International Journal of Current Trends in Pharmaceutical Research

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Research Article **Open Access**

Pharmacognostic and Phytochemical Evaluation of Leaves of *Manilkara hexandra* (Roxb.) Dubard

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A B S T R A C T

The term pharmacognosy, a constituent scientific discipline of pharmacy, has been in use for nearly 200 years and it refers to the studies on natural product drugs. The present study deals with the macroscopic and microscopic studies of *Manilkara hexandra* (Roxb.) Dubard leaf is commonly known as Rayan, traditionally used as antioxidant, antimicrobial, immune stimulant, anti ulcer, anti bacterial and antidiabetic. In the present work fresh leaf was studied for pharmaconostic evaluations, including examination of morphological and microscopic characters, physicochemical parameter, phytochemical test, extractive values and fluorescence study. The anatomy of the leaf was studied by taking transverse section which showed anomocytic stomata, unicellular trichomes, spongy parenchyma etc. Powder microscopic examination showed presence of pericyclic fibers, spiral xylem vessels, phloem fiber and stomata. Physicochemical parameters and fluorescence analysis of the powder and extract were also carried out. The phytochemical test revealed the presence of alkaloids, glycosides, tannins, flavonoids and saponin in methanol extract. The present investigation on *Manilkara hexandra* leaf might be useful to supplement information in regard to its identification parameters. Such studies are important in the way of acceptability of herbal drugs in present scenario of lacking regulatory laws to control quality of herbal drugs.

Keywords: *Manilkara hexandra*, Pharmacognostic evaluation, phytochemical test, Pharmacognosy, Anomocytic stomata

A R T I C L E I N F O

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Article History: Received 26 April 2015, **Accepted** 30 May 2015, **Available Online** 15 July 2015

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Manuscript ID: IJCTPR2571

PAPER-QR CODE

Citation: Ekta D. Patel, et al. Pharmacognostic and Phytochemical Evaluation of Leaves of *Manilkara hexandra* (Roxb.) Dubard. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(4): 984-988.

1. Introduction

Pharmacognosy has always been a translational or multidisciplinary science, most recently emphasized in the discussion of modern pharmacognosy, as described by Bohlin and co-workers [1]. In the last few decades there has been an exponential growth in the field of herbal medicine. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [2].

In older time, vaidyas used to treat patients on individual basis and prepared drugs according to the requirement of the patients. But the scene has been changed now herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems regarding the identity and purity [3,4]. *Manilkara hexandra* (Roxb.) Dubard (Syn: *Mimusops hexandra* Roxb.) (Sapotaceae) is a large evergreen tree widely distributed throughout the India and other tropical countries (Fig 1 a & b). The leaf is immune stimulant, antioxidant, antimicrobial, anti ulcer, anti bacterial and antidiabetic [5].

The therapeutic activity of herbs is because of constituents present in them and varies because the chemical constituents vary from various sources. Correct identification of the starting material is therefore an essential prerequisite to ensure reproducible quality of herbal medicine which contributes to its safety and efficacy. The present work is aimed with the pharmacognostical, physicochemical and fluorescence analysis of *Manilkara hexandra* (Roxb.) Dubard leaf [6].

Figure 1: (a) Plant Manilkara hexandra (Roxb.) Dubard
(b) Leaf of Manilkara hexandra (Roxb.) Dubard

2. Materials and Methods

Plant material authentication

The mature green leaves of *Manilkara hexandra* (Roxb.) Dubard. Were collected in the morning locally from Gandhi nagar District, Gujarat, India in the month of June 2013. The plant was identified and authenticated by the botanist, from the National institute of science communication and information resources, New Delhi, India. A voucher specimen (NISCAIR/RHMD/Consult/ 2013/ 2256/37) is deposited in NISCAIR, New Delhi. The fresh plant material collected was thoroughly cleaned. It was then homogenized to fine powder and stored in air-tight bottles for further studies.

Pharmacognostic studies

Macroscopy

Morphological observation of *Manilkara hexandra* leaf was done. It includes shape, size, surface characteristics, apex, base, margin, color, odour, taste etc. by using simple microscope.

Microscopy

Microscopic studies were done by preparing a thin transverse section of the leaf of *Manilkara hexandra* (Roxb.) Dubard. The section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid and mounted with glycerin. Powder of the dried leaves was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol and HCl solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals, trichomes and starch grains [7].

Quantitative microscopy

Physicochemical parameters were determined as per guidelines of WHO [2]. Total ash, water insoluble ash and loss on drying were determined. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble components [8,9].

Extraction and preparation of plant extract

The leaves were air-dried at room temperature for a week and powdered by grinder. Five hundred grams of the powered plant material was taken and extracted with methanol using Soxhlet extraction method. Extracts were collected, dried, weight and percentage yield was calculated. The extract was stored in a desiccator at room temperature.

Fluorescence analysis

Powdered leaf parts and methanolic extract was subjected to analysis under day/visible light and ultra violet light after treatment with various chemical and organic reagents [10].

Phytochemical screening

The crude methanolic extract of *Manilkara hexandra* was screened phytochemically for the presence of constituents by utilizing standard methods for phytochemical test [11, 12, 13].

3. Results and Discussion

The Pharmacognostic study is the major and reliable criteria for identification of plant drugs. The Pharmacognostic parameters are necessary for confirmation of the identity of the crude drug. The detailed and systematic pharmacognostic evaluation would give valuable information for future studies.

Morphological evaluation

The morphological studies revealed that the leaf is dark green in colour with characteristic odour & slight bitter taste (Table 1).

Table 1: Identification of morphological feature

S .No	Features	Observation
1	Colour (Upper surface)	Dark green colour
2	Colour (Lower surface)	Light green colour
3	Odour	Aromatic
4	Taste	Bitter
5	Shape	Ellipticle
6	Size	7-12 cm
7	Arrangement	Alternate

Macroscopical study:

The leaves are alternate, coriaceous, elliptic, oblong or obovate, emarginated at apex, glabrous, dark green in colour (Table 2).

Table 2: Botanical evaluation of *Manilkara hexandra* Leaf

S. No	Leaf Portion	Observation
1	Apex	Emarginate
2	Margin	Entire
3	Shape	Ellipticle
4	Venation	Reticulate Pinnate
5	Midrib	Continuous from base to apex
6	Dorsal Surface	Glabrous
7	Ventral Surface	Hairy
8	Petiole	Petiolate (Approx 1 cm)
10	Petiole Shape	Cylindrical
11	Colour	Olive Green
12	Leaf Base	Symmetrical

Microscopical study

The microscopic studies of leaves showed following character (Table 3). The leaf was dorsiventral. It was divided in to Lamina and Midrib portion (Figure 2a).

Midrib

The T.S. of midrib showed arc shaped collateral vascular bundle in which xylem towards the dorsal side and phloem towards the ventral side. The thick walled collenchymatous cells were present below the upper epidermis and above the lower epidermis. The xylem was lignified while phloem was non-lignified. Thick walled pericyclic fibers containing sclerenchymatous cells were present surrounding the vascular bundle (Figure 2b).

Lamina

There was single layered, straight walled upper epidermal cells covered with thin cuticle. Lower epidermis was single layered with wavy walls with anomocytic stomata and simple, unicellular, conical covering trichomes. The lower epidermis shows more number of trichomes and stomata than the upper epidermis (Figure 2c). Single layer of elongated palisade cells were present below the upper epidermis and spongy parenchymatous cells were present above the lower epidermis.

Table 3: Transverse section of leaf

Sr. no	Features	Observation
1	Trichomes	Simple, unicellular, conical and thick trichomes with single covering
2	Upper epidermis	Present
3	Mid rib	Thick walled collenchyma cells
4	Lamina (Mesophyll)	Differentiate in to palisade and parenchymatous cells
5	Vascular bundle	Collateral. i.e. xylem and phloem were present on the same radius side by side. The xylem was lignified while phloem was non-lignified.
6	Sclerenchyma cell	Pericyclic fibers containing sclerenchyma cells
7	Stomata	Anomocytic stomata

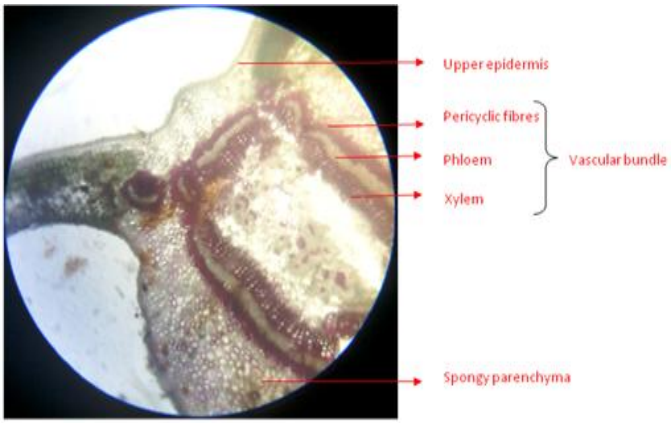

Figure 2a: Transverse section of leaf of *Manilkara hexandra* (Roxb.) Dubard.

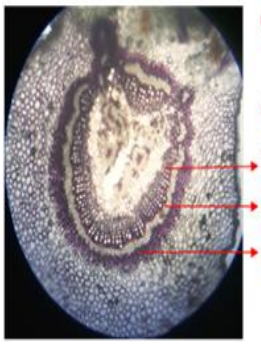

Figure 2b: Midrib portion

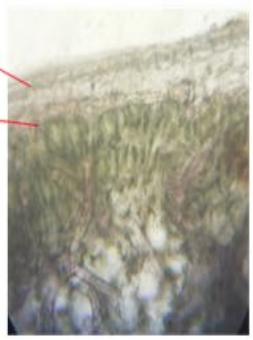

Figure 2c: Lamina portion

Powder microscopy

The crude powder study of *Manilkara hexandra* (Roxb.) Dubard leaf was dark green in colour with characteristic odour and slightly bitter in taste. Microscopy study of powder showed the presence of upper epidermis with straight walled cells, lower epidermis showed wavy walled cells with anomocytic stomata, simple covering unicellular trichome, group of pericyclic fibers and spiral xylem vessels (Figure 3).

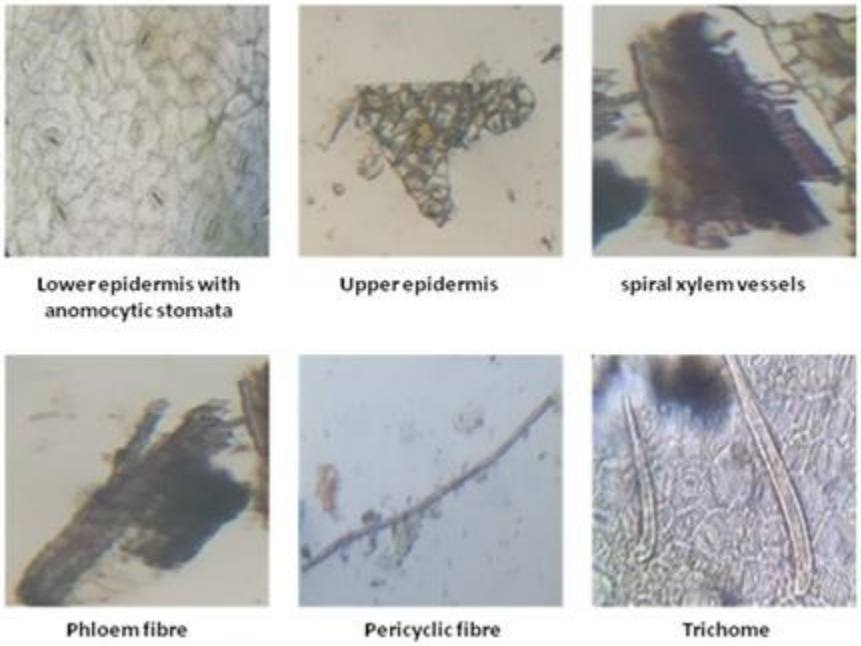

Figure 3: Microscopic character of powder of *Manilkara hexandra* (Roxb.) Dubard leaf

Table 4: Powder microscopy

S. No	Features	Observation
1	Nature	Coarse powder
2	Colour	Light green
3	Odour	Aromatic
4	Taste	Bitter
5	Covering Trichomes	Present
6	Xylem Vessel	Present (lignified)
7	Epidermal cell	Present
8	Stomata	Present (anomocytic)
9	Fibres	Present

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica [14]. The results of physicochemical parameter analysis of crude powder of *Manilkara hexandra* leaf were shown in Table 5. The average values are expressed as percentage of air-dried material.

Table 5: Data representing physiological parameter

S. No	Parameters	Value (%) w/w
1	Loss on drying	3.6 %w/w
2	Total Ash	6.4 %w/w
3	Acid insoluble Ash	1.4 %w/w
4	Water extractive value	13.1 %w/w
5	Methanolic extractive value	10 %w/w

The fluorescence analysis of the powder drug was observed in day/visible light and UV light (Table 6). The qualitative chemical test revealed the presence of alkaloids, glycosides, tannins, flavonoids and saponin in both extract.Oil is absent in methanolic extract (Table 7).

Table 6: Fluorescence analysis of powder drug

Chemical	Day Light	254 nm	365 nm
Powder	Dull green	Yellow green	Brown
Water	Dull green	Yellow green	Brown
50% HCl	Pale green	Pale green	Dark brown
50% H_2SO_4	Pale green	Pale green	Brown
1N NaOH in methanol	Red brown	Pale green	Brown

1N NaOH in water	Yellow green	Dark green	Brown
50% HNO_3	Brown	Dark green	Brown
Petroleum ether	Green	Pale green	Brown
$CHCl_3$	Pale green	Yellow	Red fluoroscenes
Picric acid	Yellow	Green	Brown
5% $FeCl_3$	Pale green	Yellow green	Brown
5% I_2	Yellow green	Yellow green	Brown
HNO3+Ammonia	Light green	Light green	Brown
Glacial Acetic Acid	Light green	Yellow green	Brown
5% KOH	Light brown	Yellow green	Brown
Methanolic Extract	Dark green	Dark green	Brown

Table 7: Data representing phytochemical screening

Plant constituent	**Test**	**Methanol extract**
Alkaloid	Dragendorff	+
	Wagner	+
Steroid	Salkowaski	+
Carbohydrate	Molish	+
Tannin	$KMnO_4$	+
	Br_2 water	+
Flavonoid	Shinoda	+
	Lead Acetate	+
Saponin	Foam Test	+
Cardiac glycoside	Keller Killani	+
Oil	Filter paper	–
	Water solubility	–

4. Conclusion

The morphological and microscopical investigations become useful elements for the quality and identification of the drugs and this is of great interest for quality control in basic research and manufacturing of ayurvedic preparation especially when raw material sold by traditional herbalists. Such studies are important in the way of acceptability of herbal drugs in present scenario of lacking regulatory laws to control quality of herbal drugs. The detailed and systematic pharmacognostic evaluation would give valuable information for future studies.

5. Acknowledgements

The authors are thankful to Ms. Kunjal Vegad for kind support and the Director, Sharda school of pharmacy, Gandhinagar for providing all the facilities for carrying out this work.

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