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# Chemical Screening and Anatomical Investigation of *Hydnocarpus macrocarpa* (Bedd.) Warb. (*Achariaceae*)

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## Abstract
*Hydnocarpus macrocarpa* contained Alkaloids, Flavonoids, Carbohydrates, Glycosides, Steroids, Phenols, Proteins, Tannis, Saponins, Terpenoids, fixed oil, trace elements and many other chemical groups. It possessed antioxidant, antimicrobial, anticancer, anti-inflammatory, and many other pharmacological effects. This paper will highlight the phytochemical screening, FT-IR, GC-MS and anatomical studies of *H. macrocarpa.* Microscopic studies revealed the presence of stomata unicellular or uniseriate covering, calcium oxalate crystals. This observation of cellular level anatomy is a major aid for the authentication of drugs. These studies will contribute further investigation on this plant.
**Keywords**
*Hydnocarpus macrocarpa*. Phytochemical profile, FT-IR, GC-MS, Microscopically studies.

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## INTRODUCTION
*Hydnocarpus macrocarpa* is a large, evergreen tree. The tree is probably harvested from the wild for its seeds, known for medicinal uses. It is an endangered tree confined to southwest India. The prime habitat of the tree has been severely fragmented because of the Kodayar Hydroelectric Project and the establishment of plantation crops. Outlying tree populations exist further north towards Annamalai. The fruits with medicinal properties are stimulants of respiration and enable digestion. In excess, however, it can cause respiratory failure and even death.

Habitat : An understory tree in low and medium elevation wet evergreen forests at elevations up to 1,000 metres Properties.

| | |
|---|---|
| **Medicinal Rating** | ++ |
| **Habit** | Evergreen Tree |
| **Height** | 15.00 m |
| **Pollinators** | Insects |
| **Cultivation Status** | Wild |

Secondary metabolites in plants are in general useful for pharmacological uses. The alkaloids, terpenoids, steroids, phenols, tannins, flavonoids, and other metabolites have medicinal properties. Plants are considered as the main source of food and rich nutrients. Traditional societies around the world had experimental knowledge of various plants and their medicinal value, though they did not possess knowledge on components present and their mode of action. In recent times there is intense research on medicinal properties of various herbs.

As said earlier in H. *macrocarpa* leaves and fruits are used as stimulants respiration and improved digestion, it is also claimed to be of benefit in the treatment of cancer. Seed powder is used against constipation, irritation other skin disease. The oil of *H. macrocarpa* plays the greatest role in medicinal field.

## MATERIALS AND METHODS

### Collection and Authentication

The plant was collected from Kerala state, India, during April 2017, and was identified by Dr. S.John Britto, Director and Head, The Rapinat Herbarium and Center for Molecular Systematics St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen RHT68237 was deposited at the Rapinat Herbarium.

### Preparation of plant extracts

20 gms of leaf powder was taken in an aspirator bottle; 150 ml of Acetone, Ethanol, Methanol and Aqueous were used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled.

### GC-MS analysis in leaf

For the GC-MS analysis, the concentrated acetone extracts of the selected plant materials were used, and was carried out at Department of Applied Chemistry, Cochin University, Kerala. The conditions and specifications were after the approved protocol with some modification in temperature. GC-MS analysis was carried out on an Agilent-7890 GC-MS System comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing given conditions. The oven temperature was programmed from 100°C (isothermal for 2 min), with an increase of 10°C/min up to 200°C, then 5°C/min up to 250°C, ending with a 9 min isothermal at 250°C. The samples were diluted to 1/10 with ethanol and 10µl of the diluted sample was injected using automatic injector (Agilent). Mass spectra of the samples were taken with GC/MSD ChemStation Software at 70eV with a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown compound was compared with the spectrum of the known compound stored in the NIST library. The names, molecular formula, molecular weight and molecular structures of the compounds of the test extracts were ascertained from the databank of PubChem and ChemSpider.

### FT-IR analysis

The powdered materials of the selected plants (section 4.2) were used for the FT-IR analysis, which was carried out at Archbishop Casimir Instrumentation Centre (ACIC) of St. Joseph's College, Trichy. The Potassium Bromide (KBr) technique and procedure of was adopted. The powdered samples were ground in an agate mortar and pestle in order to obtain fine powder. Each powdered plant material was mixed with completely dried potassium bromide (ration of 1/100), and the mixture was subjected to a pressure of 5x106 pa in an evacuated die to produce a KBr pellet for FT-IR spectrometric analysis.

The FT-IR spectrum of each sample was recorded with Perkin Elmer FT-IR Spectrum RX1. The pellets of the sampled plants were scanned at room temperature (25±2°C) at spectral range of 4000-400cm$^{-1}$. For the noise reduction of each spectrum, the spectral resolution was set to 4.0cm$^{-1}$. The number of scans was adjusted to 15 times to obtain optimum results. The spectrum of each sample was recorded with the software Spectrum version 5.0.2. Background spectra collected under identical conditions were subtracted from the sample spectrum. Interpretations of the peaks obtained in the spectrum were done by referring to standard FT-IR tables for assigning corresponding functional groups.

### Microscopic - Anatomical Studies

The fully matured leaf and stems were preserved in fixative solution FAA (Formalin-5ml +Acetic acid -5ml +70% ethyl alcohol-90ml) for more than 48 hours. The preserved specimens were cut into thin transverse section and cross section using sharp blade. The sectioned samples were observed in digital microscope attached with computer system for the distinguishing characters of the tissue system of leaf and stem. For the anatomical studies fresh plants and tissue culturally produced plants were used.

## RESULT AND DISCUSSION

The result of GC-MS analysis leads to the identification of sixty eight (Fig.1) phytochemical compounds from acetone extracts namely Geranyl acetone, Neophytadiene, phytol, isomer Isocaucolol, Lauric acid, Fernesyl acetone, Palmit aldehyde, Pentacosane, 2,6,10,15,19,23-Hexamethyl, Beta-Tocopherol, Pentatriacontane, Gamma-Tocopherolex etc. (Table-1). These compounds are used as anti-inflammatory, antioxidant, antimicrobial, anticancer, anti-inflammatory, and many other pharmacological effects.

The FT-IR spectrum was used to identify the functional group of the active components. The outcome of FT-IR functional groups is represented in Table-2. The FT-IR spectrum profile is illustrated (Fig-2). The powder of *Hynocarpus macrocarpa* (leaf) exhibited eight peaks with eight characteristic bands in the FT-IR analysis of the sample. The highest band was present at 3420.07cm$^{-1}$ indicating the presence of two compounds namely, alcohols and phenols (O-H stretch, H-bonded) and followed by 2976.29cm$^{-1}$ indicating the presence of corboxylic acid(O-H stretch), 1570.08cm$^{-11}$ indicating the presence of aromatics (C–C stretch (in-ring), 925.52cm$^{-}$ corboxylic acid(O-H bend), 780.16 cm$^{-1}$ 1°,2° amins (N-H wag), 878.27cm$^{-1}$ aromatics (C-H "oop"), 650.66cm$^{-1}$ alkyl halides (C-CL stretch) and 621.50cm$^{-1}$(–C⍰⍰ C–H: C–H bend).

The results of qualitative screening of phytochemicals of *H. pendandra* leaf showed the presence of Secondary metabolites. High concentrations of phytochemicals were found in methanolic, ethanolic, acetone and aqueous extracts while a very low concentration in chloroform and petroleum ether extracts.

**Microscopic study of the Plant Anatomy**

*Leaf:* Leaf is isobilateral and consists of upper- and lower-layers epidermis, covered with cuticle, anamositic stomata. Trichomes and stomata are present on both sides. *Mesophyll*: palisade and spongy parenchyma are compactly arranged, thin walled, and seeds play the vital role for the choolmogra oil production. *Xylem* towards upper side and phloem towards lower side surrounded by bundle sheath; xylem consists of vessels and phloem with sieve tubes and companion cells. *Sclerenchyma* cells in patches on both sides of mid rib region to give mechanical support of the vascular bundles. *Vascular bundles:* conjoint and collateral, xylem exarch, protoxylem towards endodermis, metaxylem towards pith. *Phloem* composed of sieve tubes and phloem parenchyma. *Pith*: parenchymatous filled with starch grains. It is shown in Fig: 3.

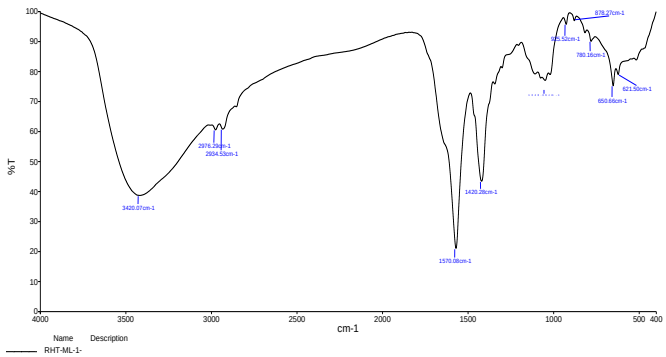
**Powder Drug Microscopy:**

Leaf was with epidermal cells, cortical tissue, scalariform, spiral and pitted vessels. Leaf powder contained paracytic stomata, vascular tissues and epidermal cells. (Fig: 4)

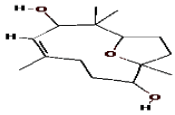
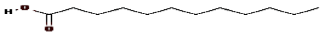
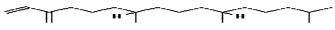
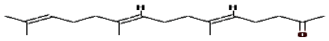
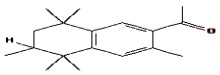
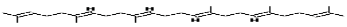
**Spectroscopic data of *Hydnocarpus macrocarpa* (leaf)** Table: 2

| S.No. | Name of the bond | Functional Group | Stretching Frequency (cm$^{-1}$) |
|---|---|---|---|
| 1 | O–H stretch, H–bonded | alcohols, phenols | 3423.07 |
| 2 | O–H stretch | Carboxylic acid | 2976.27 |
| 3 | C–C stretch (in ring) | Aromatics | 1570.08 |
| 4 | O-H bend | Carboxylic acid | 925.52 |
| 5 | N-H wag | 1°, 2° amines | 780.16 |
| 6 | C-H "oop" | Aromatic | 778.27 |
| 7 | C-CL stretch | Alkyl halides | 650.66 |
| 8 | –C⍰⍰ C–H: C–H bend | Alkynes | 621.50 |

**Fig 2. FT-IR Spectrum**



**GCMS Table: 1**

| S.No | Name | Molecular Formula | Molecular Structure | Molecular Weight | Retention Time |
|---|---|---|---|---|---|
| 1 | Geranyl acetone | $C_{12}H_{20}O_2$ |  | 196.290g/mol | 8.984 |
| 2 | Isocaucolol | $C_{15}H_{26}O_3$ |  | 254.37g/mol | 10.308 |
| 3 | Lauric acid | $C_{12}H_{24}O_2$ |  | 200.322g/mol | 10.391 |
| 4 | Neophytadiene | $C_{20}H_{38}$ |  | 276.524g/mol | 13.665 |
| 5 | Fernesyl acetone | $C_{18}H_{30}O$ |  | 262.437g/mol | 14.634 |
| 6 | Phytol | $C_{20}H_{40}O$ |  | 296.539g/mol | 17.339 |
| 7 | Palmitaldehyde | $C_{16}H_{32}O$ |  | 296.539g/mol | 17.758 |
| 8 | Pentacosane | $C_{25}H_{25}$ |  | 352.691g/mol | 23.101 |
| 9 | 2, 6, 10, 15, 19, 23-Hexamethyl | $C_{18}H_{26}O$ |  | 258.405g/mol | 30.596 |
| 10 | 2, 6, 10, 14, 18, 22-Tetracosahexaene | $C_{30}H_{50}$ |  | 410.730g/mol | 30.596 |

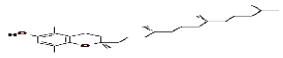
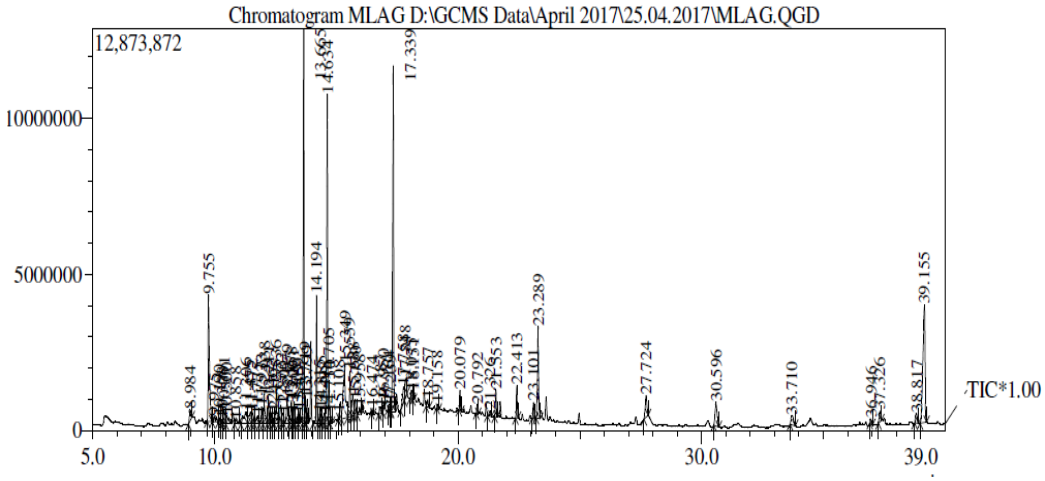
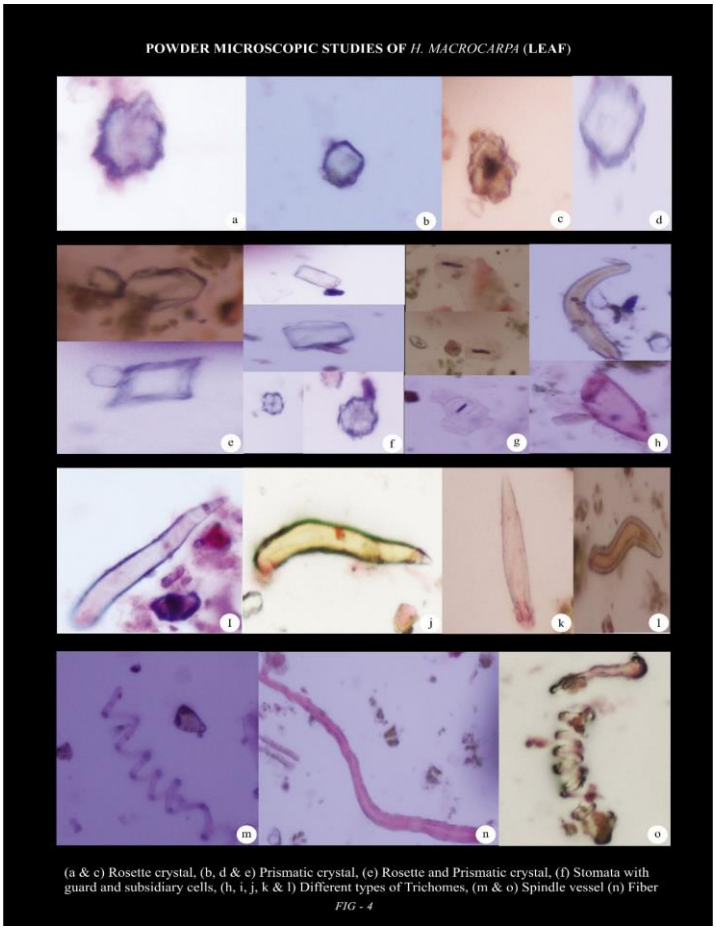
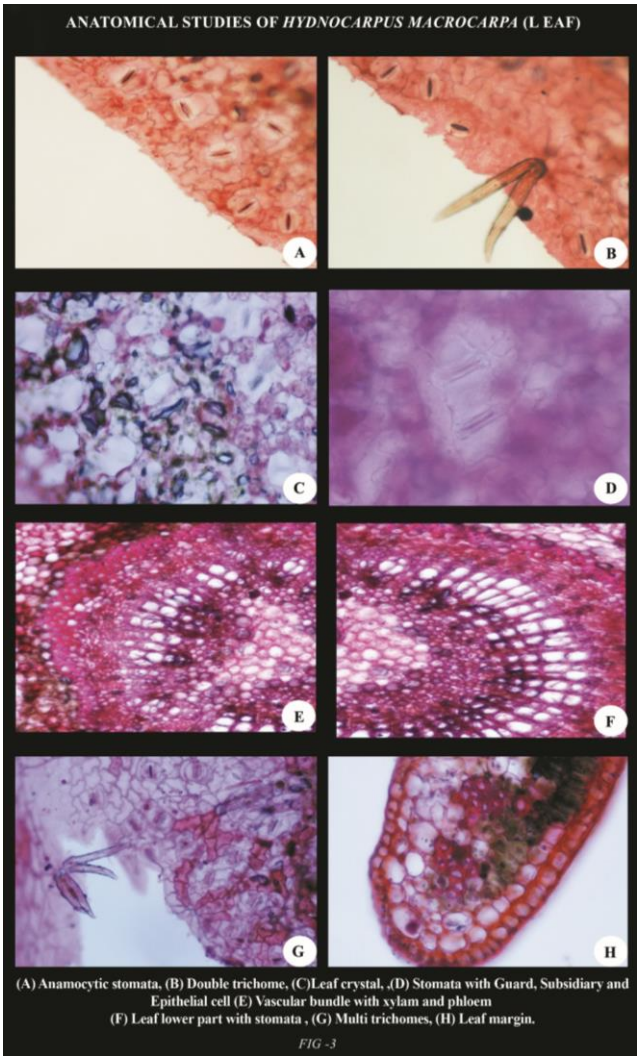
| | | | | | |
|---|---|---|---|---|---|
| 11 | Beta-Tocopherol | $C_{28}H_{48}O_2$ |  | 416.69g/mol | 36.946 |
| 12 | Gamma-Tocopherol | $C_{28}H_{48}O_2$ |  | 416.690g/mol | 37.326 |
| 13 | Pentatriacontane | $C_{35}H_{72}$ |  | 492.961g/mol | 38.817 |

**Figure-1**






## CONCLUSION

The study on the leaf of *H. macrocarpa* for its phytochemical constituents has revealed the presence of secondary metabolites. Methanol, ethanol, acetone and aqueous are good extractive solvents and the microscopically studies have shown the presence of stomata unicellular or uniseriate covering, calcium oxalate crystals, vascular bundles ex Further research on *H. macrocarpa* is necessary for elucidating the active principles and their mode of action.

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