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Research Article

Pharmacognostical and Phytochemical Investigations of the Aerial Parts of *Merremia umbellata* Linn. (Convolvulaceae)

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ABSTRACT

Pharmacognostical and Phytochemical investigations are the first step to determine the quality and purity of the crude drug. The aim of this study was to investigate the Pharmacognostical and Phytochemical properties of the rare medicinal plant - Merremia umbellata (Convolvulaceae). Merremia umbellata is a perennial climbing plant with herbaceous system 1-3 metres long that scramble over the ground and twine into other plants for support. It is a twining or prostrate herb found from Punjab eastwards to Assam, Garo, North Cachar, Aka and Lushai hills, Eastern Ghats up to Godavari, Western Ghats, Deccan and Andaman Islands ascending to 1,200m in the hills. Merremia umbellata is also known as Ipomea cymosa. The Literature review of Merremia umbellata reveals that the dried powdered leaves are sniffed up the nose for the treatment of epilepsy and used as a remedy for microbial infections. Phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and phenolic compounds. The current study describes some Pharmacognostical and Phytochemical studies on Aerial parts of Merremia umbellata.

Keywords: *Merremia umbellata, Ipomea cymosa*, Convolvulaceae, Pharmacognosy, Phytochemistry

INTRODUCTION

Merremia umbellata (L.) Hall. (Convolvulaceae) is a twinning or prostate herb (creeper). It is found from Punjab eastwards to Assam, Garo, North Cachar, Aka and Lushai hills, Eastern Ghats up to Godavari, Western Ghats, Deccan and Andaman Islands ascending to 1,200 m in the hills. Leaves variable, ovate, ovate - oblong, entire; flowers funnel - shaped, white or yellow to orange; capsules globose with 4 hairy seeds.[1] The stems sometimes become more or less woody, especially near the base for more than a year. The plant is harvested from the wild for local use as a food and medicine. It is often grown as an ornamental, valued especially for its flowers. Merremia umbellata is native in a wide range of South eastern Asia (China, India, Sri Lanka, Malaysia and Philippines[2] . The young leaves are eaten as pot - herb. The plant is considered useful in Indonesia for fistulae, pustules and tumours. Merremia umbellata is also known as Ipomea cymosa Roem. The species of Merremia containing Ornithine derived Alkaloids, 3α- Acylcloxytropane/- Nortropanes, 3β- Acycloxytropane/- Nortropanes, Pyrrolizidines, Tryptophan derived Alkaloids, Phenylalanine derived metabolites, Terpenoids (Isoprenoids), Fatty acids and their derivatives, Flavonoids and Saponins. Merremia umbellate was studied for antispasmodic, antihyperglycemic, antibacterial, antitumour, anti-inflammatory, antioxidant, cytotoxic, cancer chemoprotective and antileukaemia. Ipomea cymosa has been claimed to be useful for to be alterative, deobstruent and analgesic.1 A decoction is considered useful in the treatment of rheumatism, neuralgia, headache etc. It is also used for dropping into the ear in cases of auricular ulcers, abscesses etc. The leaves are emollient. The dried, powdered leaves are sniffed up the nose as a treatment for epilepsy. The pounded leaves are used to treat burns, abscesses, ulcers, sores and scalds. A poultice of the leaves combined with Curcuma powder (Curcuma longa) is applied on cracks on the hands and in the soles of the feet. The seeds, when soaked in water, yield mucilage that is used as an alternative in the treatment of cutaneous diseases. Tubers are mildly laxative and are widely taken as a remedy for dysentery. A decoction of the roots is drunk as a remedy for haematuria. A paste or powder made of the root, often mixed with Java flour and water, is applied as a poultice to swellings. The latex of the root is taken as a purgative. Present study was aim to investigate the anatomical characteristic of Merremia umbellata, to provide opportunities for further studies.[3]

MATERIALS AND METHODS

Collection of Plant Materials:

The fresh plant material was collected from Marthandam, Kanyakumari District in the month of February 2019. The plant material was taxonomically 3 authenticated by Prof. P. Jayaraman, Ph.D., Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai, Tamilnadu. Care was taken to select healthy plants and normal organs.

Procedure for Anatomical Studies and Staining Methods:

Standard methods of sectioning and staining were followed for preparation of semi-permanent and permanent slides. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin - 5ml + Acetic acid + 70% Ethyl alcohol - 90ml). After 24 hrs of fixing, the specimens were dehydrated with grades series of tertiary Butyl alcohol.[4] Infiltration of the

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specimens was carried by gradual addition of paraffin wax (melting point 58-60˚C) until Tertiary Butyl Alcohol solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 - 12 µm. Dewaxing of the sections was by customary procedure. [5] The sections were stained with Toluidine blue.[6] Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary, sections were also stained with Safranin and Fast-green and Iodine in Potassium iodide (for Starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration liquid were prepared. 4 Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with Sodium hydroxide and mounted in glycerin medium after staining. Different cell component was studied and measured. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books.[7, 8]

Identification of Plants constituents of *Merremia umbellata*

The various extracts of Merremia umbellata were subjected to the following preliminary phytochemical analysis.[9]

Test for Alkaloids

A small portion of solvent free petroleum ether, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid, filtered and tested carefully with various alkaloidal reagents such as

Mayer's reagent - Cream precipitate

Dragendorff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates and Glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

Molisch's test

The filtrate was treated with 2-3 drops of 1% of alcoholic alpha naphthol and 2 ml of concentrated Sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction which showed the presence of carbohydrates.

Fehling's test

The filtrate was treated with 1ml of Fehling's solution and heated. Red precipitate was obtained which showed the presence of carbohydrates.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No colour change in ammoniacal layer was observed.

Test for Phytosterols

1g of extract was dissolved in few drops of dilute acetic acid. 3 ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed oils and Fats Small quantities of various extracts were separately pressed between two filters papers. No oil stain on the paper indicated the absence of fixed oil. 5 Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with few drops of phenolphthalein. The mixture was heated on water bath for 1-2 hours. No soap formation, neutralisation of alkali indicated the absence of fixed oils and fats.

Test for Saponins

The extracts were diluted with 20ml of distilled water and it was agitated on graduated cylinder for 15min. The presence of saponins was indicated by formation of 1cm layer of foam.

Tests for Tannins and Phenolic compounds

Small quantities of various extract were taken separately in water and tested for presence of phenolic compounds and tannins with 1. Dilute ferric chloride solution (5%) - Violet color. 2. 10% Lead acetate solution - White precipitate.

Tests for Protein and free Amino acids

Dissolved small quantities of various extracts in few ml of water and treated with following:

1. Millon's reagent - Red color showed the presence of proteins and free amino acids.
2. Ninhydrin reagent - Purple color showed the presence of proteins and free amino acids.
3. Biuret test - Equal volume of 5% solution and 1% copper sulphate solutions were added. Appearance of purple color showed the presence of proteins and free amino acids.

Test for Gums and Mucilage

Powdered drug was treated with Ruthenium red solution. Reddish pink color was obtained which showed the presence of gums and mucilage.

Test for flavonoids

a) With aqueous sodium hydroxide solution blue to violet color (anthocyanins), Yellow color (flavones), Yellow to orange color (flavonones)

b) With concentrated sulphuric acid-yellowish orange color (anthocyanins) yellow to orange colour (flavones), orange to crimson (flavonones)

c) Shinoda's test - Test extracts were dissolved in alcohol, to that piece of magnesium followed by concentrated

hydrochloric acid drop wise were added and heated. Appearance of magenta color showed the presence of flavonoids.

Test for lignin

With alcoholic solution of phloroglucinol and hydrochloric acid appearance of red color showed the presence of lignin.

RESULTS AND DICUSSIONS[10-17]

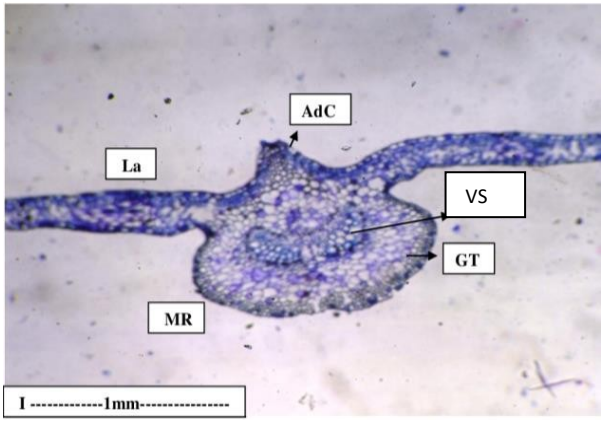

Fig.1.1 T.S of leaf through midrib

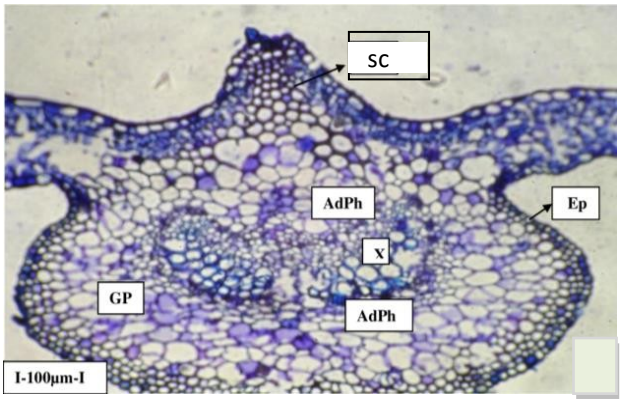

Fig.1.2 T.S of Midrib enlarged

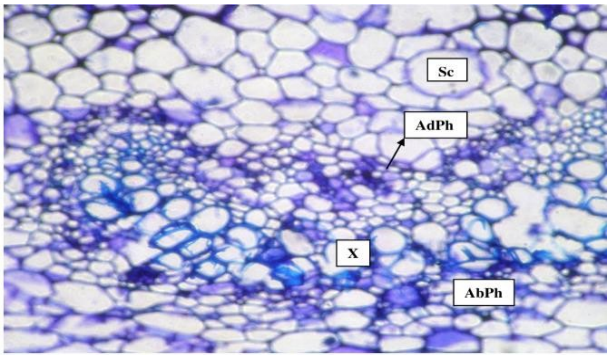

Fig.1.3 Vascular strand of the midrib

Anatomy of the Leaf: The midrib of the leaf in cross-sectional view consists of thick conical adaxial part and wide bowl shaped abaxial part.The midrib measures 2mm in vertical plane and 2.2mmin horizontal plane. The adaxial cone is 200×250µm in size. The epidermal cells of the midrib are small angular and thick walled. The inner part of the adaxial cone consist of compact mass of sclerenchymatous cells. The abaxial part of the midrib also has two or three layers of sclerenchyma cells. The ground tissue parenchymatous, angular, thin walled and compact. There is a few circular secondary cavities ensheathed externally by a layer of thin-walled sheath cells (Fig.1.1, 1.2, 2.1). The vascular system is wide, shallow is of bicollateral strand. It consists of compact are of wide, circular and thick-walled xylem elements and several isolated units of phloem located on the inner and outer parts of xylem (Fig.1.3). This situation is called bicollateral. Lamina (Fig.2.2, 3.1): The lamina is bilateral and mesomorphic. The lamina is smooth on surface. It is 200µm thick. The epidermal cells are squarish with thick walls. These are sessile glandular epidermal trichomes both on the abaxial and adaxial epidermal layers. The glandular trichome has multicellular fan shaped stalk which is burried in the concavity of the epidermal layer. The globular densely stained gland is seen in projecting out of the epidermal pit. The glandular trichome is 50µm in height and 30µm in wide. The mesophyll tissues of the lamina have adaxial part of two layers of cylindrical palisade layer and abaxial wide air chamber and reticulate spherical spongy mesophyll cells (Fig.3.1).

Stem: The stem is somewhat elliptical in cross and seen in oval outline. It measures 1.2 mm thick. The epidermal is radially oblong, thick walled and densely tanniniferous. The cortical zone is narrow, four or five layered and parenchymatous. The vascular cylinder consists of four thick cylinder of fibres which encloses all around the vascular cylinder (Fig.5). The vascular cylinder consists of outer secondary vascular cylinder and inner primary vascular cylinder. The outer secondary vascular consist of secondary xylem, ensheathed by secondary phloem tissue. The inner part includes secondary xylem the secondary xylem has isolated clusters of wide circular thick-walled vessels and secondary xylem fibres in between the vessel groups (Fig.4.2, 5). The inner zone of primary xylem consists of long radial lines of compact, thick walled xylem fibres and the fibre lines end in wide elliptical vessels comprising proto xylem and meta xylem elements (Fig.5). Inner to the primary xylem cylinder occurs thick circular units of medullary or inner phloem. Secretory cavities are also seen in the medullary phloem zone. Secondary cavities which are angular in outline and having outer layer of sheath cells (Fig.6.1, 6.2) are seen in the cortical region and phloem region.

- Powder Microscopic observation: The Powder preparation of the plant exhibits the following elements when examined under microscope
- Covering Trichome: An elongated tubular outgrowth of an epidermal cell is termed as trichome or plant hair. Elongated structures with apex, body and base.

- Thin-walled cork cell: The cork cells are rectangular, brick shaped or polygonal; phellodermcells are mostly parenchymatous in nature. Lenticels are present in the periderm, especially in the bark of old plants which are similar in function to stomata. These are open pores with absence of guard cells. The cork cells are impregnated with a layer of suberin.

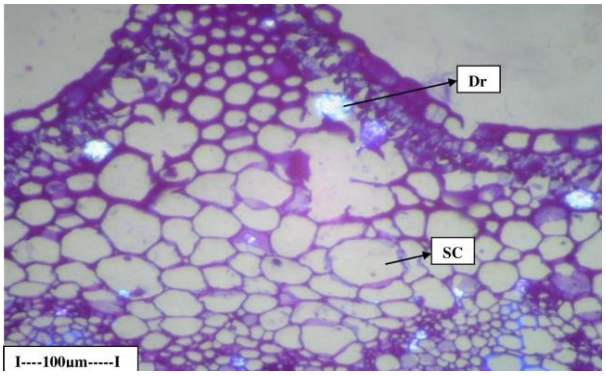

Fig.2.1 Adaxial cone of the midrib

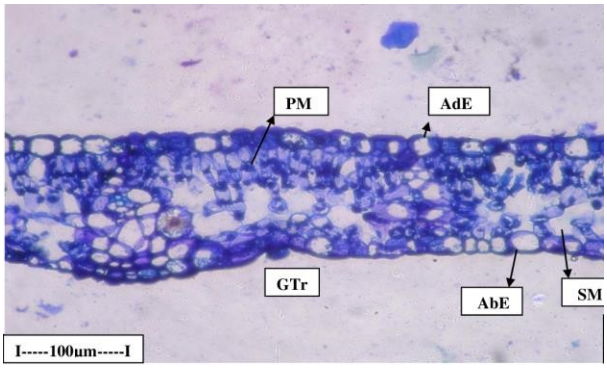

Fig.2.2 T.S of Lamina

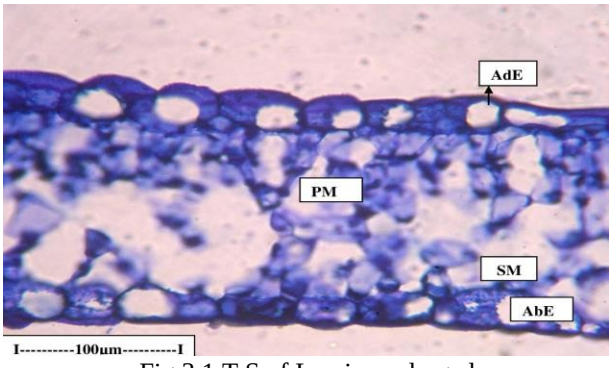

Fig.3.1 T.S of Lamina enlarged

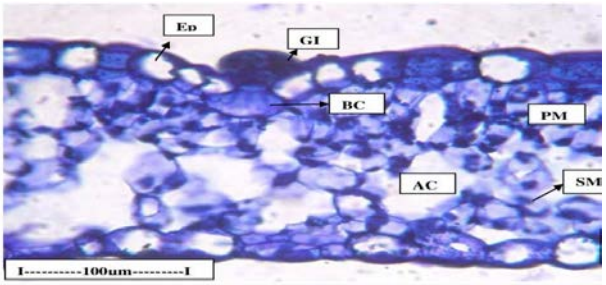

Fig.3.2 Glandular Trichome embedded in the Epidermis

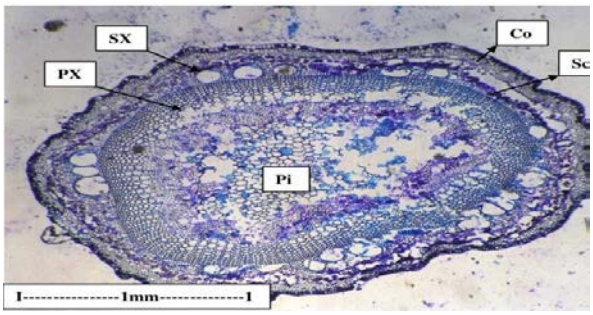

Fig.4.1 T.S of Stem – entire view

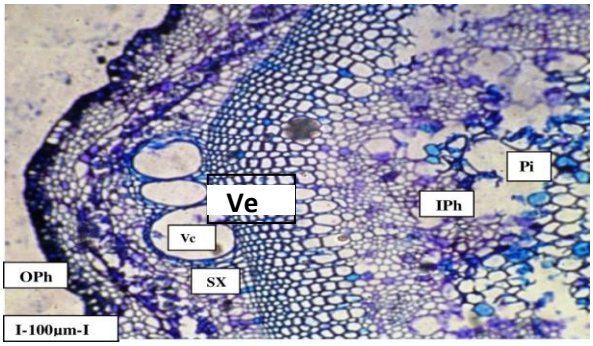

Fig.4.2 T.S of Stem – a sector enlarged

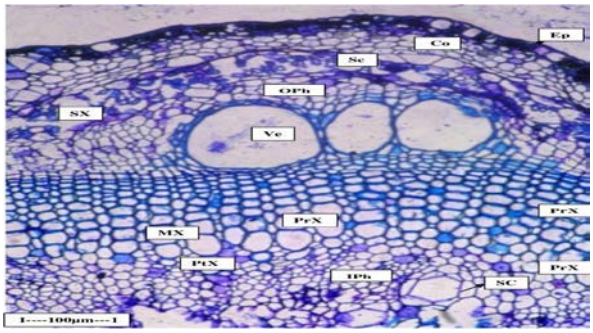

Fig.5 T.S of Stem – a sector enlarged

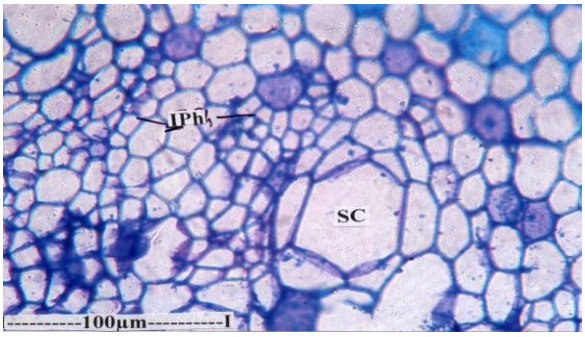

Fig.6.1 Inner Medullary Phloem

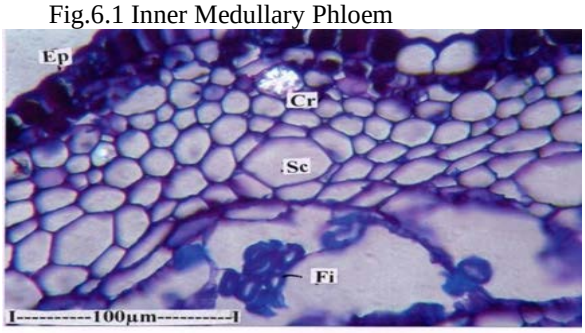

Fig.6.2 Cortical portion of the stem with calcium oxalate and a secretory cavity

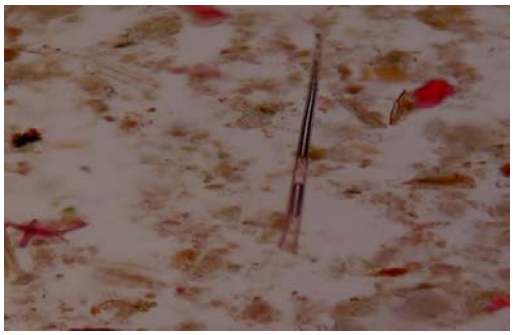

Fig. 7.1 Covering Trichome

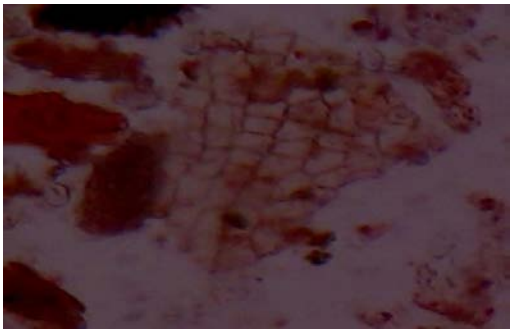

Fig.7.2 Thin walled cork cell

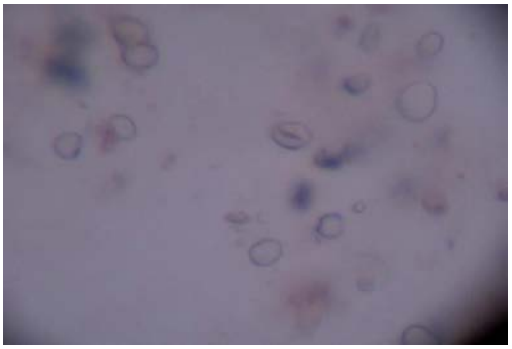

Fig.7.3 Starch grains

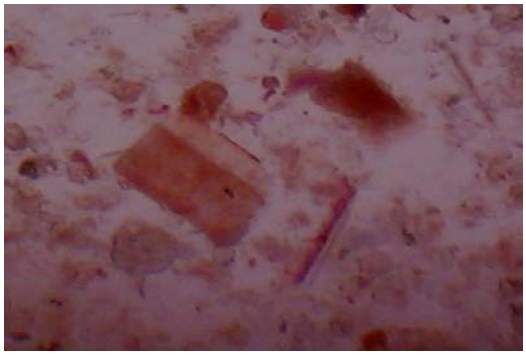

Fig.7.4 Spiral xylem vessel

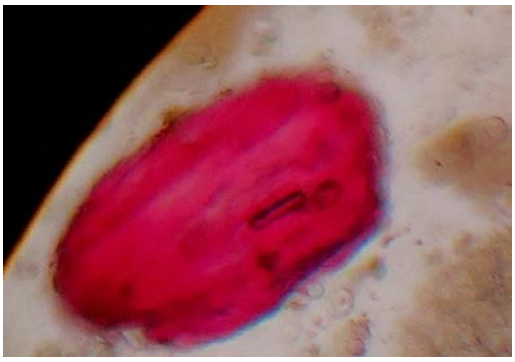

Fig.7.5 Chlorenchyma

Fig.7.6 Fibres with tracheids

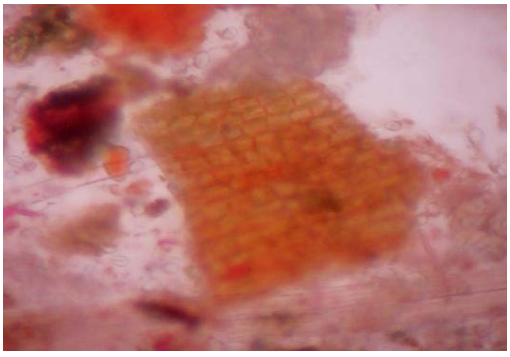

Fig. 7.7 Parenchyma sheath

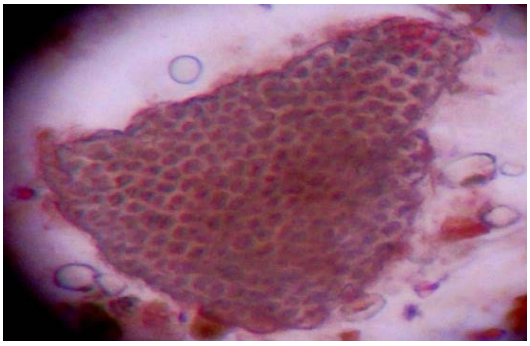

Fig.7.8 Pitted vessel with simple perforation

Table.1 Preliminary Phytochemical screening of the Crude extracts of *Merremia umbellata*

S.No.	TEST	Petroleum Ether extract	n – hexane extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	Alkaloids	_	+	+	+	_
2.	Carbohydrate	_	_	_	+	+
3.	Glycosides	_	+	+	+	+
4.	Phytosterol	+	+	_	_	_
5.	Tannins and Phenolic compounds	_	_	+	+	+
6.	Proteins	_	_	_	_	+
7.	Gums and Mucilage	_	+	_	_	+
8.	Flavonoids	_	+	+	+	+
9.	Lignins	_	_	_	+	+
10.	Fixed oils and Fats	_	_	_	_	_
11.	Saponins	_	_	_	_	+

- Fibres: The fibres are more frequent than the vessels. They are long, narrow and thick walled. The walls are lignified. They are 50µm long and 10µm wide.[18]
- Starch: Starch is present in different parts of the plant in the form of granules of varying size. Starches of different origins can be identified by their size, shape and structure, as well as, position of hilum and striations. They are spherical, 2-10 µm in dimension.
- Spiral Xylem Vessel: Spiral annular vessels are typical of protoxylem. The vessel elements very frequent seen in the powder. They are either short wide and drum shaped or long, narrow and cylindrical. They have wide circular end wall perforation.
- Pitted vessel with simple perforation: The perforation is horizontal or oblique in orientation. Pits are abundant on the lateral walls. They are circular and bordered. The vessel elements are 70 – 250µm long.
- Parenchymatous shealth: It is the simplest and the most common type of cell. The cells sre living, isodiametric with intercellular spaces, but during maturation changes in shape may occur.
- Chlorenchyma: Parenchyma containing chloroplast is called as Chlorenchyma, whereas aerenchyma is a very porous parenchyma with large intercellular spaces[19] .

CONCLUSION

There are many unknown plants with high medicinal value still that have not been recognised for their importance and brought to the light of scientific world. The present work is an attempt to compile Pharmacognostical and Phytochemical investigations of the Aerial parts of Merremia umbellata belonging to the family Convolvulaceae. The above-mentioned microscopic features and phytochemical screening can be considered as reliable and simple characters for botanical diagnosis of Aerial parts of *Merremia umbellata.*

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