ORIGINAL ARTICLE

Pharmacognostical and phytochemical study of *Musa paradisiaca* Linn. (Stmn.)

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Abstract

Background and Aim of Study: The main aim of this research is to establish a standardized data regarding its safety through including certain parameters. Kadali (*Musa paradisiaca*) Linn. (Musaceae) is a perennial herb which is like a tree and is used in Ayurvedic system of medicine to cure many diseases. **Materials and Methods:** In the present study, pharmacognostical and phytochemical standardization of plant material was performed as per the guidelines of World Health Organization. **Results:** The stamen of *M. paradisiaca* is brownish black or dull in color, slightly smooth and 0.9-1.6 cm long depending on the maturity of stamen. Powdered microscopy of stamen of *M. paradisiaca* shows some fiber vascular tissues, prism-like calcium oxalate crystal, pigmented sclereids, and spherical pollen grains. Physicochemical parameters including loss on drying (5.5% w/w), total ash (5.81%), water-soluble ash (3.79% w/w), acid-insoluble ash (2.61% w/w), water-soluble extractive (7.17% w/w), methanol-soluble extractive (2.38% w/w), hexane-soluble extractive (1.44% w/w), ethyl acetate-soluble extractive (2.24% w/w), and petroleum ether-soluble extractive (4.0% w/w) were evaluated. Phytochemical screening of aqueous and methanolic extracts showed the presence of alkaloids, carbohydrates, saponins, tannins, and phenols. Safety and efficacy of plant drug was further performed through evaluating heavy metals (Zn, Cd, and Pb). Heavy metals are discovered within the permissible limits. **Conclusion:** The diagnostic characters of stamen will give the necessary information regarding its identification. Its physiochemical and phytochemical evaluation provides information which accounts for the safety, identification, and class of chemical constituents present in this crude drug.

Key words: *Ayurveda, Kadali*, stamen, standardization

INTRODUCTION

Since a very long time, medicinal plants play an important role as therapeutic agent for a number of human aliments. Different phytoconstituents are the primary source in the pharmaceutical industry for the development of new drugs from the 19th century which was the time of rational drug discovery.[1,2] The World Health Organization (WHO) defines the herbal medicines as the materials and preparations which are derived from plants having a beneficial effect on human's health[3] and serve a valuable role in traditional systems of medicine worldwide. It is estimated that 70% people of the world depend on these herbal medicines to fulfill their basic health needs,[4,5] possibly due to local availability, high potency, cost-effectiveness, and safer drug profile of those preparations and plants.[6,7] These factors contribute to the enhancement of the demand of herbal medicines in the present scenario. Due to huge demand of crude drugs, it is moral duty of the researcher from this field to set the genuine nature and quality of crude drugs, which helps in production of standardized products. Pharmacognostical and phytochemical study of a crude drug helps in the development of standard monograph for plants. These stepwise studies provide data for the authentication as well as identification of the plant materials.

Musa paradisiaca Linn. (Family Musaceae), commonly known as "Kela" in Hindi and Kadali in Sanskrit, is a perennial herb which looks like tree, very commonly found in the tropical and subtropical area. In India, it is mostly found in Tamil Nadu, Andhra Pradesh, Bihar, Madhya Pradesh, West Bengal, Maharashtra, and Gujarat. Its fruit is

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very nutritious and tasty with high-calorie content. The plant has various pharmacological activities such as antimicrobial, antidiarrheal, antiulcerative, anthelmintic, hypoglycemic, anti-snake venom, antihypertensive, antilithiatic, wound healing, antimalarial, and diuretic.[8]

Different classes of phytoconstituents have been reported from various parts. Catecholamines such as norepinephrine,[9,10] dopamine, serotonin, acyl steryl glycosides such as sitoindoside I-IV and steryl glycosides such as myoinosityl-β-D-glucoside, sitosterol, gentiobioside,[11] crystallisable, and non-crystallisable sugars, pectin, tannin, starch, iron, vitamin B and C, albuminoids, fats, minerals, and several flavonoids and related compounds such as leukocyanidin, quercetin and its 3-O-rhamnosyl glucoside, 3-Ogalactoside[12] and 3-O-glucoside, have been reported specially in the pulp of *M. paradisiaca* fruit.[13]

A new bicyclic diarylheptanoid, rel-(3S,4aR,10bR)-8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4a,5,6,10b-tetrahydro-3H-naphtho[2,1-b]pyran 8-hydroxy-3-(4-hydroxy phenyl)-9 methoxy-4a,5,6,10b-tetrahydro-3H naphtho(2,1-b)pyran as well as four known compounds 1,2 dihydro 1,2,3 trihydroxy9-(4-methoxy phenyl), phenalene (2)-hydroxy anigorufone(3), 2-(4-hydroxy phenyl) naphthalic anhydride(4), and 1,7 bis(4-hydroxy phenyl) hepta-4(E),6(E)-diene-3-one(5) were isolated from ethyl acetate-soluble fraction of the methanolic extract of fruits.[14] Peeled fruits of *M. paradisiaca* contain two acyl steryl glycosides, Sitoindoside-III and Sitosterol-Myo-inosityl-beta-D-glucoside, have been isolated by gradient solvent extraction techniques and also with the techniques of extensive chromatography such as column chromatography, gas chromatography, thin layer chromatography, and high-performance liquid chromatography.[15]

MATERIALS AND METHODS

Plant Material

The plant *M. paradisiaca* was collected in June and July 2015, from the area Mondh located in district Bhadohi, Uttar Pradesh, India. The voucher specimen (APRL/HERB/2015-16/08) was kept securely for any further assistance in Ayurvedic Pharmacy Research Laboratory, Rajiv Gandhi South Campus, Barkachha, Mirzapur (U.P), India.

Digital microscope, compound microscope, basic instruments, and glass apparatus were used in the study. All other reagents and chemicals of analytical grade were used in the study.

The various pharmacognostical studies such as microscopic study, macroscopic study (shape, size, color, etc.), and fluorescence nature of the powder were performed.[16] The study of macroscopy and powder microscopy of the *M. paradisiaca* stamen was performed by the method given by (Brain, Khandelwal and Evans).[17-19] Many herbal drugs showed fluorescence when it is exposed under ultraviolet (UV) light, which is a very useful tool for drug identification.

Fluorescence study was performed as per the standard guidelines.[20,21] For the study, a pinch of powder was mixed with various reagents such as 1 N sodium hydroxide, nitric acid in ammonia solution, 1 N nitric acid, picric acid, acetone, 1 N hydrochloric acid, 1 N sodium hydroxide in methanol, acetic acid, and 50% sulfuric acid. The resulting solutions were seen under daylight, long UV (365 nm), and short UV light (254 nm).[22]

Physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, extractive values in different solvent, loss on drying (LOD), and foaming index were performed with shade-dried powdered drug as per the WHO guidelines and Indian Herbal Pharmacopoeia.[23]

The coarsely powdered plant material of *M. paradisiaca* was kept for 24 h in the different solvent systems such as methanol, hexane, water, and petroleum ether for cold maceration as per the WHO guidelines. After 24 h, the solutions were filtered through Whatman filter paper and resulting filtrate was concentrated on water bath and extractive values in different solvent system were calculated.

Further, the extracts were used for preliminary phytochemical screening.[18]

For the sample digestion, 2 g of the sample was taken in a Nessler's tube and it was mixed with 15 mL of 10% HNO_3 v/v and kept on water bath for 3 h at 100°C. The resulting digested solution was treated under reflux twice with HNO_3 for the analysis of lead, cadmium, arsenic, and zinc with the help of atomic absorption spectroscopy. The permissible limits of these heavy metals are as follows: Lead (10 ppm), arsenic (3 ppm) zinc (5 ppm), and cadmium (3 ppm).[24]

Bulk density is the ratio of the mass of an untapped powder and its volume including the contribution of void volume. The bulk density is defined in terms of grams per milliliter (g/mL).

Tapped density is obtained mechanically by tapping a graduated measuring cylinder containing the powder. After observing the initial powder volume, the measuring cylinder is manually tapped and volume readings were noted until further volume change is not observed.

The powder was gently filled using of funnel into a 100 ml measuring cylinder and weighed to calculate bulk density. Further, the measuring cylinder was tapped in a single platform, until the volume reading became constant, for 100 times. The Hausner ratio is the indication of the flowability of a powder and it is used in all industries. A Hausner ratio >1.25

is considered to be an indication of poor flowability. The Hausner ratio was calculated by applying following formula (H = TD/BD), and the Carr index was calculated by applying following formula (CI = TD-BD/TD*100%).

Where BD = bulk density and TD = tapped density.

RESULTS AND DISCUSSION

Pharmacognostical Evaluation

Macroscopic

Immature stamen appears creamish in color while mature stamen is rough and after shade drying it turns to dark brown and odorless. It is up to 1-3 cm long or more depending on the age of the plant.

Powder

The powder of stamen is coarse and free-flowing. Fiber vascular tissues were found in the stamen which was large and flattened. Small segment of pigmented sclereids was also observed. Calcium oxalate crystal with prism-like structure was also seen in the powder stamen of *M. paradisiaca*. Some spherical shape pollen grains were observed which is mentioned in Figure 1.

The high quality of herbal drug preparation is achieved through evaluation of some standardization parameter. Crude drugs' standardization is a necessary requirement of the Indian system of medicine in the global scenario.[23]

In the present article, different physicochemical parameters of powdered stamen were evaluated. The major drawback of the presence of moisture is the growth of microbes which may further lead to deterioration.[25] The moisture content (LOD) was performed through weighing accurately 4 g of sample and put it into crucible and dry it in oven at 110°C for 15 min, and LOD values found to be 5.5% w/w. The ash value is the key marker for evaluation of crude drugs. More amount of ash present in the sample is primarily due to the presence of calcium oxalate crystals. The earthy matter or inorganic composition or other impurities termed as Ash.[26] The ash values are categorized as physiological or natural ash which may be derived from plant itself. Whereas nonphysiological ash is the result of extraneous matter such as sand and soil adhere to the plant materials. The value of total ash may differ as per the variation of physiological ash. In acid insoluble ash, total ash was treated with acid and boiled on water bath for 5 min and then filter it due to this and then physiological ash dissolved and acid insoluble materials such as silica is remaining. This is the representation of contaminated soil in which plant grows.[27] The total ash value in the present sample was found to be 5.81% w/w as mentioned in Table 1. The extractive value is used for obtaining of concentrated extract in different solvents system. Extractive value is higher in aqueous as compared to other solvents. The extractive values are different as per the constituents present in the plant.[28] The value of foaming index is more than 100 which is the indication of high amount of saponin present in the *M. paradisiaca* stamen. Fluorescence analysis was studied under both the short UV (λ_{max} 250 nm) and long UV (λ_{max} 365 nm) as mentioned in Table 2. Powdered plant material was treated with different chemical reagents which show fluorescence of different colors indicating the presence of certain phytoconstituents.

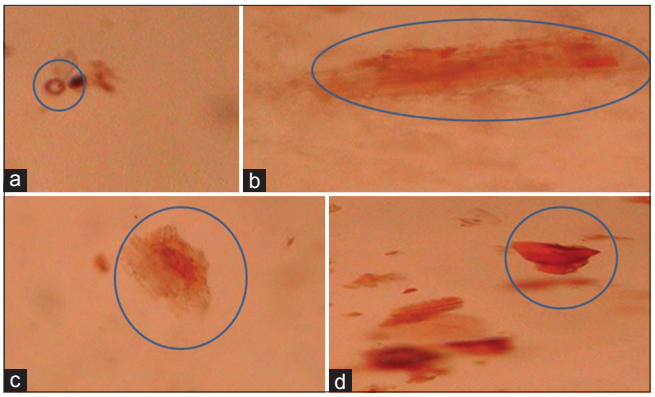

Figure 1: Powder microscopy of *Musa paradisiaca* stamen. (a) Spherical pollen grain; (b) fiber vascular tissue; (c) pigmented sclereids; (d) prism-like calcium oxalate crystal

Table 1: Phytochemical screening of different extracts

Plant constituents test/ reagent	Aqueous extract	Methanol extract
Alkaloids		
Dragendorff's reagent	-	+
Amino acids		
Ninhydrin test	-	-
Carbohydrate		
Molisch's reagent	+	+
Fehling solution	-	-
Glycosides	-	-
Flavonoids		
Zinc HCL test	-	-
Proteins		
Millon's reagent	-	-
Saponins		
Foam test	+	+
Phenolics	+	-

(+): Present; (-): Absent

Preliminary Phytochemical Evaluation

The preliminary phytochemical screening was done to find out which types of phytoconstituents were present in

Table 2: Fluorescence analysis of bark powder

Treatment	Visible light	Short UV (λ_{max} 254 nm)	Long UV (λ_{max} 365 nm)
Powder+water	Light golden rod yellow	NF	Light green
Powder+methanol	Linen	NF	Pale green
Powder+picric acid	Gold	NF	Black
Powder+ammonia	Sienna	NF	Dark sea green
Powder+nitric acid	Saddle brown	NF	Black
Powder+petroleum ether	Beige	NF	Pale green
Powder+50% HCL	Navajo white	NF	Pale green
Powder+50% H_2SO_4	Black	NF	NF
Powder+KOH	Saddle brown	NF	NF
Powder+NAOH	Saddle brown	NF	Dark sea green

NF: No fluorescence. UV: Ultraviolet

Table 3: Pharmaceutical study of stamen powder

Parameters	Values
Bulk density	0.36 g/dl
Tapped density	0.55g/dl
Hausner ratio	0.65
Carl's consolidation ratio	34.54

different extracts. Such type of parameter is essential for the identification of crude drugs.[22,29] Methanolic extracts of *M. paradisiaca* showed the presence of alkaloids. While in aqueous extracts, alkaloids were absent. Amino acid and carbohydrate were present in the aqueous extract. Proteins were absent in both the methanolic and aqueous extract [Table 1].

Safety Profile

Heavy metals are mainly stored in the plant through contaminated water, soil, and air pollution.[30,31] Some agriculture aids such as lead-based pesticides, cadmium-containing fertilizers, and contaminated irrigation water are the major source of heavy metals.[32] According to the WHO recommendations, all the herbal products as well as mineral origin drugs must be examined for the heavy metals.[33] Various heavy metals were reported for their toxic effects above certain concentration.[34-36] In many parts of Asia, Europe, and the United States, the toxicity of heavy metals has been reported.[37,38] The uses of heavy metals for longer duration of time may lead to liver toxicity, renal damage, and teratogenic effects on the fetus.[39,40] The result shows that the tested heavy metals were in the range and not exceeding up to permissible limit.

Pharmaceutical Study of Stamen Powder

Bulk density shows the mass and volume of powder, and it was found to be 0.36 g/dl. Tapped density shows the bulk volume and tapped volume of powder and it was found to be 0.55 g/dl which shows fluffy nature of powder. Hausner ratio and Carl's consolidation ratio show the flowability of powder and it was found to be 0.65 and 34.54, respectively [Table 3].

CONCLUSION

For the proper identification the characteristic feature of *M. paradisiaca,* stamen gives fundamental information. For the safety point of view, physiochemical and phytochemical evaluation gives valid information. Class of chemical constituents specially saponin and tannin present in this crude drug supports the antidiabetic activity.

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REFERENCES

1. Pferschy-Wenzig EM, Bauer R. The relevance of pharmacognosy in pharmacological research on herbal medicinal products. Epilepsy Behav 2015;52:344-62.
2. Kinghorn AD, Pan L, Fletcher JN, Chai H. The relevance of higher plants in lead compound discovery programs. J Nat Prod 2011;74:1539-55.
3. World Health Organization. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. 2000. Available from: http://www.whqlibdoc.who.int/hq/2000/WHO_EDM_TRM_2000.1.pdf. [Last accessed on 2016 Jan 10].
4. David B, Wolfender J, Dias DA. The pharmaceutical industry and natural products historical status and new

trends. Phytochem Rev 2015;14:299-315.
5. World Health Organization. WHO Traditional Medicine Strategy. 2014-2023. Available from: http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/. [Last accessed on 2016 Feb 24].
6. Cordell GA, Colvard MD. Natural products and traditional medicine: Turning on a paradigm. J Nat Prod 2012;75:514-25.
7. Cordell GA. Phytochemistry and traditional medicine - A revolution in process. Phytochem Lett 2011;4:391-8.
8. Imam MZ, Akter S. *Musa paradisiaca* L. and *Musa sapientum* L.: A phytochemical and pharmacological review. J Appl Pharm Sci 2011;01:14-20.
9. Waalkes TP, Sjoerdsma A, Creveling CR, Weissbach H, Udenfriend S. Serotonin, norepinephrine, and related compounds in bananas. Science 1958;127:648-50.
10. Vettorazz G. 5-Hydroxytryptamine content of Bananas and banana products. Food Cosmet Toxicol 1974;12:107-13.
11. Shanmugavelu KG, Rangaswami G. Tryptophan and indole compounds in banana ovaries. Nature 1962;194:775-6.
12. Lakshmi V, Agarwal SK, Ansari JA, Mahdi AA, Srivastava AK. Antidiabetic potential of *Musa paradisiaca* in streptozotocin-induced diabetic rats. J Phytopharmacol 2014;3:77-81.
13. Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 2nd ed. Dhaka, Bangladesh: The Asiatic Society of Bangladesh; 2003. p. 315.
14. Jang DS, Park EJ, Hawthorne ME, Vigo JS, Graham JG, Cabieses F, *et al.* Constituents of *Musa x paradisiaca* cultivar with the potential to induce the phase II enzyme, quinone reductase. J Agric Food Chem 2002;50:6330-4.
15. Ghosal S. Steryl glycosides and acyl steryl glycosides from *Musa paradisiaca*. Phytochemistry 1985;24:1807-10.
16. Prasad SK, Laloo D, Kumar M, Hemalatha S. Quality control standardization and antioxidant activity of roots from *Eriosema chinense*. Pharmacogn J 2013;5:149-55.
17. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceutical. Bristol: Wright Sceintechnica; 1975. p. 4-9.
18. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 17th ed. Pune, India: Nirali Prakashan; 2007. p. 149.
19. Evans WC. Trease and Evans Pharmacognosy. London: WB Saunders; 2002. p. 32-3, 95-9, 512-97.
20. Lala PK. Lab Manuals of Pharmacognosy. 5th ed. Culcutta: CSI Publishers and Distributors; 1993.
21. 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.
22. 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.
23. WHO. Quality Control Methods for Medicinal Plant Materials. Geneva, New Delhi: World Health Organization, AITBS Publishers and Distributors; 2002.
24. 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.
25. 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.
26. Ahmad A. Maurya SK, Seth A, Singh AK. Pharmacognostical evaluation of the fruit of plaksha - *Ficus lacor* Buch. Ham. Glob J Res Med Plants Indig Med 2014;3:165-74.
27. 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. Pharmacogn J 2013;5:97-103.
28. Mehara B, Maurya SK, Dwivedi KN. Pharmacognostic evaluation of Latakaranja *Caesalpinia bonduc* [L.] Roxb. Int J Green Pharm 2015;9:S63.
29. Arka G, Anindita K, Ankit S, Kumar SA, Kumar MS. Preliminary evaluation of hepatoprotective potential of the polyherbal formulation. J Intercult Ethnopharmacol 2015;4:118-24.
30. Lone MI, He ZL, Stoffella PJ, Yang XE. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. J Zhejiang Univ Sci B 2008;9:210-20.
31. Seth A, Mishra A, Maurya SK. Quality evaluation and standardization of Adraka khanda: An Ayurvedic formulation. J Chem Pharm Res 2015;7:523-9.
32. Vasudevan DT, Dinesh KR, Gopalakrishnan S. Occurrence of high levels of cadmium, mercury and lead in medicinal plants of India. Pharmacogn Mag 2009;5 Suppl S2:15-8.
33. 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.
34. Sahu AN, Hemalatha S, Sairam K, Laloo D, Patra A. Quality control studies of *Ochrocarpus longifolius* flower buds. Pharmacogn J 2010;2:118-23.
35. Laloo D, Prasad SK, Kumar M, Hemalatha S. Pharmacognostical and phytochemical standardization of the roots of *Potentilla mooniana* wight. Pharmacogn J 2014; 6:70-9.
36. Shailajan S, Swar G, Matani A, Tiwari B. Quality evaluation and standardization of a traditional Unani formulation Jawarish-e-Amla Sada. Int J Green Pharm 2015;9:21-5.
37. Sarker MR. Adulteration of herbal medicines and dietary supplements with undeclared synthetic drugs: Dangerous for human health. Int J Pharm Pharm Sci 2014;6:1-2.

38. Genuis SJ, Schwalfenberg G, Siy AK, Rodushkin I. Toxic element contamination of natural health products and pharmaceutical preparations. PLoS One 2012;7:e49676.
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;7:195-204.
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.

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