



RESEARCH ARTICLE

Anatomical Studies in the Leaves of *Hydnocarpus pentandrus* (Buch.Ham) Oken.

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Abstract

Hydnocarpus is a genus of flowering plant, commonly called chaulmoogra, belonging to the family Achariaceae. The folklore claims the use of the whole plant for curing various ailments. The oil extracted from seeds of *Hydnocarpus pentandrus* (Buch-Ham.) Oken. contains secondary metabolites such as glycosides, carbohydrates, proteins, tannins, saponins etc. The leaves are used for cardiac problems, hepatic problems, otalgia, and eruptions. Standardization is an essential measure for quality, purity & sample identification. Anatomy is very useful in the study of plant materials. Macroscopic characters, along with microscopy is one of the simplest & cheapest methods to start with for establishing and correct identity of the source materials. In the present study the sections of leaf, petiole & stem, leaf architecture, types of stomata, stomatal frequency, stomatal index of the leaf of *Hydnocarpus pentandrus* was undertaken. It showed pinnate camptodromous with festooned brochidodromous type of venation and anomocytic type of stomata. This study will be helpful in correctly identifying the plant materials which are of medicinal importance.

Keywords: *Hydnocarpus pentandrus*, Anatomy, Leaf architecture, Stomatal frequency, Stomatal index.

Introduction

Hydnocarpus pentandrus (Buch. -Ham) Oken. synonym - "Chaulmoogra" belongs to the family Achariaceae. It has about 31 genera and 197 species of trees and shrubs that are distributed in tropical and subtropical regions of Africa, Asia, and the Americas. It is Predominantly found in moist deciduous and semi-evergreen forests up to 850 m (about 2788.71 ft) in the Western Ghats, often near water bodies. In India, it is most commonly found in Maharashtra, Daman & Diu, Goa, Karnataka, Tamil Nadu and Kerala, but it is

also cultivated elsewhere (Hooker 1883). It is commonly known as, *Hydnocarpus* oil tree, chaulmoogra, kuduresotte, Garudaphala, Tuvarak etc. (Joshi and Harijan 2014). It is the largest family in the world. *Hydnocarpus pentandrus* is a medium sized evergreen deciduous tree, grown up to 15m height, bark is pale brown, mottled with occasional white patches. Leaves are simple, alternate, and stipulated. base obtuse and apex acuminate. Usually oblong to elliptic-oblong, tip pointed, the flowering takes place in the month of January to April. Flowers are borne in short cymes or solitary, in leaf axils. petals are white. The berry is woody, round 6-10 cm across usually brown tomentose, black when young, seeds numerous. Flowers are greenish white in color and grow solitary or racemes. Fruits are globose, ovoid, tomentose, rigid, protruding at apex 5-7 cm in diameter; pericarp reddish brown, scurfy, tomentose. Seeds are 15-20, ovoid-oblong, longitudinally striated, immersed in pulp. These sources emphasize the genus's effectiveness in caring for maladies such as Hansen's disease, dermatological conditions, abdominal distension with constipation, hemorrhoids, sciatic conditions, cervical lymphadenitis, abdominal lumps, fever, itching, worm infestations, inflammatory skin diseases, metabolic disorders, partial intestinal obstructions and ulcers (Varghese B *et al* 2016, David 2014).

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Materials And Methods

The plant material of *Hydnocarpus pentandrus* for the present work was collected from Dapoli, Chandra Nagar, Maharashtra, India (17°44'36.3"N 73°09'39.1"E) & authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai (Accession no.- R3060).

Macroscopy of Leaves

The morphological characters visible to naked eyes or magnifying glass of the whole plant parts were observed and described (Anonymous 2001).

Microscopy of Leaves

Free hand cut sections were taken from freshly collected material. Sections were stained with safranin and observed under an Olympus compound microscope and microphotographs were taken with the help of a mobile camera at 10X magnification (Fahn 1997, Jackson and Snowdon 1990).

Leaf Venation

For leaf clearing the method used was as described by Payne (1969) & Mohan Ram and Nayyar (1978). For the study of stomata, the method was described by Gupta (1961). The terminology used in anatomical studies is in accordance with Hickey and Wolfe (1975), Melville & Hall (1976 & 1954), Hickey (1973, 1979) and Dilcher (1974) (Figure 1).

Stomatal index

Stomatal index is the average number of stomata per square millimeter of epidermis. The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomatal index (Table 1 and Figure 2).

Stomatal Frequency

Stomatal frequency can be defined as the number of stomata per unit area of the leaf. Stomatal frequency is measured by the impression method (Weyers and Jonathan 1990).

Results

Leaf venation

Leaf organization is simple. With respect to leaf shape & size, the length of the whole leaf is 101mm and width is 42 mm. The lamina is symmetrical, base is symmetrical, form is elliptic, apex is acute, and base is acute normal. The margin is entire. The leaf texture is glabrous. There are no glands, and the petiole is normal.

The type of venation is pinnate camptodromous with festooned brochidodromous. Primary vein (1°) is massive; its course is straight and unbranched. Secondary vein (2°) is present; angle of divergence is acute moderate. The variation in the angle of divergence is nearly uniform. The relative thickness of secondary veins is moderate; its course is curved abruptly and unbranched. Intersecondary veins composite. Intramarginal vein is absent. Tertiary veins (3°) are present; angle of origin exmedial to admedial side is RR/AR/OR pattern is orthogonal reticulate. The higher order venation forms a reticulum in which vein orders are distinct. Quaternary veins (4°) are thin; its course is orthogonal. Quintenary veins (5°) are thin; its course is orthogonal. The highest vein order of leaf is 5°. The marginal ultimate venation is looped. Areoles are well developed; the arrangement is random, and shapes are quadrangular, pentagonal and polygonal.

Quantitative Microscopy

Analysis of leaf constant unveiled a stomatal index of 15.99% for leaf apex, 18.26% in the middle lamina and 19.38% in leaf base whereas stomatal frequency found in the portion of leaf apex was 161.11mm in the middle lamina of leaf was 190.72mm² & in the base it was 198.16 mm². Anomocytic type of stomata was found in the upper and lower epidermis (Photo plate-II).

Microscopic Characters

T.S. of leaf

The transverse section of the leaf is dorsiventral. The section is broadly observed in the midrib & lamina of the leaf (Figure 3).

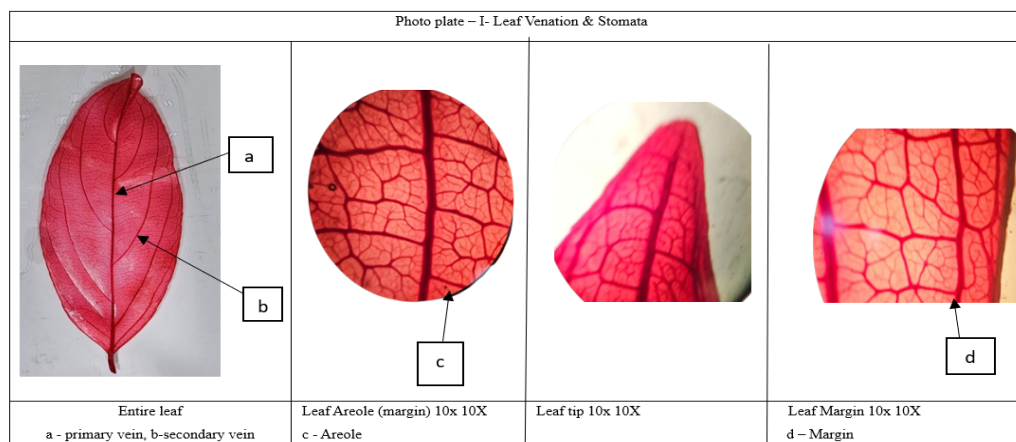
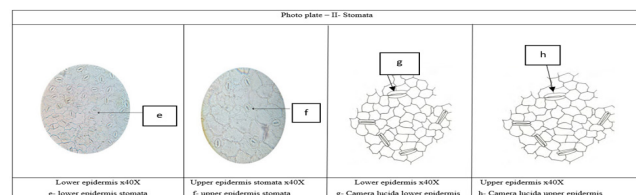
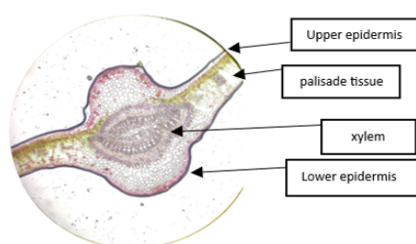
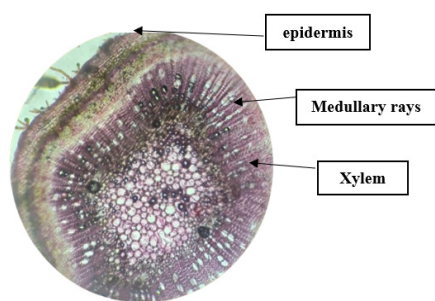


Figure 1: Leaf venation of *Hydnocarpus pentandrus*

Table 1: Leaf constants of *Hydnocarpus pentandrus*

S. No	Parameters	Mean value $\pm$ SD
1	Stomatal Index	52%
2	Stomatal frequency	19.6 mm ²

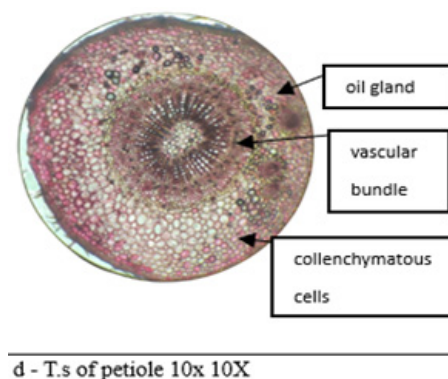
**Figure 2:** Stomata of *Hydnocarpus pentandrus***a -T. S of leaf passing through midrib 10X 10x****Figure 3:** T.S of leaf passing through midrib 10X10x**C- T.s of stem 10x 10X****Figure 4:** T.S of stem 10X10x

Midrib

Plano-convex, adaxial surface bulged, epidermal cells orbicular to barrel shaped with an even cuticle, adaxial surface hemispherical, epidermal cells orbicular with a papillate cuticle. Cortex homogenous with prismatic, rosette and druses types of crystals, vascular bundle collateral, single, urn shaped, bundle sheath is sclerenchymatous.

Lamina

Adaxial epidermal cells orbicular to barrel shaped, cuticle even, abaxial epidermal cells barrel shaped, cuticle even. heterogenous mesophyll; veinlets circular; bundle sheath sclerenchymatous; crystals rosette and druses present.

**d - T.s of petiole 10x 10X****Figure 5:** T.S of petiole 10X10x

T.S. of stem

Double layered epidermis is seen with cuticles. It also shows the presence of hypodermis which is differentiated in two zones i.e., chlorenchymatous and sclerenchymatous. Cortex is crushed. Primary vascular bundles are conjoint, collateral and arranged in a ring. Origin of xylem is endarch. Secondary growth is seen where a ring of xylem is formed with uniseriate medullary rays. The pith shows the presence of large parenchymatous cells along with which starch grains are present. In the older stem the pith disintegrates, making the stem hollow (Figure 4).

T.S. of petiole

Petiole circular to ovate, epidermal cells barrel shaped, cuticle evenly thick, heterogenous cortex, collenchyma layer 15-25 cell thick, parenchyma 4-6 layered, vascular bundle single and collateral, bundle sheath is sclerenchymatous, crystals druses, unicellular, non-glandular trichomes present (Figure 5).

Summary

The plant *Hydnocarpus pentandrus* is used for curing various illnesses. The flowers are known as "Chaulmoogra". For the correct identification, the gross macroscopical study is of great value. Type of venation is Pinnate camptodromous with festooned brochidodromous. With respect to leaf shape & size, the length of the whole leaf is 101mm and width is 42 mm. Anomocytic types of stomata were observed. In contrast to the stomatal frequency observed in the area of the leaf apex, which was 161.11 mm², the middle lamina, which was 190.72 mm², and the base, which was 198.16 mm². the analysis of the leaf constant revealed a stomatal index of 52% Anatomical sections of leaf shows that the petiole has single layered epidermis with thin cuticle, 2 - 3 layered collenchymatous hypodermis; 3-more layered thin-walled parenchymatous cells; single, bicollateral, crescent shaped vascular bundle in centre. The lamina has single layered epidermis on both the surfaces, single layered palisade followed by 3-4 layered spongy parenchyma. Stem has cork cambium, primary vascular bundle & internal phloem

with cambial ring. Xylem with fibre, wood parenchyma predominantly has paratracheal parenchyma. The said investigations will be useful in bringing these less known crude drugs to manifold. Detailed Phytochemistry and pharmacological studies are in progress.

Discussion

The microscopic characters, leaf venation, T.S of leaf, stem & petiole was studied here. It can be used to test for the purity of this drug. Since these parameters studied are constant and any change in these values are indicative of substitution and purity of the plant, *Hydnocarpus pentandrus*.

Macro & Micro morphological standards discussed here can be considered as identifying parameters to substantiate the drug.

Leaf architectural study is found to be useful for taxonomic purposes. Vein islets area as criteria for classification was first applied by Levin F. A (1929). Melville and Hall (1976) proposed veinlet termination number as a technique for testing the purity of fragments of a particular leaf type for pharmacognostical properties. Rao (1957); De Roon (1967); Rao and Das (1979) presented a resume on the morphological features of tracheoids. This may serve as a diagnostic feature in certain species. According to Metcalfe and Chalk (1950) and Kasapliligil (1951) the stomata are paracytic in Lauraceae. Ferguson and D K (1974) reported anomocytic type of stomata in the fossil leaves of the genus *Laurus*. Role of anatomy in the study of stomata in ten members of the family Arecaceae has been studied by Vaidya (2016) along with study of trichomes in some species of *Litsaea* (2016a). Anatomical studies of the medicinally important plant *Ruta graveolens* have been studied by Vaidya & Shingadia (2015). Leaf architecture in actinodaphne and *Cryptocarya* species has been studied by Chodankar & Vaidya (2022). Leaf venation Anatomy of family Lauraceae was studied by Chodankar & Vaidya (2021). Leaf architecture pattern in some members of Cucurbitaceae was studied by Vaidya & Mhatre (2021). Use of anatomy and pharmacognosy in the study of *Punica granatum* L. leaves was carried out by Vaidya & Roy (2019). Anatomical study of *Euphorbia hirta* L. was studied by Acharya & Vaidya (2023).

Conclusion

Since the plant studied is difficult to identify based on the morphological key, the present work will be a helpful tool for identification of species and play an important role in the use of anatomy in taxonomy. Here, the data gathered was helpful for further pharmacological and therapeutic evaluation of the plant material. Leaf architectural study is found to be useful for taxonomic purposes. Ayurveda is not being accepted worldwide even though it is utilized in India, there is a lack of consistency. Considering that over 7500 species are utilized in medicine, the current study is only a modest step in this direction.

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