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Evaluation of Ayurvedic compound formulations 2- *Palas'abijadi Cũrna*

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Quality assurance is an integral part of all systems of medicine to ensure quality medicament. Thus, there is an urgent need to evaluate such parameters which can be adopted by the pharmaceutical industries. In the communication, attempts have been made to evaluate *Palas'abijadi Cũrna*, an Ayurvedic compound formulation. Four samples procured from different manufacturers were subjected to physicochemical analysis, HPTLC fingerprinting, and botanical characterization, and compared using authentic ingredients as reference. It was observed that the microscopic and chromatographic analyses compliment each other in their findings, and can be used effectively for the identification of raw materials in the compound formulation (s).

Keywords: *Palas'abijadi Cũrna*, Ayurvedic formulation, Quality control parameters, Drug standardisation, Pharmacognosy

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Standardized drugs of well defined consistent quality are needed for reliable beneficial therapeutic use. Total information and controls are necessary to guarantee consistency of composition. Due to lack of proper quality control methods, there are batch to batch variations in the same product as well as variations amongst the same product obtained from different sources. The main problem encountered while working with compound formulations is that most of them consist of several ingredients, and the presence of each ingredient has to be confirmed in the final product[1]. The ongoing research has led to the development of methods for the evaluation of *Nãrãca Cũrna*[2]. The study was undertaken to develop methods for evaluation of another Ayurvedic compound formulation, viz. *Palas'abijadi Cũrna* as prescribed in the Ayurvedic Formulary II claimed to be used as *kriminaashaka* i.e. anthelmintic and which consists of a moderately fine powder of 20 gm each of *Palashbij* (*Butea monosperma* (Lam.) Taub. seeds), *Indrayav* (*Holarrhena antidysentrica* (Roth.) DC. seeds), *Vidang* (*Embelia ribes* Burm. f. fruits), *Nimba* (*Azadirachta indica* (L.) A. Juss. seeds) and *Chiraita* (*Swertia chirata* Buch.-Ham. whole plant) [3].

Methodology

Samples of *Palas'abijadi Cũrna* of one batch each; prepared by 4 different Ayurvedic pharmacies were procured. Authentic samples of *Palashbij* (*Butea monosperma* seeds), *Indrayav* (*Holarrhena antidysentrica* seeds), *Vidang* (*Embelia ribes* fruits), *Nimba* (*Azadirachta indica* seeds) and *Chiraita* (*Swertia chirata* whole plant) were used as controls. Organoleptic characters and particle size of all the samples were recorded. Quantitative analysis for total ash, acid insoluble ash, water soluble ash and sulphated ash, extractive values in n-hexane, ethyl acetate, acetone, ethanol and water, successive Soxhlet extractives in n-hexane, alcohol and water, loss on drying at 105ºC and *p*H of filtrate of 10% w/v aqueous solution were carried out in triplicate according to the prescribed standard methods in Indian Pharmacopoeia in all 4 samples of *Palas'abijadi Cũrna*[4]. Total percentage of sugar, starch and tannins were also determined in all the samples[5, 6].

For microscopic analysis a small quantity representative of the *Cũrnas*, along with the genuine samples, i.e. *Palashbij*, *Indrayav*, *Vidang*, *Nimba* and *Chiraita* well mixed with water, stained with iodine and mounted in glycerine were used to examine the starch grains and its type. Another small quantity of samples cleared by heating with chloral hydrate and mounted in glycerine was used to identify diagnostic microscopical characters of the ingredients. Further small quantity of the *Cũrnas* cleared with dilute KOH 5% was mounted in glycerine. *Kalmegh* (*Andrographis paniculata* Wall. ex Nees whole plant), which is a

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very common substitute for *Chiraita*, was also subjected to microscopic examination. For HPTLC, 2 gm of each sample was extracted with 25 ml of methanol on boiling water bath for 25 min consecutively 3 times using fresh portion of 25 ml methanol, filtered and concentrated. Similarly, methanolic extracts were prepared for all 5 ingredients, i.e *Butea monosperma* seeds, *Holarrhena antidysentrica* seeds, *Embelia ribes* fruits, *Azadirachta indica* seeds and *Swertia chirata* whole plant for use as reference. TLC of the methanolic extracts of all the samples and the reference ingredients was carried out on silica gel $60F_{254}$ precoated plates (0.2 mm thickness; Merck). Camag Linomat IV applicator was used for band application and Desaga Video documentation Unit III was used for documentation of fingerprint profiles. The mobile phase used was toluene: ethyl acetate (90:10). The plate was developed over a distance of 9 cm and visualized under visible light after spraying with anisaldehyde - sulphuric acid followed by heating at 110^0C for 5-10 min. Spiking with individual ingredients was also done before microscopical and HPTLC studies.

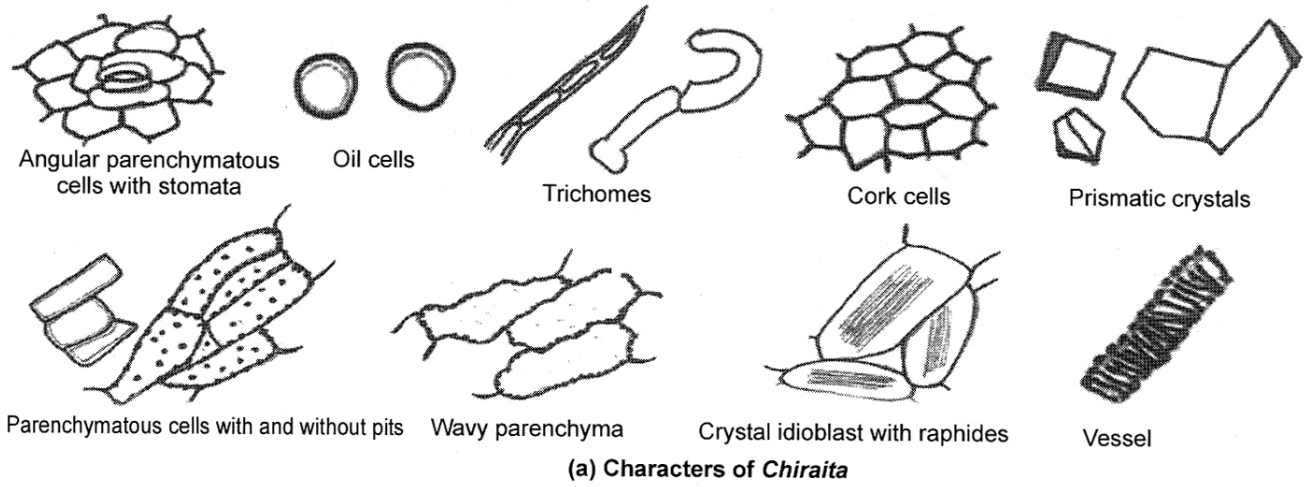
Results and discussion

Palas'abijadi Cũrna samples of 4 different manufacturers, PBC-1, PBC-2, PBC-3 and PBC-4, were subjected to analysis as above. All the samples were brown in colour, smooth with oily neem odour and bitter taste. Only 68-97% of all 4 samples passed through 60 mesh SS sieve. It was also observed that more than 50% of all 4 samples passed through 85 mesh sieve. Results of loss on drying at 105^0C, *p*H of 10% w/v aqueous solution, ash values, extractive values, successive Soxhlet extractives, sugar, starch and tannins were calculated (Table 1). Variations were observed in some of the physicochemical parameters studied. All 3 ash values, water soluble extractive as well as sugar percentage for PBC-2, hexane soluble Soxhlet extractive for PBC-3 and alcohol soluble extractive for PBC-4 were found to be lower when compared with the corresponding values for the other samples. These variations may be due to variations in the quality of raw materials used.

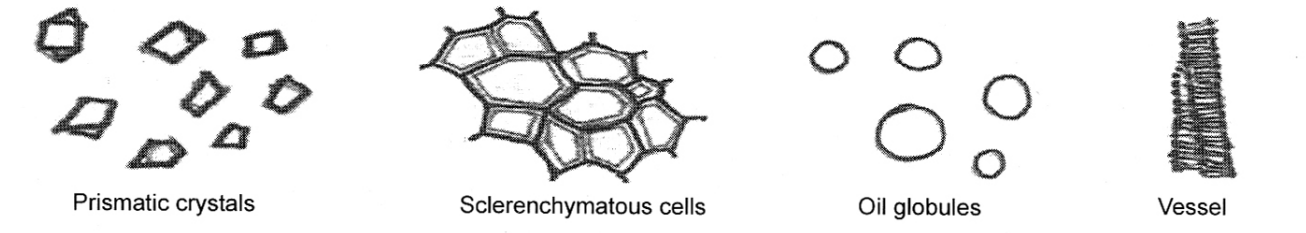
Microscopic examinations were also carried out to see the presence of the different ingredients in all 4 samples of *Palas'abijadi Cũrna* (Fig. 1). Presence of patches of yellow coloured polygonal sclerenchymatous cells of testa; orange coloured oil globules, prismatic crystals (15-20µ diameter) of calcium oxalate; fragments of narrow vessels with spiral secondary wall thickenings confirm the presence of *Indrayav*. Similarly, groups of thick walled non lignified palisade like cells (100-120µ long and 15-30µ wide); thick walled angular reddish brown cells and parenchymatous cells containing rod shaped, simple or compound (20-40µ diameter) ovoid starch grains, indicated the presence of *Palashbij*. Likewise, stone cells single or in groups, lumen broad (30-140µ diameter); prismatic crystals (70-140µ diameter); patches of parenchymatous cells having simple and compound starch grains of 15-45µ diameter; yellow coloured oil cells; scalariform vessel (25-45µ diameter) confirm the presence of neem. In case of *Vidang*, groups of polygonal, non lignified, thick walled parenchymatous cells and palisade like cells of testa (55-80µ long and 15- 30µ wide), thick walled polygonal cells filled with yellowish brown content of mesocarp (25-45µ diameter) were observed. Similarly, the presence of *Chiraita* can be detected by the presence of groups of angular parenchymatous cells with stomata; oil containing cells; two types of trichomes, unicellular (100 - 120 µ long) and multicellular, parenchymatous cells patches with or without pits (60-170µ long); cells with wavy margin (100-120µ long) and pits and prismatic crystals (140-150µ long). The adulteration/ substitution of *Chiraita* with *Andrographis paniculata* (*Kalmegh*) can be easily distinguished by the presence of trichomes and crystals. *Andrographis paniculata* showed biarmed, unicellular, multicellular and multiseriate trichomes and also has large prismatic calcium oxalate crystals (45-70µ diameter) (Fig. 2). On microscopic observations the absence of *Palashbij* (*Butea monosperma*) in PBC-1 and *Chiraita* (*Swertia chirata*) in PBC-2 were noticed. This was also supported by HPTLC as no representations of *B. monosperma* in PBC-1 and *S. chirata* in PBC-2 were observed on the plates while the samples PBC-3 and PBC-4 contained all the ingredients (Fig. 3).. Likewise, the lower values of different physicochemical parameters in sample PBC-2 may also be explained. None of the *Cũrna* samples contained *Kalmegh*. Thus the HPTLC findings (Table 2) and the microscopical examination both revealed that PBC-1 did not contain *Palashbij* and *Chiraita* was absent in PBC-2.

Conclusion

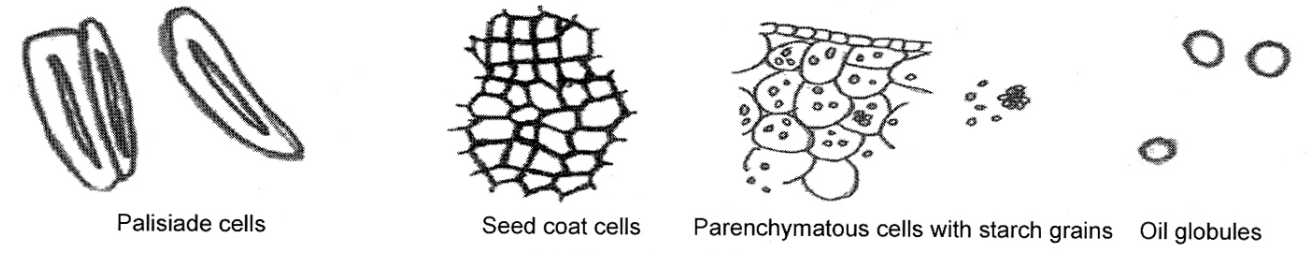
Thus from ongoing observations it can beconcluded that the characteristic microscopical



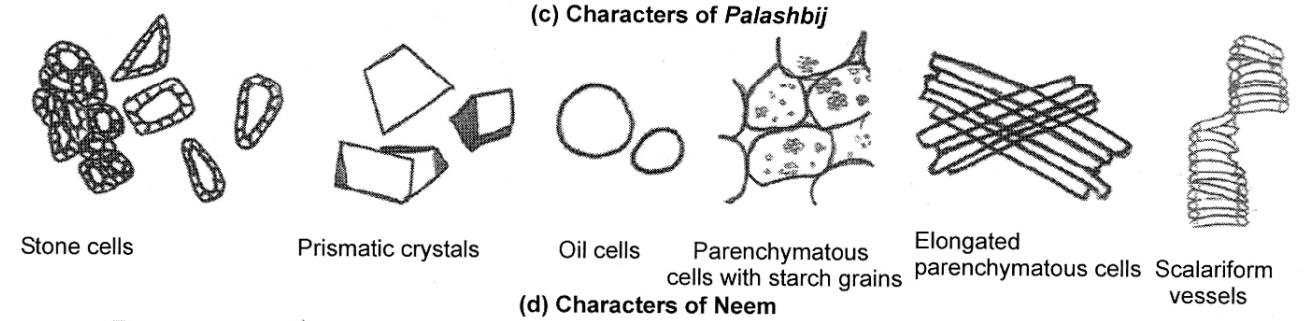
(a) Characters of *Chiraita*



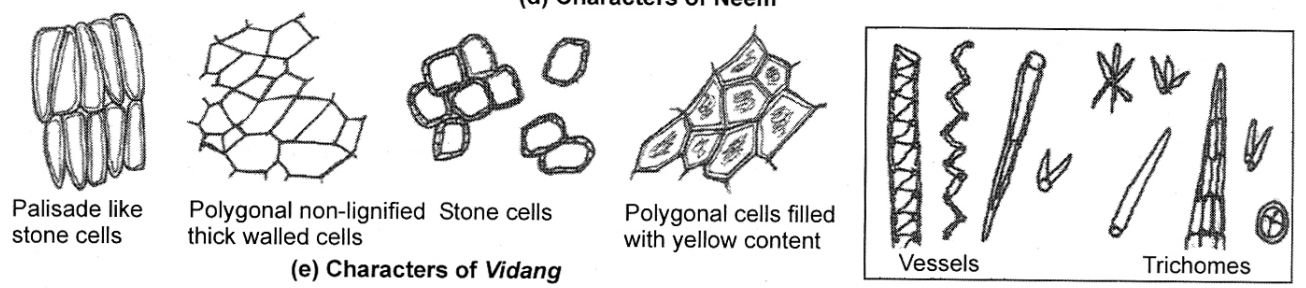
(b) Characters of Indrayav



(c) Characters of *Palashbij*



(d) Characters of Neem



(e) Characters of *Vidang*

Fig. 1 Microscopical characters of *Palas'abijadi Curna*

Fig. 2 Microscopical characters of *Kalmegh*

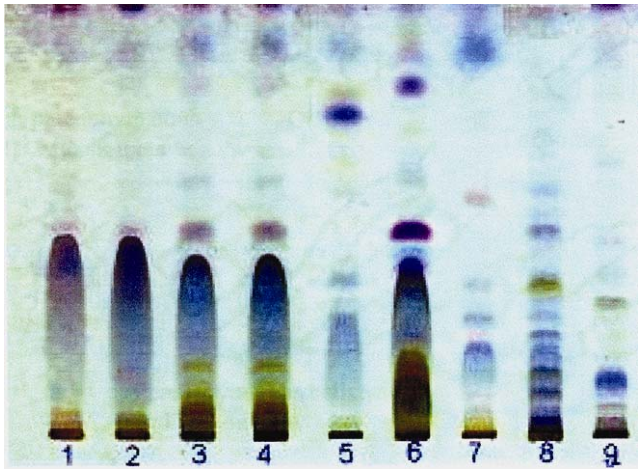


Fig. 3 HPTLC finger print profiles of *Palas'abijadi Cũrna*

features and the distinguishing bands in the HPTLC profiles may be utilized as marker parameters for monitoring the quality of the formulation. Hence the physiochemical parameters, quantitative analysis, HPTLC fingerprint profiles and the microscopic characteristics together may be used for quality evaluation and the standardization of compound formulations. Spiking of the formulations with the different genuine ingredients further confirms the presence of individual components in them.

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Table 1—Physicochemical analysis of samples of *Palas'abijadi Cũrna*

[Values are means of three determinations]

Parameters		PBC-1	PBC-2	PBC-3	PB-4
Ash values	Total ash	7.21	4.90	7.41	6.65
	Acid insol. ash	0.93	0.77	1.37	1.20
	Water sol. Ash	2.75	1.52	1.96	1.21
	Sulphated ash	9.69	6.93	9.93	9.62
Extractive values	Hexane	13.50	13.75	13.08	11.75
	Acetone	14.50	15.52	16.02	15.35
	Ethyl acetate	10.50	13.15	13.05	13.10
	Alcohol	27.33	23.40	22.50	14.73
	Water	24.50	15.31	24.91	22.70
Successive Soxhlet extractives	Hexane	13.39	13.75	8.17	15.13
	Alcohol	10.84	15.37	9.14	9.74
	Water	14.27	10.51	17.44	12.51
Loss on drying (105^0C) (w/w)		7.70	4.34	7.74	8.85
*p*H (Filtrate of 10% w/v aqueous solution)		5.38	5.56	5.22	5.69
Sugar		3.36	4.66	2.85	4.36
Starch		15.84	16.39	16.59	16.48
Tannin		0.74	0.76	0.88	0.86

Table 2—TLC fingerprint characteristics (Scanned under visible light)

Ingredients	Identifying R_fs (colour)	PBC-1	PBC-2	PBC-3	PBC-4
Palash	0.26 (light blue)	-	✔	✔	✔
Indrayav	0.41 (violet)	✔	✔	✔	✔
	0.49 (violet)	✔	✔	✔	✔
	0.82 (violet)	✔	✔	✔	✔
Vidang	0.87 (light blue)	✔	✔	✔	✔
Neem	0.15 (greenish yellow)	✔	✔	✔	✔
Chiraita	0.46 (violet)	✔	-	✔	✔
	0.64 (violet)	✔	-	✔	✔

✔ = Present; - = Absent

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