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Standardization of an Ayurvedic Pediatric Formulation *"Balachturbhadrika Churna"*

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Abstract

Ayurvedic formulations are gaining importance nowadays as they are economic, easily available and relatively free from side effects. It is important to bring the use of these remedies in existing frame work of scientific usage. Considering this, ayurvedic formulation *"Balachturbhadrika churna"* was standardized by using morphological, microscopical, physicochemical, phytochemical and chromatography parameters. Raw materials were checked for standardizing the formulation. Three market samples were collected and compared with prepared formulation.

Keywords

Balachaturbhadrika Churna, Pippali, Ativisa, Musta, Karkat sringi

INTRODUCTION

Balachturbhadrika Churna is a fine powder usually prepared by mixing equal part of *Musta* (Rhizome of *Cyperus rotundus*), *Pippali* (Fruit of *Piper longum*), *Ativisa* (Root of *Aconitum heterophyllum*), *Sringi* (Galls of *Pistacia integerrina*).

This formulation is mentioned in various ancient text like Bhaishjya Ratnavali[1], Balrogadhikara, Bhaishajya Samhita[2], Chakradatta[3], Bharat-Bhaishishajyaratnakar[4], Bhavprakash[5], Sharandhara Samhita[6], Yogratnakar[7], Gadanigraha[8], Vrinda Vidhak[9], Abhinav Bal tantra[10], Vrinda Madhavaparanama[11], Brihadyoga tarangini[12] and Ayurvedic Formulary of India, Part-I[1]3. Generally it is used for different therapeutic indication like *Atisara* (Diarhhoea), *Jwara* (Fever), *Swasa* (Asthama), *Chardi* (Nausea & vomiting) in *Balroga* (pediatric disorders).

Detailed review of scientific literature reveals no work is been done on standardization of this Ayurvedic formulation. In the present study we made an attempt to develop parameters for standardization of *Balachturbhadrika Churna.*

MATERIALS AND METHODS

Plant material

Three plants viz. *pippali, karkata sringi* and *musta* were procured from the campus of Gujarat Ayurved University, Jamnagar and *Ativisa* was collected form high altitude region of Himachal Pradesh. Authentication of plant materials were done by employing various pharmacognostical and physicochemical parameters as discussed

below along with chromatographic studies. Fresh plant materials were dried under shade and powdered (#10) for carrying out various analytical studies.

Quality control of raw material

All the powdered drugs were studied microscopically, for foreign matter, loss on drying, ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and volatile oil content (for *Musta*)[14] . Apart from the above studies qualitative phytochemical test [15-18], quantitative estimation of total alkaloid content [19] and thin layer chromatography (TLC) of methanol extract (*ativisha, pippali, karkat shringi*) and volatile oil (*musta*) were also performed.

Preparation of Methanol extract of sample

Powder drug (1 gm) was extracted by heating under reflux for 15 min. with 10 ml methanol. The filtered and filtrate so obtained were evaporated to a volume of 2 ml and used for spotting.

Preparation and analysis of formulation

A formulation was prepared in laboratory by mixing thoroughly the powdered herbs in equal proportion. Three market formulations were procured from local market of Rajkot, India. Qualitative phytochemical test [19, 21, 22], quantitative estimation of total alkaloidal content [19], TLC fingerprinting was also performed in order to standardize the formulation and to check the presence of active components in marketed formulations as well. All the four formulations were analysed for the parameters like microscopic study of the churna, particle size, loss on drying, ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive and volatile oil content of drug [20]. In chromatography, an attempt has been made to develop TLC fingerprint of the formulation through which presence of different ingredients of the sample can be checked. Hence for comparison, four more sample of *Balachturbhadrika churna* were prepared in the laboratory by omitting one of the ingredients and used in TLC.

RESULTS AND DISCUSSION

All four plants were studied for various pharmacognostic parameters for authentication. Parameters were compared with that of laid down in standards literature. Phytochemical and TLC studies confirm the presence of alkaloids in *ativisa* and *pippali* methanol extract, phenolics/tannins in methanol extract of *karkat sringi*, and terpenoids in *musta* volatile oil. The commonly used physicochemical parameters for *Balchaturbhadrika churna* were also determined as mentioned in Table 1. It was

found that there is variation in physicochemical parameters of standard churna and market samples. Loss on drying, ash value and acid insoluble ash was slightly higher in market sample than standard churna while extractive value, total alkaloid content and volatile oil content was also lower in market sample 1 & 2. In Table 2 details of solvent systems and detecting reagent used in analysis, are discussed. The TLC chromatograms of the methanol extract of the samples, obtained by using mobile phase toluene: ethyl acetate: diethyl amine (7:2:1) is presented in Fig 1. *Ativisa* shows one orange red colored spot at Rf 0.38, while standard churna and market sample 2 and 3 shows two orange red colored spot at Rf 0.38 and 0.65. But market sample 1 shows only one orange red colored spot at Rf 0.65 while the spot at Rf 0.38 is absent in market sample 1. The sample of standard churna without Ativisa shows only one orange red colored spot at Rf 0.65. Therefore, the spot at Rf 0.38 using this condition can be utilized for detecting the presence of *Ativisa* in the *Balachturbhadrika churna* sample. The chromatograms of the methanol extract of samples, obtained by using mobile phase toluene: ethyl acetate (7:3) is shown in Fig 2. Pippali, standard churna, market sample 1, 2, and 3 gave one orange red colored spot at Rf 0.44 while, the sample of standard churna without *Pippali* does not show any spot. Therefore, the spot at Rf 0.44 using this condition can be utilized for detecting the presence of *Pippali* in the *Balachturbhadrika churna* sample.

Fig 1TLC for *Ativisa*

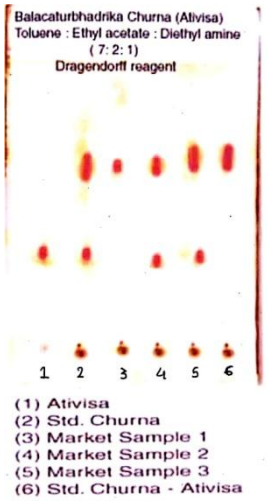
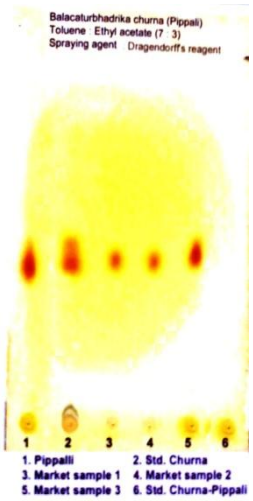


Fig 2 TLC for Pippali



The chromatograms of the methanol extract of the samples of *Karkat sringi*, obtained by using solvent system n-butanol: glacial acetic acid: water (4:1:5) is presented in Fig 3. *Karkat sringi* shows three blue colored spot at Rf 0.29, 0.56 and 0.90, while standard churna shows same three blue colored spot at Rf 0.25, 0.54 and 0.88. But the market sample 1 shows only two blue colored spot at Rf 0.25 and 0.86. In a similar manner sample 2 shows only two colored spots are Rf 0.56 and 0.92 and market sample 3 shows three blue colored spots at

Rf 0.31, 0.56 and 0.92. The sample of standard churna without *Karkat sringi* shows only one blue colored spot at Rf 0.95. It reveals that in market sample 1 spot at Rf 0.54 was absent and in market sample 2 spot at Rf 0.25 was absent. Therefore, the spot at Rf 0.29, 0.56 and 0.90 using this condition can be utilized for detecting the presence of *Karkata sringi* in the *Balachturbhadrika churna* sample.

Table 1 Physico-chemical parameters of raw materials and formulations

Parameter	**Musta**	**Pippali**	**Karkat sringi**	**Ativi-sa**	**Balachturbhadrika churna**			
					Lab. formulation	**Market Sample 1**	**Market Sample 2**	**Market Sample 3**
Foreign matter, % w/w	Nil	Nil	Nil	Nil	-----	------	------	-----
Loss on drying at 105 ^{0} C	6.20	10.70	8.60	5.80	7.8	8.0	8.5	8.1
Ash value, % w/w	4.72	7.80	5.30	3.24	5.2	6.1	5.8	5.6
Acid insoluble ash, % w/w	3.80	0.10	0.10	0.56	0.80	0.92	0.84	0.83
Water soluble extractive, % w/w	11.65	38.50	34.00	26.80	24.0	22.7	21.9	23.8
Alcohol soluble extractive, % w/w	10.20	10.30	33.40	8.60	16.0	14.3	14.0	15.6
Fineness of powder								
A pass through 60#,% w/w	----	------	-----	------	100	100	100	100
B. Pass through 85 #,% w/w	-----	------	------	------	100	85	90	100
pH (filtrate of 10% w/v aqueous solution)	-----	-------	--------	-----	4.92	5.06	5.20	5.10
Volatile oil content, % v/w	1.00	-------	--------	-------	0.60	0.20	0.40	0.60
Total alkaloidal Content	-----	0.640	-------	0.360	0.260	0156	0.169	0.200

All the results are average of three determinations

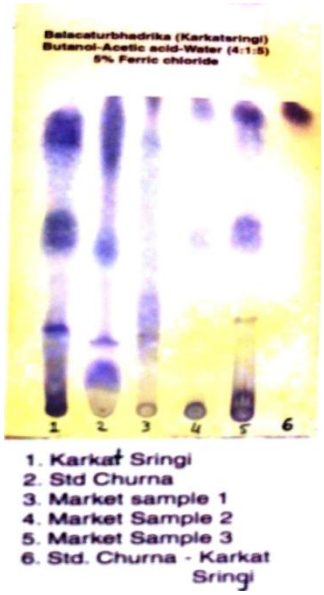
The chromatograms of the volatile oil of the *Musta, Balachturbhadrika churna* and *Balachturbhadrika churna* (without *musta*), obtained by using mobile phase toluene: ethyl acetate (93:7) is shown in Fig 4. Volatile oil of *Musta* shows nine spots at Rf 0.32 (Blue), 0.38 (Green), 0.42 (Violet),

0.48 (Blue), 067 (Magenta), 0.74 (Sky blue), 0.81 (faint), 0.88 (Grey), 0.95 (Violet). While standard churna shows eleven spots under the same condition at Rf 0.32 (Blue), 0.38 (Green), 0.42 (Violet), 0.48 (Blue), 0.54 (Brown), 0.58 (Steel grey), 0.67 (Magenta), 0.74 (Sky blue), 0.81 (Faint), 0.88 (Grey), 0.95 (Violet). Common spots have been noticed at Rf 0.32, 0.38, 0.42, 0.48, 0.67, 0.74, 0.81, 0.88, and 0.95. Two additional spots were seen at Rf 0.54 (Brown), 0.58 (Steel grey).

Table 2 Thin layer chromatography study of raw material

Drug	Stationary phase	Solvent system	Sample	Detection
Ativisa	Silica gel G	Toluene: ethyl acetate: diethyl amine (7:2:1)	Methanol Extract	Spraying with Dragendroff's reagent
Pippali	Silica gel G	Toluene: ethyl acetate (7:3)	Methanol Extract	Spraying with Dragendroff's reagent
Karkat sringi	Silica gel G	n-butanol: glacial acetic acid: water (4:1:5) upper layer	Methanol Extract	Spraying with 5% Ferric Chloride
Musta	Silica gel G	Toluene:ethyl acetate (93:7)	Volatile oil	Spraying with 1% vanilllin in sulphuric acid followed by heating at 110 ^{0}C for 5 min.

Fig 3 TLC for Karkatsringi



This sample of standard churna without *Musta* shows only seven spots at Rf 0.32 (Blue), 0.38 (Yellow), 0.42 (Violet), 0.48 (Blue), 0.54 (Brown), 0.67 (Magenta), 0.95 (Violet). The spot at Rf 0.74 (Sky blue), 0.81 (Faint), 0.88 (Grey) can be utilized for detecting the presence of *Musta* in *Balachturbhadrika churna* sample.

The chromatograms of the volatile oil of the samples are presented in Fig 5. Volatile oil of all the churna (Standard churna, market samples 1, 2 and 3) show eleven spots at Rf

0.32 (Blue), 0.38 (Green), 0.42 (Violet), 0.48 (Blue), 0.54 (Brown), 0.58 (Steel grey), 0.67 (Magenta), 0.74 (Sky blue), 0.81 (Faint), 0.88 (Grey), and 0.95 (Violet).

Fig 4 TLC for *Musta, Balachturbhadrika* Churna (BC)& *BC*(without *musta*)

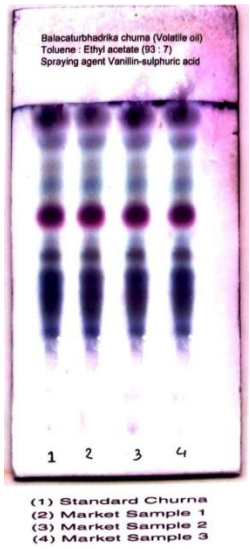
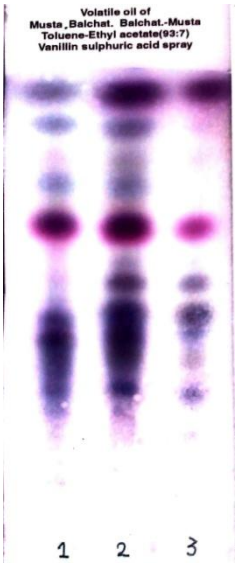


Fig 5 TLC for standard churna of Musta



So the spots at Rf 0.74, 0.81, 0.88 present in all the market samples confirm presence of Musta in all the samples. Results of various physicochemical parameters studied are recorded in Table 2.

CONCLUSION

The present study was undertaken with a view to provide the parameters for standardization of an *Ayurvedic* pediatric formulation *"Balachturbhadrika Churna"*. TLC, a simple and inexpensive technique was extensively used along with all traditional quality control tests for this purpose. The study laid down a specification regarding various qualitative and quantitative aspects of the formulation. Further studies can be extended for quantitation of markers using sophisticated instrumental techniques like HPTLC and HPLC.

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