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Studies in pharmacognostic characters of the climber *Erycibe paniculata* Roxb. of Convolvulaceae

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Abstract

The present scientific investigation deals with the pharmacognosy and preliminary phytochemical screening of the leaf and stem bark of *Erycibe paniculata* Roxb., an ethnomedicinal climber used in curing the fever, cholera, night blindness, sprains, constipation, etc. Standard methods have been followed to study the microscopic, macroscopic, physicochemical characters and fluorescence behavior of the powdered samples of this plant. Phytochemical screening including GCMS of the stem bark has also been performed. In leaf epidermal micromorphology, it was found that stomata are anisocytic type distributed in hypostomatic leaf. Stomatal Index was 13.879. Glandular unicellular type of trichomes found in both surfaces of the leaf. Non-glandular trichomes with 2 to 6-arms were observed on the epidermal surfaces of petiole and stem. Petiole in T.S. is semi-lunar in outline housing 3 vascular bundles. Total ash of the stem bark was found 9%, of which acid insoluble ash and water soluble ash values were observed 0.092% and 7.626%, respectively. Moisture content of the bark sample was 0.5%. Out of five solvent extracts of the stem bark, methanolic extract showed highest extractive value (8.672%). Important groups of phytochemicals like tannins, alkaloids, flavonoids, etc. are detected in stem bark extract. Nine bioactive compounds have been identified through GCMS analysis from the methanolic bark extract which, in turn, indicate its therapeutic properties. Present study provides the pharmacognostic characters of both leaf and stem bark of *Erycibe paniculata* which will be an instrumental in authentication and quality control of the crude drugs procured from this medicinal plant.

Keywords: Ethnomedicine, crude drug, pharmacognosy, phytochemical screening, *Erycibe paniculata*

1. Introduction

The ancient knowledge regarding the uses of natural resources, mainly plants as food, shelter and medicine is very important from the beginning of human civilization. Exploitation of medicinal plants in human society on the basis of traditional knowledge dates back more than 60,000 years^[1, 2]. Folk knowledge on uses of medicinal plants has ultimately been established as a means of disease healing through trial and errors among the people mainly those who belong to tribal communities all over the world. In course of time this traditional knowledge based on medicinal plants flourished as most vital and basic health care procedure for people from one generation to the next and for their livestock.

In recent years the reliability and demand of natural resources specially the resources of herbal origin have been increased several fold. The application of plant based medicines is now being increased in modern health care system because they are not only cost effective than modern allopathic medicines but also relatively devoid of toxicity. Day by day a general trend among the people is evident in substituting many of the allopathic medicines with the herbal ones for a long term health benefit. Thus the percentage of use of the botanicals of therapeutic importance is steadily increasing among the people all over the world^[3, 4].

It is estimated that more or less 20,000 plant based products are currently available in health care domain in the world^[5]. But scientific evaluation for identification of genuine herbal drugs and their toxicity indication are largely lacking. Toxicity data is very important to predict the physiological safety before the use of any medicine. In case of ethnomedicine, there is every possibility to form toxicity upon consumption of it in raw form or due to its adulteration. Adulteration of crude drugs may occur due to wrong identification of the source plants, inappropriate selection of drug parts and mixing of varied types of substances other than the

genuine drugs. Moreover, incorrect preparation and wrong administration process may also lead to inefficacy of the herbal drug and its adverse effect [4-6]. Thus the pharmacological and clinical studies with such adulterated drugs will be a flawed exercise in validating their efficacy against diseases. Recently, scientists are trying to develop allopolyherbal drugs by combining allopathic drugs with polyherbal preparations for treatment of diseases. It helps to reduce or eliminate the adverse effects of synthetic drug in human body⁷. Therefore, there is a need of modern scientific tools and techniques for checking the authenticity of herbal drugs before it use⁸. A big quantum of pharmacognostic research works are going on using different conventional and sophisticated techniques for standardization and quality control of herbal drugs. Pharmacognostic studies covering preliminary and advanced phytochemical screening like microchemical tests and several chromatography techniques such as Thin Layer Chromatography, GCMS, HPLC are reliable methods which provide standard information about crude drugs for its proper identification and subsequent standardization of desired bioactive phytochemicals from respective plant sources⁹. Pharmacognostic study is also instrumental to develop the pharmacopoeial standards for successful authentication of the crude drugs.

Survey of literature on pharmacognosy and phytochemistry has elucidated that there are several ethnomedicinal plants which have been systematically studied for their pharmacognostic and phytochemical profiles [3, 10-13]. Crude drugs of a large number of ethnomedicinal plants remain uninvestigated scientifically. Keeping this scenario in mind present study has been planned to explore scrupulous pharmacognostic characters including macro and microscopic characters, physico-chemical values and phytochemical profile of the plant *Erycibe paniculata* Roxb. No work has been done on pharmacognosy of this plant but it is used in ethnomedicines of different states of India for treatment of several health problems like cholera, fever, diarrhea, constipation, etc. [14-16]

2. Material and methods

2.1. Material

Erycibe paniculata Roxb. (Fig.1)



Fig 1: Twigs of the *Erycibe paniculata* Roxb.

a. Tribal name-Keri (Santali)

b. Botanical characters

An evergreen, large, woody climber with several young branches, branches with densely rusty brown tomentum. Stem woody, herbaceous towards tip, terete, younger parts greenish and covered with reddish-brown indument, older stem part faint-green to purple in color. Leaves 4-8 × 2.5 – 5cm,

broadly elliptic or elliptic-ovate, coriaceous, apex obtusely acuminate, entire, glabrous; lateral veins 5-6 pairs; petiole very short, 0.5-1cm long. Inflorescence paniculate cymes, axillary and terminal, 10-20cm long. Flowers numerous, yellowish white, 6-8 mm across; pedicel 3-4mm; calyx tube 1.5-2mm long, lobes 5, ovate, densely brown tomentose, sepals 3mm long, suborbicular; corolla campanulate – rotate; 6mm long, yellowish white; lobes thick, fleshy, hairy on the back; stamens 10, exerted; ovary 1-celled, ovules 4; style absent; stigma subglobose. Fruits berry, subglobose, 1.2cm across, black when ripe. Seeds ovoid or elliptic, 1mm in diam.

c. Flowering and Fruiting times–April to June.

d. Medicinal uses

- i) Bark is used in muscular pain and headache during menstruation cycle by the Santal tribal people of Birbhum district, West Bengal (Author's personal observation).
- ii) Stem bark paste is used as poultice on the affected body parts in case of Ricket [17].
- iii) Leaf and stem syrup of this plant is used orally for treatment of sprains of the cattle by the ethnic people of Purulia district of West Bengal [18].
- iv) Bark decoction is traditionally used by the people of Nawarangpur district of Odisha (India) for the treatment of fever and headache [15].
- v) Folk people of Chhattisgarh state of India use extract of the young leaf in treatment of night blindness [19].
- vi) Root extract is administered to cure fever [14].
- vii) Ripe fruits are eaten in constipation whereas extract of aerial part shows diuretic activity [14].
- viii) Bark is used by the tribal people of Odisha as ethnomedicine to cure diarrhea [16].

2.2 Methods

a. Plant collection

Flowering twigs of the species were collected from the forest areas of Chorchor, Birbhum district, West Bengal, India. The plant species has been identified and authenticated with the help of standard flora [20]. The herbarium specimen of the plant has been deposited at the Visva-Bharati herbarium, Department of Botany, Visva-Bharati, Santiniketan, West Bengal for future reference.

b. Foliar micromorphology

Leaf pieces were cut from apical, middle and basal portions of the lamina and pieces were cleared following the Bokhari's method [21]. Aqueous safranin (1%) of few drops were applied to the cleared samples and mounted it with 10% glycerine on a slide for observation under compound light microscope [Olympus microscope, Model- CH-20i fitted with camera]. Photomicrographs of the leaf parts were taken by the camera attached with the microscope and measurement of the plant cells were taken with the standardized ocular micrometer.

c. Vegetative anatomy (stem and petiole)

Transverse sections (T.S.) of stem and petiole of the selected plant were stained suitably following standard staining method [22] and observed under compound light microscope. Photographs of the suitable sections were taken. Scanning Electron Microscope (SEM) photographs of the stem (T.S.) were also taken.

d. Xylem maceration

In this study, 1cm long stem pieces were macerated following

the standard wood maceration technique^[23]. Small amount of boiled stem piece was washed thoroughly in distilled water and a bit of boiled sample taken on a glass slide, teased with needles, mounted with 10% glycerine and observed under microscope.

e. Organoleptic study

Study of crude drugs using sensory organs was performed following the standard methods²⁵ which includes basic characters of the crude drug sample like morphology, colour, odour, taste, etc.

f. Physicochemical evaluation

In this evaluation, moisture content, ash value and extractive value of the plant sample were determined as per guidelines of Indian Pharmacopoeia and WHO^[8, 24].

Moisture content study

About 5g. of fresh stem weighed and subjected it to dry in shade for few days. Dried sample was then incubated at 80°-90°C for one hour. Post incubation weight of the sample was taken and calculated the percentage of moisture content.

Ash value determination

The residue left after incineration of the crude drug is designated as ash which usually represents the inorganic salts naturally occurring in the drug and adhering to it.

Determination of total ash value: 5g. of air dried powdered drug was taken in a tared silica crucible and incinerated at 650°C in the muffle furnace for 6 hours to make it dull red hot until free from moisture and carbon. Ash was cooled and weighed, and the percentage of total ash was calculated with reference to the air-dried drug sample by the following formula:

$$\text{Total ash value (\%)} = \frac{\text{Weight of the ash}}{\text{Weight of the plant drug taken}} \times 100$$

Determination of acid-insoluble ash value: The total ash was boiled with 30 ml of 2N HCL for 5 minutes. Diluted ash was then filtered using Whatman 41 filter paper. The insoluble matter was collected from filter paper, completely dried, weighed and calculated the percentage of acid insoluble ash with reference to the air-dried drug.

Determination of water-soluble ash value: The total ash was boiled with 30 ml of water for 5 minutes. The insoluble matter was collected on Whatman 41 filter paper. The matter was ignited for 15 minutes until loss of all moisture of it. The percentage of water soluble ash was calculated with reference to the air-dried drug.

g. Fluorescence analysis

Here in this study, different chemical reagents were mixed with the powdered drug and observed distinctive colour changes under UV light (355nm and 254nm). Very distinct color changes were recorded and compared those with the colours of powdered drugs as seen under visible light^[25].

h. Determination of the extractive value

Different solvents like water, methanol, chloroform, benzene, petroleum ether separately taken in 100 ml conical flasks and with each of which 10 g dried plant powder was added. All mixture were subjected to occasional stirring with glass rod

for 6 hours and left for overnight. After filtering the solvent extracts with Whatman No.41 filter paper, all the extracts were then left separately to dry at room temperature. Finally weight of each solvent extract was noted and extractive value was calculated following the formula:

$$\text{Extractive value (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

i. Histochemical study

Transverse sections of the stem were made and mounted in glass slides. One to two drops of reagents like Wagner's, Dragendroff's, Mayer's, Lugol's, Millon's, 1% lead acetate, Phloroglucinol, Ferric chloride, etc. were applied to the sections. Specific reaction between reagents applied and phytochemical groups present in specific tissue locations makes the change of colour in the cells of the stem sections. The changes were observed under compound light microscope and recorded^[24, 26].

2.3 Phytochemistry

j. Preliminary phytochemical screening:

Dried stem bark powder was extracted with methanol solvent and filtered it. Then different chemical reagents were added to a certain volume of bark extract separately for colour reaction tests. Different phytochemical groups present in the powdered sample were detected following standard methods^[26, 27].

k. Gas Chromatography-Mass Spectrometry:

Extract preparation

5g of dried bark powder was macerated in 100ml of methanol. The solution was subjected to occasional stirring for 10 days and the extract was filtered using Whatman No. 41 filter paper. Then the crude extract was completely concentrated by evaporating the methanol in a thermostat water cabinet at 40 °C for few hours. Then 1g of dried extract sample was dissolved in 10ml methanol. The stock solution of extract was then transferred to air-tight container and kept in the refrigerator at 4 °C until required for the experiment.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

This was carried out on a Mass Spectrophotometer (GCMS-model number Agilent 6890-MS). The sample solution was taken for GC-MS analysis after filtering it in sterilized milipore filter (0.22µ). 1µl of the sample was injected in to a GC equipped with a MS and a non-polar capillary column HP-5 (30m × 0.25 mm; 0.25µm). The oven program had an initial temperature of 110° for 2 min., transfer line temperature was 200° C. Then its temperature was increased to 250° C for 2 min at the rate of 5° C/min. Finally temperature was raised to 280° C at the rate of 6° C/ min for 7 min. Total run time was 50 min. The detector temperature were set at 250° C. As carrier gas Helium (purity 99.999%) was used at a flow rate of 1ml/min. The sample was injected in the split mode(1:10). The electron impact ionization (EI) mode was set in 70eV. The mass range scanned was 50- 550 m/z.

Identification of compounds

The phytochemical constituents present in the bark sample were identified consulting the mass spectral library of NIST. The spectra of reference compounds were extracted from the NIST database and then matched with the spectra of unknown phytochemical compounds present in the bark extract sample. Percentage of peak areas of the individual chemicals was

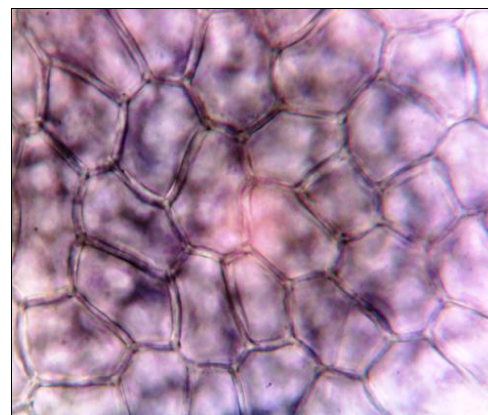
determined in respect of total peak area of all chemical compounds detected from the chromatogram.

3. Results

3.1 Foliar Micromorphology

Characteristic features along with measurement of the epidermal cells, stomata and trichomes of the investigated plant are given below.

a. Epidermis: Cell shapes are irregular and cell walls are straight in upper epidermis and it is slightly wavy in lower epidermis. Upper epidermal cell size is $45.38 \mu\text{m} \times 27.33 \mu\text{m}$ and on lower surface, the size is $50.88 \mu\text{m} \times 28.58 \mu\text{m}$. Frequency of the epidermal cell is $784.15 /\text{mm}^2$ and $817 /\text{mm}^2$ on the upper surface and lower surface, respectively. Palisade ratio is 6.62 (Table-1; Fig.-2A).



2A

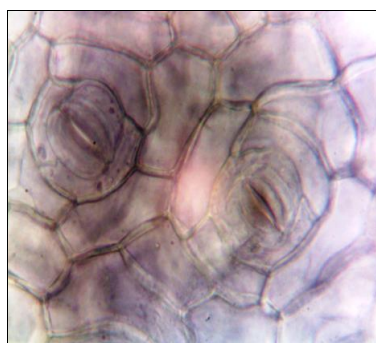
Fig 2A: Upper epidermis of the leaf

Table 1: Foliar epidermal cell characters of the investigated species

Leaf surface	Cell shape	Cell length (μm)	Cell width (μm)	Cell frequency (No./ mm^2)	Cell wall out line	Palisade ratio
Upper	Irregular	45.384	27.330	784.15	More or less straight	6.62
Lower	Irregular	50.881	28.582	817	Slightly wavy	

b. Stomatal complex: Leaves are hypostomatic and stomata are of anisocytic type with distinct subsidiary cells. Size of the stomata is $32.747 \mu\text{m} \times 19.06 \mu\text{m}$.

Stomatal frequency and stomatal index are $80.99 /\text{mm}^2$ and 13.87%, respectively (Table- 2; Fig.- 2B).



2B

Fig 2B: Lower epidermis with anisocytic stomata

Table 2: Stomatal features of the investigated plant species

Leaf surface	Type	Length (μm)	Width (μm)	Stomatal index (%)	Stomatal frequency (No./ mm^2)
Upper	-	-	-	-	-
Lower	Anisocytic	32.747×19.064	19.064	13.879	80.99

c. Trichomes: Upper and lower epidermal surfaces of the leaf ornamented with unicellular, glandular type of trichomes. Size of the glandular trichomes of upper epidermis is $32.042 \mu\text{m}$ in diameter and it is $31.092 \mu\text{m}$ in lower epidermis. Frequency of the trichome is $5.23 /\text{mm}^2$

on the upper surface and it is $12.70 /\text{mm}^2$ on the lower surface. Trichome indices are 4.72 % and 3.20 % on the upper and lower surfaces, respectively (Table-3; Fig.- 2C).

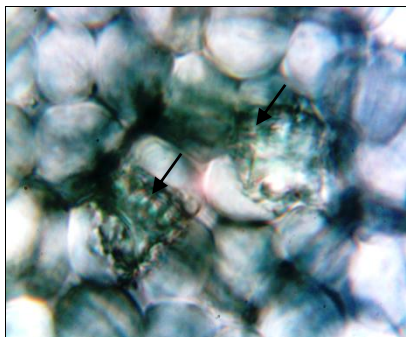


Fig. 2C: Sessile glandular trichome

Table 3: Trichome characters of the investigated plant species

Leaf surface	Trichome type	Trichome size (µm)	Trichome Index (%)	Trichome frequency (No./mm ²)
Upper	Glandular	32.042	4.72	5.23
Lower	Glandular	31.092	3.205	12.704

d. Crystals: Clustered crystals of calcium oxalate (Sphaeraphides) are present in upper leaf surface. The diameter of the crystals ranges from 30 to 35.36µm (Table- 4; Fig. -2D).



2D

Fig 2D: Crystals of calcium oxalate (Sphaeraphides)

Table 4: Crystal characters of the plant species

Leaf surface	Type	Diameter (µm)	Dissolved in
Upper	Calcium oxalate (Sphaeraphide)	30 - 35.36 µm	Dil. HCl
Lower	-	-	-

3.2 Vegetative anatomy

a. Stem-

The transverse section of the stem is circular to oval in outline and showed the following anatomical features (Fig.-3A₁, 3A₂, 3A₃).

Cork layer: The cork layer is thick measuring 390 µm – 460 µm with a homogeneous zone of 2-3 rows of brownish somewhat tangentially elongated cells.

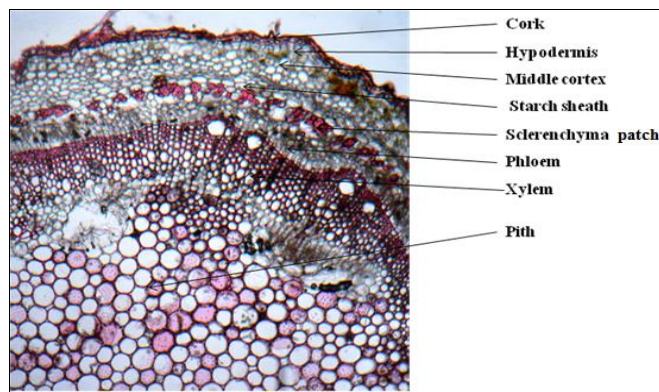
Epidermis: It consists of single layer, barrel shaped cells, with cuticularized outer wall. Cells are rectangular, compactly arranged. Non-glandular, 2-6 armed trichomes were distributed throughout the epidermis, each of 55 to 520 µm in length. Some multicellular glandular trichomes are also present on the stem epidermis, each about 220 µm in length.

Cortex: The cortex is massive and differentiated into three zones- 1) 2-3 layered collenchymatous hypodermis, 2) 4-5 layers of parenchymatous middle cortex, and 3) One cell layered discontinuous layer of starch sheath.

Sclerenchyma zone: Discontinuous patches of sclerenchyma cells are present just on periphery of the phloem.

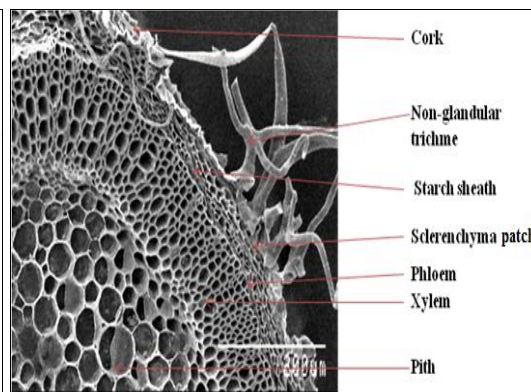
Vascular bundle: Vascular bundles are conjoint, collateral, open type and form a continuous vascular cylinder. Phloem scanty followed by xylem layer. In the phloem zone, there is a band of 3-5 layers of sclerenchyma. Xylem endarch. Cambium is not distinct.

Pith: It is large, occupying central part of the stem. It is composed of parenchymatous cells.



3A₁

Fig 3A₁: T.S. of stem (Light microscopic photograph)



3A₂

Fig 3A₂: T.S. of stem (SEM photograph)

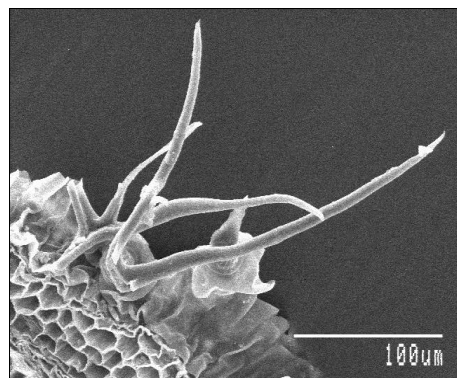


Fig 3A₃: Epidermal trichome (magnified, SEM photograph).

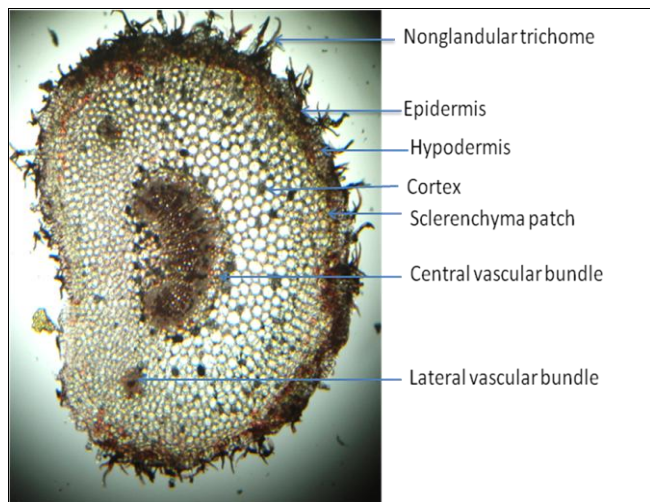
b. Petiole

The transverse section of the petiole is more or less semi-lunar in outline and shows the following anatomical features (Fig.-3B).

Epidermis: It is uniseriate, cuticularized, with 2 to many-armed non-glandular trichomes.

Ground tissue: It is a massive, 13-15 cell layered zone and is composed of large, isodiametric parenchymatous cells with profuse intercellular spaces.

Vascular bundle: Three vascular bundles are present. Out of three 2 bundles are smaller in size, present on each side of 1 large, centrally located vascular bundle which is semilunar in shape.



3B

Fig 3B: T. S. of petiole

3.3 Xylem elements

Description and measurement of xylem elements, their size, pit, perforation plates of vessel elements, side wall thickening of tracheids, fibre size and nature, etc. of the investigated plant have been represented in Table- 5.

The vessel elements are long, cylindrical with simple, circular perforation plate oriented obliquely at the end wall. Pits are bordered and opposite on the lateral walls. Vessel elements have long pointed tails at both or in one end. Size of the vessel element is $493.974 \mu\text{m} \times 52.102 \mu\text{m}$ and frequency is $1.326/\text{mm}^2$ (Fig.-4A).

Tracheids are very long and with spiral side wall thickening. Diameter and frequency are $32.6\mu\text{m}$ and $17/\text{mm}^2$, respectively (Fig.-4B).

Fibres are typically libriform type; ends are narrow, tapering, pointed. Pits are simple, present throughout the length of the fibre. Fibre size is of $749.193 \times 23.748\mu\text{m}$ and frequency is $10.754/\text{mm}^2$ (Fig.-4C).



Fig 4A: A vessel element



Fig 4B: A portion of tracheid



Fig 4C: A portion of fibre.

Table 5: Xylem element characters of the investigated plant species

Structure	Character	Type/ measurement
Vessel elements	Type of perforation plate	Simple
	Arrangement of perforation plate	Oblique
	Pits	Bordered
	Tail	Present
	Length (μm)	493.974
	Breadth (μm)	52.102
Tracheids	Frequency (No./ mm^2)	1.326
	Wall thickening	Spiral
	Diameter (μm)	32.6
Fibres	Frequency (No./ mm^2)	17
	Ends	Pointed
	Pits	Present
	Length (μm)	749.193
	Diameter (μm)	23.748
	Frequency (No./ mm^2)	10.754

3.4 Organoleptic features of the stem bark powder

Stem bark part: Colour- Grey; Odour-Not specific; Taste-Pungent; Texture- Finely fibrous (Table- 6; Fig.- 5A,5B)



5A

Fig 5A: Dried stem bark



5B

Fig 5B: Powdered stem bark

Table 6: Organoleptic characters of the bark powder of the investigated plant

Plant part	Colour	Odour	Taste	Texture
Stem bark	Grey	Not specific	Pungent	Finely fibrous

3.5 Physicochemical analysis of the bark powder

For physicochemical characterization of the powdered plant samples moisture content, total ash content, acid insoluble ash, water soluble ash, extractive value, were determined and results are presented below (Table-7).

a. Moisture content

The moisture content of stem bark is 0.5 %.

b. Ash value

Total ash of the stem bark were recorded 9%, of which acid

insoluble ash is 0.092% and water soluble ash is 7.626%.

c. Extractive value

Extractive value of the drug sample varies according to the nature of the solvent. It is found that methanol soluble extractive value is highest (8.627%) among the other solvents. Benzene soluble extractive value is 0.8 % which is found to be lowest here. The extractive values for the solvents of water, chloroform and petroleum ether are 7 %, 4.56 % and 1.856 %, respectively.

Table 7: Physico-chemical properties of powdered stem bark of the investigated plant

S. No.	Physico-chemical parameters	Value (%)
1.	Moisture content (w/w)	0.5
2.	Ash value (w/w)	09
	a. Total ash	
	b. Acid insoluble ash	0.092
	c. Water soluble ash	7.626
3.	Extractive value (w/w)	
	a. Water soluble extract	07
	b. Methanol soluble extract	8.672
	c. Chloroform soluble extract	4.56
	d. Benzene soluble extract	0.8
	e. Petroleum ether soluble extract	1.856

3.6 Fluorescence analysis

Drug powder of the stem bark treated with different chemical reagents emitted distinguishing colour when observed under different wave length of UV light (355nm and 254 nm). In some cases the colour changes were markedly distinct in comparison to the colours observed under ordinary light

(Table-8; Fig.-6A, 6B). The natural colour of the bark powder is creamy white. When it was observed under UV light it fluoresces green. The bark powder treated with 50 percent nitric acid showed reddish orange colour and the powder treated with same reagent fluoresced black to deep green under UV light.



Fig 6: UV- fluorescence study of powdered stem bark: A- Under visible light; B- Under UV light (355 nm)

Table 8: UV-Fluorescence behavior of the stem bark powder

Material and treatment	Under UV light		Under visible light
	(355nm)	(254nm)	
Bark powder as such	Fluorescent deep green	White green	White
Powder treated with 50% Nitric acid	Black	Deep green	Reddish orange
Treated with Acetone	Fluorescent creamy green	Green	Deep brown
With Methanol	Fluorescent green	Deep green	Reddish brown
With Ethanol	Fluorescent green	Deep green	Light brown
With Picric acid	Black	Green	Creamy yellow
With 80% Sulfuric acid	Blackish green	Blackish violet	Dark coffee
With Acetic acid	Fluorescent creamy white	Green	Deep brown
With 1(N) Hydrochloric acid	Blackish green	Olive green	Pale orange
With Antimony trichloride	Fluorescent deep green	Green	Pale brown
With 5 % Sodium hydroxide	Black	Blackish green	Reddish black
With 5% Ferric chloride	Deep green	Greenish black	Blackish yellow
With Water	Fluorescent green	Green	Deep brown

3.7 Histochemical study

Different phytochemical groups like, tannins, proteins, alkaloids, flavonoids, glycosides, lignin, etc. have been

detected in various tissue zones such as vascular bundles, cortex, pith, etc. through histochemical localization study of the stem part (Table -9).

Table 9: Histochemical localization test of the stem of the investigated species

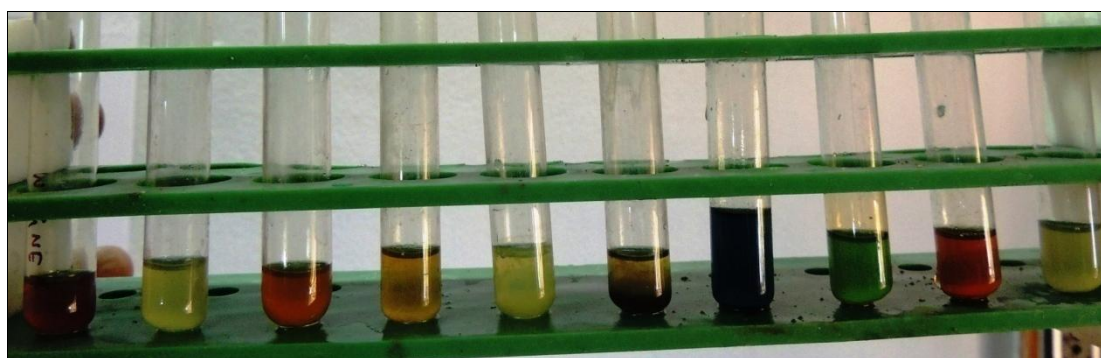
Test for	Test/ reagents	Histological location
Alkaloids	Mayer's reagent	Vascular bundles
	Wagner's reagent	Vascular bundle, pith
	Dragendroff's reagent	Epidermis, vascular bundle
Flavonoids	10% NaOH	Phloem, cortex
Reducing sugars	Fehling's reagent	Epidermis, hypodermis, some cells of cortex, xylem and phloem
	Benedict's reagent	No tissue zone detected
Proteins	Millon's reagent	Phloem, cortex including hypodermis
	Lugol's reagent	Few cells of cortex
Tannins	10% Lead acetate solution	Vascular bundles
	5% Ferric chloride solution	Hypodermis
	10% Potassium di-chromate	Epidermis, chlorenchyma, cortex and some cells of pith
Lignin	Phloroglucinol	Sclerenchyma tissue including xylem and phloem fibres
Glycosides	Keede reagent	Xylem
Carbohydrates	Molish's test	Xylem, phloem and pith

3.8. Preliminary phytochemical screening of the powdered sample of bark

a. Microchemical colour reaction tests

Preliminary chemical screening of the methanol extract of the investigated plant showed presence of alkaloids, flavonoids,

tannins, saponins and other major phytochemical groups. Presence of these groups in the stem bark indicates that the plant is possibly enriched with a good number of important phytochemical groups which are basically responsible for its wide range of medicinal properties (Table- 10; Fig.-7).

**Fig 7:** Microchemical colour reaction test.**Table 10:** Microchemical tests of the methanolic extract of stem bark

Test/Reagent	Test for	Nature of colourchange	Degree of colourchange
Dragendroff's reagent	Alkaloids	Orange brown ppt.	++
Wagner's reagent	Alkaloids	Orange brown ppt.	+++
Mayer's reagent	Alkaloids	White/ Cream ppt.	+
Hager's test	Alkaloids	Yellow ppt.	+++
Shinoda test	Flavonoids	Magenta colour	++
10% NaOH	Flavonoids	Yellow colour	+++

Salkowski test	Steroids and Triterpenoids	Reddish-blue and green fluorescence	+++
Kedde reagent	Glycosides	Violet to blue colour	+++
Keller-Killiani test	Cardiac Glycosides	Reddish-brown at junction of two liquid	+++
Benedict's reagent	Reducing sugar	Brick red ppt.	-
Fehling's reagent	Reducing sugar	Brick red ppt.	-
Molish's test	Gums	Red-violet ring	+++
10% aqueous potassium dichromate solution	Tannins	Yellowish-brown ppt.	+++
10% aqueous lead acetate solution	Tannins	White ppt.	+++
5% aqueous ferric chloride solution	Tannins	Greenish-black colour	++
1% lead acetate solution	Saponins	White ppt.	+++
Bontrager's test	Anthraquinones	Pink colour	+++
Ninhydrin test	Amino acids	Purple colour	-
Phloroglucinol test	Lignin	Red colour	+
Millon's reagent	Proteins	White ppt.	+
Lugol's reagent	Proteins	Faint yellow colour	++

+ = present; - = absent

b. Gas Chromatography Mass Spectroscopy

The methanolic extract of *E. paniculata* was subjected to GC-MS analysis for identification of different phytochemical compounds present in it. A total of nine chemical compounds have been elucidated for the first time from the bark extract of this ethnomedicinal plant. Out of nine, two compounds are of fatty acid esters viz., 10-Heptadecen-8-ynoic acid, methyl ester, (E)- and Cyclopropanedodecanoic acid, 2-octyl-, methyl ester; three compounds are of carboxylic acid esters viz., 1,2-Benzenedicarboxylic acid, butyl octylester; Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester and 2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester; two are coumarin derivatives (benzopyrone family member with lactone-like chain), viz. scoparone and scopoletin; 2-Hexadecanol, a phenol and remaining one aromatic ketone(ether) viz. 2,4,6-Trimethoxyacetophenone.

The area percent of a peak in respect of the total area of all peaks in the chromatogram represents the abundance of a

particular compound in the tested extract. Thus, based on abundance, highest peak in the chromatogram has been detected for Scopoletin (31.788%) and lowest peak was recorded for 2-Hexadecanol (3.995%). Second and third highest abundances have been recorded for Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (18.546%) and 2,4,6-Trimethoxyacetophenone (14%), respectively. Rest of the five compounds which showed less than ten percent of peak area in the chromatogram viz., Scoparone (8.864%); 1,2-Benzenedicarboxylic acid, butyl octyl ester (7.181%); 10-Heptadecen-8-ynoic acid, methyl ester, (E)-(5.788%); 2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester (5.102%) and Cyclopropanedodecanoic acid, 2-octyl-, methyl ester (4.665%). Name of the phytochemicals along with their retention time, peak percent, molecular formula, molecular weight, chemical structures and reported biological roles have been presented in a tabular form (Table-11; Fig.-8).

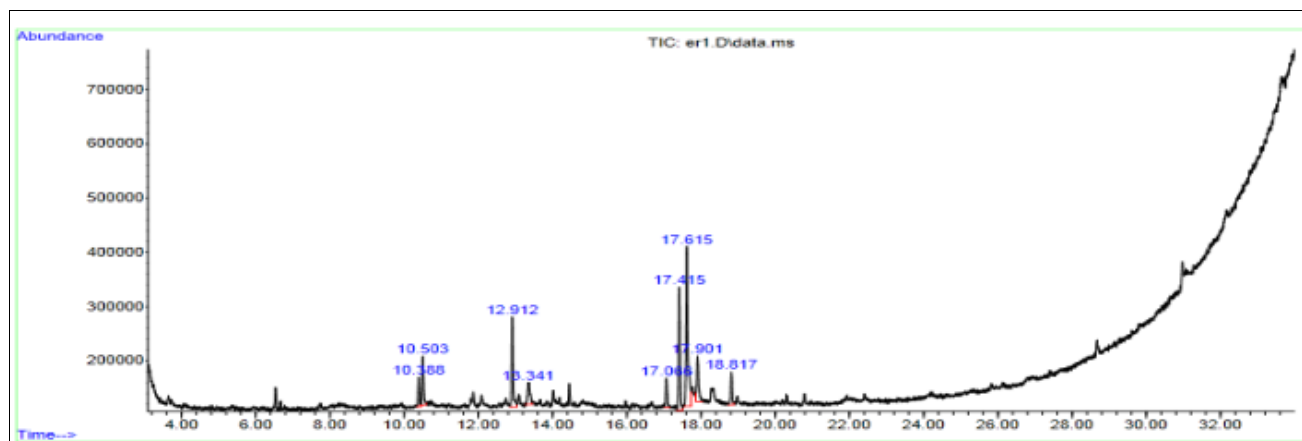
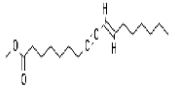
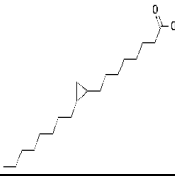
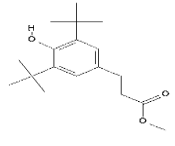
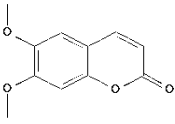
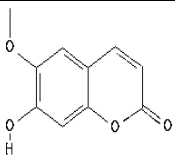
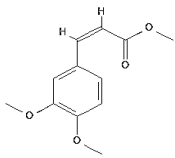
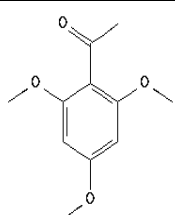


Fig 8: GCMS chromatogram of the methanolic extract of stem bark.

Table 11: Gas Chromatography-Mass Spectroscopy (GCMS) analysis of stem bark extract of *Erycibe paniculata*

Class of the chemical compound	Name of the compound	RT (min)	Peak area (%)	Molecular Formula; Molecular weight (g/mol.)	Structure	Biological activity reported earlier
Phenol (pheromone)	2-Hexadecanol	10.388	3.995	C ₁₆ H ₃₄ O; 242		Not reported
Dicarboxylic acid-ester	1,2-Benzenedicarboxylic acid, butyl octyl ester	10.503	7.181	C ₂₀ H ₃₀ O ₄ ;334		Antimicrobial, antifouling (Adeyemi M A et al., 2017) [54]

Ester derivative of fatty acid	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	13.34 7	5.788	C ₁₈ H ₃₀ O ₂ ;278		Anti-inflammatory (Ibraheam <i>et al.</i> , 2017) [59]
Ester derivative of fatty acid	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	17.07 2	4.665	C ₂₄ H ₄₆ O ₂ ;366		Anticancer, antitumor, antiestrogenic, antimicrobial (Manjamalai, 2011) [57]
Phenolic ester	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	17.41 5	18.546	C ₁₈ H ₂₈ O ₃ ;292		Antifungal, antioxidant (Bashir <i>et al.</i> 2012) [55]
Coumarin derivative	Scoparone	17.90 1	8.864	C ₁₁ H ₁₀ O ₄ ;206		Antiacylcholinesterase activity (Mogana <i>et al.</i> , 2014) [62]
Coumarin derivative	Scopoletin	17.61 5	31.788	C ₁₀ H ₈ O ₄ ;192		Antileishmanial activity (Mogana <i>et al.</i> , 2014) [62]
Benzene carboxylic acid ester having two ether group	2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester	18.82 2	5.102	C ₁₃ H ₁₆ O ₅ ;252		No activity reported
Ether of trihydroxyacetophenone	2,4,6-Trimethoxyacetophenone	12.91 7	14	C ₁₁ H ₁₄ O ₄ ; 210		Antiinflammatory (Chairadia <i>et al.</i> , 2008) [58]

4. Discussion

In the present study some characters obtained from foliar micromorphology, anatomy of petiole and stem, preliminary phytochemical screening and physicochemical evaluation, are found very distinct and can be used as marker in identification of *Erycibe paniculata* in its fresh as well as dried from. The study is the firsthand information since no pharmacognostic work has been done earlier on this plant.

According to WHO (1998), the macroscopic and microscopic characterization of plants is the first and basic criterion to confirm identity and confer purity of drug of it [8]. In this study it has been observed that epidermal cells are irregular in shape. Very distinct type of cell wall out line is observed between the epidermal cells of upper and lower surfaces of leaf. Cell wall outline is straight in upper epidermis and it is slightly wavy in lower epidermal cells. The palisade ratio for this species is 6.62 which distinguishes it among the species of Convolvulaceae studied for their palisade ratio. Taxonomically and pharmacognostically the study of stomata is very important to identify the medicinal plants [24, 26, 27]. The foliar micromorphology of the present investigated plant showed anisocytic type of stomata which conforms the stomatal type reported in earlier studies in the genus *Erycibe* [28]. Although paracytic, anisocytic and anomocytic types of stomata have been found in different members of Convolvulaceae [28-34]. Similarly, Stomatal Index (SI) of *E.paniculata* was determined as 13.87 which is distinct to this

species of genus *Erycibe* and makes it unique among other members of the same family [29-33].

Foliar trichome feature is considered as one of the valuable taxonomic markers as well [34-37]. It has been reported that, some species under the family Convolvulaceae showed the trichomes of unicellular, glandular type and both uni- and multicellular, non-glandular trichomes with two arms [28]. Here in case of *Erycibe paniculata*, trichomes of the leaf are of unicellular glandular type. However, typical 2-6-armed, non-glandular types were recorded in the petiole and stem epidermal surfaces of this taxon. Such type of multi armed nonglandular trichome structure has not been recorded earlier from the members of the genus *Erycibe* [28, 35, 38]. Therefore, the trichomes with many arms will serve as marker character here in identification of the shoot part of this investigated plant.

Petiole anatomy is employed as an efficient tool in plant species identification as well as leaf drugs. Anatomy of petiole of this plant is found very distinct among the other taxa of the family Convolvulaceae so far studied. In our investigation, petiole showed a semi-lunar outline in its transverse section and it contains three vascular bundles, of which central one is semilunar in shape, larger than other two. Chemical analysis and biological assay are two important aspects which are employed in Pharmacognosy for proper evaluation of crude drug procured from the medicinal plants [39]. Preliminary phytochemical analysis highlights the

chemical nature of crude drug and its valuable phytoconstituents. The important phytochemical groups detected in the stem bark of the present investigated plant are alkaloids, anthraquinones, saponins, tannins, glycosides, steroids, etc. Histochemical study gives a scientific impression about the localizations of alkaloids, saponins and tannins in the vascular bundles, sclerenchyma and cortical zone of the stem part. This information proves the therapeutic utility of shoot bark of this medicinal climber as anatomically bark includes the tissues of cork, cortex and phloem part of the vascular bundles. Compounds of various phytochemical groups are generally stored and/ or synthesized mainly in the phloem, cortex, cork layers, pith zone and definitely in the leaf tissues. This scientific reality has also been realized here in histochemical study of stem where cortex, pith, vascular bundles and cork zone have been detected as active sites of many phytochemical groups localized.

Therapeutic properties of different phytochemicals have already been studied and well documented from some medicinal plants which are traditionally used for treatment of tumour, inflammation, diarrhea, malaria, diabetes, rheumatic pain, sexual diseases, etc. [40-43] In our study, microchemical colour reaction tests confirm the presence of different phytochemical groups like alkaloids, flavonoids, saponins, steroids and triterpenoids, tannins, etc. Presence of such important phytochemical groups in *E. paniculata* clearly indicates its therapeutic properties and also its possibility towards scientific validation of different ethnomedicinal uses. In pharmacognosy, physicochemical characters play a crucial role in establishing fingerprint of some standards of a crude drug and it helps in detection of adulterants also. Moisture content, ash value, extractive value and UV-fluorescence characters are some of the pharmacognostic parameters commonly employed in crude drug evaluation [9, 25]. In this study, a negligible content of moisture (0.5%) was found in bark powder which can be considered as an official standard of this bark drug.

Among the physical constants, ash value is considered as a fixed character which varies from one species to another. It is applied as one of the important diagnostic tools in crude drug study. The value also considered as an indicator of presence of inorganic matters in the crude drugs [44]. It has been found that the ash value of bark is 9% and it is very distinct as well which indicates that bark part of this medicinal plant consists of a good amount of inorganic minerals like carbonate, oxalate, phosphate including silica and siliceous earthy matters. Values for various parameters like acid insoluble ash and water soluble ash observed in the bark part of this plant are different and distinct from one another which can be used as identifying marker in authentication and quality control of the crude drug obtained from the bark of the plant *E. paniculata*.

The fluorescence analysis of the drug powder is very useful to distinguish genuine drug from the adulterated one [44, 45]. Some chemicals present in the plant drug powder fluoresce differently under different wavelength of UV-light. In present investigation, creamy white colored bark powder showed the green fluorescence under UV light. When the same powder is treated with 50 percent nitric acid it exhibited reddish orange colour under ordinary light and fluoresced black colour in UV light. These marked changes in colour are found very convincing here in identification of the bark in its powdered form.

Extractive value indicates the amount of extractive substance released in a given solvent from a crude drug. This value

plays a decisive role in selection of standard extractive solvent for estimation of maximum phytochemicals present in a powdered drug [24]. In our study, methanol exhibited highest extractive value (8.672%) among other solvents used like benzene, Petroleum ether, chloroform. In benzene, the plant drug yields lowest extractive value (0.8 %). So, it indicates that the chemicals present in drug sample of bark are highly dissolved in polar organic solvents like methanol than the non-polar solvents like petroleum ether, benzene, etc. The study revealed that methanol, being a highly polar solvent, extracted out the highest amount of chemical constituents from the bark of *E. paniculata*. The extractive values for all four solvents taken here gave a distinct pattern which will be instrumental in quality control of the bark drug.

Several standard techniques are used in qualitative and quantitative estimation of crude drugs for their various chemical constituents. GCMS is one of such important techniques for the analysis, characterization and identification of chemical compounds of low molecular weight. In present study GCMS of methanol extract of *E. paniculata* bark has been carried out and it revealed nine compounds which belong to the groups of phenolics, fatty acid esters, coumarins, carboxylic acid esters, and ether of trihydroxyacetophenone. The obtained chromatogram can be considered as the chemical fingerprint of the crude bark of this medicinal plant.

Perusal of literature revealed that among the nine identified phytochemical compounds from the bark extract, seven compounds show a wide range of biological actions such as anti-inflammatory, anticancer, antioxidant, antimicrobial, antiparasitic, etc. (Table -10). Out of seven phytochemicals, two compounds, viz. an aromatic carboxylic acid ester, Benzenedicarboxylic acid, butyl octyl ester-(peak area = 7.181%) and a phenolic ester, Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester have been reported as antimicrobial agents in earlier studies [46, 47]. Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester has also a good applications in food industry to maintain the flavor, quality, fragrance, taste. GCMS chromatogram of the investigated plant drug showed second highest concentration of this compound (peak area=18.546%).

It has been reported that the crude extract of the *E.paniculata* is effective against some gram positive and gram negative pathogenic bacteria [48] which again supports the presence of antimicrobial agents in the plant extract. Another compound of fatty acid ester namely Cyclo propanedodecanoic acid, 2-octyl-, methyl ester was reported to have both antimicrobial and anti-inflammatory activities [49]. Reports on such biological activities of these compounds identified from the bark extract support the traditional uses of root and bark decoction of this investigated plant by the people of Orissa in curing cholera, fever and diarrhea [14-16] as many microbial organisms are responsible for such disease as well as health conditions.

Other two chemical compounds identified here viz., 2,4,6-Trimethoxyacetophenone (a ether of trimethoxyacetophenone) and 10-Heptadecen-8-ynoic acid, methyl ester, (E)- (a fatty acid ester) have been reported as potent anti-inflammatory compounds [50, 51]. GCMS chromatograms showed the third and sixth highest concentrations of these two compounds in the bark extract (14% and 5.788%, respectively). Anti-inflammatory substances are very helpful in several diseases and ailments like cancer, rheumatism, cardiovascular problems, immune

related problems, etc. [52, 53] Presence of such anti-inflammatory chemicals in the bark of this plant is correlated with its ethnomedicinal uses for headache (during menstruation cycle).

Two different coumarin derivatives have been detected in this study, viz., scopoletin and scoparone. It is reported that Scopoletin shows potent antileishmanial [54] and antispasmodic activity [55]. In our study, scopoletin exhibited the highest concentration (peak area=31.788%) in the crude bark extract. Other coumarin compound, scopoletin has been established as the cures of a wide range of health disorders like mental retardation, cretinism, nerve deafness, scrotal dermatitis and different inflammatory conditions including painful sores of mouth, swelling of tongue, etc. [56] Application of scoparone (peak area=8.864%) stimulate the inhibition of synaptic transmission during muscular pain, helps to mobilize calcium in muscle and reduce skeleton-muscular problems including muscular pain and spasm [54, 56, 57]. Presence of scopoletin and scoparone in greater quantities in the bark clearly justify the traditional uses of stem bark of this plant for curing muscular spasm, headache and pain during menstruation because of the active role played by these two coumarin chemicals in inhibiting the synaptic transmission occurs in those health problems [15-17].

It is interesting to mention here that two phytochemicals belonging to the groups of phenol and benzene carboxylic acid ester viz., 2-Hexadecanol (peak area= 3.995%) and 2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester (peak area=5.102%), respectively have been detected in the GCMS study of bark of this plant for which the biological and pharmacological activities have not been reported till date. So, there is a scope to find out the biological roles of these two compounds obtained from this plant.

Presence of different types of phytochemical compounds in the crude bark of *E. Paniculata* make this plant very promising candidate for its further chemical and biological activity studies. Systematic investigations of the parts other than bark are also to be carried out for scientific validation of ethnomedicinal claims attached to this important medicinal plant.

5. Diagnostic characters of the investigated plant

A. Foliar epidermal micromorphology :

1. Cells are irregular in shape and cell walls are more or less straight
2. Anisocytic type of stomata and stomatal index is 13.87.
3. Trichomes are unicellular, glandular type
4. Presence of clustered crystals in upper epidermal cells

B. Vegetative anatomy:

- I. Multi-armed (2-6), non-glandular trichomes teeming on petiole and stem.
- II. Petiole with 3 vascular bundles, the central one is larger and semi-lunar in shape

C. Physical evaluations

- Moisture content-0.5% (stem part)
- Total ash content- 9%
- Water soluble ash- 7.626%
- Acid insoluble ash- 0.029%
- Methanol soluble extractive value- 8.762%
- UV-fluorescence character- Methanol treated bark powder appears pinkish brown colour in visible light and in UV light it shows fluorescent blue colour.

6. Conclusion

The diagnostic characters on foliar epidermal micromorphology and physicochemical constants investigated here in the pharmacognostic study of *E. paniculata* will be very useful in proper identification of the crude drugs obtained from its different parts. The data of the pharmacognostic study will also be helpful in quality assurance of the drugs obtained from this ethnomedicinal plant. A wide range of therapeutically important phytochemical groups like alkaloids, flavonoids, fatty acids, phenolics, glycosides, etc. have been detected in methanolic extracts of stem bark powder of this medicinal climber which basically highlights its various therapeutic properties.

GCMS study of the bark extract revealed the existence of nine phyto-compounds and of which, seven chemicals have been identified by other workers as very potent in a wide panorama of pharmacological functions including antimicrobial, anti-inflammatory, antispasmodic, anticancer, etc. This information clearly focuses the promising domains of phytochemical and pharmacological studies of present investigated plant. Present investigation also highlights the scientific basis regarding traditional uses of this medicinal plant for various healing purposes. Finally, it may be concluded that *E. paniculata* needs further investigation in the line of phytochemistry and pharmacology for identification of the lead molecules and for development of potent bioactive natural products.

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