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Original Article

QUALITY CONTROL STANDARDIZATION OF THE BARK OF *MORINGA* OLEIFERA LAM.

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ABSTRACT

Objective: *Moringa* oleifera Lam. (Morangaceae) is a large glabrous tree, found throughout India. It is well known for curing a variety of ailments such as wound Healing, dysentery, fever, diarrhea and urinary problems. The present study highlights the pharmacognostical and phytochemical parameters of the bark of *M.* oleifera.

Methods: The quality control standardization was performed following the standard parameters prescribed in World Health Organization guidelines and Indian Herbal Pharmacopoeia.

Results: Standardization parameters show that it contains calcium oxalate crystal, starch grains and stone cells. High ash value may be due to presence of calcium oxalate crystals. Extractive value is high for water as compared to other solvent which accounts for the presence of comparatively more polar compounds. It contains different chemical constituents as carbohydrate, amino acid, terpenoid and saponin. Total saponin content was found to be 3.022 mg/g equivalent to diosgenin.

Conclusion: The present observations will aid in the botanical identification and standardization of the drug in crude form and will help to distinguish the drug from its other species.

Keywords: *M.* oleifera, Pharmacognosy, Calcium oxalate crystals, Saponin.

INTRODUCTION

Moringa oleifera is commonly known as horse radish tree, ben oil tree or drumstick tree and ranges in height from 5 to 10 m. It is found mostly in India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean Islands [1]. It is known for several medicinal uses like diuretic [2], antiurolithiatic [3], hepatoprotective [4], antidyslipidemic, antifertility [5] and antidiabetic [6] with high nutritional value. All parts of the plant (bark, fruit, leaves, nuts, seeds, tubers, roots, flowers) have rich traditional medicinal values against a variety of ailments viz. fever, abscess, wound, infections and UTIs.

The drumstick tree was used as food and medicine since centuries. Varieties of phytoconstituents such as alkaloids (moringine and moringinine), phenolics [7], several procyanidin [8] and 4–(–l–rhamnopyranosyloxy)–benzylglucosinolate [9] were reported in the plant. The present study focuses on the pharmacognostical and phytochemical parameters of the new annual bark of *M.* oleifera. The same plant material was found to be effective in the management of urinary tract infection [10]. Previously pharmacognostical study of the mature bark was performed [11]; therefore, we wish to reveal that in our study the bark before the secondary growth has been taken which is entirely different from the previous study.

MATERIALS AND METHODS

Plant material

The bark of *M.* oleifera was collected during the month of October 2012 from Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur (Uttar Pradesh) (25°10′, 82°37′ longitude and altitude, 147 m ASL). The plant was identified and authenticated by Prof. Anil Kumar Singh, Professor, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University Varanasi. The specimen of the plant was deposited for the further reference at the Department of Dravyaguna, Faculty of Ayurveda, Banaras Hindu University, Varanasi, India.

Pharmacognostical evaluation

The macroscopy and microscopy of the *M.* oleifera bark was studied according to the method of Khandelwal (2007) [12]. The microscopic powder analysis was done according to the method of Kokate (1986) [13]. Microphotographs were taken using Magnus microscope image projection system (MIPS). Many herbs show fluorescence behavior when exposed to UV light and the fluorescence pattern was utilized in the identification of crude drugs. To study the fluorescence nature of powder, the powder drug was treated with different chemical reagents *viz.* 1N sodium hydroxide, 1N nitric acid, 1N hydrochloric acid, acetone, 1N sodium hydroxide in methanol, picric acid, acetic acid, 50% sulphuric acid, nitric acid in ammonia solution and observed under short UV (λmax 254 nm) and long UV (λ_{max} 366 nm) as per the standard procedures [14, 15, 16]. The identification and comparison of the colors were done using the standard colour index chart.

Physico-chemical evaluation

Crude powdered drug (bark) of *M.* oleifera was used for the determination of physico-chemical analysis i. e. percentage of ash values, extractive values, foaming index, swelling index, volatile oil content and pesticide contamination were performed according to the official methods [17, 18].

Preliminary phytochemical evaluation

The dried plant material was made into coarse powder using mechanical grinder, passed through mesh sieve (20 #). The homogenous powdered drug (5 g) was extracted separately with different solvents such as methanol, hexane, chloroform, ethyl acetate 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). The extracts were filtered through Whatmann No. 1 filter paper and concentrated using rotary evaporator (Perfit India, Pvt. Ltd.) below 60°C to generate the crude extracts of *M.* oleifera bark and was finally stored in dessicator for further studies. Preliminary phytochemical screening for the presence of various phytoconstituents such as alkaloids, carbohydrate, steroids, glycosides, saponins, terpenoids, phenolics, flavonoids and protein were carried out by using standard procedures [13, 19].

Thin layer chromatography

Preliminary phytochemical were further confirmed using thin layer chromatography (TLC). Silica gel 60 F_{254} was used as stationary phase [20] and mixture of different solvents as mobile phases.

Quantitative estimation of total saponin

The total saponin content was estimated as per the methods proposed by Hiai, et al., (1976) [21]. For the preparation of standard curve, from the stock solution of standard diosgenin (0.5 mg/ml) various dilutions were prepared at different concentrations (25 µg, 50 µg, 75 µg, 100 µg and 125 µg). Pipette standard saponin with concentration of 0.05 ml, 0.10 ml, 0.15 ml, 0.20 mL and 0.25 mL and then respectively add methanol at concentration 0.20 ml, 0.15 ml, 0.10 ml, 0.05 mL and 0 mL at room temperature. The tubes were then transferred in ice-water bath and 0.25 mL of stock solution of vanillin reagent (0.08mg/ml) were then added to all the test tubes. The rack containing test tubes were placed on a magnetic stirrer and sulphuric acid was added up to 2.5 mL while it is being stirred. The mixture was then warmed in a water bath at 60°C for 10 min, and then cooled in ice-cold water bath. The absorbance was measured at 544 nm. The absorbance was stable for 2 hr when the reaction mixture was kept in an ice-water bath. Absorbance was measured immediately after taking out the tubes from the ice-bath. Standard and unknown samples were analysed simultaneously (from mixing of sulphuric acid to heating at 60°C, cooling and recording of reading). A blank solution was prepared excluding the standards.

Microbial contamination

Plant material (10 g) was homogenized in buffered lactone broth (100 ml) (for evaluation of *E. coli*, *Salmonella* spp. & total aerobic count) and later buffered in sodium chloride and peptone solution (for evaluation of *Pseudomonas aeruginosa* & *Staphylococcus aureus*). The solutions were inoculated into Mac. Conkey broth and incubated at 43-45°C for 18-24 hrs for evaluation of *E. coli*. For the evaluation of *Salmonella* spp the inoculated were prepared on Mac. conkey broth and incubated at 35-37°C for 5-24 hr. However, for *Pseudomonas aeruginosa* and *Staphylococcus aureus* the inoculated material was composed of 100 mL soyabean-casein digest medium and incubated at 35-37^{0}c for 24-48 hrs. The plates were placed on a colony counter and the number of colony forming units was determined [18].

Total viable aerobic count

10 mL of the respective samples as prepared above in the assessment of the microbial contamination were taken and transferred to the respective membrane filter apparatuses for filtration. The samples were then filtered and the membranes were washed accordingly. The membrane filters were then transferred to the plate with casein Soybean digest agar. The plates were incubated at 30- 35^{0}C for five days. The numbers of colonies formed were counted and the number of microorganism per gram of the material was evaluated. [18]

Heavy metal analysis and pesticide residue evaluation

For digestion, 2 g of the sample was taken in a Nesseler's tube and was treated with 15 mL of 10% HNO_3 v/v. It was then kept in a water bath at 100°C for 3 h. For the analysis of mercury, the digested solution was analyzed through hydro vapor generator of AAS (Atomic absorption spectroscopy) after reduction with $NaBH_4$. The digested sample solutions were treated twice under reflux with concentrated HNO_3 for the analysis of cadmium and lead, which was then analyzed by AAS. Each sample was tested thrice. The limits of quantification will be Lead (10 ppm), Arsenic (3 ppm), Mercury (3 ppm) and Cadmium (3 ppm) [22]. Pesticide residue was evaluated as per WHO guideline [18, 23].

RESULT AND DISCUTION

Pharmacognostical evaluation

a) Macroscopic

Mature bark was found to be rough, deeply cracked, grey or dark green; young bark, greenish to greenish-brown, 1 to 3 cm thick or more, depending upon the age of plant; taste, bitter and pungent.

b) Microscopic

Cork region was very wide, composed of 15 to 20 layers, thin-walled, radially arranged, rectangular cells with coloured contents; cork cambium consists of a single row of thin-walled, rectangular or tangentially elongated cells; secondary cortex very wide, composed of nearly cubical to rectangular, thin-walled parenchymatous cells containing a few rosette and cubical, rhomboidal or hexagonal crystals of calcium oxalate; several groups of thick walled, lignified, elongated to polygonal stone cells with striations and wide as well as narrow lumen present; a few small, simple, round to oval, starch grains measuring 5 to 14 µ in diameter, with concentric striations and hilum, and a few oil globules scattered in cortical region; secondary phloem consists of thin-walled, oval to polygonal parenchyma, fibres, and phloem rays; phloem parenchyma cells adjoining the sclerenchyma cells containing small rhomboidal or cubical crystals of calcium oxalate and many large lysigenous mucilage cavities filled with mucilage; groups of lignified fibres form nearly concentric, discontinuous zones, separated by phloem rays; rays many, 2 or 3 seriate, occasionally uniseriate; towards the inner phloem regions they are radially elongated but, become tangentially elongated in the outer phloem; most of the cells loaded with simple, starch grains and crystals of calcium oxalate. The characters observed were in agreement with the Moringaceae family [24].

Powder characteristic

Light brown, fragments of thin-walled, polygonal, sometimes rectangular cork cells; groups or single, thick-walled, lignified, elongated to polygonal stone cells with striations and lumen; a few rhomboidal, rosette crystals of calcium oxalate; a few oil globules; numerous starch grains which was not easily seen due to very small size these particle were simple, oval to round with concentric striations and narrow hilum; pieces of phloem parenchyma, lignified phloem fibres and ray cells were present. During powder microscopy, the presence of crystals confirmed by the reacting with dilutes NaOH which dissolved the crystal completely.

Physico-chemical evaluation

In the present study, we have attempted to evaluate the various physicochemical parameters on the bark of *M.* oleifera (Table 1). Foreign matter was found to be of low percentage (1.0%) which depict, that the plant material collected was free from contamination with unwanted substance. Loss on drying was found to be 3.0%, which was attributed to the amount of moisture and volatile matter present in the plant. As per WHO the determination of loss on drying (LOD) is important since LOD reveals the percentage of moisture present in the drug. Presence of high amount of moisture in any drugs may facilitate the enzyme hydrolysis or enhance the growth of microbes which leads to deterioration [18].

The result of the present study showed that the plant contains a high percentage of total ash (11.43%) which can be predicted mainly due to the presence of an enormous amount of calcium oxalate crystals. Ash value of the drug gives an idea of the earthy matter or inorganic composition or other impurities present along with the drug. The determination of ash values are important since ash may be derived from the plant itself (physiological or natural ash) as well as from the extraneous matter, like sand and soil adhere to the surface of the plant material (non physiological ash).

The total ash may vary within wide limits for specimen of genuine drug due to variable natural or physiological ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid- insoluble ash which represents most of the ash from the contaminating soil. Hence, the determination of ash value can serve as an important diagnostic parameter for the evaluation of crude drugs [25]. Extractive value is highest in water (5.60%) and decreases as the polarity of the solvent decreases which shows that plant contains polar compound more in quantity in comparison to the 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. The foaming index of the drug was less than 100, thus indicating that the plant *M. oliefera bark* was having less quantity of saponin. The haemolytic index was also found to be low with a hemolytic value of 10.5 units/g. In addition, the plant also showed low swelling index indicating the absence of gum, mucilage, pectin or hemicelluloses in the sample.

Fluorescence analysis of powdered drug was studied in both UV. The powder showed different color fluorescence when made to react with various chemical reagents which suggested that there might be presence of certain phytoconstituents possessing chromophore group in the bark (Table. 2).

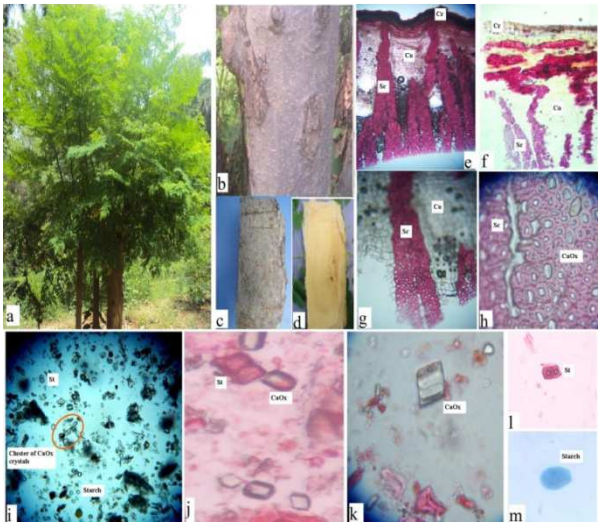

Fig. 1: Pharmacognostical study [a]: *Moringa* oleifera Lam. Whole plant, [b-c]: bark outer surface, [d]: bark inner surface, [e]: T. S. of Stem bark (Diagrammatic), [f]: T. S. of Stem bark (Outer region), [g]: T. S. of Stem bark (Inner region), [h]: T. S. of Stem bark (Sclerenchyma cells containing small rhomboidal or cubical crystals of calcium oxalate), [i]: Very small, numerous, simple, oval to round starch grains and crystals in 10x, [j]: Calcium Oxalate Crystal and Stone Cell, [k]: Sclerenchyma cells and crystal of calcium oxalate) in 40x, [l]: Stone Cell [m]: Single Starch Grain, Simple, Oval To Round. [Cr.]: cork region; [Co.]: cortex region; [Sc.]: Sclerenchyma cells; [CaOx]: Calcium Oxalate Crystal; [St.]: Stone Cell

Most of the authors have reported that the plant material has the capability to produce fluorescence pattern when made to react with various reagents either of acidic or basic media [26, 27].

Preliminary phytochemical evaluation

The preliminary phytochemical screening was performed to identify the nature of the phyto-constituents present in various extracts. This can serve as essential diagnostic parameters for the identification of crude drugs [23]. Hexane and chloroform extracts showed the presence of steroids. Ethyl acetate extract was found to contain flavanoids. Carbohydrate and amino acid were present in the aqueous extract. Methanolic extract showed the presence of proteins. The Preliminary phyto-chemical screening shows that Shigru contain different chemical constituents viz. carbohydrate, amino acid, terpenoid and steroid which indicate the presence of both polar and non polar compounds in the drug.

To further confirm the presence of various phytochemicals, thin layer chromatographic analysis was performed on the various tested extract. For analysis of amino acids, solvent system for the development of chromatogram was composed of a solvent mixture (Butenol -Acetic acid-Water, 4: 2: 1). After derivatization with Ninhydrin reagent, methanolic extract showed three prominent spot at Rf value 0.69, 0.91 & 0.82, whereas aqueous extract showed a single spot with Rf value 0.64.

For the evaluation of triterpenoid, solvent systems selected were toluene- chloform- ethanol (4:4:1) and the chromatograms were derivatized using anisaldehyde-H_2SO_4. The results showed the presence of three prominent spots for methanolic extract (Rf value 0.32, 0.49, 0.64), two spots for ethyl acetate extract (Rf value 0.46, 0.64) and a single spot for chloroform extract (Rf value 0.62). Furthermore, TLC for carbohydrate analysis (Butanol-Acetic acid-Water, 4: 2: 1) after spraying with benzidine-sodium metaperiodate reagent produces two spots (0.68, & 0.82) for methanolic extract and a single spot (0.68) for aqueous extract.

Analysis of phytochemicals on the basis of TLC provides an essential knowledge on the type and classes of phytochemicals, since TLC operates on the separation of the different classes of phytochemicals which can be easily identified [28].

Table 1: Physicochemical evaluation

S. No.	Parameter	Results
1.	*Foreign matter (% w/w)*	Not more than 1.0 %
2.	*Loss on drying (% w/w)*	Not more than 3.0 %
3.	***Ash Values***	
	Total ash (% w/w)	Not more than 11.43 %
	Water soluble ash (% *w/w)*	Not more than 10.2 %
	Acid insoluble ash (% *w/w)*	Not more than 0.85 %
4.	***Extractive values***	
	Water	Not less than 5.6 % (w/w)
	Methanol	Not less than 1.7 % (w/w)
	Ethyl Acetate	Not less than 0.40% (w/w)
	Hexane	Not less than 0.22% (w/w)
5.	*Foaming index*	Not more than 100
6.	*Hemolytic index*	10.2 units/g
7.	*Swelling index*	Not more than 1
8.	Pesticide residue	
	Chlorinated pesticide residue	
	TS1 (First elute)	Not more than 0.0010 mg/kg
	TS 2 (Second elute)	Not more than 0.015 mg/kg
	Phosphated pesticide residue	
	TS1 (First elute)	Not more than 0.017 mg/kg
	TS 2 (Second elute)	Not more than 0.013 mg/kg
	TS 3 (third elute)	Not more than 0.011 mg/kg
9.	Heavy metals	
	Lead (Pb)	Not more than 0.011 ppm
	Cadmium (Cd)	Not more than 0.0002 ppm
	Zinc (Zn)	Not more than 0.115 ppm
	Mercury (Hg)	Not more than 0.156 ppm

Table 2: Fluorescence analysis of bark powder

Treatment	Long U. V. (λ_{max} 365 nm)	Short U. V. (λ_{max} 254 nm)
NaOH + Methanol	Cade blue	NF
NaOH + Water	Medium aquamarine	NF
HNO_3 + Methanol	Light state gray	NF
HNO_3 + Water	Light state gray	NF
HCL + H_2O	Light state gray	NF
HCL + Methanol	Medium purple	NF
Iodine solution	NF	NF
NH_3	NF	NF
KOH	Lime green	NF
Glacial acetic acid	Medium slate blue	NF

NF: No Fluorescence

Table 3: Phytochemical screening of different extracts

Plant Constituents Test / Reagent	Aqueous extract	Alcohol extract	Ethyl acetate extract	Hexane extract
Alkoloids				
Dragendroff's reagent	+	-	-	-
Amino acids	+	+	-	-
Carbohydrate				
Molisch's reagent	+			-
Fehling solution				
Reducing sugar test				
Cardiac glycosides	-	-	-	-
Flavonoids				
Shinoda/Pew test	-	-	-	
Lead acetate test				
Zinc HCL test				
Alkaline reagent test				
Proteins				
Millon's reagent	+		-	-
Ninhydrin reagent				
Saponins				
Foam test	+	-	-	
Sodium bicarbonate test				
Steroids				
a)Salkowski	-	-	+	+
b)libermanns			-	

(+): Present; (-): Absent

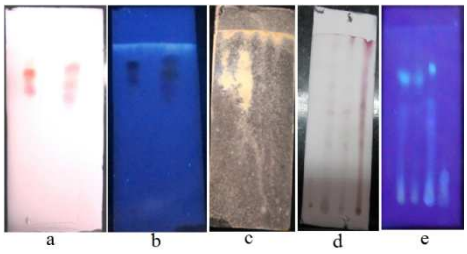

Fig. 2: Thin layer Chromatographic study [a]: Amino acids under visible light, [b]: Amino acids under UV light, [c]: Carbohydrates under visible light, [d]: Triterpenoids under visible light, [e]: Triterpenoids under UV light

Quantitative estimation of phytoconstituents

Quantitative estimation of saponins in moringa bark was found to be less with 3.022 mg/g equivalent to diosgenin. Estimation of phytoconstituents in the extract/plant material is an important step prior to conduct any isolation of active biochemical markers since it gives a brief idea about different classes of chemical constituent's present in the extract.

Evaluation of microbial content

Total Aerobic organisms (cfu/g) was found to be 1.7 x 10^3 but no visible microbes were observed. Plant products usually are contaminated with bacteria and moulds from soil and atmosphere. According to European Pharmacopoeia (2007) [29] limits of microbial contamination are: total aerobic bacteria: 10^5cfu/g, yeasts and moulds: 10^3cfu/g, *Enterobacteria* and other Gram negative organisms: 10^3cfu/g and *E. coli* and *Salmonella* should be absent. In traditional system of medicine the provision of sterilization of the crude drugs is nonexistent and contaminated medicinal products sometimes produce undesirable adverse effects as well as their efficacy may be considerably reduced [30]. Some serious infections have been reported with the use of contaminated raw materials of plant [31]. Therefore it becomes necessary for the manufacturers to make sure the lowest possible microbial contamination in the raw materials to maintain appropriate quality, safety and efficacy of the finished products.

Heavy metals analysis

Result shows that all tested samples were within the limit as prescribed by an official monograph. Heavy metal toxicity with plant products have been reported in many parts of Asia, Europe and the United States [32-35]**.** Plants may contain heavy metals as they grow in the soil including contamination of the plant material with soil, water or air [36]. High levels of toxic metals can occur when the plants are grown in polluted areas, such as places near the roadways or metal mines and smelting units [37]. Also the source of heavy metals includes some agriculture aids like cadmium containing fertilizers (eg. Rajphose), lead based pesticides, and contaminated irrigation water [38]. Long term exposure to lead and cadmium can cause renal damage as well as lead is reported to cross the placental barrier causing teratogenic effects on the fetus [39, 40]. Herbal drug preparations are supposed to be produced with high quality. Quality encompasses all the properties of the final product which makes it optimally suitable for its intended use. Reproducible quality is a goal, which among others is achieved by the process of standardization. The quality requirements for orthodox drug preparations are stringent in terms of content of active principles and toxic materials.

Pesticide residue

Pesticide residue estimated in *M.* oleifera was found to be below the permissible limit of WHO standard which depicts that the plant is safe for use [41].

CONCLUSION

The pharmacognostical study of *M.* oleifera was done for the purpose of correct identity and standardization. Standardization of medicinal plants is a multifaceted task due to their heterogenous composition, which is in the form of whole plant, plant part/extracts obtained there of. The characters which are observed in microscopic and powder microscopical studies can be considered as anatomical markers. 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 *M. oleifera.*

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Morton JF. The horseradish tree, *Moringa pterigosperma* (Moringaceae) A boon to arid lands. Econ Bot 1991;45:318–33.
2. Caceres, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Anti–inflammatory, diuretic & antiurolithiatic activity. J Ethnopharmacol 1992;36(3):233–7.
3. Fahad J, Vijayalakshmi, Kumar MCS, Sanjeeva, Kodancha GP, Adarsh B, *et al*. Antiurolithiatic activity of aqueous extract of bark of *Moringa* oleifera (Lam) in rats. Health 2010;2:352–5.
4. Kurma SR, Mishra SH. Hepatoprotective principles from stem bark of *Moringa pterygosperma*. Pharm Biol 1998;36:295–300.
5. Senecha C, Shama KP, D'Souza UP, Shastry CS. Anticholesteremic and antilipidemic activity of stem bark extracts of *Moringa* oleifera in diet induced hyperlipidemia model in rats. Int J Pharm Chem Sci 2012;1(3):567–4.
6. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003;84:105–8.
7. Kumbhare MR, Guleha V, Sivakumar T. Estimation of total phenolic content, cytotoxicity and *in–vitro* antioxidant activity of stem bark of *Moringa* oleifera. Asian Pac J Trop Disease 2012;2(2):144–50.
8. Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, *et al*. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa* oleifera Lam. J Med Food 2010;13(3):710–6.
9. Bones AM, Rossiter JT. The myrosinase–glucosinolate system its organization and biochemistry. Physiol Plantarum 1996;97:194–208.
10. Maurya SK, Singh AK. Clinical efficacy of Moringa *oleifera* Lam. stems bark in urinary tract infections. Int Scholarly Res Notices 2014.
11. Sholapur HPN, Patil BM. Pharmacognostic and phytochemical investigations on the bark of *Moringa* oleifera Lam. Indian J Nat Prod Res 2013;4(1):96–101.
12. Khandelwal KR. Practical pharmacognosy: Techniques and Experiments. 17th ed. Pune India: Nirali Prakashan; 2007.
13. Kokate CK. Practical pharmacognosy 1st ed. New Delhi, India: Vallabh Prakashan; 1986. p. 15-30.
14. Lala PB. Lab manuals of pharmacognosy. 5th ed. Calcutta: CSI publishers & distributors; 1993.
15. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharmacol Assoc 1949;38(6):324-31.
16. Ahmad A, Maurya SK, Seth A, Singh AK. Pharmacognostical evaluation of the fruit of Plaksha-*Ficus lacor* Buch. Ham. Global J Res Med Plants Indigen Med 2014;3(4):112–26.
17. Indian Pharmacopoeia, 4th ed. Vol. II. Government of India, Ministry of Health and Family Welfare, The Controller of Publication: Civil lines, New Delhi; 1996. p. A53–A4.
18. WHO. Quality control methods for medicinal plant materials. World Health Organization, Geneva. New Delhi: AITBS Publishers and Distributors; 2002.
19. Harborne JB. Methods of extraction and isolation. In: Phytochemical methods. London: Chapman and Hall; 1998. p. 60-6.
20. Wagner H, Bladt S. Plant drug analysis: A Thin Layer Chromatography Atlas. 2nd ed. New York: Springer–Verlag Berlin Heidelberg; 1984.
21. Hiai S, Oura H, Nakajima T. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Med 1976;29:116–22.
22. Chow PYT, 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.
23. Prasad SK, Laloo D, Kumar M, Hemalatha S. Quality control standardization and antioxidant activity of roots from *Eriosema chinense*. Phcog J 2013;5(4):149–55.
24. Metcalfe CR. Chalk L. Anatomy of dicotyledons Vol. II. Oxford, England: Clarendon Press; 1950.
25. Laloo D, Kumar M, Prasad SK, Hemalatha S. Quality control standardization of the roots of *Potentilla fulgens* Wall: A potent medicinal plant of the Western Himalayas and North-eastern India. Phcog J 2013;5:97–103.
26. Laloo D, Sahu AN, Hemalatha S, Dubey SD. Pharmacognostical and phytochemical evaluation of *Cinnamomum wightii* Meissn flowers. Indian J Nat Prod Resour 2012;3(1):33–9.
27. Sahu AN, Hemalatha S, Sairam K, Laloo D, Patra A. Quality control studies of *Ochrocarpus longifolius* Benth flowers buds. Phcog J 2010;2(6):118–23.
28. Sharma R, Saxena N, Thakur GS, Sanodiya BS, Jaiswal P. Conventional method for saponin extraction from *Chlorophytum borivilianum* Sant et Fernand. Global J Res Med Plants Indigen Med 2014;3(2):33–9.
29. European Pharmacopoeia Directorate for the Quality of Medicines of the Council of Europe, 5th Edition. Strasbourg, France; 2007.
30. 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.
31. Gupta DK, Sharma RD, Gupta R, Tyagi S, Sharma KK. Evaluation of some herbal solid dosage forms for microbial contamination. Int J Pharm Pharm Sci 2012;4(4):261–3.
32. Vaijayanthi P, Roy R, Roy B. Strategic marketing model for practice of ayurvedic medicine–a case-study of tiruchirapalli and thanjavur districts, tamilnadu. Int J Pharm Pharm Sci 2012;4(2):172–9.
33. Sarker MR. Adulteration of herbal medicines and dietary supplements with undeclared synthetic drugs: dangerous for human health. Int J Pharm Pharm Sci 2014;6(4):1–2.
34. Nwoko CO, Mgbeahuruike L. Heavy metal contamination of ready-to-use herbal remedies in south eastern Nigeria. Pak J Nutr 2011;10(10):959–64.
35. Genuis SJ, Schwalfenberg G, Siy A-KJ, Rodushkin I. Toxic element contamination of natural health products and pharmaceutical preparations. PLOS One 2012;7(11):e49676.
36. Lone MI, He ZL, Stoffella PJ, Yang X. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. J Zhejiang Univ Sci B 2008;9(3):210–20.
37. Prakash NKU, Deepa S, Sripriya N, Bhuvaneswari S. Quality assessment for the presence of heavy metals in herbal materials from the markets of Chennai, India. Int J Pharm Pharm Sci 2014;6(8):574–8.
38. Vasudevan DT, Dinesh KR, Gopalakrishnan S. Occurrence of high levels of cadmium, mercury and lead in medicinal plants of India. Phcog Mag 2009;5 Suppl S2:15–8.
39. 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(3):195–204.
40. 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.
41. Singh NK, Ghosh A, Laloo D, Singh VP. Pharmacognostical and physicochemical evaluation of *Croton Bonplandianum*. Int J Pharm Pharm Sci 2014;6(3):286–90.