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Morpho-Anatomical and Physicochemical Standardization of *Diospyros malabarica* (Desr.) Kostel *S*tem Bark

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Abstract

Diospyros malabarica (Desr.) Kostel synonym Diospyros peregrina Gurke (Family: Ebenaceae) is a medium sized evergreen tree commonly known as Kendu in Assamese. The plant has ethnomedicinal significance and used by various ethnic communities of North-East India to treat various disorders like dysentery, diabetes, diarrhea malaria, ulcer and wounds. However, detailed scientific information is not available to identify the plant material, in order to ascertain its quality and purity. Therefore, the present work was carried out to perform morpho-anatomical and physicochemical analysis of D. malabarica stem bark. The bark is externally dark brown to black in color and rough having characteristics odor and astringent taste. The microscopy of bark reveals the presence of polygonal thick walled cork, 3-4 layered phellogen, 7-8 layered phelloderm, stone cells, phloem fibers and medullary rays. Stem bark powder showed thick walled cork cells, thick walled elongated phloem fibers, lignified stone cells and rhomboidal crystals of calcium oxalate. Further, physicochemical analysis of the bark power showed loss on drying, total ash, water soluble ash, and acid insoluble ash as 6.2, 5.6, 1.1, 2.45, and 5.7% w/w respectively. The alcohol and water soluble extractives values of the stem bark were 10.6 and 16.8% w/w respectively. The result of preliminary phytochemical screening indicates presence of triterpenoids, saponins, tannins, flavonoids and sterols. The findings of this study will facilitate pharmacognostic standardization of the plant material and aid in the preparation of an herbal monograph for the species.

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Introduction

Diospyros malabarica (Desr.) Kostel synonym *Diospyros malabarica* Gurke, commonly known as Kendu in Assamese, is a small middle sized evergreen tree belongs to family Ebenaceae (Fig. 1). The plant is distributed throughout the tropics and has various ethno -medicinal significances. It is used for the treatment of dysentery, fever, malaria, diabetes, ulcer and wounds [1-3]. The fruit and stem bark possess astringent property and are traditionally used for the treatment of dysentery, diarrhea and intermittent fever & the macerated matured unripe fruit is successfully used in costal West Bengal of India for treatment of diabetes [4, 5]. The fruits are also used to dye fishing nets for its durability and attract fishes [6]. The alcoholic extract of stem bark has been reported to possess hypoglycemic, diuretic, antidiarrheal, antiplasmodial and antitumor activities. [7-9]. The active constituents of this plant are mostly triterpenes, alkanes, flavonoids, and tannins. Various bioactive compounds have been isolated from this plant including dihydroflavonol glycoside, anthrocyanin, taraxerone, sitosterol, gallic acid, peregrinol, hexacosane, hexacosanol, b-sitosterol and betulinic acid [10, 11]. However, available literature reveals that no pharmacognostic study had been carried out on the plant; therefore the present investigation was undertaken. The object of the present study is to evaluate various pharmacognostical parameters such as macroscopic, microscopic, physicochemical, fluorescence and phytochemical studies of the stem bark of *D. malabarica*.

Materials and Methods

Plant Material

The fresh stem bark of *D. peregrina* was collected from the homestead garden of Ashrang village, Dimahasao district, Assam, India in the month of July 2017. The specimen was identified by Taxonomist, TERI -Northeastern Regional Centre, Guwahati and later specimen was confirmed in BSI, Shillong. The voucher specimen was deposited in herbarium section of TERI-Guwahati for future reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in a grinder for physico-chemical and phytochemical studies.

Pharmacognostic Study

Fresh stem bark was taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters, physicochemical parameters, and phytochemical investigation. For the microscopical studies, transverse sections of stem bark were prepared and stained as per standard procedure [12-14]. The powder microscopy was performed according to the method used by Khandelwal [14].

Physicochemical and Phytochemical Analysis

Physicochemical values such as the percentage of foreign organic matter, moisture content, ash values, extractive values, foaming index, swelling index and pH were determined according to the well established official method and procedure [14-16]. Preliminary phytochemical screening was carried out using the standard procedure described by Khandelwal [14].

Florescence Analysis

Powdered stem bark material was treated with various chemical reagents and exposed to visible, ultraviolet light to study its fluorescence behavior [17].

Results

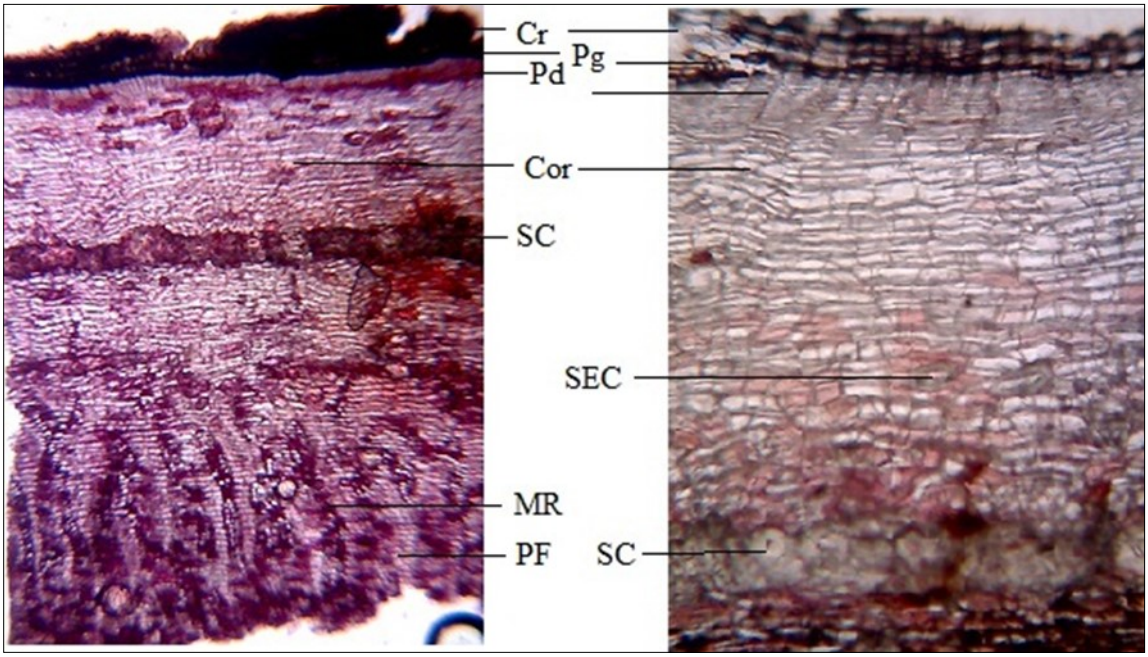
Macroscopic Characteristics

The bark is externally dark brown to black in color and rough, internally light brown and smooth having characteristics odor and astringent taste. Pieces of the bark are curved or occur in the form of flat pieces about 1 to 2 cm thick and 10 to 12 cm long.

Microscopical Characteristics

The transverse section of *D. malabarica* bark shows the presence of cork, phellogen, phelloderm, cortex and secondary phloem (Fig. 2). Cork consists of several layers of radially arranged rows of polygonal thick walled parenchymatous cells filled with dark brown contents. Phellogen is 3-4 layered of rectangular, thin walled cells. Phelloderm consists of 7-8 layers having rectangular thick walled parenchymatous cells, arranged radially without any content. Cortex is composed of several layers of tangentially elongated thin walled cells with few cells containing brown matter. Beneath the secondary cortex, a large group of stone cells, arranged in a tangential manner forming continuous band are

Figure 1. *Diospyros malabarica* tree



(10X) **(40X)**

Figure 2. Transverse section of the *Diospyros malabarica* stem bark (cr: Cork; Pg: Phellogen; Pd: Phelloderm; Cor: Cortex; SC: stone cells; SEC: Secretory gland; PF: Phloem fibres; MR: Medullary rays)

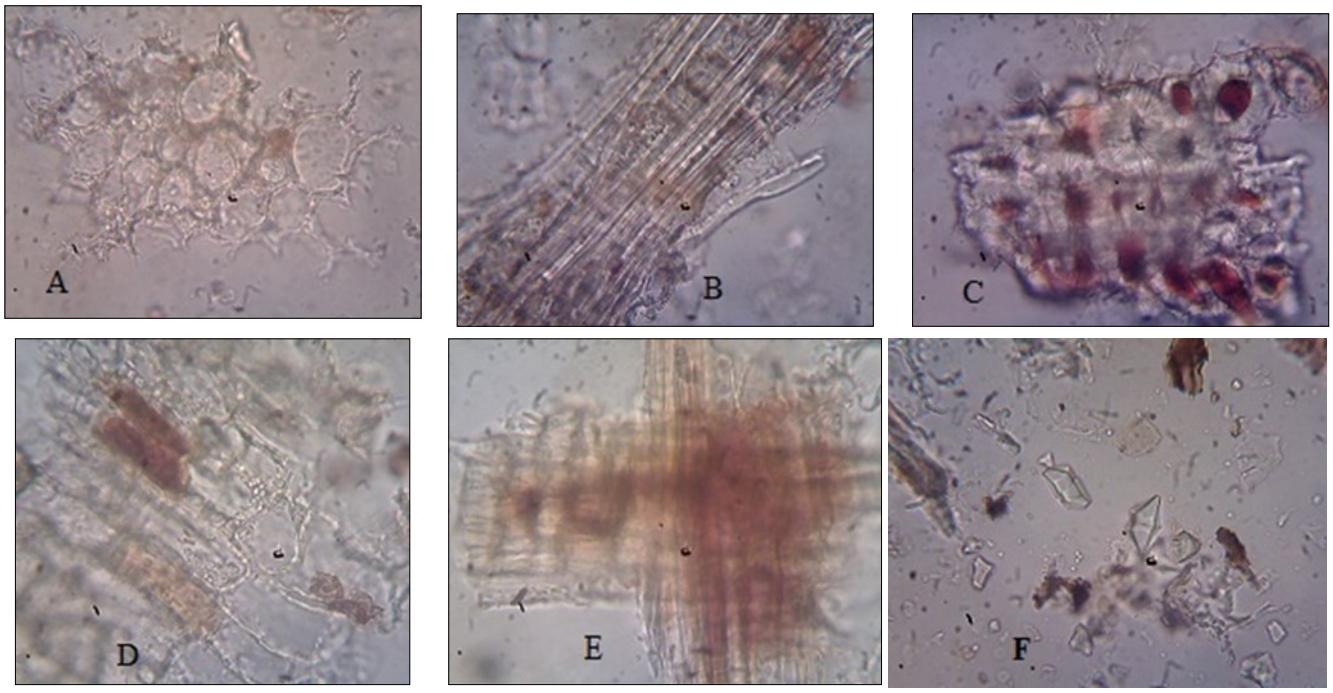


Figure 3. Powder characteristics of *Diospyros malabarica* stem bark

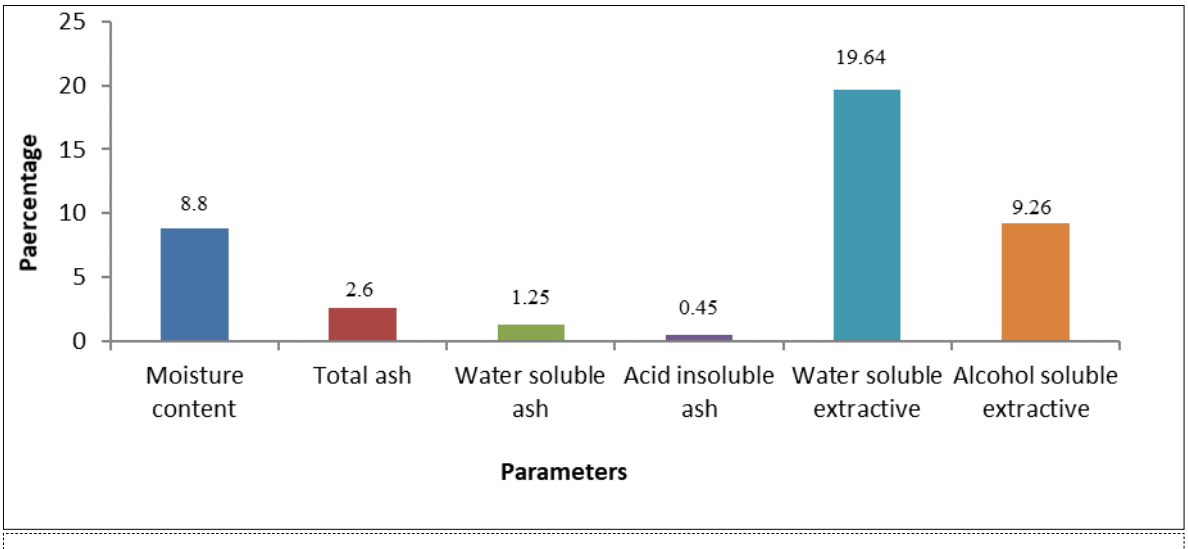


Figure 4. Results of physicochemical parameters of *Diospyros malabarica* stem bark

Table 1. Fluorescence analysis of *Diospyros malabarica* stems bark powder

Treatment	Day light	Under U.V. light	
		Short wave-length	**Long wavelength (365nm)**
Powder as such	Reddish brown	Light brown	Brown
Powder + 1N NaOH (aq.)	Dark brown	Forest green	Black
Powder + 1N NaOH (alc.)	Brown	Brown	Dark brown
Powder + 1N HCl	Brown	Olive	Chocolate
Powder + HNO_3 (1:1)	Reddish brown	Greenish brown	Chocolate
Powder + H_2SO_4 (1:1)	Brown	Green	Dark brown

present. Some of the cortical cells are filled with microsphenoidal crystals of calcium oxalate. Secondary phloem is composed of phloem parenchyma, phloem fibers and medullary rays which are not uniformly arranged. Phloem fibers are intermingled with phloem parenchyma and in between medullary rays. Medullary rays appear to run in different directions, 1-2 celled wide and extend up to cortex.

Powder Microscopic Characters:

The stem bark powder is brownish in color showing fragments of cork cells (Fig. 3A), elongated phloem fibers (Fig. 3B), stone cells (Fig. 3C), parenchyma with simple pits, a few containing crystals (Fig. 3D), medullary rays crossing fibers (Fig. 3E), and rhomboidal crystals of calcium oxalate (Fig. 3F).

Preliminary Phytochemical Screening:

The Preliminary phytochemical screening reveals the presence of triterpenoids, flavonoids, saponins, tannins and sterols.

Physicochemical Parameter:

Physicochemical parameters of stem bark *viz.* percentage of moisture content, ash value and extractive values are presented in Fig. 4. The foreign organic matters was absent in the dried plant material. Swelling index of powdered bark material was found to be 0.6 ml. Foaming index of dried bark plant material was found to be less than 100 because the height of foam in each test tube was less than 1.0 cm. pH of 1% solution of dried bark of plant was found to be 7.79. The fluorescence characteristics of crude powdered drugs with different reagent were observed under UV radiation of long and short wavelength and day light. The color changes for the crude powder were distinctive and data is presented in table 1.

Discussion

Owing to the wide use of this plant by local people in various disease conditions, standardization becomes an important measure for ensuring quality, purity, and authenticity of the crude drugs. First step in this regard is the authentification of plant species. For this purpose, morpho-anatomical studies are the simplest and cheapest methods to start with for establishing the correct identity of the source materials [18, 19]. In this context, the study was undertaken to set the standardization parameters for establishing the quality of the stem bark of *D. malabarica*. Physicochemical studies of stem bark acts as

a reliable tool for detecting adulteration. Thus moisture content, foreign matter, ash value, extractive value, foaming index, swelling index, pH and fluorescence study determined here signifies standard parameters to ensure the quality and purity of the crude drug [20, 21]. The total ash indicates the presence of inorganic matter present in a plant material. Extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents [22]. Swelling and foaming index also provide useful information that help in quality control of drugs. The fluorescent analysis under day light and UV light by treatment with different chemical reagents showed different color. This analysis suggests that, extract of *D. malabarica* stem bark probably contain active agent(s) and this provides the basis for their folkloric use as a cure for many human ailments.

Conclusion

Ethnomedically, the *D. malabarica* plant is widely used by villagers in various disease conditions without standardization. Standardization and quality control for herbal drugs are need of the hour and an integral part for establishing its correct identity. So the information obtained from present work will be useful in identifying this drug and differentiating it from other related species. In addition, analytical parameters are useful in determining the quality and purity of the commercial samples and detection of adulterations.

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Conflict of interest

The authors declare no conflicts of interest.

References

1. Kirtikar, K. R. and Basu, B. D. (1935) Indian medicinal plants, 2nd Ed., 1st Vol. Lalit mohan Basu Allahabad, India, p. 785-788.
2. Anjaria, J., Parabia, M., Bhatt, G. and Khamar, R. (2002) A Glossary of selected indigenous medicinal plants of India, 2nd Ed., SRISTI Innovations, Ahmedabad, India, p. 26.
3. Asolkar, L.V., Kakkar, K. K. and Chakre, O. J. (1992) Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Part-I (A–K). Council of Scientific and Industrial Research (PID) Publication, New Delhi, India, p. 27.
4. Joshi, S. G. (2000) Medicinal Plants. Oxford and IBH Publishing Co Pvt. Ltd India, p. 173.
5. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) Glossary of Indian Medicinal plants, Council of Scientific and Industrial Research, New Delhi, India.
6. Kar, A. and Borthakur, S. K. (2008) Dye yielding plants of Assam for dyeing handloom textile products. Indian J Tradit Knowl. v. 7 p. 166-171
7. Alex, A.T., Nawagamuwa, N.H., Joseph, A., Rao, J.V. (2012). *In vitro* anti-cancer and antioxidant activity of different fractions of *Diospyros peregrina* unripe fruit extract. Free Radicals and Antioxidants. v. 46 p. 49-53.
8. Kaushik, V., Saini, V., Pandurangan, A., Khosa, R. L. and Parcha, V. (2012). A review of Phytochemical and biological studies of *Diospyros malabarica*. Int J Pharm Sci Lett. v. 2 p. 167-169.
9. Dewanjee, S., Bose, S, K., Sahu, R. and Mandeal, S. C. (2008). Antidiabetic effect of matured fruits of *Diospyros peregrina* in alloxan-induced diabetic rats. Int J Green Pharm. v. 2 p. 95-99.
10. Jain, N. and Yadav, R. N. (1994). Peregrinol, a lupane type triterpene from the fruits of *Diospyros peregrina*. Phytochem. v. 35 p. 1070–2.
11. Misra, P. S., Misra, G., Nigam, S.K. and Mitra, C.R. (1971). Constituents of *Diospyros peregrina* fruit and seed. Phytochem. v. 10 p. 904-905.
12. Brain, K. R. and Turner, T. D. (1975). The Practical Evaluation of Phytopharmaceuticals, Wright-Scientechnica, Bristol.

13. Kokate , C. K. (1994). Practical Pharmacognosy, 4th Ed. Vallabh Prakashan, Delhi, India.
14. Khandelwal, K. R. (2008) Practical Pharmacognosy, 19th Ed. Nirali Publication, Pune, India.
15. World Health Organisation (1992). Quality Control Methods of Medicinal Plant Material, Organisation Mondiale De La Sante, Geneva.
16. Anonymous (1966). Indian Pharmacopeia, 4th Ed. Ministry of Health and Family Welfare, Controller of Publications, New Delhi, India.
17. Pratt, R. J. and chase, C. R. (1949). Fluorescence of powdered vegetable drug with particular reference to development of a system of identification. J Am Pharm Assoc. v 38 p. 324-333.
18. Singh, S., Machawal, L. and Chauhan, M. G. (2010). Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. J Pharmacognosy Phytother. v. 2 p. 71-75.
19. Gupta, P. C., Sharma, N. and Rao, C. V. (2012). Pharmacognostic studies of the leaves and stem of *Careya arborea* Roxb. Asian Pac J Trop Biomed. v 2 p. 404-408.
20. Raj, R. S. N. and Radhamany, P. M. (2012). Pharmacognostic and physicochemical analysis on the leaves of *Brunfelsia americana* L. Asian Pac J Trop Biomed. v. 2 p. 543-546.
21. Zhao, Z., Liang, Z. and Guo, P. (2011). Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. J Ethnopharmacol. v. 131 p. 556-561.
22. Gupta, P. C. and Rao, C. V. (2012). Pharmacognostical studies of *Cleome viscosa* Linn. Indian J Nat Prod Resour. v. 3 p. 527-534.