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Pharmacognosy and phytochemical evaluation of *Gynochthodes umbellata* (L.) Razafim. and B .Bremer (Rubiaceae) a potent dye yielding medicinal plant

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ABSTRACT

Gynochthodes umbellata is a pretty woody climber with bright orange fruit having potent medicinal properties. Traditionally it is used for treating dysentery and diarrhoea. The present study is focussed on the pharmacognostic characters of the plant and its bioactive compounds. A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. Various physicochemical parameters like ash values, extractive values, and moisture content were investigated. Preliminary analysis showed the presence of various groups of phytochemicals like anthraquinone, terpenoids, flavonoids, steroids, glycosides, phenols in various plant extracts. These studies revealed the presence of various important bioactive compounds and proved that the plant is medicinally important. These results may help in standardization, identification and in carrying out further research in *G. umbellata*.

Key words: *Gynochthodes umbellata, Morinda umbellata*, pharmacognostical evaluation, phytochemical screening.

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases[1]. Plants have been the source of many important drugs because they are able to produce various chemical entities and bioactive molecules through the process known as metabolism. Most of the pharmaceutical industry is highly dependent on wild populations for the supply of raw materials for extraction of medicinally important compounds. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. Pharmacognosy is the study of the structural, physical, chemical and sensory characters of crude drugs of animal, plant or mineral origin and phytochemical evaluation detects the presence / absence of bioactive compounds in the crude extracts of plant[2].

Gynochthodes umbellata (Syn: *Morinda umbellata*) was selected for the present study. *G. umbellata* is a woody climber. It is native from Southern India and the Deccan peninsula to Burma, China, Sri Lanka, Southeast Asia, Philippines, Northern Australia and Japan[3, 4]. *G. umbellata* commonly known as (Common Indian Mulberry) Vomit vine[5]. Traditionally it is used for treating dysentery and diarrhoea[6]. The leaves are used to expel intestinal worms. Fruit juice expels toxins, regularises menstruation cycle[7]. This plant is a rich source of many class of biologically active compound. But studies for the identification of biologically active compounds for this species are very less. So it is important to evaluate the phytochemicals present in this plant species. The present study is focussed on the pharmacognostic characters of the plant and its bioactive compounds. Pharmacognostical evaluation includes morphological and microscopical characters, physicochemical and fluorescence properties.

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MATERIALS AND METHODS

Collection of plant material

Preliminary phytochemical analysis of leaf, stem and root of *G. umbellata* were done in the present work. Fresh plant materials were collected from University of Kerala Kariyavattom Campus, Thiruvananthapuram, Kerala. The plant was properly identified with the help of authentic literature[8, 9] and documented with their characteristic features.

Macroscopic study

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug**.** The fresh materials like, leaf, stem, flower, fruits of

G. umbellata collected and observed with the help of a dissection microscope. The characters were analysed and photographs of various plant parts were taken.

Microscopic studies

The fresh plant materials were collected for microscopic studies. Free hand sectioning of root, leaf and stem were done and observed under the microscope. Microphotographs at different magnifications were taken with an image analysing system (Olympus-BX51TF, Japan) to study the anatomical characters.

Stomatal number

Peel out upper and lower epidermis of leaf separately by means of forceps. Thin transparent region of epidermis is mounted in glycerine (5%) on a clean slide. Draw a square of 1mm by means of a ocular micrometer. Place the slide with cleared leaf on the stage. Trace the epidermal cell and stomata. Count the number of stomata present in the area of one square mm, include the cell if at least half of its area lies within the square. Record the results and calculate average number of stomata per square mm.

Stomatal index

Stomatal index is the percentage, which the number of stomata / unit area and the total number of epidermal cells were counted. Each stomata will counted as one cell. Stomatal index can be calculated by the following formula

$$I = S/E+S$$

(I = stomatal index; S= No. of stomata per unit area; E= No. of epidermal cells in the same area)

Qualitative analysis

Physico-chemical analysis

Physico – chemical parameters such as total ash value, acid-insoluble ash, water soluble ash, extractive values, alcohol soluble extractive, cold water extractives and hot water extractives of root, stem, leaf powders of *G. umbellata* were determined according to standard procedures[10.11,12,13]. Moisture content, crude fiber content, fluorescence analysis and organoleptic analysis of root, stem, leaf powders were also done.

Phytochemical screening

A systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. Ethanolic extracts of various plant parts like root, stem and leaf of *G. umbellata* were taken by using Soxhlet extraction method[14]. These extracts were then subjected to preliminary phytochemical screening for the identification of various classes of active chemical constituents using the various standard procedures[14, 15].

RESULTS AND DISCUSSION

Morphology

G. umbellata is a sprawling or climbing shrub with long, slender branches (Fig: 1 & 2). Leaves opposite, oblong, round, narrow, cuspidate, solid, dense, glabrous. The mid-rib, standing out on both sides, gives out many smaller ones in an obliquely circular fashion. Flowers arise at the tip of the shoots above the origin of leaves; four from each node on five petioles and on each petiole, five, six or seven in number arise, and are small buds without calyx, four petalled, on the outside light green, on the inside totally beset with white hairs (Fig: 3). Stamens are four in the middle, short, slender, with whitish apices. The style more thick, green, stands out in the middle, starting from a little smooth, green head which is the rudiment of the fruit.

Fig: 1 & 2: Habit of *Gynochthodes umbellata*

Fig: 3: Infloresence of *G. umbellata*

Fruit which in aggregates of six or seven are seen at the tip of shoots, are berries, round in shape (Fig:4), unripe fruits are green in colour when they reach maturity colour become yellow then to reddish orange, on the outside marked with six or seven lattices on the rind, from each of which a light yellow eye bulges out (Fig:5). The flesh inside is dense, yellow and more humid, of sweet taste tearing (biting) the tongue more than the leaves, has in itself many small from yellow to whitish, smooth, oblong and narrow stones, with hard shell, and whitish kernel. Inside the fruit there present small seeds about 12 seeds/fruit (Fig: 6)

Fig: 4: Unripe fruit of *G. umbellata*

Fig: 5: Ripened fruits of *G. umbellata*

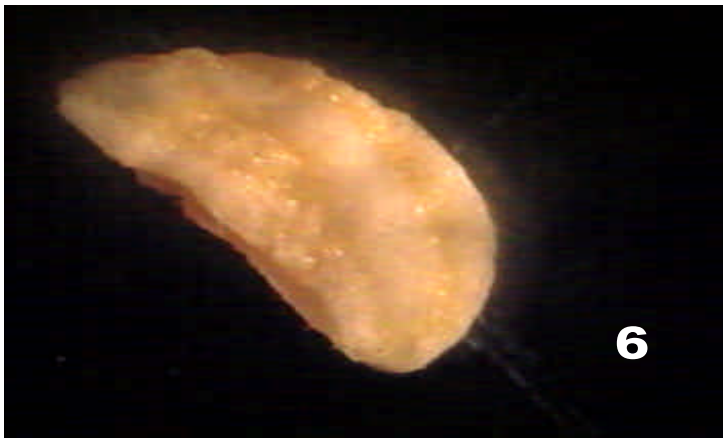

Fig: 6: Seeds of *G. umbellata*

Microscopic studies

Microscopic studies of leaf, stem and root were done. The stomata is paracytic, stomatal frequency is 4-5/mm^2 on the adaxial side and on the abaxial side it is 18-20/mm^2. The stomatal index on adaxial side is 10.2% and 22.8% on the abaxial side (Fig: 7). The anatomical studies of stem (Fig: 8,9), leaf (10,11) and root (12) were done. Epidermal hairs are present in stem and leaf (Fig: 8,11).

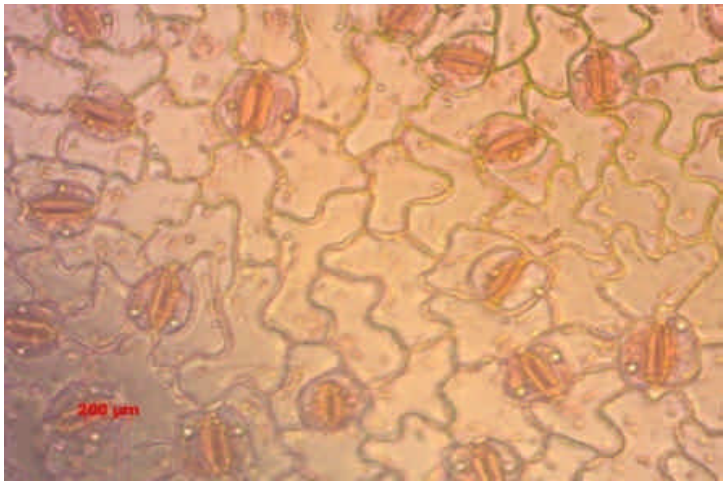

Fig: 7: Picture of Stomata

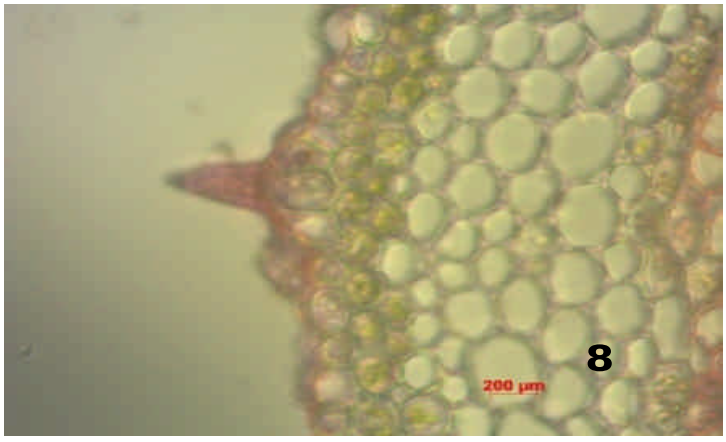

Fig: 8: Unicellular hair present in stem of *Gynochthodes umbellata*

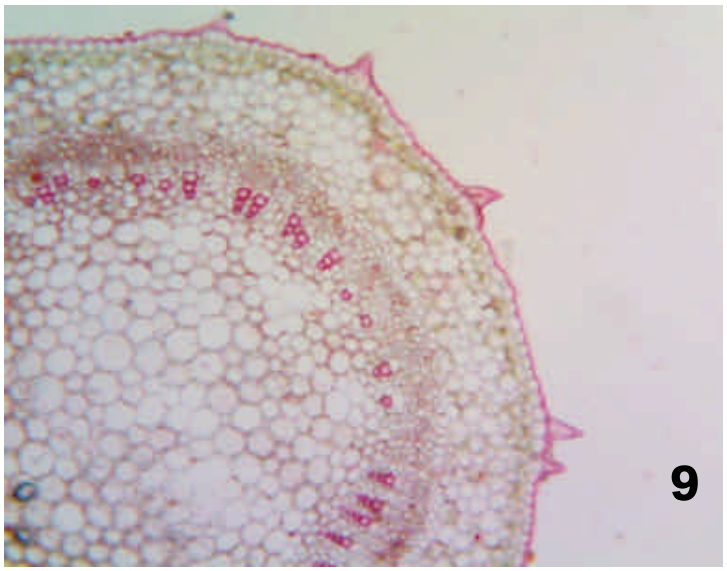

Fig: 9: C. S. of stem

Fig: 10: C. S. of leaf

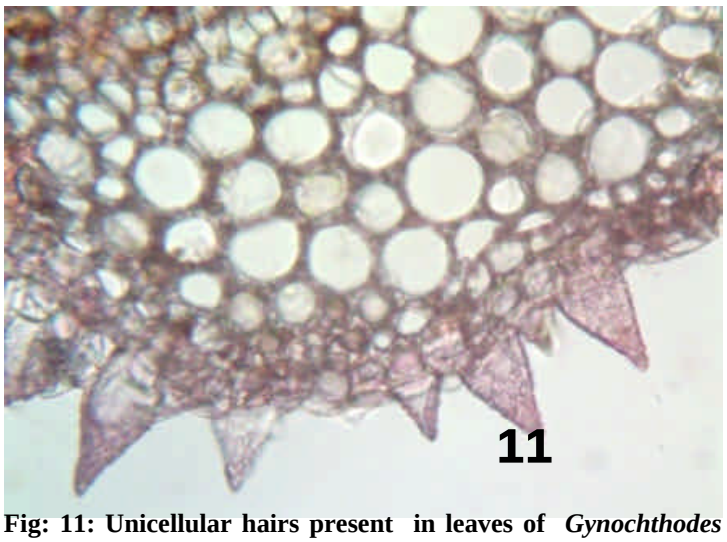

Fig: 11: Unicellular hairs present in leaves of *Gynochthodes umbellata*

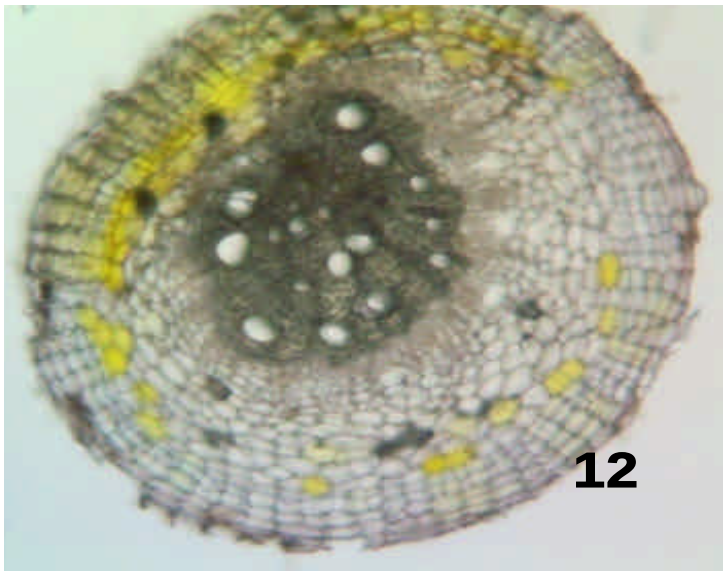

Fig: 12: C. S. of root

Physico-chemical analysis

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physicochemical parameters like ash values, extractive values, moisture content were investigated and the results are presented in Table: 1. Ash values of the drug give idea about earthy matter or inorganic composition and other impurities present along with the drug.

Table: 1. Physico-chemical parameters of *G. umbellata*

Parameters	Root powder	Stem powder	Leaf powder
Moisture content	8.98	12.23	14.79
Alcohol soluble extractives	2.5	5.65	8.6
Water soluble extractives			
a) Cold water soluble extractives	5.45	8.65	9.72
b) Hot water soluble extractives	4.68	11.5	11.79
Fibre content	48.85	53.33	12.37
Total ash	3.99	2.93	6.05
Acid-insoluble ash	0.44	0.24	0.46
Water soluble ash	1.75	1.34	2.75
Sulphated ash	7.01	6.48	11.64

Fluorescence analysis

Crude drugs show their own characteristic fluorescence when exposed to ultra violet radiation and is dependent on its chemical constituents. Fluorescent analysis of the drug powder with different solvents is an important pharmacognostic tool in checking adulterants[2]. The powdered root, stem and leaf of *G. umbellata* were boiled with different solvents according to their increasing polarity. The boiled powders with solvents were examined under short UV (254nm), long UV (365nm) and visible light (Table: 2, 3 & 4).

Table: 2. Results of fluorescent analysis of leaf powder

Sl.No.	Treatment	Colour		
		Visible light	Short UV	Long UV
1	Powder as such	Willow green	Leek green	Ivy green
2	Powder + 50% NaCl	Salmon	Sage green	Paris green
3	Powder + Ab. Alcohol	Parsley green	Willow green	Porcelain rose
4	Powder + n-Hexane	Fern green	Chinese yellow	Carmine rose
5	Powder + n-Butanol	Willow green	Sap green	Porcelain rose
6	Powder + 1N NaOH	Pansy purple	Ivy green	Nickel green
7	Powder + Ethyl Acetate	Willow green	Parsley green	Porcelain rose
8	Powder + Petroleum Ether	Lettuce green	Sky grey	Empire rose
9	Powder + Toluene	Scheeles green	Lettuce green	Camellia rose
10	Powder + Benzene	Lettuce green	Naples yellow	Venetian pink
11	Powder + Chloroform	Willow green	Pod green	Orient Pink

Table: 3. Results of fluorescent analysis of stem powder

Sl.No.	Treatment	Colour		
		Visible light	Short UV	Long UV
1	Powder as such	Empire yellow	Sage green	Willow green
2	Powder + 50% NaCl	Egyptian buff	Primrose yellow	Cyprus green
3	Powder + Ab. Alcohol	Canary yellow	Sap green	Cobalt blue
4	Powder + n-Hexane	Sap green	Capri blue	Princes blue
5	Powder + n-Butanol	Pea green	Uranium green	Aster violet
6	Powder + 1N NaOH	Cardinal red	Cyprus green	Paris green
7	Powder + Ethyl Acetate	Aureolin	Sap green	Paris green
8	Powder + Petroleum Ether	Nickel green	Viridian green	French blue
9	Powder + Toluene	Uranium green	Cyprus green	Chrysocolla green
10	Powder + Benzene	Sulphur yellow	Pea green	Nickel green
11	Powder + Chloroform	Pod green	Lettuce green	Verdigris

Table: 4. Results of fluorescent analysis of root powder

Sl.No.	Treatment	Colour		
		Visible light	Short UV	Long UV
1	Powder as such	Straw yellow	Lavender green	Parsley green
2	Powder + 50% NaCl	Naples yellow	Pod green	Nickel green
3	Powder + Ab. Alcohol	Sulphur yellow	Cyprus green	Jade green
4	Powder + n-Hexane	Empire yellow	Jade green	Langite green
5	Powder + n-Butanol	Mimosa yellow	Naples yellow	Ethyle blue
6	Powder + 1N NaOH	Chrysanthemum crimson	Leek green	Nickel green
7	Powder + Ethyl Acetate	Canary yellow	Chartreuse green	Veronese green
8	Powder + Petroleum Ether	Verdigris	Veronese green	Hyacinth blue
9	Powder + Toluene	Sap green	Primrose yellow	Verdigris
10	Powder + Benzene	Primrose yellow	Dresden yellow	Veronese green
11	Powder + Chloroform	Canary yellow	Pea green	Agathia green

Table: 5. Results of organoleptic analysis

Sl. No.	Solvents	Colour obtained		
		Leaves	Stem	Root
1	5% $FeCl_3$	Light brown	Golden yellow	Golden yellow
2	50% NaOH	Light red	Dark red	Blood red
3	NH_4 solution	Light brown	Orange red	Dark red
4	Glacial acetic acid	Brownish green	Golden yellow	Golden yellow
5	50% HNO_3	Light green	Green	Light brown
6	Con. HNO_3	Green	Brown	Dark brown
7	50% HCl	Light brown	Light green	Light green
8	Con. HCl	Dark green	Dark brown	Dark brown
9	50% H_2SO_4	Dark green	Light brown	Light brown
10	Con. H_2SO_4	Dark violet	Dark brown	Dark violet

Organoleptic analysis

The plant powders were treated with various chemicals ; the reaction colour were noticed and results were listed in Table 5.

Preliminary phytochemical analysis

Determination of phytochemical profile of plants is an indication of the class of compounds present in the plant[2]. Preliminary phytochemi-

Table: 7. Results of preliminary phytochemical screening of ethanolic extracts of root, stem and leaf of *G. umbellata*

Sl.No.	Compound	Leaf	Stem	Root
1	Glycoside	-	-	+
2	Phenols	+	-	-
3	Steroid	+	+	+
4	Saponins	+	+	
5	Flavanoids	+	+	+
6	Terpenoids	+	+	+
7	Alkaloids	-	+	+
8	Fixed oil	-	-	-
9	Quinones	-	+	+
10	Anthraquinones	-	+	+

cal analysis of ethanolic extracts of root, stem and leaf were done by using various preliminary analysis tests. Preliminary phytochemical analysis is done to identify the major groups of phytochemicals present in the plant samples. Results of preliminary screening are shown in table (Table: 6). Preliminary analysis showed the presence of various groups of phytochemicals like anthraquinone, terpenoids, flavonoids, steroids, glycosides, phenols in the various plant extracts. Anthraquinones are present in the root and stem. But the leaf lack the presence of anthraquinone. The curative properties of plants are perhaps due to the presence of various secondary metabolites such as flavanoids, alkaloids, anthraquinones etc. They were known to show medicinal as well as physiological activities[16].

CONCLUSION

Pharmacognostic studies and phytochemical screening can serve as a basis for proper identification of a plant. Before any drug can be included in the pharmacopoeia, these standards must be established[2]. A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. In the present study, a set of pharmacognostical standardization parameter studies were conducted on *G. umbellata* root, stem and leaves as per Pharmacopoeia and WHO guidelines. These studies revealed the presence of various important bioactive compounds and proved that the plant is medicinally important. These results may help in standardization, identification and in carrying out further research in *G. umbellata*.

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