

Macro and Microscopic Characters of *Mitracarpus Hirtus*

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Abstract

Plants are a great source of medicines, especially in traditional medicine, which are useful in the treatment of various diseases. Standardization of a compound Ayurvedic formulation is a critical and essential issue to be considered in assuring the therapeutic efficacy and safety and to rationalize their use in the health care. The paper deals with a detailed investigation on the leaves of traditional medicinal plant *Mitracarpus hirtus* belonging to the family Rubiaceae, which includes its morphological, anatomical and powder analysis. The leaf amphistomatic, Vascular bundles are occupied in mid rib region and lamina portion consists of both spongy and palisade parenchyma. Leaf epidermis also has trichomes. Most of the trichomes are multicellular. Both tapering and slightly curved trichomes are present in leaf and The powder microscopic and histological characters are also presented in this study. This study would help as an appropriate source for authentication of the present studied drug.

Keywords: *Mitracarpus hirtus*, Rubiaceae, powder analysis, organoleptic characters, macro- micro studies.

Introduction

India is one of the worlds 'mega diversity' countries. It is ranked ninth in the world in terms of higher plant species richness. At the ecosystem level, India is also well endowed, with ten distinct biodiversity zones. It also contains two of world's 25 biodiversity hotspots, because of their extraordinarily high levels of species richness and endemism, and threatened status. As a consequence of both the diversity of these conditions and of various ethnic populations living in India, the country has become an important centre of diversity of a great many domesticated species including medicinal and aromatic plants.

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Avijgan *et al.*, 2010). Early humans recognized their dependence in nature in both health and illness. Led by instinct test and experience, primitive people treated illness by using plant, animal parts and minerals that were not

part of their diet (Anwannil *et al.*, 2005). The medicinal value of plants is related in their phytochemical components which produce definite physiological actions on human body.

The use of plant- based medicinal therapeutics by primitive man is as the history of the man himself. As civilization advances these practices tend to disappear from our sight. There for, a great deal of awareness is generated among the scholars of focus immediate attention on plant based crude drugs and validate their folk claims for their phytochemical and pharmacological properties.

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant

chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000).

These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Narasinga rao, 2003).

Recently, it is clearly known that they have roles in the protection of human health and are not essential nutrients and not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease because of this property; many researchers have been performed to reveal the beneficial health effects of phytochemicals. More than 4,000 phytochemicals have been catalogue and About 150 phytochemicals have been studied in detail and are classified by protective function, physical characteristics and chemical characteristics (American cancer society, 2000). The most important of these components are alkaloids, tannins, flavonoids and phenolic compounds etc. Thus, screening medicinal plants for their therapeutic properties have increased greatly with the aim to find new treatment against various existing and emerging diseases.

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250000-500000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously crucial factor for ultimate success of the investigation. Still

there are many unexplored traditionally important plant species as mentioned above one amongst them is a medicinal herb called *Mitracarpus hirtus*.

The genus *Mitracarpus* consists of 115 species as reported in The Plant List (theplantlist.org); of these two are unresolved, 67 accepted and 46 synonyms. *M. hirtus* prefers to grow in tropical and sub-tropical regions and belongs to the Rubiaceae family. It is commonly distributed throughout gardens, farms and fields in tropical and neotropical regions. Common uses for this plant include use as an insecticide, antibiotic and antidote for insect stings and bites (Burkill, 1970).

Up to my knowledge scarcity of scientific report is noticed on *M. hirtus*, thus limiting the information on this traditional plant and on its potential usefulness. In this regard, the present study is aimed to screen the followings.

Objectives of the Study

- To study the organoleptic and anatomical characteristics of the *M. hirtus*
- To evaluate the histo-chemical reactions of the *M. hirtus*

Study Area

The selected plant *Mitracarpus hirtus* was collected from Kozhikode district in the northern part of Kerala. The city of Kozhikode is also known as Calicut. Keezhariyur is a small village situated in Kozhikode district, Kerala state, India. It comes under Keezhariyur panchayath. . It is located 35 Km from District headquarters Kozhikode. Keezhariyur (Figure-1) connect to other part of India through Koyilandy town. The average annual rainfall of the state ranges from 101.6 to 362cm. The district is lies between latitudes of 11°08' N and 11°50' N and longitude of 75°30' E and 76°8' E with a tropical wet and dry climate. The highest temperature recorded was 39.4°C in March and the lowest was 14°C on December. The district has a generally humid climate with a very hot season extending from March to May. The rainy season is during the south west monsoon which sets in the first week of June and extends up to

September. The district is a vast expanse of fertile plains interspersed with hills, rivers, mountain streams and forests

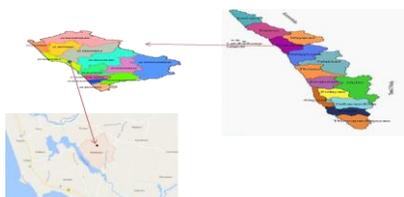


Figure 1 Study area

Plant Description

Systematic Position

- Division : Phenerogamae
- Class : Dicotyledon
- Sub class : Gamopetalae
- Series : Inferae
- Order : Rubiales
- Family : Rubiaceae
- Genus : Mitracarpus
- Species : hirtus

Common Name: Tropical Girdle Pod

Vernacular Name: Gothia gobi (odia), thaval (Malayalam)

Synonym: *Mitracarpus villosus*

Distribution

Grow in tropical and sub-tropical regions namely Central America, Mexico, Argentina, Caribbean Islands, Asia, Africa and Pacific Islands (de Souza, De Sales., 2001).

Status: Native

Habit: Trailing herb

Habitat: Degrade moist deciduous forests and wastelands

Medicinal Uses

M. hirtus L. has also been widely used to treat ringworm, rashes, toothache, itch, eczema, venereal diseases (B/kudu *et al.*, 2018), and as an antidote for bites and stings (Pansuksan *et al.*, 2014). Its edible leaves have been used as livestock feed (Idris *et al.*, 2015) and its dried leaves have been used for rapid healing of old ulcers

(Uphof, 1959).

A paste prepared from the plant is used to heal bone fractures. Leave decoction is claimed to treat hysteria, mental disorders, fever, skin diseases, and leprosy (Quattrocchi, 2012). The plant can also be used as an antiseptic, antibiotic and antidote against insect bites (Pansuksan *et al.*, 2014).

Materials and Methods

Collection and Authentication of Plant Material

The plant leaf of *Mitracarpus hirtus* was collected during the month of December, 2021, Keezhariyur, Kozhikode district, Kerala. The authenticity of the selected plant materials were duly identified and confirmed by Botanical Survey of India, Coimbatore (Vide No: BSI/SRC/5/23/2021/Tech./343, dated 16.12.2021). Fresh and healthy plant leaf of *M. hirtus* was harvested shade dried and coarsely powdered for cold extraction.

Pharmacognostic Study

Macroscopic Evaluation

The morphological features such as habit, stem characters, leaf characters, root characters, floral characters and their arrangement in plant were observed with help of hand lens and naked eye.

Microscopic Evaluation

Transverse section of stem and leaf were made by free hand section method using sharp blade. The thin section were stained with safranin and mounted with glycerine on the slide and slide was covered with the cover slip and observed under the compound microscope.

Organoleptic Evaluation

Various sensory parameters of the plant material such as color, odour, size, shape and taste were studied by organoleptic evaluation.

Histochemical Colour Reaction (Khandelwel *et al.*, 1996)

The histochemical colour reactions of plant samples were performed separately in order to identify major cell

components by chemical reagents. The following table represents the procedure of histo-chemical reaction and some other procedures are also followed.

Table 1 Histochemical Color Reaction Study

S.No.	Reagents used	Test	Colour formation
1.	T.S. of plant parts + iodine solution	Starch	Blue
2.	T.S. of plant parts + iodine solution + H ₂ SO ₄	Cellulose	Bright yellow
3.	T.S. of plant parts + safranin	Lignin	Red
4.	T.S. of plant parts + methylene blue	Mucilage	Deep violet
5.	T.S. of the plant part + aqueous solution of 0.1% toluidine blue	Tannin, phenol, pectin	Bluish green
6.	T.S. of the plant part + 10% aq. potassium hydroxide + 1% copper sulphate	Protein	Violet
7.	T.S. of the plant part + Mayer's reagent	Alkaloid	Grey
8.	T.S. of the plant part + 2,4 di nitrophenyl hydrazine	Terpenoid	Golden yellow

Powder Microscopy (Kokate, 1986; Anonymous, 2008)

The dried leaf powder studied under microscope with suitable reagents (iodine solution, safranin). The characteristic structures and cell components were observed and their photographs were taken.

Acid Value

Take 1 g plant sample and then dissolve it in 50 mL of equal volume of ethanol (95%) and petroleum ether. Then filter the sample using Whatman No. 1 filter paper. Then add few drops of phenolphthalein and then titrate it with 0.1 M potassium hydroxide until it remained faintly pink after shaking for 30 min.

pH of Plant Powder

Take 1 g of plant powder in the conical flask. Add 10 mL of distilled water to the conical flask and blend it. Then allow it to stand for 5 min at room temperature. Measure the pH of sample using pH meter (Indian Pharmacopoeia, 2010).

Result and Discussion



Figure 2: a) Habit of the plant b) leaf c) Flower and Inflorescence d) Stem and trichome Macroscopic Evaluation

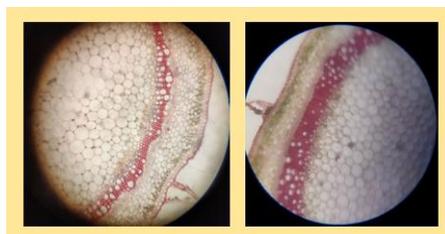


Figure 3: T.S of stem

Morphology of Leaves

Morphological characters of the plant is represented in plate 1. Leaf blades 1–6 x 0.3– 2.3 cm., elliptic, subacute at the apex, cuneate at the base, scabrid-pubescent in

nature, petiole 1 mm long, often densely pubescent and with ciliate margins; stipule sheath 1–3 mm. long, divided into 6–9 often colleter-tipped fimbriae, 1–5 mm. long, ciliate. Plant grow in wet region have broad leaves and narrow leaves in dry region. Deeply veined leaves are present.

Morphology of Stem

Stem is cylindrical, highly pubescent and it has a brown colour in mature state.

Morphology of Inflorescences

Inflorescences numerous, present in most axils, subglobose, 0.8–1.8 cm. in diameter.; flowers sessile or almost so; bracteoles filamentous, white in colour, 1–2 mm long.

Morphology of Flowers

Flowers are white, whorled in fascicles in leaf-axils, tetramerous, bracteoles are thread-like. Sepal-tube is small, unequally 4-lobed, larger 2 sepals about 1 mm long. Flowers are about 1.5 mm long, funnel-shaped; petals 4. Stamens are 4, attached at throat. Ovary is 2-locular, ovule 1-per locule. Capsule is about 1 mm long, ovoid, seeds 2, minute.

Morphology of Fruits Capsule, straw-coloured.

Microscopic Evaluation

Stem

The anatomical features of stem is represented in Figure-3, The major anatomical peculiarity observed in stem is it has a large parenchymatous pith region. Epidermis is covered by thick cuticle followed by chlorenchymatous layer and then parenchymatous cortex is present. Vascular bundles are conjoint, collateral, open and endarch. Long trichomes are present in stem.

leaf

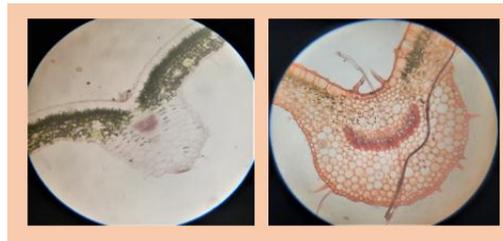


Figure 4: T.S of leaf

In leaf both lower and upper epidermis is present. Figure -4 Lower epidermis posses more number of stomata than upper epidermis. Vascular bundles are occupied in mid rib region and lamina portion consists of both spongy and palisade parenchyma. Leaf epidermis also has trichomes.

Trichomes

Most of the trichomes are multicellular. Both tapering and slightly curved trichomes are present in leaf and stem. Figure-5

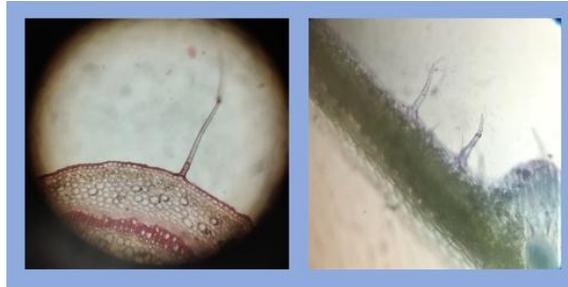


Figure 5: Microscopy of Trichomes

Organoleptic Evaluation

Various sensory parameters of the plant material such as colour, odour, size, shape and taste were studied.

Table 2 Organoleptic characters of *Mitracarpus hirtus*

Parameters	Leaf	Stem
Colour	Green	Green-young Brownish-mature
Shape	Slender and cylindrical	Elliptical and sub-acute apex

Taste	Slightly bitter	Slightly bitter
Odour	Odourless	Odourless
Texture	Rough, deeply veined and slightly pubescent	Highly pubescent

Histochemical Colour Reaction

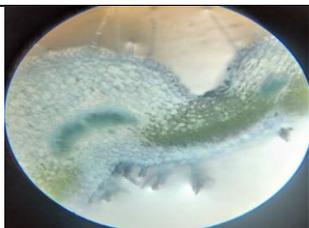
The result of histochemical colour reaction data is shown in the Table-3 and Figure-6 which shows the presence of constituents such as starch, cellulose, lignin and mucilage in stem and leaf sections

Table 3 Histochemical colour reaction of *M.hirtus*.

SL NO.	Phytochemical test for	Reagent used	Colour	Zone of colour formation	T.S of Stem	T.S of Leaf
1.	Starch	Iodine solution	Blue	Spongy parenchyma	-	+
2.	Cellulose	Iodine solution +H ₂ SO ₄	Brightyellow	Chlorenchyma	+	+
3.	Lignin	Safranin	Red	Vascular zone	+	+
4.	Mucilage	Methylene blue	Deepviolet	Spongy parenchyma	-	+
5.	Tannin, Phenol, Pectin	Toludine Blue	Bluishgreen colour	----	-	-
6.	Protein	10% aq. KOH and 1% CuSO ₄	Violet	----	-	-
7.	Alkaloids	Mayer's reagent	Grey colour	----	-	-
8.	Terpenoids	2,4-dinitrophenyl hydrazine	Goldenyellow	-----	-	-

+ presence, -Absent

Histochemical colour reaction (Microscopic view) of *Mirtacarpus hirtus*



Blue colour in spongy parenchyma (test for starch)

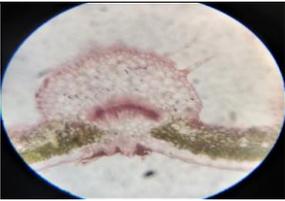
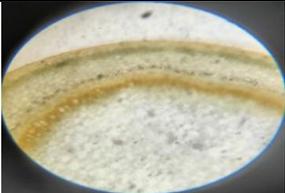
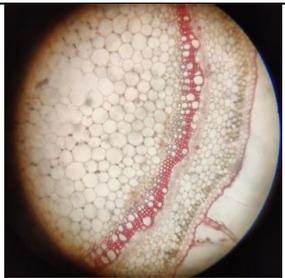
	Red colour in vascular zone (test for lignin)
	Yellow colour in chlorenchyma (test for cellulose)
	Yellow colour in chlorenchyma (test for cellulose)
	Red colour in vascular zone (test for lignin)

Figure 6: Histochemical study of *Mirtacarpus hirsutus*

Powder Microscopy

Presence of xylem tracheids and thickenings were observed in the powdered sample of *M. hirtus*

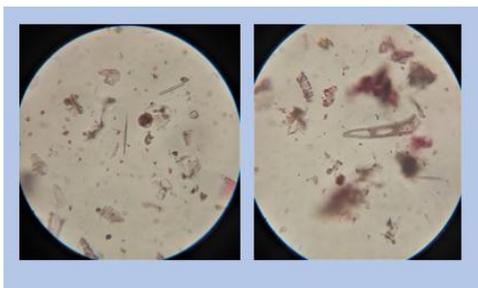


Figure 7: Powder microscopy of *Mirtacarpus hirsutus*

Acid Value

Acid value is calculated by the formula

$$\text{Acid Value (mgKOH/g)} = \frac{\text{titer value} \times \text{N of KOH} \times 5.61}{\text{Weight of the sample (g)}}$$

$$= \frac{5.61 \times 12.1}{1}$$

pH of the plant powder = 67.881

pH of the plant leaf powder is 4.58 . It is slightly acidic

Summary and Conclusion

The ability to provide timely, accurate and reliable data is an essential part of discovery, development and manufacture of Pharmaceuticals. The present study has been carried out on the pharmacognostic, character of

Mitracarpus hirtus. The plant is native to Kerala and in other European countries. The reported number of studies on the plant is very limited. Macroscopic, microscopic, organoleptic, histochemical colour reaction, acid value and pH values were studied under pharmacognostic evaluation. Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants and any other quality control of the drugs. It's a simple and reliable tool, by which the complete information of the crude drugs can be obtained. The macro and microscopic features and organoleptic characters along with the anatomical studies are diagnostic and establish in the standards for the plant leaf drug. The information provided by this study may be useful to carry out further study of Ayurvedic drugs of traditional medicinal practice of present era.

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