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Comparative standardization of a polyherbal Ayurvedic formulation *Talishadi Churna*

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India is a land mark for traditional system of medicine from the past few centuries. Most of the traditional systems of medicine are effective but only one major drawback is lack of standardization. So, there is a need to develop a standardization technique to mingle this system of medicine in the main stream of health sciences. Central Council for Research in Ayurveda and Siddha (CCRAS) has given preliminary guidelines for standardizing these conventional formulations. The present paper reports on standardization of *Talishadi churna*, an Ayurvedic formulation. Three marketed samples and in-house preparation were subjected to organoleptic study, physical characteristics, physiochemical screening and High Performance Thin Layer Chromatography (HPTLC) chromatogram. It was observed that all commercial samples and standard are similar in their organoleptic and qualitative chemical analysis but physical characteristic, fluorescence analysis and High Performance Thin Layer Chromatography (HPTLC) chromatogram of various 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. This study provides ready reference for the selection of an appropriate formulation in the clinical practice and hence effective rational therapy, the overall theme of health sciences.

Keywords: *Talishadi churna*, Ayurvedic formulation, Standardization, Chromatography

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Herbal formulations have been used by the majority of Indians since ancient times. In recent years, there has been an increased inclination towards the herbal formulations due to the trend towards the natural sources and a healthy life style. Moreover, the complexity, side effects and costly treatment associated with the allopathic drugs have caused both the healthcare practitioners and the majority of world populations to turn towards alternative therapies, more likely towards the herbal medicines[1]. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important[2]. Botanicals constitute of major part of these traditional medicines. With the emerging world wide interest, in adopting traditional practices, in the healthcare systems by exploiting there potential, the evaluation of the botanicals in these systems of medicine in India is utmost essential. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage but also to rationalize the use of natural products in the healthcare[3, 4]. Standardization is a system to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect[5]. In this aspect standardization of herbal formulations is essential in order to assess the quality of drugs.

Talisadi churna is a classical preparation from the text *Astanga Hridaya - Rajayakshma Chikitsa*, which consists of fine powders of *Talishpatra* (*Taxus baccata* Linn., Family:Taxaceae), *Trikatu* (*Piper nigrum* Linn., Family: Piperaceae) (*Piper longum* Linn., Family: Piperaceae) and (*Zingiber officinalis* Rosc., Family: Zingiberaceae) in the ratio 1:1:1, *Banshlochan* (*Bambusa arundinacea* Wild., Family: Poaceae), *Ela* (*Elettaria cardamomum* Maton., Family: Zingiberaceae), *Dalchini* (*Cinnamomum zeylanicum* Blum., Family: Lauraceae) and sugar. It is best remedy in acute, chronic and allergic bronchitis. It is very useful in acute exacerbation of asthma. In chronic asthma it reduces the frequency and severity of asthmatic attacks.

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A number of polyherbal formulations are widely marketed in the name of *Talisadi churna* claiming to be very effective. But, the documented reports authenticate that, plants vary in the content of secondary metabolite with the time of collection and geographical variation[6]. Emphasizing these perplexing reports, present study was planned to evaluate the organoleptic study, physical characteristics, qualitative chemical screening and High Performance Thin Layer Chromatography (HPTLC) chromatogram.

Methodology

The samples were collected from the physicians and retailers of Ranchi of 3 companies namely Dabur (A), Baidyanath (B), Zandu (C). For In-house preparation (D), the ingredients of *Talisadi churna* were procured from raw traders of Ranchi and Tamil Nadu, India and were aunthenticated by Dr S Jha, Department of Pharmaceutical Science, BIT, Mesra. A voucher specimen (PP2801-2807) of the same has been deposited in the Museum of the Department of Pharmacognosy, Guru Ghasidas University (Central University), Bilaspur for further reference. The *churna* was prepared as per the procedure and used as control.

Organoleptic characters such as particle size, colour and odour of all samples were recorded. Physical analysis for total ash, water soluble ash, acid insoluble ash, water soluble extractive value, ethanol soluble extractive value, ether soluble extractive value and loss on drying at 70^0C were carried out in triplicate in all four samples of *Talishadi churna* according to the prescribed methods as per CCRAS guidelines[7].

Preliminary phytochemical tests were carried out on methanolic extract for the presence/ absence of phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, saponns, sterols, terpenes and tannins[8].

Fluorescence analysis were carried out in 1N sodium hydroxide in methanol, 1N sodium hydroxide in water, 50% HCl, 50%HNO_3, 50% H_2SO_4, petroleum ether and chloroform at 254nm and 366nm[9].

HPTLC chromatogram profiles were determined in all the samples. For HPTLC, 5 gm of each sample were extracted with 30ml of methanol by a sonicator for 20 minutes, filtered and concentrated[10]. The chromatograph was performed by spotting extracted samples on precoated silica gel aluminium plate 60-F-254 (10 cm×10 cm with 250 µm thicknss) using Camag Linomat IV sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 5 mm, were spotted 15 mm from the bottom, 15 mm from left margin of the plate and 10 mm apart, at a constant application rate of 150 nl/s using nitrogen aspirator. Plates were developed using a mobile phase consisting toluene-ethyl acetate (9:3 v/v). Linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 15 min at room temperature. The length of chromatogram run was 7 cm, 10 ml of the mobile phase (5 ml in trough containing the plate and 5 ml in other trough) was used for development, which required 8 min. It resulted in better apparent resolution with more convenient capability of the detecting device to perform integration of peak area. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer. The slit dimension settings of length 5.00 mm and width 0.45 mm, and a scanning rate of 20 mm/s was employed. The monochromator band width was set at 20 nm. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/ reflectance mode at 260 nm and operated by win CATS Planar chromatography version 1.1.4.0. The source of radiation utilized was halogen tungsten lamp.

Results

Talisadi churna samples of different manufacturers and in house preparation were subjected to analysis. Organoleptic evaluation of all samples shows that all are brown in colour with pleasant odour and very fine. From the results outlined in the Tables 1-3 the following can be deduced. Physical analysis data of various samples A, B, C and D shows different value as they are claimed to be of same plant material in same quantity. Qualitative phytochemical analysis shows there is presence of all active ingredients in all samples. On fluorescent analysis different sample shows there is different in colour even if in same wave length and same developing solvent. Methanolic extract of samples subjected to HPTLC chromatogram with various mobile phase systems, in which toluene-ethyl acetate (9:3 v/v) shows maximum number of spots, shown in Figs. 1-4. The

Table 1—Physical analysis of various samples of *Talisadi churna*
[Values are % mean of determinations ± SEM (standard error of the mean)

Parameters		Sample A	Sample B	Sample C	Sample D
Ash value	Total ash	3.66±0.045	3.75±0.047	3.45±0.034	3.56±0.043
	Water soluble ash	1.75±0.022	1.67±0.014	1.73±0.015	1.80±0.019
	Acid insoluble ash	0.79±0.007	0.90±0.006	0.83±0.004	0.88±0.003
Extractive value	Water soluble extractive value	12.32±0.670	10.23±0.320	11.35±0.534	9.87±0.675
	Ethanol soluble extractive value	11.37±0.345	10.53±0.330	11.47±0.546	10.17±0.564
	Ether soluble extractive value	9.45±0.334	8.46±0.356	8.49±0.450	8.03±0.320
Loss on drying	Loss on drying at 70^0c	9.21±0.603	9.13±0.453	8.35±0.332	9.45±0.564

Table 2—Qualitative phytochemical analysis of various samples of *Talishdi churna*

Constituents	Sample-A	Sample-B	Sample-C	Sample-D
Alkaloids	†	†	†	†
Tannins	†	†	†	†
Carbohydrates	†	†	†	†
Reducing Sugars	†	†	†	†
Terpenes	†	†	†	†
Sterols	†	†	†	†

†Sign denotes presence of the active ingredient.

Table 3—Fluroscent analysis of various samples of *Talisadi churna* in different ultraviolet lights

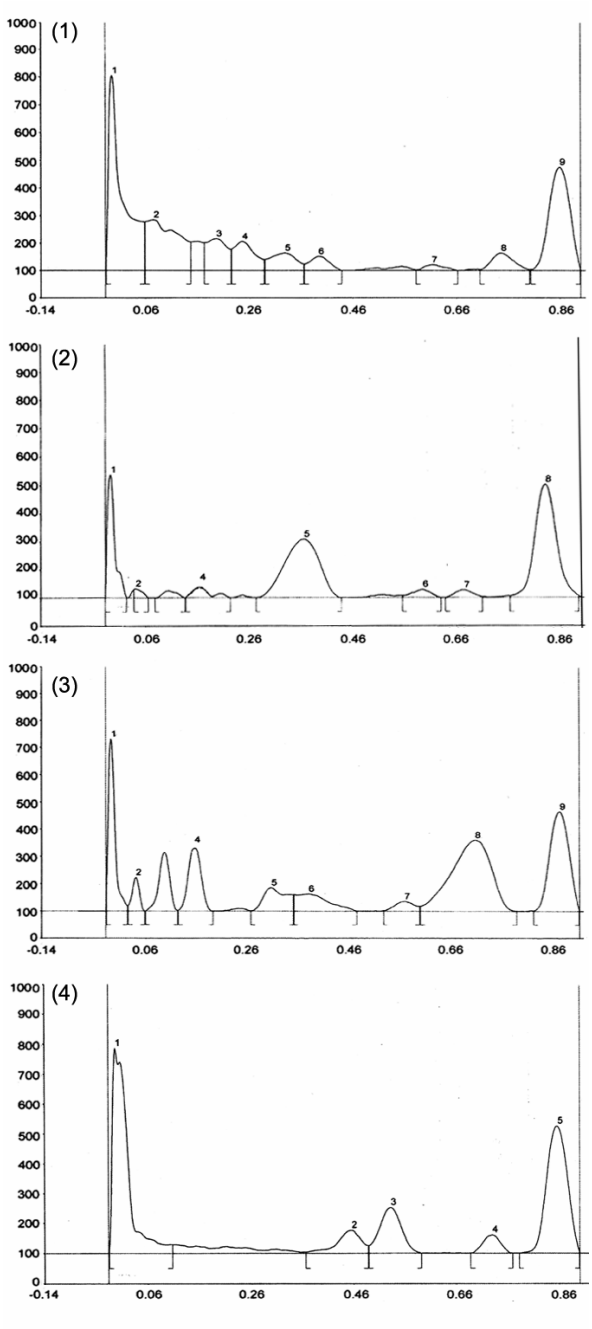
Treated chemical	Sample A		Sample B		Sample C		Sample D	
	254 nm	366 nm	254 nm	366 nm	254 nm	366 nm	254 nm	366 nm
1N Sodium hydroxide in methanol	BR	LB	BR	BY	LB	LY	LY	PY
1N sodium hydroxide in water	GR	LG	BR	BY	LB	BR	LY	PY
50% HCL	BR	BB	BR	LB	LB	BR	LY	GY
50%HNO_3	LB	BR	LY	PY	LY	PY	BR	LB
50% H_2SO_4	BR	BB	BR	LB	LY	PY	BR	LB
Petroleum ether	LB	BR	LB	BY	BR	LB	LY	PY
Chloroform	LY	BR	LB	BY	BR	LB	LY	PY

LG: Light green, GY: Greyish yellow, GR: Green, LB: Light brown, BY: Brownish yellow, BB: Brownish black, LY: Light yellow, PY: Pale yellow, BR: Brown

densitometric scanning was performed on developed methanolic extract with Camag TLC scanner III in the absorbance/reflectance mode at 260 nm. Sample A shows 9 spots, sample B shows 8 spots, Sample C shows 9 spots and Sample D shows 5 spots.

Discussion

Ayurvedic formulations claimed to be made according to CCRAS guidelines are effective but it is very difficult to maintain uniformity in formulations which is may be due to the natural heterogeneity, the quality of herbal starting material obtained from wild collections shows more and more fluctuations[2]. Which can be depicted from our experimental data. Organoleptic evaluation shows similarity between formulations. Physical analysis data depicts there is no uniformity in collection of crude drugs for manufacture of all formulations. Qualitative phytochemical analysis shows there is presence of alkaloids, tannins, carbohydrates, reducing sugars, terpenes and sterols in all samples which can be further studied for quantification of it. Fluorescent analysis data justifies there is difference in content of fluorescent compound in all samples, which can be further investigated quantitatively. HPTLC chromatogram in methanolic extract of samples shows maximum resolution in solvent system toluene-ethyl acetate (9:3 v/v). Depending on the number of spots we can depicts what is the number of active principles present. Samples A, B, C and D shows varies number of spots with some spots in same Rf value but intensity (area under curve) and some are in different Rf value which depicts there is no uniformity in content of active ingredient.



Figs. 1—4: 1 HPTLC Chromatogram of sample A. Number of peaks corresponds to number of active ingredients separated which is shown in chromatogram with number of peaks; 2 HPTLC Chromatogram of sample B. Number of peaks corresponds to number of active ingredients separated which is shown in chromatogram with number of peaks; 3 HPTLC Chromatogram of sample C. Number of peaks corresponds to number of active ingredients separated which is shown in chromatogram with number of peaks; 4 HPTLC Chromatogram of sample D. Number of peaks corresponds to number of active ingredients separated which is shown in chromatogram with number of peaks.

Conclusion

The result of the present study clearly indicate that there is no uniformity in preparation of formulations which is may be due to varied geographical locations where these plants grow, coupled with the problem of different vernacular names these plants are known by, a great deal of adulteration or substitution is encountered in commercial market[2]. It might be a useful contribution to the selection of an appropriate formulation in the clinical practice and hence effective rational therapy; the overall theme of health sciences. So, further, it can be studied for comparative pharmacological evaluation.

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