ORIGINAL ARTICLE

Pharmacognostic evaluation of Latakaranja (*Caesalpinia bonduc* [L.] Roxb.)

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Abstract

Background: Latakaranja [*Caesalpinia bonduc* (L.) Roxb. (Caesalpiniaceae)] is a prickly shrub used to cure number of diseases in Ayurveda. **Method:** The present study has been carried for pharmacognostical and phytochemical standardization of plant material as per WHO guidelines. **Result:** Seeds of *C. bonduc* are greenish-grey to bluish grey in color, globous or rounded, smooth, shiny, 1.2 o 2.5 cm in diameter. Powdered seed showed the presence of columnar palisade cells, bone-shaped thick-walled, parenchymatous cells with resinous contents and roundish to polygonal bearer cells. TLC of the seed alcoholic extract was also performed in the present study. Physicochemical standards quantified includes foreign organic matter (1.0% w/w), loss on drying (2.88% w/w), total ash (10.4% w/w), water soluble ash (10.2% w/w), acid insoluble ash (3.2% w/w), water soluble extractive (14.6% w/w), alcohol soluble extractive (26.68% w/w). Phytochemical screening of aqueous extracts showed the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, proteins, saponins, steroids, tannins and glycosides. Safety profile of plant material was established by quantify microbial load, pesticide residue content and heavy metals (Hg, Zn, Cd and Pb) analysis. Total aerobic organisams (cfu/g) was found to be 1.75 x 103 but no visible microbial growths were observed in seed sample. Pesticide residue content and heavy metals were found to be present within the permissible limits. **Conclusion:** In conclusion, the diagnostic characters obtained from the seed of *C. bonduc* will provide necessary information in identifying the crude drugs and comparing this plant from other closely related *Caesalpinia* species.

Key words: Ayurveda, *Caesalpinia bonduc,* Latakaranja, pharmacognostical, standardization

INTRODUCTION

At the moment, scientific research on medicinal plants is being carried out most intensely in research institutes, universities, and pharmaceutical laboratories as well as in the clinics of many developed countries. Since ancient time, plants have been used to cure various diseases in human as well as animals. Latakaranja mentioned in Ayurveda classics is an important plant and different parts of the plant, i.e. nuts, root, bark and leaves are used by health workers in colic fever, intermittent fever, malaria, menstrual complaints, pneumonia, skin diseases, swelling, fever, pulmonary tuberculosis, and edema.[1] Though the drug is not found in any ayurvedic ancient treatise *viz. Brihattrayi* (*Charaka Samhita* [2nd BC], *Sushruta Samhita* [2nd AD] and *Ashtanga Hridaya* [5th AD]), and *Astanga samgraha*. There was much confusion regarding this drug. Therefore, authentication of this drug in need of today.

Caesalpinia bonduc (L.) Roxb. (Caesalpiniaceae) commonly known as Latakaranja. It is a prickly shrub distributed throughout the hotter parts of India and Sri Lanka at an altitude of 1,000 m and tropical countries of the World. It is reported to have multiple therapeutic properties such as adaptogenic,[2] immunomodulatory,[3] antiulcer,[4] anti-diabetic,[5] and anticonvulsant[6] several isolated compounds were reported from different parts of the plant.[1]

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The present study focuses on the pharmacognostical and phytochemical parameters of the leaves, stem and seeds of *C. bonduc*.

MATERIALS AND METHODS

Plant Material

The seeds of *C. bonduc* were procured from an authentic shop from the Gola Deenanath Market of Varanasi. Fresh stem and leaves were collected from Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh (25°20' N, 83°00' E, 80.71 m. ASL). The plant was identified and authenticated by Prof. S. D. Dubey, Retired Professor, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University Varanasi.

Pharmacognostical Evaluation

The pharmacognostical evaluation of different part of the *C. bonduc* were studied according to the method of Khandelwal (2007),[7] while the method of Kokate[8] was used for the microscopic powder analysis. To study the fluorescence nature of powdered plant material, different chemical reagents *viz.* 1 N nitric acid, 1 N hydrochloric acid, acetic acid, acetone, nitric acid in ammonia solution, 1 N sodium hydroxide in methanol, 1 N sodium hydroxide in water, picric acid, 50% sulfuric acid was used to treat the powder of drug and observed under ordinary light, short ultraviolet (UV) (λ_{max} 254 nm) and long UV (λ_{max} 365 nm) as per the standard procedures.[9-11] The fluorescence was compared with the standard color index chart.

Physicochemical Evaluation

Different physiochemical parameters such as percentage of ash values, extractive values and pesticide contamination of crude powdered drug (seed) of *C. bonduc* were performed as per standard methods using.[7,12]

Preliminary Phytochemical Evaluation

Coarse powder of the dried seeds was made using a mechanical grinder and passed through a mesh sieve (20 #). 5 g of the powdered drug was subjected for the extraction separately with different solvents such as methanol and water (100 mL each) using cold maceration process for 24 h (shaking frequently for 6 h and the allowed to stand for 18 h). crude extracts of *C. bonduc* seed was obtained after concentrating the filtrate using rotary evaporator (Perfit India, Pvt. Ltd.) below 60°C to generate the and were finally stored in dessicator for further studies. The extract was used for the preliminary phytochemical screening. Tests for the various phytoconstituents such as alkaloids, steroids, carbohydrate, glycosides, terpenoids, saponins, flavonoids, phenolics, and protein were carried out by using standard pharmacopeial procedures.[8,13]

Thin Layer Chromatography (TLC)

Preliminary phytochemical were further confirm using TLC. Aluminum sheet with silica gel 60 F_{254} plate was used as stationary phase and solvent system (toluene:ethyl acetate:methanol:formic acid [5:4.5:0.5:0.5]) used as a mobile phase for the development of chromatogram.[14]

Microbial Contamination

For evaluation of *Escherichia coli*, *Salmonella* spp. and total aerobic count 10 g of powdered seed was homogenized in buffered lactone broth (100 mL). For evaluation of *Salmonella* spp., the prepared solutions were subjected for inoculation into MacConkey broth and incubated at 35-37°C for 5-24 h while for the evaluation of *E. coli* the inoculated were prepared on MacConkey broth and incubated at 43-45°C for 18-24 h. However, sodium chloride with peptone solutions were used as buffer for evaluation of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. After that the solution was inoculated with 100 mL of soybean-casein digest medium and incubated at 35-37°C for 24-48 h. The number of colony forming units was determined using a colony counter.[12]

Total Viable Aerobic Count

Respective samples (10 mL) prepared for microbial contamination assessment were filter through the respective membrane filter apparatuses. After filtration, the membranes were washed accordingly. The membrane filters were inoculated with casein soybean digest agar and incubated at 30-35°C for 5 days. The numbers of colonies formed were counted, and the number of microorganism per gram of the material was estimated.[12]

Heavy Metal Analysis and Pesticide Residue Evaluation

2 g of the seed powder was digested with 15 ml of 10% HNO_3 v/v in a Nesseler's tube on a water bath at 100°C for 3 h. The digested solution was analyzed through hydro vapor generator of atomic absorption spectroscopy (AAS) after reduction with $NaBH_4$ for analysis of Hg for the analysis of Cd and Pb, the digested sample solutions were treated twice under reflux with concentrated HNO_3 before analysis by AAS. All the results were recorded in triplicate. The limits of quantification will be Pb (10 ppm), arsenic (3 ppm), Hg (3 ppm) and Cd (3 ppm).[15] Pesticide residue was evaluated as per WHO guideline.[12,16]

RESULT AND DISCUSSION

Pharmacognostical Evaluation

Leaf

Macroscopic: Leaves are very large 30-40 cm long widely spreading abruptly bipinnate stipules large, composed of 2 or 3 rounded segments, rachis stout cylindrical, pubescent, main rachis 5-7 inches hooked usually in pairs, 6-8 pairs of pinnae, 1.5-2.5 inches. Spreading nearly at right angles, coming off from the upper surface of the rachis, all with numerous hooked-prickles usually in pairs on the under surface. Leaflets numerous 10-14 (5-7 opposite pairs) to each pinna, long, with two hooked prickles at the base of each pair very shortly stalked 2.5-4.5 cm long, elliptical to elliptically oblong rounded at base bluntly pointed and with a sharp micro at the apex, entire, pubescent at the margin, and on the midrib or all over beneath [Figure 1].

Microscpoic: Transverse section through midrib demonstrate an upper and lower single layered epidermis which was covered with cuticle, few modifications of epidermal cells such as uni-seriate, 2-3 celled trichomes, 2-3 layered collenchymatous and 4-7 layered round, thin-walled parenchymatous cells was also observed. Vascular bundles were arranged in a ring, having secretory cavities in phloem, each bundle covered externally with sclerenchymatous sheath, vascular bundle vascular bundle, collateral, conjoint [Figure 2].

Stem

Microscpoic: Transverse section of mature stem shows 1.5-2 cm thick epidermis with 2-3 celled uniseriate trichome. Cortex consists of 3-5 layers of parenchymatous cells, some cells thickened, pericyclic fiber all around the stem; phloem consists of sieve elements, parenchyma, a few fibers; xylem consists of vessels, tracheids, parenchyma, fibers, and traversed by xylem rays, central region occupied by a large pith [Figure 2].

Seed

Macroscopic: Seeds globous or rounded, smooth, shiny, 1.2-2.5 cm in diameter: Slightly flattened on one side due to close pressing of adjacent seed: Hilum and micropyle close together: Hilum surrounded by a dark area around 4 mm in diameter, usually with a whitish remnat to funiculus: Micropyle near the periphery of dark area: Seeds coat greenish - gray to bluish gray, lineat, shiny: 100 seeds weigh from 150 to 250 g [Figure 1].

Microscopic: Section of testa demonstrates an outermost compact and single row arrangement of very narrow, transluscent, radially elongated arranged cells which forms a palisade layer (Malpighian layer). In surface view, these cells appear hexagonal containing pectin rich thick walls. 2 or 3 layers sub-epidermis of consists of thick walled bearer cells, followed by multilayered osteosclereids. The size of osteosclereids cells gradually increases, elongate laterally; intercellular spaces toward the inner side also increases. a brown substance was observed in the outer few osteosclereids layers; laterally elongated vascular elements tissues were observed in the lower part of this zone. The cells inner to vascular tissues steadily compacted and rounded toward the inner margin; cotyledons illustrate an outer single layer of epidermis containing small, isodiametric cells and inner parenchymatous ground tissue cells rich in fixed oil, and having empty cavities uniformly distributed in them [Figure 3].

Powder: Color light yellow through mustard to brown, coarse and free-flowing; bitter in taste and possessing tamarind-like odor. Columnar palisade cells, bone-shaped thick-walled, empty parenchymatous cells, bone-shaped thick-walled parenchymatous cells with resinous contents, thick-walled compressed parenchymatous cells, thick-walled small, roundish to polygonal bearer cells, in surface view with dark brown cell contents in the center of the cells, thick-walled parenchymatous cells slightly larger than the bearer cells, with brown cell contents, thin-walled, roundish to polygonal cells with round starch grains taking bluish black stain with Iodine solution and identical rounded colorless oil globule

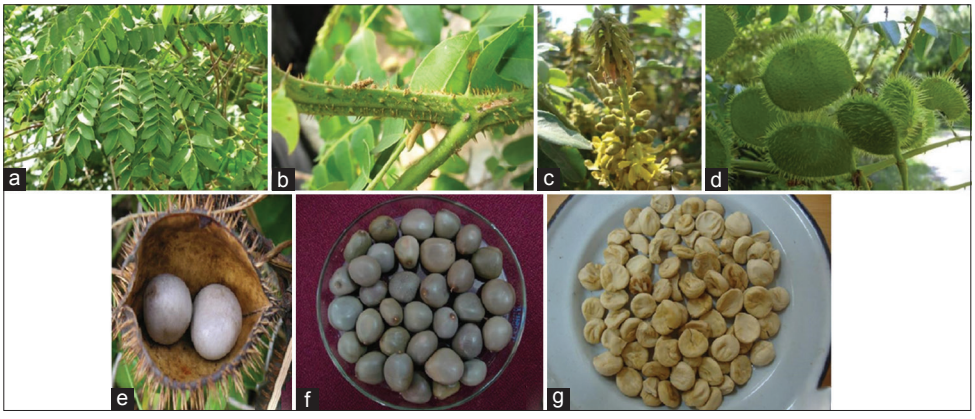

Figure 1: Latakaranja (*Caesalpinia bonduc* (L.) Roxb.). (a) Leaf, (b) stem, (c) flower, (d) fruits, (E) fruit with seed, (F) seeds, (g) cotyledons

and yellowish brown resinous masses of irregular shape and size [Figure 3].

Physicochemical Evaluation

In the present study, we have attempted to evaluate the various physicochemical parameters on the seed of *C. bonduc* [Table 1]. 1% foreign matter was found in the seed powder shows that the sample was free from contamination by unnecessary matters. Loss on drying was found to be 2.8%, which is indicative of the amount of moisture and volatile matter present in the plant material. The presence of high quantity of moisture in any drugs may facilitate the hydrolysis of enzymes or microbial growth enhancement which directed to deterioration of the material with time passed.[12] Ash value is an indicator of the earthy matter or inorganic composition (physiological ash) or other impurities present in the drug. It also shows the presence of some extraneous matter, like sand and soil adhere to the surface of the plant material (nonphysiological ash). Therefore, the determination of ash value can serve as an important diagnostic parameter for the evaluation of crude drugs[17] 10.4% total ash of seeds predicted that seed contains an enormous amount of calcium oxalate crystals. More or less similar extractive value

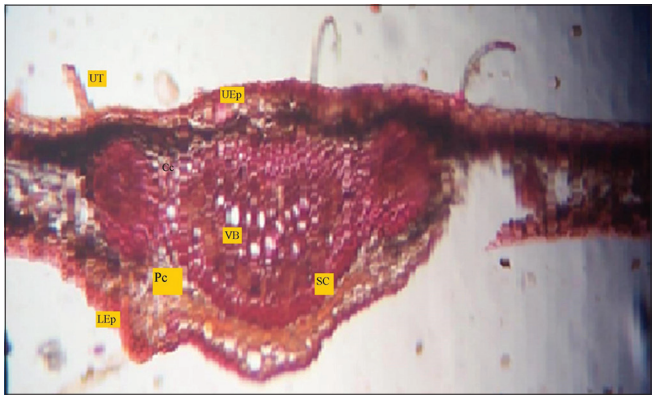

Figure 2: Microscopic character of Latakaranja leaf. UEp: Upper epidermis, LEp: Lower epidermis, UT: Uni-seriate trichomes, VB: Vascular bundles, SC: Sclerenchymatous sheath, Cc: Collenchymatous, Pc: Parenchymatous cells

Table 1: Physicochemical evaluation

Parameter	Results (%)
Foreign matter (% w/w)	Not more than 1.0
LOD (% w/w)	Not more than 2.8
Ash values	
Total ash (% w/w)	Not more than 10.4
Water soluble ash (% w/w)	Not more than 10.2
Acid insoluble ash (% w/w)	Not more than 3.2
Sulphated ash	Not more than 11.15
Extractive values	
Water	Not <26.68 (w/w)
Methanol	Not <25.22 (w/w)
Pesticide residue	
Chlorinated pesticide residue	
TS1 (first elute)	Not more than 0.0010 mg/kg
TS2 (second elute)	Not more than 0.0013 mg/kg
Phosphated pesticide residue	
TS1 (first elute)	Not more than 0.016 mg/kg
TS2 (second elute)	Not more than 0.018 mg/kg
TS3 (third elute)	Not more than 0.016 mg/kg
Heavy metals	
Pb	Not more than 0.009 ppm
Cd	Not more than 0.0001 ppm
Zn	Not more than 0.103 ppm
Hg	Not more than 0.004 ppm

LOD: Loss on drying, Pb: Lead, Cd: Cadmium, Zn: Zinc, Hg: Mercury

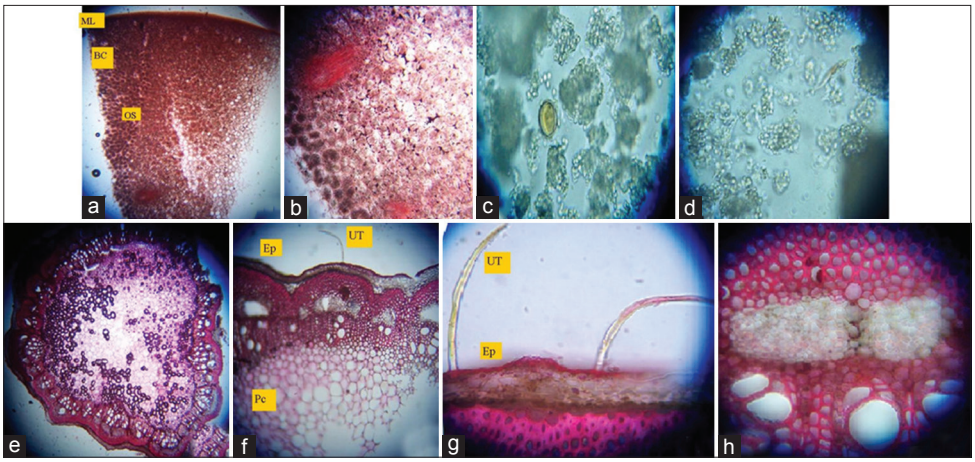

Figure 3: Microscopic character of Latakaranja (*Caesalpinia bonduc* [L.] Roxb.). (a) Microscopical characters, (b) TS through vascular strands of testa, (c, d) microscopy of powder of cotyledon, (e) TS stem (diagrammatic), (f) TS stem (A portion), (g) outer region with unicellular trichome, (h) phloem and xylem. ML: Malpighian layer, BC: Bearer cells, OS: Osteosclereids, UT: Uniseriate trichome, Ep: Epidermis, Pc: Parenchymatous cells

in water (26.68%) and in methanol (25.22%) shows that seed hold polar as well non-polar compounds. Extractive values generally correspond to the constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs [Table 1].

Fluorescence Analysis

Fluorescence analysis of powdered drug was studied in ordinary light and long U.V. (λmax 365 nm). The powder showed different color fluorescence when made to react with various chemical reagents which suggested that there might be the presence of certain phytoconstituents possessing chromophore group in the seed [Table 2]. Most of the authors have reported that the plant material have the capability to produce fluorescence pattern when made to react with various reagents either of acidic or basic media.[18,19]

Preliminary Phytochemical Evaluation

The preliminary phytochemical screening was performed to identify the nature of the phytoconstituents present in various extracts. This can serve as essential diagnostic parameters for the identification of crude drugs.[16] Aqueous extracts showed the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, proteins, saponins, steroids, tannins and glycosides [Table 3]. The preliminary phytochemical screening shows that *C. bonduc* contains different chemical constituents which indicate the presence of both polar and non-polar compound in the drug. TLC analysis of alcoholic extract of *C. bonduc* seed performed with solvent system (toluene:ethyl acetate:methanol:formic acid [5:4.5:0.5:0.5]) showed 10 spots at Rf values 0.15, 0.33, 0.38, 0.43, 0.48, 0.55, 0.63, 0.67, 0.73 and 0.80 after derivatization with Iodine while two prominent spots at Rf values 0.35 (blue) and 0.50 (green) were seen in UV 365 [Figure 4].

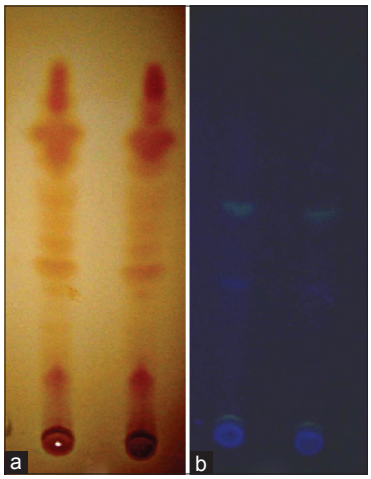

Figure 4: Thin layer chromatographic analysis of alcoholic extract of Latakaranja seed. (a) Visible light, (b) under ultraviolet light

Table 2: Fluorescence analysis of seed powder

Treatment	Under ordinary light	Long UV (λ_{max} 365 nm)
Drug as such	Cream	White
Drug+nitrocellulose	Yellowish brown	Whitish yellow
Drug+picric acid	Yellow	Brown
Drug+HCl	Gray	Brown
Drug+H_2SO_4	Brown	Reddish brown
Drug+HNO_3 (50%)	Dark brown	Brown
Drug+1 N NaOH in MeOH	Cream	Whitish yellow
Drug+1 N NaOH in water	Cream	Whitish yellow
Drug+NH_4OH	Whitish cream	Whitish yellow
Drug+$FeCl_3$	Green	Brown
Drug+glacial acetic acid	Cream	Whitish yellow
Drug+Sudan-III	Cream	Whitish yellow

NF: No fluorescence, UV: Ultraviolet

Table 3: Phytochemical screening of aqueous extracts

Plant constituents test/reagent	Aqueous extract
Test for alkaloids	
Dragendorff's test	+
Hager's test	+
Result: Wagner's test	+
Mayer's test	+
Test for carbohydrates	
Anthrone test	+
Benedict's test	+
Fehling's test	+
Molisch's test	+
Test for flavonoids	
Shinoda's test	+
Test for triterpenoids	
Liebermann-Burchard's test	+
Test for proteins	
Biuret's test	+
Millon's test	+
Test for resins	+
Test for saponins	+
Test for steroids	+
Test for tannins	+
Test for starch	+
Test for glycosides	+

(+): Present; (−): Absent

Safety Profile

In Ayurveda and other traditional medicine systems the plants were used in the crude form and there is always a chance of microbial, heavy metal and pesticide contamination these crude drugs.[20] Such types of products may produced adverse effects or diminish the effect.[21] Therefore, it essential to determine safety profile before manufacturing the medicine with the crude drugs to maintain appropriate quality, safety and efficacy of the finished products. Total aerobic organisms (cfu/g) were found to be 1.75×10^3. No visible microbial growths were observed in seed sample. While heavy metal content and Pesticide residue were found within the permissible limit. Different heavy metals have been reported for their toxic effects in all part of the world.[22-25] Long-term exposure to heavy metals can cause renal damage, liver toxicity and may be teratogenic effects on the fetus.[26,27]

CONCLUSION

The pharmacognostical study of *C. bonduc* was done for the purpose of correct identity and standardization. Standardization of crude drugs and formulations are the most essential requirement of traditional medicine systems in the current global scenario. Different anatomical markers were established in this study by microscopic and powder microscopical evaluation. Hence, from the overall investigation it can be concluded that any pharmacognostical character that deviates from the data reported in the present study can be treated as adulteration/substitution of *C. bonduc.*

REFERENCES

1. Moon K, Khadabadi SS, Deokate UA, Deore SL. *Caesalpinia bonducella* F. - An overview. Rep Opin 2010;2:13-23.
2. Kannur DM, Hukkeri VI, Akki KS. Adaptogenic activity of *Caesalpinia bonduc* seed extracts in rats. J Ethnopharmacol 2006;108:327-31.
3. Shukla S, Mehta A, John J, Mehta P, Vyas SP, Shukla S. Immunomodulatory activities of the ethanolic extract of *Caesalpinia bonducella* seeds. J Ethnopharmacol 2009;125:252-6.
4. Javed AA, Shafique A, Jameel M. Effect of *Caesalpinia bonducella* on ulcer and gastric secretions in pylorus ligated rat model. JDDT 2012;2:102-4.
5. Kannur DM, Hukkeri VI, Akki KS. Antidiabetic activity of *Caesalpinia bonducella* seed extracts in rats. Fitoterapia 2006;77:546-9.
6. Ali A, Rao NV, Shalam MD, Gouda TS, Kumar SM. Anticonvulsive effect of seed extract of *Caesalpinia bonducella* (Roxb.) Int J Pharmacy Tech 2009;8:51-5.
7. Khandelwal KR. Practical pharmacognosy: Techniques and Experiments. 17th ed. Pune, India: Nirali Prakashan; 2007.
8. Kokate CK. Practical Pharmacognosy. 1st ed. New Delhi, India: Vallabh Prakashan; 1986. p. 15-30.
9. Lala PB. Lab Manuals of Pharmacognosy. 5th ed. Calcutta: CSI Publishers & Distributors; 1993.
10. Chase CR Jr, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc Am Pharm Assoc 1949;38:324-31.
11. Ahmad A, Maurya SK, Seth A, Singh AK. Pharmacognostical evaluation of the fruit of Plaksha-*Ficus lacor* Buch. Ham Glob J Res Med Plants Indigen Med 2014;3:112-26.
12. WHO. Quality Control Methods for Medicinal Plant Materials. Geneva, New Delhi: World Health Organization, AITBS Publishers and Distributors; 2002.
13. Harborne JB. Methods of extraction and isolation. In: Phytochemical Methods. London: Chapman and Hall; 1998. p. 60-6.
14. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. New York: Springer–Verlag, Berlin Heidelberg; 1984.
15. Chow PY, Chua TH, Tang KF. Dilute acid digestion procedure for the determination of lead, copper and mercury in traditional Chinese medicines by atomic absorption spectrometry. Analyst 1995;120:1221-3.
16. Prasad SK, Laloo D, Kumar M, Hemalatha S. Quality control standardization and antioxidant activity of roots from *Eriosema chinense*. Phcog J 2013;5:149-55.
17. Laloo D, Kumar M, Prasad SK, Hemalatha S. Quality control standardization of the roots of *Potentilla fulgens* Wall: A potentmedicinal plant of the Western Himalayas and North-Eastern India. Phcog J 2013;5:97-103.
18. Laloo D, Sahu AN, Hemalatha S, Dubey SD. Pharmacognostical and phytochemical evaluation of *Cinnamomum wightii* Meissn flowers. Indian J Nat Prod Resour 2012;3:33-9.
19. Sahu AN, Hemalatha S, Sairam K, Laloo D, Patra A. Quality control studies of *Ochrocarpus longifolius* Benth flowers buds. Phcog J 2010;2:118-23.
20. Kumar S, Maurya SK, Seth A, Singh AK. Quality control standardization of the bark of *Moringa oleifera* Lam. Int J Pharm Pharm Sci 2015;7:56-60.
21. Nakajima K, Nonaka K, Yamamoto K, Yamaguchi N, Tani K, Nasu M. Rapid monitoring of microbial contamination on herbal medicines by fluorescent staining method. Lett Appl Microbiol 2005;40:128-32.
22. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 2014;7:60-72.
23. Singh R, Gautam N, Mishra A, Gupta R. Heavy metals and living systems: An overview. Indian J Pharmacol 2011;43:246-53.
24. Nwoko CO, Mgbeahuruike L. Heavy metal contamination of ready-to-use herbal remedies in South Eastern Nigeria. Pak J Nutr 2011;10:959-64.
25. Genuis SJ, Schwalfenberg G, Siy AK, Rodushkin I. Toxic element contamination of natural health products and

pharmaceutical preparations. PLoS One 2012;7:e49676.
26. Samuel JB, Stanley JA, Princess RA, Shanthi P, Sebastian MS. Gestational cadmium exposure-induced ovotoxicity delays puberty through oxidative stress and impaired steroid hormone levels. J Med Toxicol 2011;7:195-204.
27. Benitez MA, Mendez-Armenta M, Montes S, Rembao D, Sanin LH, Rios C. Mother-fetus transference of lead and cadmium in rats: Involvement of metallothionein. Histol Histopathol 2009;24:1523-30.

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