaturbhadra Churna was based upon the composition given in the book of *Bhaisajyaratnavali,* All the crude drugs were washed , dried and examined for the presence of the foreign matters and were weighed as prescribed under the formula. All of the drugs are grinded separately and powdered and mixed each after passing through sieve 60#.

1. Physical Parameters

Determination Of Bulk Density, Tapped Density, Compressibility, Hausner's Ratio: [4][5]

About 10 gm of powder of both the standard and test formulation were weighed and filled into graduated cylinder of densitometer. Measure the volume initially as bulk volume. Put the cylinder on the densitometer and set the parameter for 100 tapping. Measure the volume and further tapping was done until constant volume was obtained. After these bulk density, tapped density, compressibility, hausner's ratio is calculated using following equation

Initial Bulk density ($\rho 0$) = weight / Initial volume

Tapped bulk density (ρt) = weight / tapped volume

Huusner's ratio = Initial volume/ final volume

Carr's index = $\left(\rho t - \frac{\rho 0}{\rho t}\right) \times 100$

To determine ash value 2-3 g of accurately weighed formulation was incinerated in a tared silica crucible at a temperature not exceeding 450°C in a muffle furnace until white ash has been obtained indicating the absence of carbon. If carbon free ash can not be obtained in this manner, cool the crucible and exhaust the residue with about 2 ml of water and collect the residues on the ashless filter paper. Incinerate the residue and filter paper , add the filtrate to crucible, evaporate to dryness, and ignite at the temperature not exceeding 450°C.It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried powdered drug.

1.2 Determination of angle of repose:

A glass funnel is held in place with a clamp on a ring support over a glass plate.The glass plate is placed on a micro lab jack.Approximately 100 g of powder is transferred into the funnel.Keeping the orifice of funnel blocked by the thumb.As the thumb is removed, the lab jack is adjusted so as to lower the plate and maintain about a 6.4 mm gapbetween the bottom of the funnel stem and the top of the powder pile.When the powder is emptied from the funnel,the angle of the heap to the horizontal plane is measured.The height of the pile(h) and the radius of the base®is measured with a rular.The angle of repose thus estimated.

$$\tan \Theta = \text{height of pile funnel/radius}$$

2. Pharmacognostical Study of crude drugs, standard and market formulations of Balchaturbhadra churna

2.1 Macroscopical and Microscopical Study

All powdered crude drug ingredients, standard and market formulations of Balchaturbhadra churna were examined for their morphological and microscopical characters and quantitave microscopical studies.

3. Physico-chemical parameters

Physico-chemical parameters like ash value, extractive value loss on drying and p^H were determined for standard and market formulations of Balchaturbhadra churna.

3.1 Determination of Ash Values [6]

2-3 g of accurately weighed formulation was incinerated in a tared silica crucible at a temperature not exceeding 450°C in a muffle furnace until white ash has been obtained indicating the absence of carbon. If carbon free ash can not be obtained in this manner, cool the crucible and exhaust the residue with about 2 ml of water and collect the residues on the ashless filter paper. Incinerate the residue and filter paper , add the filtrate to crucible, evaporate to dryness, and ignite at the temperature not exceeding 450°C.It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried powdered drug.

3.2 Determination of Extractive Values [6]

Extractive values of standard and market formulations of Balchaturbhadra churna were determined by following methods:

A. Determination of Water Soluble Extractive

5g of the air-dried powdered material was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently for 6 hours. It was then allowed to stand for 18 hours and filtered rapidly taking precaution against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred porcelain dish and dried at 105 ˚C to a constant weight. It was then weighed and the percentage of water soluble extractive was calculated with reference to the air-dried powdered drug.

B. Determination of Alcohol Soluble Extractive

5 g of the air-dried powdered materials was macerated with 100 ml of alcohol in a closed flask for 24 hours, shaking frequently for 6 hours. It was then allowed to stand for 18 hours and filtered rapidly taking precaution against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred porcelain dish and dried at 105 ˚C to a constant weight. It was then weighed and the percentage of alcohol soluble extractive was calculated with reference to the air-dried powdered drug.

3.3 Determination of pH: [7]

The pH of formulations was measured by making 1% w/v solution of water soluble portions was determined using standard glass electrode at 24ºC according to the prescribed standard method in Indian Pharmacopoeia.

3.4 Determination of loss on drying and moisture content (7)

10gm of drug (without preliminary drying) was taken in a tared evaporating dish.After placing the above said amount of the drug in the the tared evaporating dish,dried at 105ºC for 5 hrs, and weighted.continue the drying and weighted at one hour interval until difference between two successive weighing corresponds to not more than 0.25%.Constant weight was reached when two consecutive weighing after drying for 30 minutes and cooled for 30 minutes and cooled for 30 minutes in a desiccators,show not more than 0.01 gm difference.

4. Phytochemical Screening [8] [9]

Standard and market formulations of Balchaturbhadra churna were subjected to the following tests separately for the presence of various phytoconstituents like alkaloids, flavonoids, saponins, carbohydrates, sterols and terpenoids, anthraquinone glycosides, coumarins, tannins and phenolic compounds.

A) Test for Alkaloids: 1 g of the formulation was extracted with 20 ml alcohol by refluxing for 15 min and filtered and the filtrate was evaporated to dryness. The residue was dissolved in 15 ml of 2N H_2SO_4 and filtered. Filtrate was made alkaline and extracted with chloroform. The chloroform extract was evaporated to dryness on the water bath. The residue left after evaporation was tested for the presence of alkaloids with Dragendorff's reagent. Formation of orange coloured precipitates indicates the presence of alkaloids.

B) Tests for Flavonoids

Fluorescence Test: 1 g of the formulation was extracted with 15 ml methanol for 2 min on a boiling water bath, filtered while hot and evaporated to dryness. To the residue, 0.3 ml 3% w/v boric acid solution and 1 ml 10% w/v oxalic acid solution were added. The mixture was evaporated to dryness and the residue was dissolved in 10 ml ether. Observe fluorescence in the ethereal layer under U.V. light. Development of greenish fluorescence in ethereal layer under U. V. indicates the presence of flavonoids.

C) Test for Saponins

Froth Test: 0.1 g of STD and market formulation powders of Balchaturbhadra churna were vigorously shaken with 5 ml of distilled water in a test tube for 30 sec and was left undisturbed for 20 min. Persistent froth indicates the presence of saponins.

D) Tests for Carbohydrates

a)Molisch's Test : To the ethanolic extracts of formulation,α-napthol and concentrated H_2SO_4 were added.Development of purple colored ring indicates the presence of carbohydrates.

b)Fehling's Test : To 1 ml of ethanolic extract of formulation, 1 ml of the Fehling solution(Fehling A + Fehling B) was added.The mixture washeated on boiling water bath for 5-10 min.Developement of yellow precipitates, changing to brick red precipitates indicates the presence of reducing sugars.

E) Tests for Phenolic Compounds

Test with $FeCl_3$: To the methanolic extracts of the formulation, a drop of freshly prepared $FeCl_3$ solution was added. Development of brownish green color indicates the presence of phenolics.

F) Tests for Tannins

Test with Lead acetate: To 2-3 ml of aqueous extracts of the formulations, 2 ml of 10 %w/w solution of lead acetate was added. Formation of heavy dull yellowish precipitates indicates the presence of tannins.

G) Tests for Coumarins

a) With Ammonia: A drop of ammonia was taken on a filter paper. To this, a drop of aqueous extracts of STD and market formulation of Balchaturbhadra churna, was added. Appearance of fluorescence indicates the presence of coumarins

b) With Hydroxylamine hydrochloride: To the ethereal extracts of STD and market formulation of Balchaturbhadra churna, add a drop of saturated alcoholic hydroxylamine hydrochloride solution and a drop of alcoholic potassium hydroxide. The mixture was heated, cooled and acidified with 0.5N Hydrochloric acid and a drop of 1% w/v $FeCl_3$ solution was added. Development of violet color indicates the presence of coumarins.

5. Estimation of phytoconstituents

5.1 Estimation of Flavonoids ($AlCl_3$ method) [10]

I) Preparation of test extract

1 g of the formulation was extracted with 25 ml of 95% ethanol for 24hrs. After filtration the filtrate was adjusted to 25 ml.

II) Calibration curve and estimation

0.5 ml of above extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV-160A spectrophotometer. Percentage of total flavonoid was calculated from calibration curve of rutin (200-700 µg/ml), plotted by using same procedure. Results were expressed in g/100g of formulation.

5.2 Estimation of Sodium and Potassium ion salts: (Flamephotometric method) [11]

I) Preparation of test solution

1 g accurately weighed formulation was incinerated in a tared silica crucible at a temperature not exceeding 450°C in a muffle furnace until white ash has been obtained indicating the absence of carbon. If carbon free ash can not be obtained in this manner, cool the crucible and exhaust the residue with about 2 ml of water and collect the residues on the ashless filter paper. Incinerate the residue and filter paper, add the filtrate to crucible, evaporate to dryness, and ignite at the temperature not exceeding 450°C.It was then cooled and weighed. The ash so obtained was dissolved in 100 ml of water and from that 0.5 ml of the solution was withdrawn and diluted up to 100 ml.

II) Preparation of standard solution

Standard stock solution for Na^+ ion was prepared by dissolving 25.42 mg of sodium chloride, previously dried to constant weight at 130^0C, in distilled water and make up the volume was made up to 100ml. The solution thus prepared represents 100µg/ml Na^+ ion concentration.

Standard stock solution for K^+ ion was prepared by dissolving 19.06 mg of potassium chloride, previously dried to constant weight at 130^0C, in distilled water and make up the volume was made up to 100ml. The solution thus prepared represents 100µg/ml K^+ ion concentration.

III) Method

Series of the dilutions were prepared from the stock solutions of Na^+ ion and K^+ ion by diluting 1, 2, 5, 10, 20, 50, 100 ml of stock solution to 100ml so as to get 1, 2, 5, 10, 20, 50, 100 µg/ml concentrations. Flame Photometer was adjusted for appropriate filter or monochromator of desired wavelength. Initially distilled water was sprayed through flame and reading at the galvanometer was adjusted to 0. Then highest concentration of the solution was sprayed through the flame and reading at the galvanometer was adjusted to 100. Again the reading at the galvanometer was adjusted to 0 and readings of all the samples were taken in increasing order of their concentration with the washing of distilled water each time between different solutions. Reading of the test samples was taken in a similar way. Calibration curve was prepared for both ions by plotting concentration Vs galvanometer reading to estimate Na^+ and K^+ ions.

5.3 Determination of crude fibre content: [10]

2 gm of drug was taken in a beaker and 50ml of 10% nitric acid was added.It was heated to boil with stirring(30 sec).This was strained through fine cloth on a buchner funnel.The residue was washed with boiling water and transferred to a beaker.50ml of 2.5% v/v sodium hydroxide solution was added.It was strained and washed with hot water.The residue was transferred in a clean dried crucible.The rtesidue was weighted and crude fibre content was determined.

5.4 Estimation of Tannin: (Titrimetric method) [12]

A. Method

2 g of the accurately weighed formulation was taken and 50 ml of boiling water was poured in it. The mixture was heated for 30 minutes on the water bath under frequent shaking. After 30 minutes the solution was allowed to settle for few minutes and flitered cautiously through cotton wool in to a 250 ml volumetric flask, taking care that no particles of drugs should get on cotton wool. The drug in the flask was extracted once more with boiling water as indicated above and the liquid was filtered in to the same flask. The extraction procedure was repeated several times till the solution gives the negative test for tanning substances (tested with ammonium ferric alum).The liquid in the volumetric flask was cooled and the volume was made up to 250 ml mark. 25 ml of liquid was placed in the 1000 ml of the conical flask. To this 750 ml of distilled water and 25 ml of indigo sulphonic acid solution were added and the content of the conical flask was titrated against 0.1 N $KMnO_4$ under constant stirring until golden yellow colour was obtained.z1 ml 0.1 N $KMnO_4 \approx$ 0.004157 g of tanning substances calculated as tannin.

A blank was carried out simultaneously, titrating 25 ml of the indigo sulphonic acid in 750 ml of water.

B. Preparation of the Indigo sulphonic acid

Dissolve 1 g of Indigocarmin in 25 ml of concentrated H_2SO_4. Add another 25 ml concentrated H_2SO_4 in the same flask. Dilute the solution with distilled water to produce 1000 ml solution.

6. Fluorescence analysis [22]

0.5 g of STD and market formulation of Balchaturbhadra churna were macerated with 5 ml 1N H_2SO_4, 1N HNO_3, 1N aq. NaOH, 1N ethanolic NaOH, 1N HCl,I_2, KOH, NH_3 solution separately for one hour and extract was filtered. Filtrate was analyzed for their colour and fluorescence in day and UV light.

RESULTS

TABLE- 3: Physical Parameters

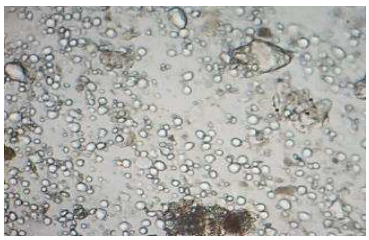
Parameter	Balchaturbhadra Churna	
	Standard Preparation	Market Preparation
Bulk Density(gm/ml.)	0.5882	0.434
Tap Density(gm/ml)	0.8	0.625
Carr's Index	26.47	30.43
Hausner's Ratio	1.36	1.43
Angle of Repose	32.32	34.43

TABLE 4: Morphological study of standard and market formulations of Balchaturbhadra churna

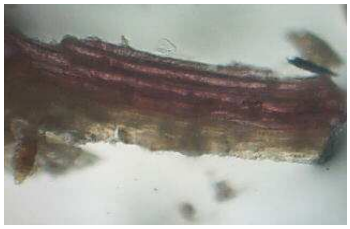
PARAMETERS	Balchaturbhadra churna	
	Standard	Market
State	Fine	Very fine
Colour	Brown	Greyish brown
Odour	Pleasant	Pleasant
Taste	Bitter	Bitter

2.1 Microscopical characters of STD Balchaturbhadra churna

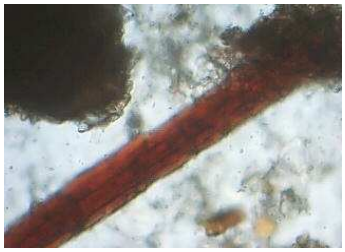
(a) Spiral xylem vessel



(b) Starch grain

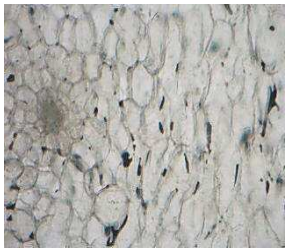


(c) Pitted xylem vessel



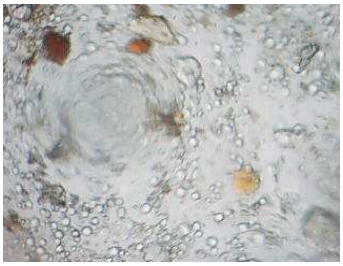
(d) Lignified fibre

(e) Cork cells



(f) Parenchymatous cells

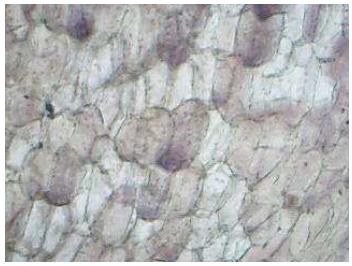
2.2 Microscopical characters of Test Balchaturbhadra churna



(a). Starch grains

(b). Pitted xylem vessel

(c).Spiral xylem vessel



(d) Parenchymatous cells

(e). Lignified fibres

TABLE 5: Quantitative Analysis of microscopic constituent of standard and market formulations of Balchaturbhadra churna

Microscopic chahacter	Size (in µm)	
	Standard preparation	Market preparation
Starch Grains	3.43	3.24
Xylem vessels	55.12	52.56
Fibres (length)	170.76	176.34

TABLE- 6: Physical parameters of standard and market formulations of Balchaturbhadra churna

QUALITY PARAMETERS	FORMULATION	
	STD(w/w)	MARKET(w/w)
Total ash	5.1%	4.8%
Extractive value(% w/w)		
Water soluble extractive	18%	16%
Alcohol Soluble extractive	22%	16%
Loss on drying	0.12 %	0.18 %
pH	5.59	5.58

TABLE 7: Screening of phytoconstituents in standard and market formulations of Balchaturbhadra churna

PHYTOCONSTITUENTS	FORMULATION	
	STD	MARKET
Alkaloids	√	√
Flavonoids	√	√
Phenolics	√	√
Carbohydrates	√	√
Tannins	√	√
Coumarins	X	X
Sterols and triterpenoids	√	√
Saponins	X	X

√**:** Present; X**:** Absent

TABLE 8: Estimation of phytoconstituents in standard and market formulations of Balchaturbhadra churna

Sr. NO	PHYTOCONSTITUENTS	FORMULATIONS (% W/W)	
		Std	Market
1	Flavonoids	0.107%	0.096%
2	Na^+ ion salts	0.84%	0.76%
	K^+ ion salts	0.62%	0.38%
3	Tannins	2.91 %	3.74 %
4	Crude fibre content	15.5%	13%

TABLE 9: Fluorescence analysis of standard and market formulations of Balchaturbhadra churna

TREATMENT OF POWDER	WAVELENGTH IN nm	FORMULATIONS	
		STD	MARKET
1N HCL	Day Light	Brown	LightBrown
	UV Light	Dark Green	Light Green
1N H_2SO_4	Day Light	Light Brown	LightBrown
	UV Light	Light Green	Light Green
1N HNO_3	Day Light	Yellowish Brown	Brown
	UV Light	Light Green	Light Green
Aq. NaOH	Day Light	Dark Brown	Dark Brown
	UV Light	Dark Green	Dark Green
Alcoholic NaOH	Day Light	ReddishBrown	Reddish Brown
	UV Light	Dark Green	Dark Green
I_2	Day Light	Greenish Brown	Dark Brown
	UV Light	Dark Green	Dark Green
KOH	Day Light	Dark Brown	Reddish Brown
	UV Light	Green	Dark Green
NH_3	Day Light	Reddish Brown	Reddish Brown
	UV Light	DarkGreen	Green

DISCUSSION

Physical parameters like bulk density, tap density, Carr's index , Hausner's ratio and angle of repose were obtained for STD and Market formulations of balchaturbhadra churna respectively. STD showed comparatively better flow property with compare to Market Preparation.

Quantitative determination of starch grains, xylem vessels and fibres of standard and market formulations of Balchaturbhadra churna has been done.

The total ash content was found to be more in STD Balchaturbhadra churna than market formulation.This shows that the STD preparation of Balchaturbhadra churna contain more amount of inorganic matter than market preparation. The water soluble extractive value was found to be more in STD Balchaturbhadra churna than market formulation and alcohol extractive value was also found more in STD Balchaturbhadra churna than market formulation . The higher value of water soluble extractive value shows that STD formulation has more amount of polar constituents than market preparation .Loss on drying at 105° C was performed and the value of LOD was found to be more in market preparation than in STD preparation.This shows that the market preparation has more moisture content than the STD preparation of Balchaturbhadra churna. pH of the STD and test formulation was find

out. This values shows that both the preparation of Balchaturbhadra churna are slightly acidic in nature.

Phytochemical analysis showed the presence of alkaloid, flavonoids Tannin, phenolics, carbohydrate and Sterols and triterpenoids and absence of coumarins and saponins.

The amount of flavonoids,crude fibre content and Na^+ ,K^+ content were found to be more in standard than market formulation where tannins were found to be less in standard than market preparation.

Fluorescence analysis was performed by observing the powder of the standard and market formulation of Balchaturbhadra churna with 1N HCL , 1N H_2SO_4, 1N HNO_3 , aquous and alcoholic NaOH, Iodine solution, KOH and NH_3 and the fluorescence in day light and in UV light was compared for both the preparation of Balchaturbhadra churna.

Conclusion

After analysis of Standard (As per in *Bhaisajya Ratnavali)* and Market Ayurvedic formulation of Balchaturbhadra churna by different parameters such as total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and loss on drying at 105°C,microscopic analysis phytochemical analysis, it can be concluded that the market formulation was comparable with Standard formulation.

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