

Pharmacognostic Evaluation of *Manilkara zapota* (L.) P. Royen Root

Rupesh Pingale*¹, Gouri Kumar Dash²

¹Department of Pharmaceutical Sciences, NIMS University, Shobha nagar, Jaipur- India.

²Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, 30450 Ipoh, Malaysia.

Available Online: 7th April, 2015

ABSTRACT

Manilkara zapota (L.) P. Royen (Family: Sapotaceae), commonly known as 'Sapodilla' is very popular in India with a long history of traditional and folklore medicinal uses. Traditionally, different parts of the plant are used in treating cough, cold, diarrhoea, hemorrhage ulcer and gonorrhoea. A variety of phytoconstituents like lignans, flavonoids, coumarins, steroids, terpenes, fatty acids, anthraquinone glycosides and aliphatic long-chain compounds have been isolated from the plant. The plant is reported to possess anti-inflammatory, analgesic, antidiarrheal, antimicrobial, antioxidant, and insecticidal activities. The present study was carried out to investigate macroscopical, microscopical and physiochemical parameters of *M. zapota* roots. Some of the diagnostic features of the roots were studied. All the parameters were studied according to WHO guidelines. The determination of these characters will help future researchers in phytochemical as well as pharmacological analysis of this species.

Keywords: *Manilkara zapota*, Sapotaceae, Sapodilla, Root, Microscopy

INTRODUCTION

Manilkara zapota (L.) P. Royen (Family: Sapotaceae) is a large, evergreen tree, more than 30 m in height and with a diameter up to 1.5 m. The leaves are simple, elliptic or oblong, apex obtuse to shortly acuminate, glabrous, spirally arranged and clustered at the shoot tips. Flowers are green in colour, solitary, with a brown pubescent peduncle, 6 sepals and 6 corolla lobes. Fruit is ovoid to globular berry with a rough brown skin, containing 1-12 shining, brown or black seeds (frequently 5), surrounded by a brownish, sweet, juicy, scented flesh. 'Manilkara' is a common name for a member of the genus in Malabar. The common name 'Sapodilla', by which the fruit is known, is taken from the Spanish 'Zapotillo' meaning 'Small zapote'¹.

Traditionally, almost every part of the plant is used for its medicinal activity. To name a few, the leaf juice is used to treat common cold, fever, wounds and ulcers^{2,3}. The fruits are believed to be beneficial for the treatment of diarrhoea and pulmonary diseases^{4,5}. The infusion and decoction of the seeds are used as diuretic⁶⁻⁸. In Nicaragua, the decoction of dried sap is taken orally for the treatment of aches and applied externally for treating skin rashes and sores⁹. In Guatemala, the infusion of the bark is reported to be used orally for the treatment of gonorrhoea¹⁰. Several biological studies such as analgesic, anti-inflammatory, antipyretic¹¹, antimicrobial¹²⁻¹⁵, antioxidant¹⁶⁻¹⁹, antitumor²⁰ and antidiabetic activity²¹ have been reported by different authors. The ground fresh roots along with water is taken orally by the Kondh tribes of Ganjam district of Odisha to treat diabetes mellitus. The present study was carried out to investigate macroscopical, microscopical

and physiochemical parameters of *M. zapota* roots. The studies were carried out in accordance with WHO General Guidelines for Herbal Drug Standardization methodologies²².

MATERIALS AND METHODS

Plant material

The plant specimens for the study were collected from the Junnar, (Pune, India) and were positively identified and authenticated by the Botanist Dr. Jayanthi, Botanical Survey of India, Pune. The voucher specimen No. RUPMANZ2, dated 31 / 07 / 2012 is preserved in the herbarium for future reference. Care was taken to select healthy fully grown plants with normal organs. The samples of root were cut suitably removed from the plant, thoroughly washed with water to remove the adherent impurities, and dried in sunlight. The roots which were used for the extraction process were primarily collected from local areas of Junnar. Further, these roots were subjected to air drying for about two weeks and were used for the extraction.

Macroscopy

The size, color, odour and taste of the roots were studied.

Microscopy

Sectioning: Selected samples of the dried root were stored in a solution containing formalin (5 ml), acetic acid (5 ml), and 70% v/v ethyl alcohol (FAA) (90 ml). After 24 hours of fixing, the specimens were dehydrated with a graded series of tertiary-butyl alcohol as per the method²³. Infiltration of the specimens was carried out by gradual addition of paraffin wax (50 – 60°C m.p.) until the tertiary-butyl alcohol solution attained supersaturation. The



Figure 1 : External morphology of the *M. zapota* root

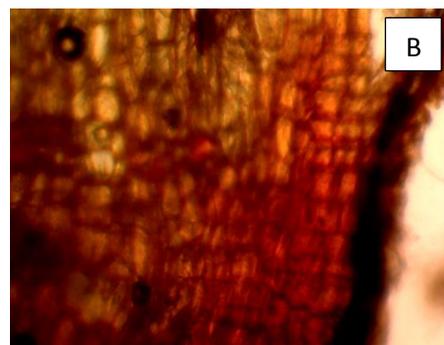
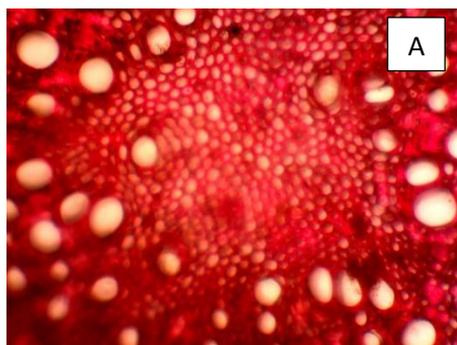


Fig.2: Microscopical view of the T. S. of the *M. zapota* root (a) xylem, phloem, vascular bundle and sclerenchyma (b) the cortex and cork, enlarged at $\times 10 \times 45$



Lignified fibres



Cork cells



Calcium oxalate crystals



Starch grains



Parenchyma



Phloem fibre

Fig.3: Powder microscopy of *M. zapota* root

Table 1: Results of physicochemical analysis

Parameter	Value
Total ash	7.79%
Acid-insoluble ash	2.19%
Water soluble ash	3.99%
Water-soluble extractive	8.93%
Alcohol-soluble extractive	6.27%

specimens were casted into paraffin blocks. The paraffin embedded specimens were sectioned with the help of a Senior Rotary Microtome, RMT-30 (Radical Instruments, India). The thickness of the sections was kept between 10 and 12 μm . The dewaxing of the sections was carried out as per the procedure described by Johanson²⁴. The section was stained with phloroglucinol-hydrochloric acid (1 : 1) and mounted in glycerin.

Photomicrograph: Microscopic descriptions of the selected tissues were supplemented with micrographs. Photographs were taken with digital camera. For normal observations, a bright field was used. For the study of crystal, starch grains, and lignified cells, polarized light

was employed. As these structures have a birefringent property under polarized light they appear bright against a dark background²⁵.

Physicochemical evaluation

Physicochemical parameters of *M. zapota* root powder were determined²⁶ and reported as total ash, water-soluble ash and acid-insoluble ash. Alcohol and water-soluble extractive values were also studied.

Preliminary phytochemical screening

The coarse root powder (25 g) was subjected to soxhlet for solvent extraction using Pet. Ether, Chloroform and Methanol. The extract was concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents^{27,28}.

RESULTS

Macroscopy

The root was long, hard, about 10 to 12 cm in length, and 10 to 20 mm in breadth. The outer surface appeared light brown and the inner was brown in color. The taste was astringent and odor was none. The fracture was fibrous [Figure 1].

Microscopy

Table 2: Preliminary phytochemical analysis of *Malinkara zapota* roots

Test	Pet. Ether	Chloroform	Methanol	Aqueous
Steroids and sterols	+	+	-	-
Triterpenoids	+	+	-	-
Alkaloids	-	+	+	-
Saponins	-	-	+	+
Flavonoids	-	-	+	+
Carbohydrates	-	-	-	+
Proteins and amino acids	-	-	-	+
Tannins and phenolic compounds	-	-	+	+

+ve = Detected, -ve = Not detected

The transvers section of the root was circular in outline and showed the outer cork, cortex, and stellar regions [Figures 2 (a) and 2 (b)]. Abundant clustered crystals of calcium oxalate were present in the cortex region.

Centrally, the stellar region was present with well-developed xylem and phloem. The medullary rays were multiseriate and well-developed. The cork was six-to-eight layered and the cortex was narrow, five-to-seven layered.

Powder microscopy

The dried root powder was screened through sieve no. 40 was used for the powdered drug analysis. The specimens were separately treated with glycerin, N/20 iodine solution (for detection of starch grains) and phloroglucinol-hydrochloric acid (1:1) for detecting lignin. After staining, the samples were observed under a compound microscope. The diagnostic microscopic features observed in the powder of *M. zapota* root are depicted in fig. 3.

Physicochemical parameters

The results of physicochemical evaluation are presented in Table 1.

Preliminary phytochemical studies

The results of preliminary phytochemical analysis of *M. zapota* roots are summarized in Table 2.

DISCUSSION

The macroscopic study of the root indicated that its color, odor, and taste may be an important characteristic feature for identifying the plant. The microscopic study of the powder revealed the presence of cork cells, parenchymatous tissue, lignified fibres, crystals of calcium oxalate and starch grains.

The physical constant evaluation of drugs is an important parameter in detecting adulteration. The total ash is particularly important in the evaluation for the purity of the drugs than acid insoluble and water soluble ash. The ethanol-soluble extractive was less than the water-soluble extractive.

Preliminary phytochemical analysis showed the presence of various phytoconstituents in the extract such as Carbohydrates, alkaloids, glycosides, terpenoids, tannins, flavanoids and saponins [Table 2]. The pharmacognostic constants for the roots, the diagnostic microscopic features, and the numerical standards reported in this study can be useful for the compilation of a suitable monograph of *M. zapota* for its proper identification.

CONCLUSION

The present study on the pharmacognostic evaluation of the roots of *M. zapota* will be useful with regard to its identification and standardization.

ACKNOWLEDGMENT

The authors are thankful to the Management of NIMS University, Jaipur for their constant help and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

- Kirtikar KR, Basu BD., Indian medicinal plants. Vol. II International book Distributers, Dehradun; 2005: 1486-1487.
- Jain PK, Soni P, Upmanyu N, Shivhare Y. Evaluation of Analgesic Activity of *Manilkara Zapota*(Leaves). European J of Expt Bio. 2011; 1(1):14-17.
- Howard BV. Lipoprotein metabolism in diabetes mellitus. J Lipid Res 1987; 28:613.
- Raju VS, Reddy KN. Ethnomedicine of dysentery and diarrhea from Khammam district of Andhra Pradesh. Indian J of Traditional Knowledge 2005; 4:4:443-447.
- Kothari V, Seshadri S. In vitro antibacterial activity in seed extracts of *Manilkara zapota*, *Anona squamosa* and *Tamarindus indica*. Boil Res. 2010; 43:165-168.
- Simpson GE. Folkmedicine in Trinidad. J Amer Folklore 1962;75:326-340.
- Nadkarani KM. Indian Materia Medica. Vol I, Bombay Popular Prakashan pvt.Ltd: Bombay; 1976:331-333.
- Singh PDA, Simon OR, West ME. Acute toxicity of seeds of the Sapodilla (*Achras sapota* L.), Toxicon 1984; 22:1:145-147.
- Murillo G, Hirschelman WH, Ito A, Moriarty RM, Kinghorn AD, Pezzuto JM.Mehta RG. Zapotin, a phytochemical present in a Mexican fruit, prevents colon carcinogenesis. Nutr Cancer. 2007; 57(1):28-37.
- Caceres A, Menedez H, Mendez E, Samayao BE, Jauregui E, Peralta E, Carrillo G. Antigonorrhoeal activity of plants used in Guatemala for the treatment of sexually transmitted diseases. J Ethnopharmacol 1995; 48(2):85-88.
- Pal SC,NandyA. Antiinflammatory,analgesic and antipyretic activity of *Achras sapota* Linn. Leaf extracts and isolated compounds.Indian Drugs 1999 ; 36(2):106-114.

12. Kaneria M, Baravalia Y, Vaghasiya Y, Chanda S. Determination of antibacterial and Antioxidant potential of some medicinal plants from Saurashtra Region, India. *Indian J PharmSci.* 2009;71(4):406-412.
13. Mirimanoff A, Ihanes ML. Active principles of the bark of *Achras zapota*. *Pharm Acta Helv.* 1961;36 :97-102.
14. Muelas-Serrano S, Nogal JJ, Martinez-diaz RA, Escario JA, Martinez-Fernandez AR, Gomez- Barrio A. In vitro screening of American plant extracts on Trypanosome cruzi and Trichomonas vaginalis. *J Ethnopharmacol.* 2000;71(1/2):101-107.
15. Nakanishi K, Sasaki SI, Kiang AK, Goh J, Kakisawa H, Ohashi M, Goto M, Watanabe JM, Yokotani H, Matsumura C, Togashi M. Phytochemical survey of Maiaysian plants. Preliminary Chemical and pharmacological screening. *Chem Pharm Bull.* 1965;13(7):882-890.
16. Linda SE, Reynertson KA, Xiao-Dongluo A, Basileb MJ, Kenneliya EJ. Anthocyanin Antioxidant from edible fruits. *Food Chemistry.* 2004;84: 23-28.
17. Jamuna KS, Ramesh CK, Srinivasa Tr, Raghu KL. In vitro antioxidant studies in some Common fruits. *International Journal of Pharm and Pharm Sci.* 2011;3:1.
18. Rafikul Islam MD R, Mst. Shanaj Parvin, MD. Raqibul Hasan, Md. Ekramul Islam. In vitro and In vivo antioxidant activity of ethanolic extract of *Manilkara zapota* bark. *Journal of Global Pharma Tech.* 2010;2(11):23-30.
19. Dey M, Debnath SK, Chaulya NC, Baile SB. Phytochemical screening and pharmacological evaluation of the modified stem of *Achras sapota* linn. *Phcog J.* 2010;2:5.
20. Osman MA, Rashid MM, Aziz MA, Habib MR, Karim MR. Inhibition of Ehrlich ascites Carcinoma by *Manilkara zapota* L. stem bark in Swiss albino mice. *Asian Pacific J of Tropic Biomedicine.* 2011;1(6):448-451.
21. Muhtadi A, Sumiwi S, Lestari K. Study on the antidiabetic activity extract of *Achras zapota* Linn. Roots in rats. *Bionatura.* 2000;2(2):60-65.
22. Shrikumar S, Maheswari MU, Suganthi A and Ravi TK. WHO Guidelines for herbal drug Standardisation. *Pharmainfo.net.*, [cited 2004 September 19]. Available from www.pharmainfo.net/reviews/whoguidelines-herbal-drug-standardization.
23. Sass JE. New York: Mc Graw Hill; 1940. Elements of Botanical Microtechnique.
24. Johanson DA. New York: Mc Graw Hill; 1940. Plant Microtechniques.
25. Esau K. New York: John Wiley and Sons; 1964. Plant Anatomy.
26. The Ayurvedic Pharmacopoeia of India. 1999;1:191-2.
27. Khandelwal KR. Pune, India: Nirali prakashan; 2005. Practical book of Pharmacognosy.
28. Harborne JB. London: Chapman and Hall; 1998. Phytochemical Methods