ORIGINAL ARTICLE

Comparative standardization of different market samples of ayurvedic formulation – *Balachaturbhadra Churna*

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Abstract

Background: Standardization of the herbal formulation is a critical and essential issue to be considered in assuring the therapeutic efficacy and safety and to rationalize their use in the health care. It is crucial to assess the quality and purity standards of the drug. *Balachaturbhadra* (BCB) *Churna* is a reputed poly-herbal formulation of Ayurveda for pediatric disorders. It is prescribed for the treatment of respiratory disorders, fever, diarrhea, and vomiting of children. **Objective:** The present study is aimed at comparison of physico-chemical standards of *Balachaturbhadra Churna* prepared In-house by following standard operative procedures and three marketed brands of the same. **Materials and Methods:** The selected samples were subjected to pharmacognosy study, phytochemical characteristics, physiochemical screening, and high-performance thin layer chromatography (HPTLC) as per standard procedures. **Results:** It was observed that two market samples and standard are almost similar in their organoleptic and qualitative chemical analysis with the In-house preparation except for one sample. Again HPTLC of these formulations are not matching with each other, and it may be due to the raw material collection time, geographical variation, etc., which can be further investigated for its pharmacological activity. **Conclusion:** The data evolved from this study can be adopted for laying down the standards for the manufacturing of BCB *churna*.

Key words: *Balachaturbhadra Churna*, high-performance thin layer chromatography, market samples, pharmacognosy, physico-chemical, phytochemical

INTRODUCTION

Ayurveda has stood against the test of time and is proving to be an effective remedy for most of the ailments. However, poor quality control measures and lack of standardization have been the major cause for its backdrop in the modern scientific world. The World Health Organization (WHO) recommends the herbal/traditional remedies in national healthcare programs in view of their low cost and better safety profiles along with people's faith in such remedies.[1] To promote the safety of herbal medicines, the WHO has committed itself to develop the necessary new guidelines and to update existing ones relating to the quality assurance and control of herbal medicines.[2] The WHO assembly in a number of resolutions has emphasized the need to ensure quality control of medicinal plant products using modern techniques and applying suitable standards.[3]

Children being more vulnerable, special care have to be taken in selecting the drugs and formulations. *Balachaturbhadra* (BCB) *Churna* is an important

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formulation in Ayurveda particularly in *kaumarbhritya* (branch of pediatrics) which is indicated for respiratory disorders, fever, diarrhea, and vomiting of children. It is a powder formulation containing four drugs namely *Musta* (*Cyprus rotundus* Linn.), *Pippali* (*Piper longum* Linn.), *Ativisha* (*Aconitum heterophyllum* Wall.), and Karkatashringi (*Pistacia integerrima* Stew.).[4]

Herbal medicines are being manufactured on the large scale in pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs, and formulation and quality control parameters. The increasing demand for the raw materials and unavailability of the resources compel many manufacturers to go for substitutes and adulterants which result in the substandard products. The quality of finished products vary from one pharmacy to another also there is no consistency in batch to batch production of herbal drugs. It is important to ensure the standard and quality right from the raw drugs to the finished product. The present study is targeted at the same objective.

There is a work already published regarding pharmacognostical and pharmaceutical assay of BCB *Churna*.[5] Here, an attempt has been made to study and compare three market samples of BCB *Churna* with the In-house preparation by means of pharmacognostical, preliminary phytochemical, physico-chemical parameters, and high-performance thin layer chromatography (HPTLC) fingerprints, thereby developing standards for this very popular pediatric medicine of Ayurveda.

MATERIALS AND METHODS

Drug Source

The In-house sample is coded as sample 1. The In-house sample 1 preparation was done as per the standard procedure and used as control. The main four ingredients of BCB *Churna* [Table 1] rhizome of *C. rotundus* Linn., fruits of *P. longum* Linn., roots of *A. heterophyllum* Wall., and gall of *P. integerrima* Stew. are collected from the pharmacy of institute. Drugs are dried properly by shade drying, powdered by micro–pulverizer, and stored in an air-tight container. The BCB *Churna* is prepared by mixing the powder of above four ingredients in equal proportions.

Three different market samples were collected from local retailers. These three market samples were coded as sample 2, sample 3, and sample 4. All the samples were subjected to pharmacognostical, preliminary phytochemical, physico-chemical analysis, and the parameters were compared.

Microscopic Study of BCB *Churna*

The study was carried out at pharmacognosy laboratory of the institute. 2 g of each sample was separately mixed with distilled water and was mounted on slides for the study. All the characters were studied with and without staining. Staining was done with phloroglucinol and conc. HCl. Microphotographs were taken using a Carl zeiss trinocular microscope.[6]

Analytical Study

An analytical study was carried out at the Pharmaceutical Chemistry Laboratory of the institute. Organoleptic parameters were assessed. Preliminary phytochemical investigations such as Molisch's test, Salkowski test, Keller-Killiani test, Foam test, Flavonoid test, Dragendorff's test, and test for tannins and phenols were performed.[7] Physico-chemical analysis like loss on drying, ash value, water and alcohol soluble extract, pH value, and particle size were carried out by following standard procedure.[8] HPTLC studies were carried out with acid hydrolysed methanolic extract.[9] The methanol extract of BCB *Churna* was spotted on pre-coated Silica Gel GF_{254} Plates by means of Camag Linomat V sample applicator. The mobile phase consisted of Toluene: Ethylacetate:Acetic acid a ratio of 7:2:1 v/v/v. After development, the densitometric scan was performed with a camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under the control of Wincats software (version 1.2.1.camag). The source of radiation utilized was deuterium and tungsten lamp emitting a continuous spectrum between 200 and 700 nm.

Then, the plate was sprayed with vanillin-sulfuric acid followed by heating and then visualized. The R_f values were recorded.

Table 1: Ingredients and the part used of *Balachaturbhadra Churna*

Name of drug	Botanical name	Part used	Proportion
Musta	*Cyperus rotundus* Linn.	Rhizome	1 part
Pippali	*Piper longum* Linn.	Fruit	1 part
Ativisha	*Aconitum heterophyllum* Wall.	Root	1 part
Karkatashringi	*Pistacia integerrima* Stew.	Gall	1 part

RESULTS

Organoleptic Characters

All the samples were assessed for organoleptic characters such as taste, smell, color, and touch. The observations made are listed in [Table 2].

Microscopic Characters

The list of all microscopic characters observed in all the samples is summarized in [Table 3]. Powder microscopy of *Churna* shows striking characters of all four individual constituents *Musta*, *Pippali*, *Ativisha,* and *Karkatashringi* of BCB *Churna* in sample 1 [Figure 1] and sample 2 [Figure 2]. The characters such as Cork cells of *Ativisha,* Scalariform cells, Oleo resins and Lignified fibers of *Musta,* Oil globules of *Pippali* were absent in sample 3 further no characters pertaining to *Karkatashringi* were identified in sample 3 [Figure 3]. From all observed characters, sample 4 [Figure 4] had features of all the four drugs except prismatic crystals of calcium oxalate of *Ativisha,* Lignified fibers of *Musta,* Starch grains of *Pippali* without hilum, and Epidermal cells of *Karkatashringi.*

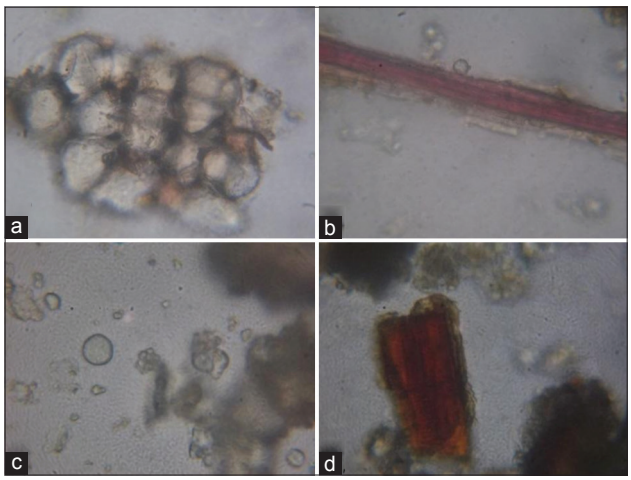

Figure 1: Powder microscopy of sample 1. (a) Parenchymatous cells of *Ativisha.* (b) Lignified fibers of *Musta.* (c) Oil globules of *Pippali.* (d) Vascular bundle along with tannin cells of *Karkatashringi*

Table 2: Organoleptic characters of samples of *Balachaturbhadra Churna*

Characters	Sample 1	Sample 2	Sample 3	Sample 4
Taste	*Kashaya, Tikta*	*Kashaya, Tikta*	*Kashaya, Tikta*	*Kashaya, Tikta*
Smell	Spicy pungent	Irritable, pungent	Not irritable, pungent	Irritable, pungent, causes sneezing
Color	Light brown	Light brown	Light brown	Light brown
Touch	Fine	Fine	Fine coarse	Fine coarse

Table 3: Observation of various microscopic characters of different samples of *Balachaturbhadra Churna*

Characters	Sample 1	Sample 2	Sample 3	Sample 4
Simple and compound starch grains with hilum of *Ativisha*	+	+	+	+
Cork cells of *Ativisha*	+	+	-	+
Prismatic crystals of calcium oxalate of *Ativisha*	+	+	+	-
Parenchyma cells of *Ativisha*	+	+	+	+
Scalariform cells of *Musta*	+	+	-	+
Starch grains of *Musta*	+	+	+	+
Oleo resins of *Musta*	+	+	-	+
Dark brown coloring matter of *Musta*	+	+	+	+
Annular vessels of *Musta*	+	+	+	+
Lignified fibers of *Musta*	+	+	-	-
Starch grains of *Pippali* without hilum	+	+	+	-
Stone cells of *Pippali*	+	+	+	+
Oil globules of *Pippali*	+	+	-	+
Tannin content material of *Karkatashringi*	+	+	-	+
Fragments of pitted vessels of *Karkatashringi*	+	+	-	+
Vascular bundle along with tannin cells of *Karkatashringi*	+	+	-	+
Parenchyma cells of *Karkatashringi*	+	+	-	+
Epidermal cells of *Karkatashringi*	+	+	-	-

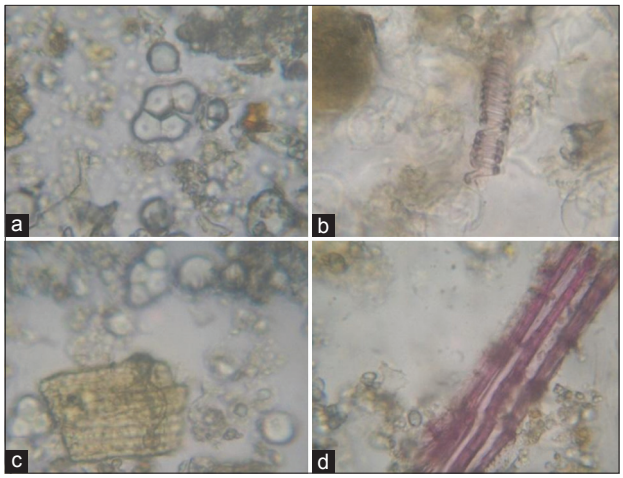

Figure 2: Powder microscopy of sample 2. (a) Compound starch grains with hilum of *Ativisha*. (b) Annular vessels of *Musta*. (c) Stone cells *Pippali*. (d) Fragments of pitted vessels of *Karkatashringi*

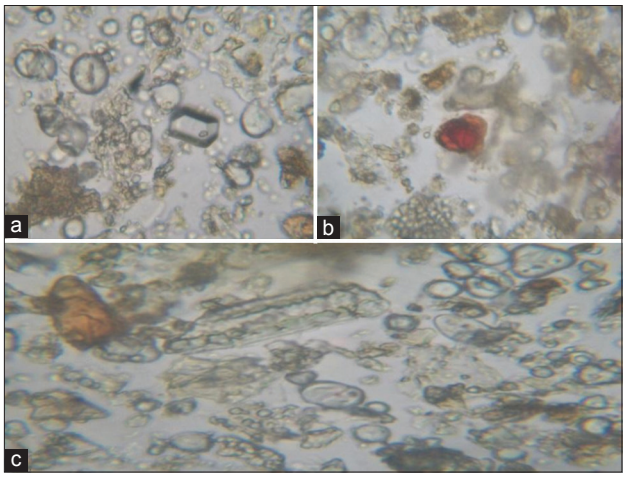

Figure 3: Powder microscopy of sample 3. (a) Prismatic crystals of calcium oxalate of *Ativisha*. (b) Coloring matter of *Musta*. (c) Stone cells of *Pippali*

Pharmaceutical Study of BCB *Churna*

Pharmaceutical studies have been done on four samples. The list of tests performed and observational value obtained are listed in [Table 4].

Phytochemical Screening for Organic Constituents

Various preliminary phytochemical tests were carried out to analyze all samples of BCB *Churna*. Qualitative test for various functional groups for all samples revealed the presence of carbohydrates, steroids, cardiac glycosides, alkaloids, and tannins in the formulations. Saponin was absent in all samples of *Churna*. Flavonoids were found in all samples except sample 3.

HPTLC Details

On performing HPTLC for sample 1, the chromatogram showed 17 peaks at 254 nm; while at 366 nm, the chromatogram showed 14 spots [Table 5 and Figure 5]. For sample 2 of BCB *Churna*, 25 spots were obtained when the HPTLC plate was visualized at 254 nm UV light, whereas 22 spots were obtained when visualized at 366 nm UV light [Table 6 and Figure 6]. For sample 3 of BCB *Churna* when the HPTLC plate was visualized, 23 spots were obtained at 254 nm UV light and 20 spots were obtained at 366 nm UV light [Table 7 and Figure 7]. HPTLC plates for Sample 4 of BCB *Churna* visualized 22 spots and 26 spots at 254 nm and 366 nm UV light, respectively [Table 8 and Figure 8].

DISCUSSION

The multifaceted drug in ayurvedic pediatric practice BCB *Churna,* which is widely used, has been analyzed in present work. Study on three market samples with In-house sample

Table 4: Observation of various physico-chemical parameters of different samples of *Balachaturbhadra Churna*

Name of test	Sample 1	Sample 2	Sample 3	Sample 4
Loss on drying (%w/w)	4.7	5.69	7.1	4.52
Ash value (%w/w)	5.75	5.75	6.1	3.9
Water soluble extract (%w/w)	29.3	28.6	27.3	28.2
Alcohol soluble extract (%w/w)	27.3	22.9	29.1	22.5
pH value	6 (acidic)	6 (acidic)	6 (acidic)	6 (acidic)
Particle size	10.069	10.171	10.061	10.010
mesh>60	3.209	0.580	2.437	0.787
mesh 61-85	2.345	1.740	2.596	4.980
mesh 86-120	1.360	1.979	2.635	1.432
mesh>120	3.019	5.735	2.072	2.738

of BCB *churna* is a step toward pharmacognostical and pharmaceutical standardization of the drug.

In powder microscopy, the presence of characteristic features of all the raw drugs in final product shows the purity and the quality of In-house BCB *Churna* (sample 1, Sample 2, and sample 4). No characters related to the drug *Karkatashringi* were found in sample 3. The inadequate characters relating to raw drugs prove the inaccuracy of sample 3.

The physico-chemical parameters of all samples obtained were as per API standards.[10] The loss on drying of any sample is directly related to its moisture content. The less value of moisture content could prevent bacterial, fungal, or yeast growth.[11] Percent weight loss on drying or moisture content was found to be less in sample 1, sample 2, and sample 4 compared to sample 3. The total ash value was relatively high in sample 3 which may be due to high content of inorganic and salt materials in the sample. Ash value is useful in determining authenticity and purity of drug, and also, these values are important quantitative standards.[12]

The extractive values, such as water-soluble and alcohol-soluble, indicate the amount of active constituent and the bioavailability of the plant. A lower value indicates the presence of the exhausted material. In the present study, In-house preparation had maximum water soluble extractive values and sample 3 had maximum alcohol soluble extractive values. On analyzing both water-soluble and alcohol-soluble extractive values, the In-house *Churna* was better compared to all other market samples. pH of all the *Churna* samples

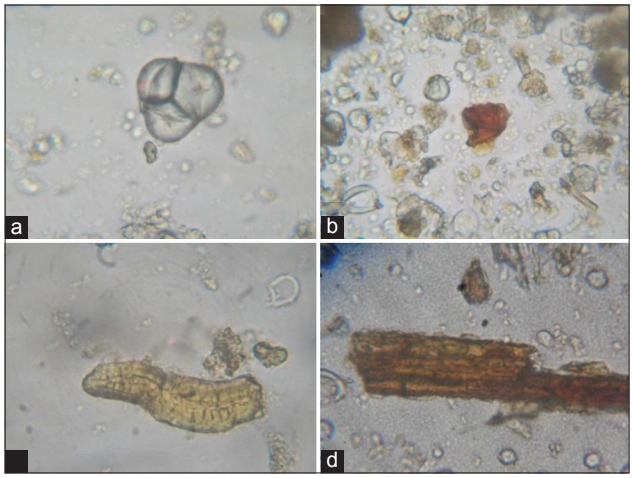

Figure 4: Powder microscopy of sample 4. (a) Starch grains of *Ativisha*. (b) Oleo resins of *Musta*. (c) Stone cells *Pippali*. (d) Tannin content material of *Karkatashringi*

Table 5: R_f values of sample 1

Visualizing condition	Number of spots	R_f value
254 nm	17	0.01, 0.05, 0.14, 0.18, 0.23, 0.29, 0.34, 0.35, 0.45, 0.47, 0.53, 0.58, 0.61, 0.65, 0.73, 0.88, 0.95
366 nm	14	0.01, 0.14, 0.18, 0.23, 0.29, 0.34, 0.35, 0.43, 0.47, 0.53, 0.58, 0.66, 0.71, 0.80

Table 6: R_f values of sample 2

Visualizing condition	Number of spots	R_f value
254 nm	25	0.01, 0.03, 0.05, 0.08, 0.12, 0.15, 0.19, 0.22, 0.30, 0.35, 0.37, 0.41, 0.44, 0.48, 0.50, 0.52, 0.53, 0.54, 0.62, 0.64, 0.68, 0.74, 0.81, 0.84, 0.90
366 nm	22	0.01, 0.03, 0.05, 0.15, 0.22, 0.30, 0.3, 0.37, 0.41, 0.48, 0.50, 0.52, 0.59, 0.62, 0.64, 0.69, 0.73, 0.80, 0.84, 0.87, 0.93, 0.94

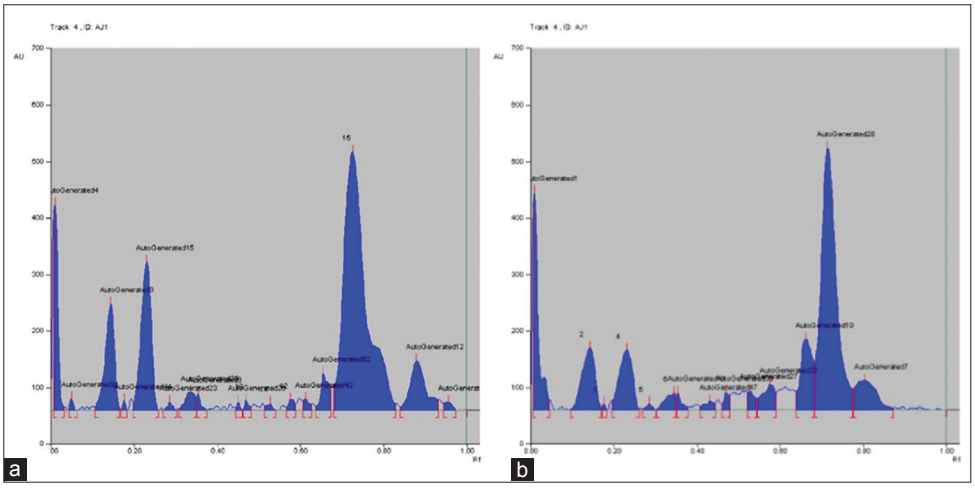

Figure 5: Densitogram for sample 1. (a) At 254 nm peak display. (b) At 366 nm peak display

Table 7: R_f values of sample 3

Visualizing condition	Number of spots	R_f value
254 nm	23	0.01, 0.03, 0.08, 0.11, 0.13, 0.17, 0.20, 0.24, 0.28, 0.34, 0.36, 0.40, 0.45, 0.48, 0.59, 0.61, 0.63, 0.64, 0.71, 0.76, 0.86, 0.92, 0.97
366 nm	20	0.01, 0.08, 0.13, 0.20, 0.24, 0.28, 0.34, 0.36, 0.40, 0.45, 0.48, 0.63, 0.65, 0.70, 0.73, 0.76, 0.84, 0.86, 0.92, 0.97

Table 8: R_f values of sample 4

Visualizing condition	Number of spots	R_f value
254 nm	22	0.01, 0.04, 0.15, 0.19, 0.24, 0.29, 0.35, 0.43, 0.46, 0.48, 0.50, 0.53, 0.58, 0.60, 0.64, 0.66, 0.68, 0.71, 0.80, 0.85, 0.87, 0.94
366 nm	26	0.01, 0.04, 0.15, 0.19, 0.25, 0.29, 0.32, 0.35, 0.38, 0.43, 0.46, 0.49, 0.50, 0.53, 0.58, 0.60, 0.65, 0.68, 0.71, 0.79, 0.83, 0.85, 0.87, 0.91, 0.97, 0.98

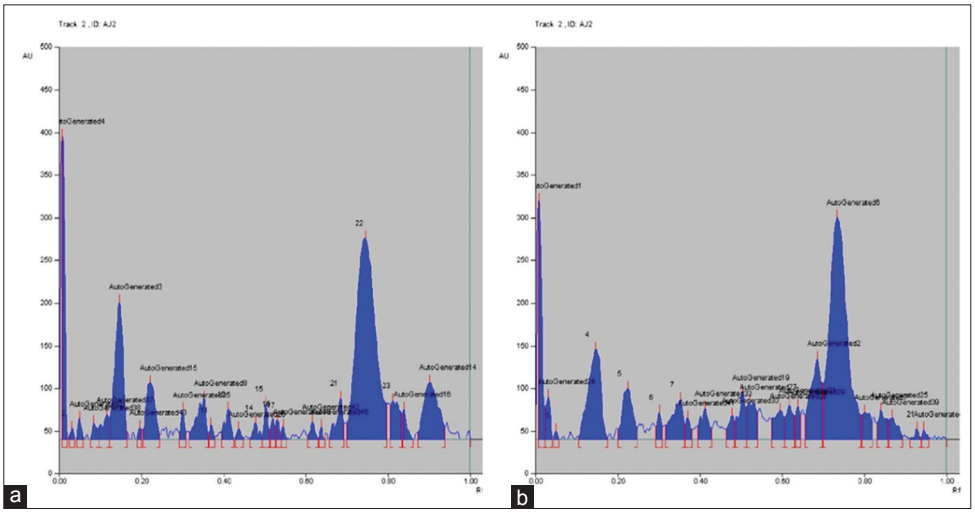

Figure 6: Densitogram for sample 2. (a) At 254 nm peak display. (b) At 366 nm peak display

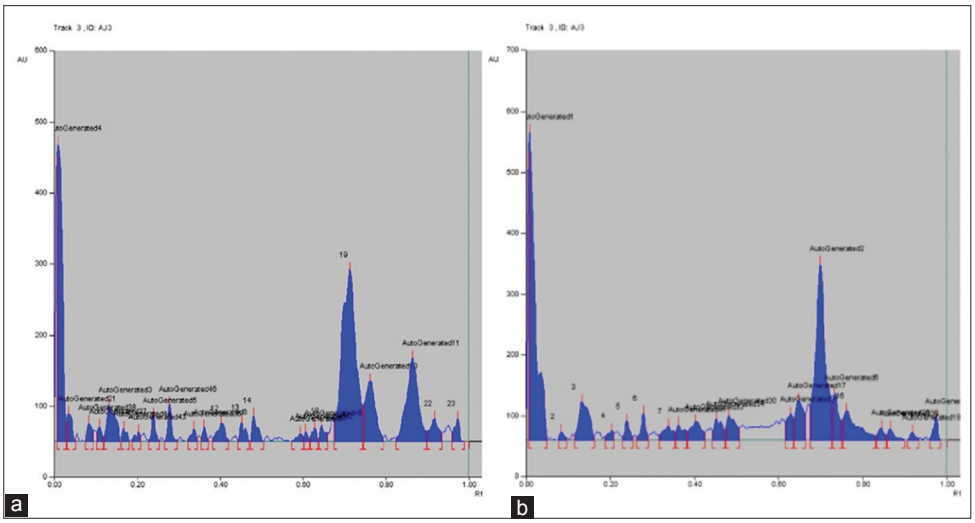

Figure 7: Densitogram for sample 3. (a) At 254 nm peak display. (b) At 366 nm peak display

was 6.00 which indicates all are acidic in nature. In general, *Churna* for internal use is advocated to be superfine to facilitate quick absorption. Most fine particle size, i.e., >120 mesh was observed in the sample 2 and least in sample 3.

The phytochemical parameters also reveal the presence of common functional groups in all the samples of *Churna*. Only flavonoids were an exception which was present in all samples except sample 3. These findings once again query the legitimacy of sample 3.

HPTLC in the methanol extract of samples shows maximum resolution in solvent system toluene-ethyl acetate-acetic acid (7:2:1 v/v/v). Depending on the number of spots, we

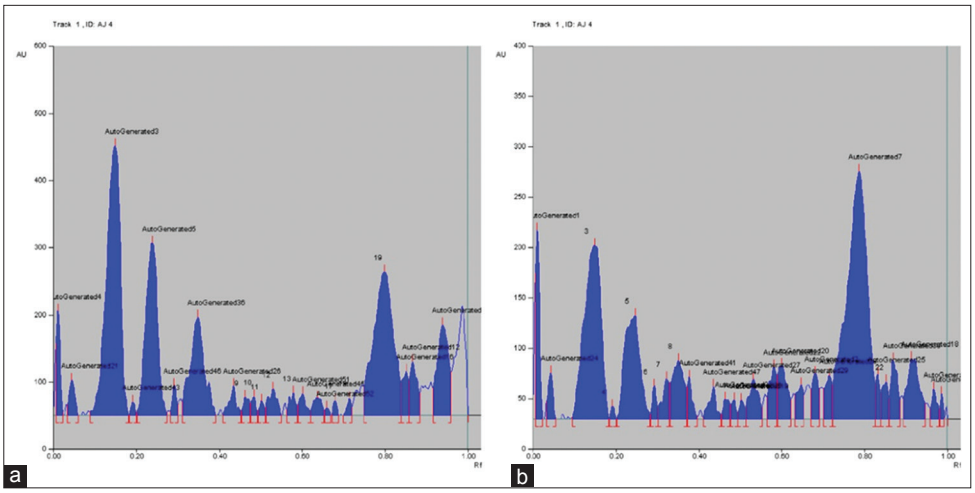

Figure 8: Densitogram for sample 4. (a) At 254 nm peak display. (b) At 366 nm peak display

can depict what is the number of active principles present. Samples 1, 2, 3, and 4 shows varied number of spots. R_f value at 0.01 was common active principle found in all samples. In these samples, some spots are in same R_f value but the intensity (area under curve) differs and some are in different R_f values. This clearly depicts there is no consistency in the content of active ingredient. Again in sample 3, the intensity of all peaks is minimal in comparison to other samples.

CONCLUSION

The result of present study based on pharmacognostical, preliminary phytochemical, and physico-chemical parameters indicates that the In-house sample and two market samples (sample 2 and sample 4) were more or less uniform in its attributes, but there was a visible difference in sample no 3. Sample 3 had inadequate characters related to the formulation. The HPTLC study shows no uniformity in the content of active ingredient except one at R_f 0.01. The experimental differences in these formulations may be due to vary geographical locations where these plants grow, the ambiguous identity of these plants, a great deal of adulteration or substitution encountered in the commercial market. The parameters of this study can be used for the authentication and further research. This study may be a useful contribution in overcoming the hurdles encountered in standardization and drug manufacturing of BCB *Churna*, thereby selection of an appropriate formulation in the clinical practice and hence provide effective treatment to the patients.

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