Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(10); 1309-1313
doi: 10.25258/phyto.v9i10.10454
ISSN: 0975-4873

Research Article

Pharmacognostical, Phytochemical, Physio-Chemical and Acute Toxicity Studies on the Leaves of *Manilkara zapota (l)* Van Royen var. PKM1

Swarnakumari S[1*], Premanand S[2]

[1]*Department of Pharmacognosy, Karpagam College of Pharmacy, Coimbatore, Tamil Nadu, India*
[2]*Department of Mechatronics, Sri Krishna College of Engineering And Technology, Coimbatore, Tamil Nadu, India*

Received: 10th Aug, 17; Revised 12th Sept, 17, Accepted: 14th Oct, 17; Available Online:25th Oct, 17

ABSTRACT

The present research work was extend out for the leaves of *Manilkara zapota* (L) Van Royen var. PKM1 to investigate the pharmacognostical characters, physiochemical constants, phytochemical studies for the presence of various secondary metabolites and its toxicity was screened using brine shrimp lethality assay. In pharmacognostical work, both macroscopical and microscopical characters were examined. Physiochemical constant like ash value, loss on drying and extractive values of successive solvents with increasing order of polarity were analysed. Preliminary phytochemical screening were conducted with various solvent extracts, exposes the presence of carbohydrates, proteins and amino acids, flavonoids, terpenoids, tannins and phytosterols and wherein ethanolic extract of leaves indicates the presence of flavonoids. Further investigation on toxicity was carried out with the ethanolic extract of the leaves, showed 100% mortality in the range of 1500 ppm/ml compared to standard podophyllotoxin which showed lethality at 3ppm level. This work will aid future investigators in their pharmacological analysis of this particular species which is imbibed with medicinal values.

Keywords: *Manilkara zapota*, microscopical, physiochemical, acute toxicological studies, brine shrimp.

INTRODUCTION

Traditionally medicinal plants are used worldwide to prevent/ cure diseases/ meloriate health hence appeals more attention towards it[3,4]. Scientific documentation of herbal remedies had proved to be beneficial in the further multidirectional researches including newer drug development[5]. Medicinal plants and traditional health care system in solving the health care problems is gaining progressive attention. Most of the developing countries have adopted traditional medical practice as an integral part of their culture.

Manilkara zapota (Sapotaceae) is commonly known as sapodilla, fairly slow-growing, long lived tree with ornamental leaves. The plant grows up to 60ft (18m) high in cultivation but reaches 100ft (30m) when crowded in forest. Sapota is indigenous to Caribbean island, Central America, and South Mexico. It is commercially cultivated in India, Malaysia, Bangladesh and Cambodia for edible fruits and latex used in preparation of chewing gum[1,2]. Traditionally young leaves and shoots are used either in raw or cooked condition as a food and decoction from leaves were used in treatment of fever, haemorrhage, wounds, ulcer. Caution has to be taken in usage of older leaves since it contains poisonous alkaloids and also seeds contain hydrocyanic acid[1]. However very few reports available for the phytochemical and pharmacological investigation. So it will be worth-while to make some preliminary research works[6].

MATERIALS AND METHODS

Collection and Authentication of the Plant material

The leaves of the plant *Manilkara zapota* Van Royen var. PKM1 was collected from The Department of Horticulture, Agriculture University, Madurai, Tamilnadu, India and were authenticated by The Department of Horticulture, Agricultural University, Madurai, Tamilnadu, India and by Dr. Stephan, Department of Botany, American College, Madurai, Tamilnadu, India.

Preparation of plant extracts

Fresh leaves were thoroughly washed with running tap water for 3-4 times then ultimately with sterile water preceded by shade drying at room temperature for 15-20 days. The dried plant material was pulverized, sieved and utilized for extraction. Fine powder (5gm) of leaves was extracted incessantly in Soxhlet apparatus for six hours, individually with different solvents of increasing order of polarity (petroleum ether, ethyl acetate, ethanol and water). The extract was filtered through Whatman filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) under reduced pressure. Further, the dried residue was preserved in airtight container and kept at 4-5°C until further use. Extractive value for each solvent was calculated and the residue prevailed was used for the phytochemical screening and Preliminary acute toxicity studies.

Macroscopic analysis

**Author for Correspondence: swarnapremanand@gmail.com*

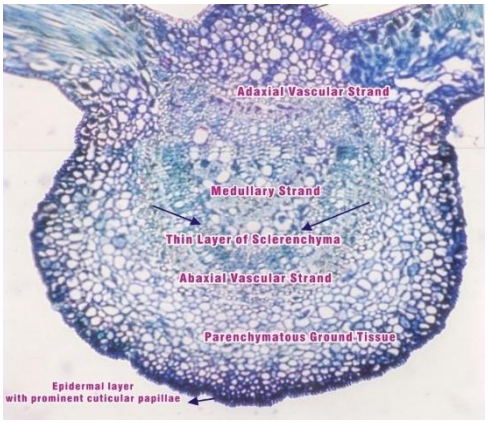

Figure 1: T.S of midrib of leaves *M.zapota.*

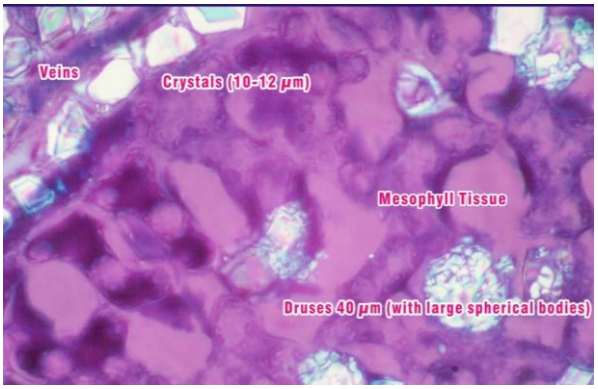

Figure 2: Mesophyll Tissues Showing Druses.

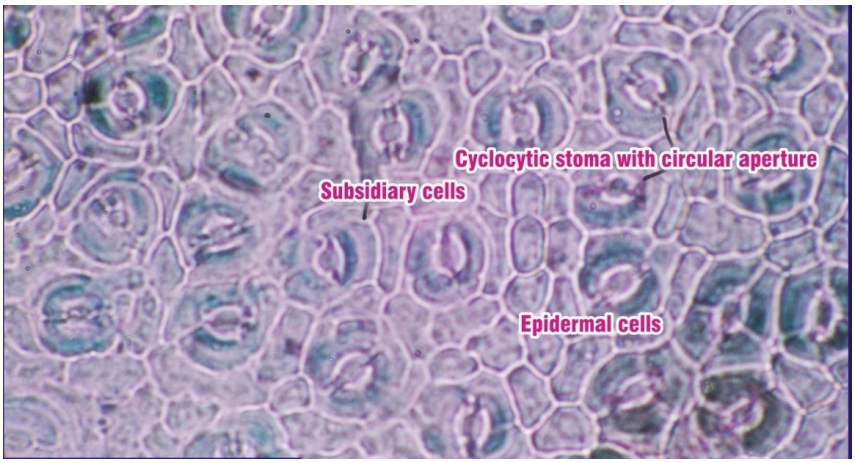

Figure 3: Stomatiferous Abaxial Epidermis.

Table 1: Quantitative Microscopical Character.

Parameters	Minimum	Average	Maximum
Vein islet Number	2	5.7	12
Vein Termination Number	1	3.2	6
Stomatal number (Lower epidermis)	19	20.9	23
Stomatal index(Lower epidermis)	11.1	12.0	12.93

Table 2: Physiochemical constants.

Parameters	(% w/w)
Moisture content	6.33 ± 0.40
Total ash	4.45 ± 0.45
Acid insoluble ash	0.63 ± 0.04
Water soluble ash	1.36 ± 0.29

The macroscopical characters (size, shape, colour, odour, texture, margin, base, apex and petiole) of the leaves were ascertained[4,7].

Microscopic analysis

The required samples of leaves of the plant were cut and immediately fixed into fixative fluid- FAA, formalin + Acetic acid + 70% Ethyl alcohol in a ratio 5:5:90. Specimen was prepared according to the methods of Evans; sections were prepared and stained with Toluidine blue (polychromatic stain, which renders pink colour to the cellulose walls and blue to the lignified cells). Where ever necessary sections were prevailed with safranin and iodine. Photographs of different magnification were taken with Nikon Labphot[8,9,10].

Then powder was identified with routine reagents to study the lignified cells, trichomes, stomata, fibres etc. Quantitative microscopy like measurement of vein islet number, vein termination number, Stomatal number and Stomatal index were determined[11,12].

Physiochemical analysis

The physiochemical parameters like Ash value (total ash value, water soluble ash value, acid insoluble ash value), loss on drying, and extractive values were carried out[13]. Extractive value ascertained for individual solvents and successive solvents with increasing order of polarity[17].

Phytochemical analysis

Preliminary phytochemical investigations for secondary metabolites were conducted on different extracts obtained from leaves of *Manilkara zapota* and examined for metabolites like carbohydrates, alkaloids, glycosides, tannins, protein and amino acid, saponins[14,15].

Preliminary Acute toxicological study using brine shrimp (Brine shrimp lethality assay BSLA)

Table 3: Results for the preliminary phytochemical screening of leaf extracts of *Manilkara zapota*

Sl. No.	Tests	Petroleum ether extract	Ethyl Acetate extract	Ethanolic extract	Aqueous extract
I.	Alkaloids				
a)	Mayers Reagent	-	-	-	-
b)	Dragendorff's Reagent	-	-	-	-
c)	Hager's Reagent	-	-	-	-
d)	Wagner's Reagent	-	-	-	-
e)	Purine alkaloid Murexide test	-	-	-	-
II.	Carbohydrates				
a)	Molisch's test	-	-	-	+
b)	Fehling's test	-	-	-	+
c)	Benedict's test	-	-	-	+
III.	Glycosides				
a)	Anthraquinone Glycosides				
i)	Borntrager's test	-	-	-	-
ii)	Modified borntager's	-	-	-	-
b)	cardiac Glycoside				
i)	Keller killiani test	-	-	-	-
ii)	Raymond test	-	-	-	-
iii)	Legal's test	-	-	-	-
c)	Cyanogenetic glycoside	-	-	-	-
d)	Coumarin glycoside	-	-	-	-
IV	Sterols				
a)	Sallkowski's test	+	+	+	-
b)	Libermann – burchard	+	+	+	-
vii)	Saponins	-	-	-	+
viii)	Tannins				
a)	$Fecl_3$ test	-	-	-	+
b)	Gold beater's skin test	-	-	-	+
III	Proteins and free amino acids				
a)	Million's test	-	-	+	-
b)	Biuret test	-	-	+	-
c)	Ninhydin test	-	-	+	-
XII	Mucilages	-	-	-	-
X	Terpenoids	-	-	-	-
ix.	Flavonoids				
a)	Shinoda test	-	-	+	-
b)	Alkali test	-	-	+	-
c)	Acid test	-	-	+	-
d)	Zn-Hcl test	-	-	+	-
XI	Volatile oil	-	-	-	-
IV	Fixed oils	-	-	-	-

BSLA is a pandemic bioassay and also an index for general toxicity studies[16]. The toxicity study on leaves of *M.zapota* was performed using Brine Shrimp Lethality bio assay, based on its ability to kill laboratory cultured brine shrimp (*Artemia nauplii*). This assay was proposed by Michel (1956) later developed by Vanhaecke et al (1981) and Sleet and Brendel (1983). This assay is a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials. This method is captivating because it is very simple, affordable and low toxin mounts are sufficient to perform the test in the microwell scale.

Production of Atrtemia strain

Artemia is a non-selective filter feeder of organic detritus, micro algae and bacteria. *Artemia* are naturally found in saltpans, hypersaline lakes, coastal lagoons as well as manmade saltpans. When the cysts are inoculated in sea water for 24 hrs, the free swimming *nauplii* are hatched out.

Cytotoxic bioassay

10 *nauplii* were drawn through glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5ml of the extract was added to 4.5ml of brine solution and maintained at room temperature for 24 hrs under the light and surviving larvae were counted. Experiments were conducted along with the control, different concentration of the extract (100 to 3000 ppm) in triplicates per dose[16].

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control vials. LC_{50}

Table 4: Effect of various concentrations of leaves *M. zapota* on *Artemia Nauplii*

Concentration (ppm)	Number of Larvae released	Number of Larvae dead after 24 hrs.	Mortality	Corrrected (%) Mortality using Abbot's formula
	10	1	10	
100	10	2	20	20
	10	3	30	
	10	3	30	
200	10	2	20	23.3
	10	2	20	
	10	2	20	
400	10	2	20	26.6
	10	4	40	
	10	4	40	
600	10	5	50	43.3
	10	4	40	
	10	5	50	
800	10	5	50	50
	10	5	50	
	10	8	80	
1000	10	6	60	70
	10	7	70	
	10	10	100	
1500	10	9	90	90.66
	10	9	90	
	10	-	-	
Control	10	-	-	-
	10	-	-	

100% Mortality was observed at 3ppm level for the standard drug podophyllotoxin

value was obtained from the best fit line, plotted concentration versus percentage lethality. Podophyllotoxin was used as a positive control in the bio assay. The death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

The percentage lethality was calculated from the mean survival larvae of various extract treated tubes and control. The corrected (%) mortality was calculated by using Abbot's formula.

$$\text{Corrected Mortality (\%)} = \frac{\text{Test Mortality (\%)} - \text{Control Mortality(\%)}}{100 - \text{Control Mortality (\%)}}$$

RESULTS AND DISCUSSION

Macroscopic analysis

Leaves of *Manilkara zapota* are highly ornamental, glossy, alternate, spirally clustered at the tips of the forked twigs.

Colour :Dorsal view- Dark green, Ventral view- pale green

Size :7.5 -11.5 cm long and 2.5- 4 cm width, slender petiole

Shape :Ovate elliptic, pointed at both the ends.

Marigin :Entire or emarginated

Midrib :Prominent.

Microscopic analysis

Transverse section shows that leaf is dorsi-ventral. Epidermal cells are polygonal with straight or wavy anticlinal walls. The upper epidermis is apostomatic where else lower epidermis is stomatiferous of cyclocytic type. Lamina slightly differentiated into palisade and spongy parenchyma. The mesophyll region has druses (40µm) containing spherical bodies and calcium oxalate prismatic crystal (10-12µm). It also contains dense tannin content. (Figure 2). The leaf exhibit characteristic vascular bundle system with an *adaxial* concave strand in upper side and bowl shaped *abaxial* strand in lower side. The entire system appears shield shaped outline surrounded by thin layer of sclerenchyma. They appeared bright because of the lignified walls of the xylem and sclerenchyma boundary. (Figure 1)

Venation pattern is another character for segregating the original specimen from the adulterants. The venation architecture in leaves prove to be of importance either from a descriptive or systematic strand point of study. The vein Islets and vein termination have been shown to be species specific and this parameter can be employed for identification of co-species. Reticulate type of venation and the major lateral veins running parallel to each other were ascertained. The space between them divided into 3 or more lines of vein Islet producing rectangular (or) polygonal shape in parallel rows. The vein terminals were not distinct. Among the cell inclusion, lactiferous cells, calcium oxalate crystals and druses are readily observable characters. The plant drugs are generally used in the powdered form where the macro morphology pattern was completely destroyed, so the diagnosis of the plant through the microscopical character becomes crucial one. The powdered crude drugs can be identified based on the presence (or) absence of different cell types. In powdered microscopy, we have observed cyclocytic stomata with the round stoma and round aperature (figure 3), xylem vessels, phloem cells and lactiferous cells, abundant calcium

oxalate prismatic crystals, epidermal cells – surface view, sclerenchyma fibres, and parenchyma cells. The above features provide complementary evidence in diagnosis of the plant in powder condition. Quantitative microscopical characters like Vein islet number, Vein Termination Number, stomatal number and stomatal index were analysed and repoted in Table 1

Physiochemical analysis

Physiochemical parameters were analysed and reported in Table 2,

Phytochemical analysis

The leaves powder of *Manilkara zapota* were extracted with various solvent and subjected to preliminary phytochemical screening and presented in Table 3. The preliminary phytochemical screening reveals the presence of carbohydrates, proteins and amino acids, flavonoids, terpenoids, tannins and phytosterols. Alkaloids, glycosides, saponins, volatile oil, fixed oil were found to be absent.

Preliminary Acute toxicological study using brine shrimp (Brine shrimp lethality assay BSLA)

It is necessary to evaluate safety of botanicals before screening for various activities. Hence Brine shrimp lethality assay was applied to screen toxicity. Acute toxicological screening was carried out by using Brine shrimp Lethality assay (BSLA). BSLA is a bench top assay using sensitive brine shrimp invertebrate for the mass screening of drugs, to minimize animal usage. Investigation was imparted using ethanolic extract of leaves and shows 100% mortality in the range of 1500 ppm/ml compared to podophyllotoxin which showed lethality at 3ppm level. The values are reported in Table 4.

CONCLUSION

Pharmacognostical studies provide credentials for proper botanical identity of the plant taken for research work.. *Manilkara zapota* PKM 1 a hybrid variety showed no morphological changes except small variation in size of the leaves. Micromorphological characters shows usual leaf characters along with cyclocytic stomata with circular aperature, a venation architecture of lateral vein with rectangular (or) polygonal vein Islets. Cell inclusion includes laticiferous cells, calcium oxalate crystals and druses. Powder microscopy provides complementary evidence in diagnosis of the leaves in powder condition. Quantitative leaf microscopy and physicochemical analysis were also studied and presented, as a basis for judging the identity and purity of the drug. Preliminary phytochemical screening with various extracts indicates the presence of Carbohydrates, Proteins and Amino Acids, Flavonoids, Terpenoids, Tannins and Phyto Sterols. Alkaloids, glycosides, saponins, volatile oil, fixed oil were found to be absent. The preliminary acute toxicological study using brine shrimp lethality assay proved that ethanol extract of the leaves to be safe and non – toxic particularly to soft tissues.

REFERENCES

1. http://www.worldagroforestry.org/ useful tropical plants database, 2014 .
2. Sohini Bhowal, Rishov Mukhopadhyay, Sanjib Bhattacharya, Moulisha Biswas. Acute and sub-chronic toxicity study of *Manilkara zapota* leaf extracts in mice. American-eurasian journal of toxicological sciences, 2014; 6(3): 58-61.
3. Farnsworth N R, Morris RW. Higher plants-the sleeping giant of drug development. Am J Pharm Sci. Support Public Health. 1976; 148(2):46–52
4. Trease and evans. Pharmacognosy. 15th edition. Saunders Publications, New Delhi. 2005. Pp. 3,13
5. Sathiyabalan G, Paulpriya K, Tresina P S, Muthukumarasamy S, Mohan V R. Pharmacognostical, Physicochemical and Phytochemical Standardization of Petiveria alliacea L. International Journal of Pharmacognosy and Phytochemical Research 2017; 9(2); 233-241
6. Seedi H R E, Nishiyama S. Chemistry of Bioflavonoids. Indian J. Pharm Educ. 2002; 36(4): 191-202.
7. Kokate C K, Purohit A P, Gokhale S B. Pharmacognosy. 32th ed., Pune; Nirali Prakashan. 2005; pp. 99-100.
8. Shanti Bhushan Mishra, Alok Mukerjee, M. Vijayakumar. Pharmacognostical and Phytochemical Evaluation of Leaves Extract of Jatropha curcas Linn. Pharmacognosy Journal. 2010; 2(15): 11
9. Johansen DA. "Plant Micro technique" McGraw Hill Book Company. 1940; pp. 27-93, 126-154.
10. Purvis MJ, Collier DC, Wallis D. Laboratory Techniques in Botany. 2nd edn. Butter worth & Co Ltd. 1966: 82 -169.
11. Wallis TE. Analytical Microscopy. 3rd edn. J&A Churchill Ltd. 1965: pp. 180, 183, 184.
12. Iyengar MA, Nayak SGK. Pharmacognasy Lab Manual. 1994: pp. 78-87
13. Kokate C K. Practical Pharmacognosy. New Delhi; Vallabah prakashan:1994, pp. 54
14. Harborne J B. Phytochemical Methods. London; Chapman and Hall: 1973, pp. 56,71
15. Wallis T E. Practical Pharmacognasy. 6th ed., J& A Churchill Ltd: 1953, pp. 170-175
16. Lilybeth F. Olowa and Olga M. Nuñeza. Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. International Research Journal of Biological Sciences, 2013; 2(11): 74-77.
17. Ramasubramania Raja R, Sreenivasulu M, Lakshmi Priyanka S, Lavanya K, Mohammad Anees. Phytochemical screening and pharmacognostical evaluation of collected crude drug of pongamia pinnata (fabaceae) leaf. European journal of pharmaceutical and medical research, 2016; 3(9): 236-242.