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Standardization of ayurvedic formulation caturjata churna using modern techniques

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Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. The present paper aims to standardize the ayurvedic formulation of caturajata churna containing four ingredients, Darusita, *Cinnamomum zeylanicum*, Ela, *Elettaria cardamomum*, Patra, *Cinnamomum tamala*, Nagakesara, *Mesua ferrea* using modern technique such as ash values, extractive values moisture content by carrying out all four ingredients of the formulation. Preliminary phytochemical screening of caturjata churna was carried out using methanol as solvent. The study was carried out in marketed formulation against in house formulation of caturjata churna. It was observed that almost all the parameters are comparable with the marketed formulation. Some are not due to the geographical variation of the raw materials.

Keywords: Caturjata Churna, Micromeritic properties, Mineral estimation

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Standardization of crude drugs is not an easy procedure because almost all crude drugs are of natural origin. In the case of natural drugs, the therapeutic efficacy is a total effect of its chemical constituents, so the quality and purity refer to the profile of the drug rather than any of its characters so a multidimensional approach is essential for the standardization of crude drugs, then only the total profile of the crude drug is established. For the purpose of above, all the aspects of crude drugs are to be considered in details. This multidimensional approach should cover every minute aspect of the crude drugs specifically the name, botanical name, geographical source, morphology, anatomy, physical and chemical references[1]. Standardization of herbal raw drugs includes passport data of raw plant drugs, botanical authentication, microscopic & morphological examination, identification of chemical composition by various chromatographic techniques and biological activity of the whole plant. Macroscopic and microscopic evaluation and chemical profiling of the herbal materials for quality control and standardization have been reported by various workers. The macroscopic identity of plant materials is based on sensory evaluation parameters like shape, size, color, texture, and taste while microscopy involves comparative microscopic inspection of powdered herbal drug. Further, advances in microscope technology have enhanced the accuracy and capabilities of microscopy as a mean of herbal crude material identification due to the implication of light and scanning electron microscopes (SEM) in herbal drug standardization. Furthermore, various advanced methods such as chromatographic, spectrophotometric and combination of these methods, electrophoresis, polarography and the use of molecular biomarkers in fingerprints are currently employed in the standardization of herbal drugs. Ayurveda is an original holistic system of medicine, whose principles of therapeutics are applicable universally[2]. Caturjata churna is one of the important formulations of ayurvedic formulations which contains four major ingredients, Darusita, *Cinnamomum zeylanicum*, Ela, *Elettaria cardamomum*, Patra, *Cinnamomum tamala*, Nagakesara, *Mesua ferrea*. It is used to treat cough, cold, piles and other various diseases. Due to change in the present scenario of globalization the importance of standardization has been more stressed out than before. Based on this motivation this work provides a brief insight into the

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ayurvedic formulation and in particular caturjata churna. The objective of present study is to develop standardization profile of caturjata churna which includes pharmacognostical profile of individual drug present in the formulation, in-house formulation and marketed formulation and development of the analytical method for estimation of some important constituent of the formulation.

Material and methods

Raw materials were collected from the local market of Ranchi and were authenticated by Botanical Survey of India, Kolkata (BSI/CNH/SF/ Tech/2012). One marketed sample of VHCA herbals labeled as A was used, and one in- house formulation B was prepared. Microscopical study was carried out using powders as well as transverse section of each individual drug to identify and confirm the presence of these ingredients in the formulation of caturjata churna, as it is a mixture of four ingredients[3]. Physical evaluation was carried out as per CCRAS parameters[4].

The ash values, extractive values and moisture content were carried out of all four ingredients of the formulation. Preliminary phytochemical screening of caturjata churna was carried out using methanol as solvent[5].

Evaluation of micromeritic properties of caturjata churna was done by using various parameters such as bulk density, tapped density, flow properties of powders, angle of repose, flow rate, true density, percentage porosity and estimation of caturjata churna using cinnamaldehyde[6]. Mineral elemental analysis was performed by ICP-AES spectrometry. Mineral composition is a function of the soil on which plants grow and may therefore vary within the samples obtained from the same and between the species[7]. HPTLC method was used for qualitative analysis of cinnamaldehyde. The parameters checked were precision, reproducibility, limit of detection, limit of quantification and recovery. A stock solution of 1 mg/mL cinnamaldehyde was prepared in methanol. The different volumes of stock solution 1,4,7 and 10 µg were spotted in duplicate on HPTLC plates concentrations of 1,4,7 and 10 µg per spot of cinnamaldehyde, respectively. The data of peak height/area versus drug concentration were treated by linear least- square regression. Calibration curve of cinnamaldehyde shows a good linear relationship over the concentration range 1-12 µg per spot with respect to peak height (n=3). No significant difference was observed in the slopes of standard curve. After the method validation the standard and sample solutions were prepared and spotted on 20×20 silica plate in 7 tracks and content of cinnamaldehyde was estimated[8].

Results

The collected raw material and in-house preparation were analysed using ICH guidelines. Transverse section of cinnamon bark with cork and cortex shows, except at certain places, pericyclic sclerenchyma, 3 or 4 rows of isodiametric cells. It contains starch grains, small groups of pericylic fibres, phloem fibres with very thick walls, up to 30 µ in diameter, isolated or in short tangential rows, sieve tubes narrow with transverse sieve plates, collapsed in outer periphery, medullary rays of isodiametric cells, mostly 2 cells wide, cortical parenchyma and medullary rays containing small starch grains mostly below 10 µ in diameter, Minute acicular crystals of calcium oxalate were also present. The powder samples of cinnamon showed abundant starch grains, simple, ovoid or sack shaped, and had a distinct eccentric hilum. Separate fibres in group were associated with vessels, fibres mostly non lignified. Similarly powder characteristics of Patra showed light moss green shows fragments of epidermis, satellite trichomes, oil globules and round to oval starch grains. Ela powder showed light greenish in colour, and under microscope showed a sclerenchymatous layer, epidermis of the testa, perisperm cells and arillus. Powdered samples Nagakesara of showed the brownish to light brownish parenchymatous fragment; vascular bundles (9 to 10 in a circle), pith parenchymatous with oil cells, many oil cells. Different Powder characteristic of In house formulation sample showed light brown parenchymatous cells, numerous fibres with pointed tips, numerous oil cells and calcium oxalate crystals. Different Powder characteristic of marketed formulation showed light brown parenchymatous cells, numerous fibres with pointed tips, numerous oil cells, calcium oxalate crystals and parenchymatous pith. The ash values, water soluble ash, acid insoluble ash were carried out of all ingredients, in house preparation and marketed formulation (Table 1) The phytochemical study showed the presence of reducing sugars, tannin, resin, terpenoid and starch. The micromeritic properties of marketed and in house formulation of caturjata churna were also analysed (Table 2 & Fig. 1).

Mineral elemental analysis was performed by ICP-AES spectrometry. The experimental result indicates

Table 1 — Physical chacterstic of various samples of various ingredients of Caturjata churna and marketed sample and In-house prepared sample of Caturjata churna [Values are % mean determination ± Standard error of the maen (SEM)]

PARAMETERS		A	B	C	D
ASH VALUE	Total ash	1.52±0.058	3.9±0.059	3.7±0.048	3.4±0.036
	Water soluble ash	1.7±0.018	0.95±0.020	1.5± 0.021	1.78± 0.022
	Acid insoluble ash	0.85± 0.005	0.75± 0.007	0.82± 0.004	0.75± 0.003
Extractive value	Water soluble	12.60± 0.028	10.5± 0.025	11.31± 0.032	18.12± 0.028
	Methanol soluble	15.50± 0.028	7.91± 0.025	16.31± 0.032	25.27± 0.028
Moisture content at 105º C		8.11 ± 0.228	6.31± 0.125	8.21± 0.0332	6.39± 0.328

A: Dalchini powder B: Cardamom powder C: Tezpatra powder D: Nagkesara powder

PARAMETERS		A	B
ASH VALUE	Total ash	2.52±0.056	3.6±0.059
	Water soluble ash	1.5±0.018	0.75±0.020
	Acid insoluble ash	0.85± 0.005	0.75± 0.007
Extractive value	Water soluble	30.02± 0.018	31.5± 0.065
	Methanol soluble	21.60± 0.028	22.50± 0.025
Moisture content at 105º		8.30 ± 0.223	8.01± 0.325

A-MARKETED SAMPLE
B-IN-HOUSE PREPARED SAMPLE

Table 2 — Micromeritic properties of various Caturjata Churna samples

S. No.	Parameters	Formulation-A Mean±SEM	Formulation-B Mean±SEM
1.	Bulk density	0.357±0.02	0.360±0.03
2.	Tapped density	0.389±0.012	0.40±0.023
3.	Angle of repose	26.56±5.2	33.69±6.2
4.	True density	0.96±0.4	0.93±0.5
5.	Flow rate	0.224±0.01	0.23±0.02
6.	Carr's index	8.57±1.2	11.11±2.6
7.	Hausner's ratio	1.08±0.9	1.00±0.84
8.	Porosity	62.8±9.1	61.2±8.2

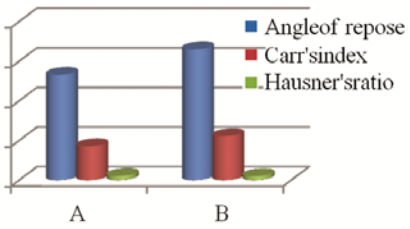

Fig. 1 — Flow properties of Formulations A-MARKETED SAMPLE B-IN-HOUSE SAMPLE

Table 3 — Mineral profile of the churna formulation, as determined by ICP-OES

Element	Formulation A Concentration. (mg/g.)	Formulation B Concentration. (mg/g.)
Ca	137.82	146.54
Cd	0.00	0.00
Co	0.006	0.004
Cr	0.098	0.078
Cu	0.132	0.122
Fe	0.134	0.143
Mn	0.480	0.378
Ni	0.02	0.012
Pb	0.098	0.054

Table 4 — Analysis of Cinnamaldehyde in Caturjata Churna by HPTLC

Drug	R*f*	Amount found in mg	Present in 100 mg	% Drug found
T1	0.34	0.00414233	0.424233	97.54
T2	0.35	0.00455112	0.455112	102.27
T3	0.35	0.0043222	0.43222	98.63
T4	0.34	0.00446434	0.446434	99.12
IH	0.37	0.000434848	0.043484809	1.67
MKT	0	0	0	0

that the churna has high contents of calcium, manganese and lower concentration of chromium, cobalt, lead and nickel (Table 3). Mineral composition is a function of the soil on which plants grow and may therefore vary within the samples obtained from the same and between the species. The HPTLC procedure was optimized for qualitative study of cinnamaldehyde in a herbal formulation. The mobile phase Ethyl acetate: n-hexane (1:1) gave good resolution with R*f* value 0.45 for cinnamaldehyde. Under the chromatographic condition employed, standard compound, cinnamaldehyde and the formulation have shown sharp peaks and good separation (Table 4 & Fig. 2-6).

Discussion

With the change in the present scenario of globalization the importance of standardization has been more stressed out than before. India has rich wealth of traditional knowledge of Ayurveda, Siddha,

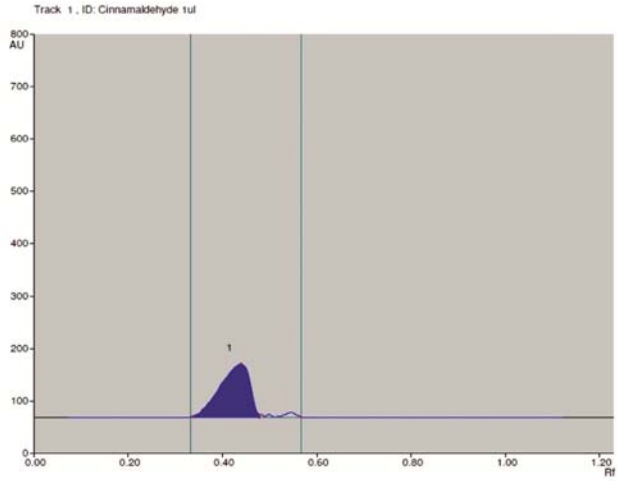

Fig. 2 — HPTLC chromatogram of standard cinnamaldehyde

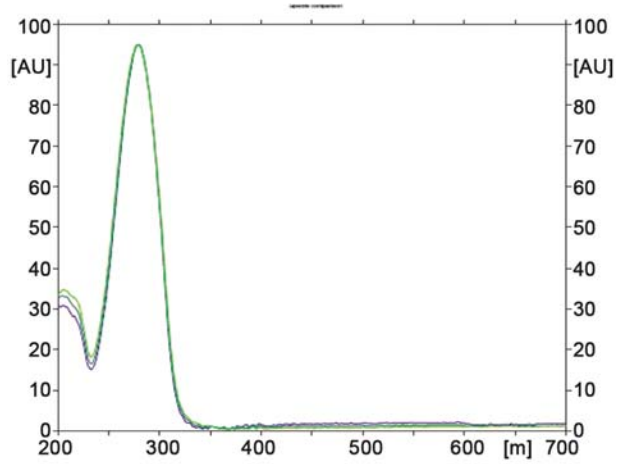

Fig. 3 — HPTLC chromatogram showing purity spectra of Cinnamaldehyde in first three tracks

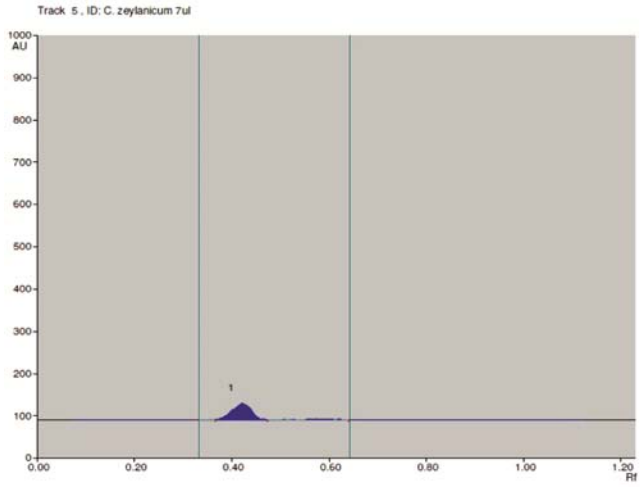

Fig. 4 — HPTLC chromatogram of C. Zeylanicum methanolic extract

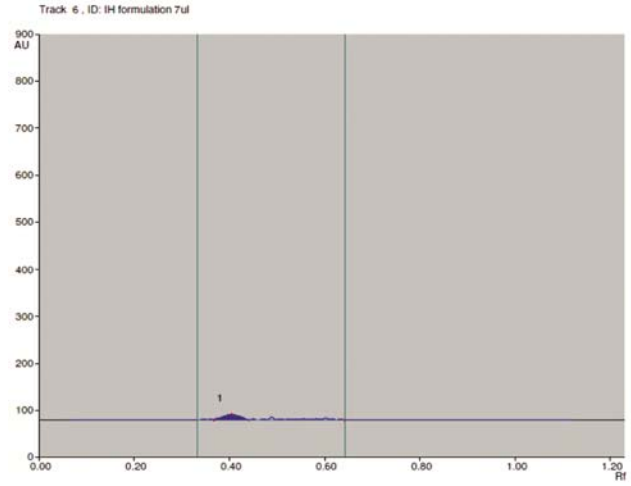

Fig. 5 — HPTLC chromatogram of methanolic extract of in house formulation

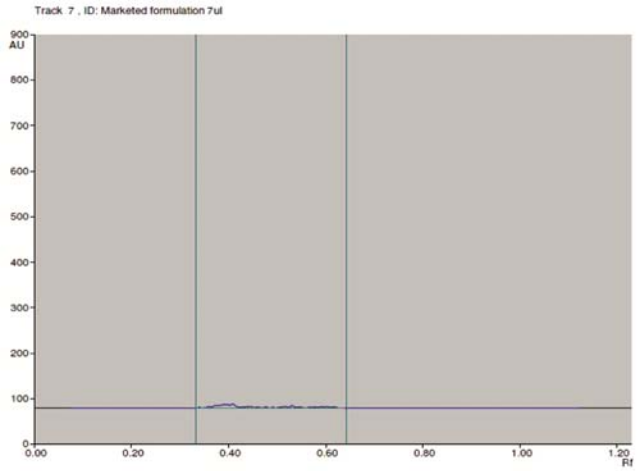

Fig. 6 — HPTLC chromatogram of methanolic extract of marketed formulation

Unani and other traditional medicine. In order to market our products outside the country and also to maintain the quality of these medicines when shifted to industrial production requires validated analytical methods for maintaining the quality of these products. So in this direction we have chosen an Ayurvedic formulation caturjata churna. Caturjata churna contains four major ingredients. All ingredients were analysed using various parameters of CCRAS guidelines. In house preparation was made using the procured raw materials. Marketed sample of the churna was procured from local market. Mineral study showed that the difference in result may be due to the presence of soil in the samples. The results of micromeritic properties showed the flow charecterstic of particle. The HPTLC procedure was optimized for qualitative study of

cinnamaldehyde in an herbal formulation. The mobile phase Ethyl acetate: n-hexane (1:1) gave good resolution with R*f* value 0.45 for cinnamaldehyde. The results of study help in standardization of ayurvedic formulation.

Conclusion

Comprehensive standardization of a powdered herbal formulation requires the use of microscopical investigation and chemo finger printing. Microscopical investigation revealed the diagnostic characters which help to find the adulterants and substituent. Preliminary standardization can only be carried out using CCRAS guidelines. Mineral analysis is very helpful because minerals influences the action of drug and it also vary according to geographical variations and climate factors. Analytical method for estimation of phytochemical marker helps to find the exhausted drugs. So, it may be concluded that the supply of raw materials to the herbal industry must be under strict control government so that manufactures could get authentic raw materials containing the adequate amount of chemical constituent.

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- Conflict of interest: none

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