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Anatomical studies and preliminary phytochemical analysis in cucumis dipsaceous ex. spach. ehrenb

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Abstract
The present studies are focused on the anatomical sectioning and phytochemical screening of the plant *Cucumis dipsaceous* Ex. Spach. Eherb. belongs to the family Cucurbitaceae. Anatomical studies revealed that the tissue differentiation and number of layers of cells in the transverse sections of stem and leaf. There were variations in the vascular bundles of stems which are bicollateral. The phytochemical screening helps to identify the secondary metabolites in the plant which is used for the pharmacology. It contains secondary metabolites like tannin, alkaloids, saponins, flavonoids, resins, steroids and carbohydrates.

Keywords: *Cucumis dipsaceus*, common name, botanical description, anatomical studies, phytochemical screening

Introduction
The comparative study of plant structure, morphology and anatomy, has always been the backbone of plant systematics, which endeavours to elucidate plant diversity, phylogeny and evolution. The second half of the 20th century has been a fascinating period in which systematics and structural studies greatly profited from new techniques and methods. The state of the art in plant systematics and structural research in the mid-20th century was shown in a detailed review by Constance (1955). The presence of more than one floral archetype among extant angiosperms, indicating different evolutionary origins, was hypothesised by these botanists. They are based on their ideas in comparisons of form of mature structures and often gave special emphasis to vascular pattern. The phytochemistry can be considered sub-fields of botany or chemistry. Activities can be led in botanical gardens or in the wild with the aid of ethnobotany. The applications of the discipline can be for pharmacognosy, the discovery of new drugs, or as an aid for plant physiology studies.

Fig 1: Morphological features of the *Cucumis dipsaceous*. Ex. Spach. Ehreb.

Distribution
It is cultivated in tropical region and Subtropical regions. It is found to be native in Tanga region, Uganda, Kenya, Africa, Ethiopia, Somalia, Sudan and Southern Egypt. Flowering season is September – November. Fruiting season is November – January. Its common name is hedge hog cucumber.

Botanical Description
It is a climbing herb by axillary tendrils, 1.5 m in length. Stems have branched, slender, angular, sulcate, tendrils simple. Leaves are simple, alternate, ovate, lobes obtuse, base cordiform, apex obtuse, margins dentate or entire. Inflorescence is solitary and axillary Flowers are unisexual, monocieous, Berry ellipsoid or globose, densely spiny (spines ca. 1 cm long), 3-6.5 cm long, pale yellow; seeds numerous, elliptical, cream-colored, 4-5 mm long. IUCN Status is Exotic, naturalized, uncommon. Flowering season is September – November and fruiting season is November – January. But is Common in dry bushyland, especially like in disturbed woodland and wooded grassland and a weed of cultivation about 400 - 1,800 m.

Materials and Methods
The fresh plant materials are collected in the kallipalayam, Coimbatore (Dt), Tamilnadu, India.

Anatomical sectioning using microtome
In order to study the variation of internal structural in the leaves, stems of *Cucumis dipsaceous*, microtome study was undertaken and microscopic anatomical observations were carried out.

Plant Materials
The plant materials are collected washed with water dried and the stem and leaves were cut into small pieces.

Killing and Fixing
The specimens were killed and fixed in formalin acetic acid (FAA) for at least 24 hours (FAA: 10:50:5:35 proportion of formalin, alcohol, acetic acid and water). The plant specimens were washed in distilled water three times allowing a time of 20 minutes for each wash.

Dehydration
After killing and fixing the plant specimens, were transferred to a series of different alcoholic concentrations (50 %, 70 %, 90 %, and 95%) and then were left overnight or more in each concentration.

Clearing
The plant specimens were cleared using two mixtures, with different ratios for different times:

- **Mixture I** composed of absolute alcohol: cedar wood oil (100: 0 for 24hr, 50: 50 for 3hr, 25 : 75 for 3 hr and 0 : 100 for 24 hr) respectively.
- **Mixture II** composed of cedar wood oil : xylene (100 : 0 for 24hr, 50 : 50 for 3hr, 25 : 75 for 3 hr and 100 for 24 hr), respectively.

sEMBEDDING
Next to the clearing process, the plant specimens were embedded in wax with melting point of 60 º C. The plant specimens were placed in closed vials containing 1:1 xylene and melted wax and put in the oven for 45 minutes. The wax was later replaced by pure wax twice after 45 minutes. The vials were left open to get rid of the xylene vapour. The specimens were transferred from the vials to the mold containing pure melted wax. Each specimen was pressed gently against the peripheral part of the mold. The wax was left to consolidate.

Sectioning
The paraffin embedded specimens were sectioned with the help of a rotary microtome. The thickness of the stem sections was 14 micrometer (µm) while the leaves and the roots were 9 and 12 µm thick, respectively. The ribbons were mounted on slides flooded with distilled water and placed on a hot plate to flatten the sections.

Staining
The double staining process was employed for this purpose. The steps of this process included dewaxing, rehydration, staining, and dehydration of material. In the dewaxing process the slides were passed twice through xylene, each for 3-5 minutes. Rehydation was carried by passing the dewaxed slides through a series of different concentrations of ethanol in this order: absolute, absolute, 95%, 90%, 70% and 50% and were then immersed in safranine, each for 2-3 minutes. Dehydration was carried out by passing the slides through different concentrations of ethanol (50%, 70%, 90%, 95% and absolute) each for 2-3 minutes. The slides were then immersed in fast green stain for one and a half minutes. The slides were then cleaned by passing through absolute ethanol and xylene.

Mounting
The material was mounted in D.P.X and covered with cover slip. The slides were kept in an oven at 60 ºC and left for 3 days, before examining under a light microscope at 10x.

Phytochemical analysis (Balasundrum *et al.*, 2005)
The methanolic extracts of samples by using the soxhlet apparatus was treated with different reagents and the result showed the presence of targeted secondary metabolites.

Table 1: Preliminary phytochemical screening

Phytochemicals	Tests	Reagents
Alkaloids	Dragendroff test Wagners test Mayers test	Dragendroff reagents Wagners reagents 1%HCl, Mayers reagents
Flavanoids	Ammonium test Sodium hydroxide test	1% NH3 20% NaOH, HCl
Tannins	Ferric chloride test	5% FeCl3
Phenolic compounds	Gelatin test	1% gelatin solution containing 10% NaCl
Saponins	Lead acetate test Foam test	10% lead acetate 20ml Distilled water. Mixed vigorously
Terpenoids	Salkowsi test	0.5 ml chloroform 1ml Concentrated Sulphuric acid.
Carbohydrates	Molish test Fehlings test	4% NaOH, 1% copper sulphate
Proteins	Biuret test	Pyridine, sodium nitroprusside
Glycosides	Legals test Keller killani test	Glacial acetic acid

Result
The anatomical study helps us to find out the variations present inside the vascular bundle and arrangements of each cell and its variations of the features in the stem and leaf of the plant.

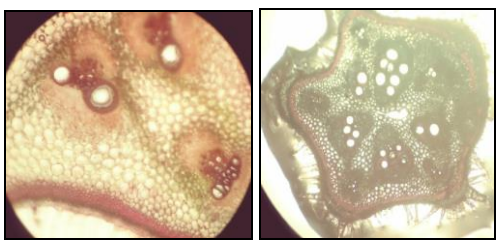

Fig 2: T.S. of stem cucumis dipsaceous. ex. spach. ehreb.

Stem: The stem of *Cucumis dipsaceous* is hollow with 5 ridges and furrows. The vascular bundles are arranged in 2 rows those of the outer rows correspond to the ridges and those of inner to the furrows. The epidermis is single layered passing over the ridges and furrows. The hypodermis or collenchymas lies below the epidermis and consists of 5-6 layers. These cells contains chloroplast. The cortical parenchyma which lies below the collenchyma, forms a narrow zone in the middle. The endodermis is the innermost layer of the cortex, lies immediately outside the pericycle of the schlerenchyma. It is the single layered and rich in starch. It is 4-5 layered thick which is made up of thick walled, lignified which are polygonal. There is a continous mass of thin walled, parenchymatous cells and extend from schlerenchyma to the pith. The vascular bundles are bicollateral and arranged in 2 rows. Each bundle consisting of xylem, two strips of cambium and two patches of phloem. Xylem tissue occupies in the centre. It has protoxylem, which are smaller vessels inner side

and metaxylem, which is slightly bigger and seen higher up. The outer and the inner phloem is plano convex and the inner one is semi lunar. Each patch of phloem contains of sieve tubes companion cells and phloem parenchyma. The stele is siphnostele.

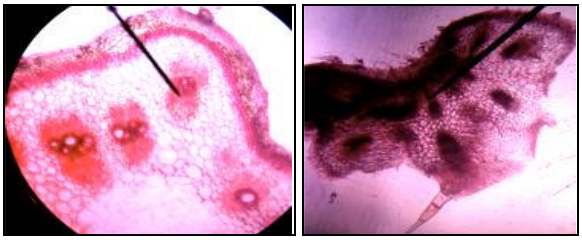

Fig 3: L.S. of Leaf Cucumis Dipsaceous. Ex. Spach. Ehreb.

Leaf: The upper epidermis of the leaf is single layered with cuticle which checks for the evaporations of water from the surface. The lower epidermis of the leaves is thin walled. There are 2-3 layered of palisade parenchyma in all the species which is elongated, cylindrical cells, closely packed together, with narrow intercellular spaces and rich in chloroplasts. The spongy parenchyma cells are irregular in shape, loosely arranged, toward the lower epidermis with intercellular spaces in them. There are about 8 vascular bundles which are bicollateral. Each consists of xylem which is present in the upper epidermis, phloem present in the lower epidermis. Xylem consists of vessels, tracheids and xylem parenchyma and phloem consists of sieve tubes, companion cells and phloem parenchyma.

Phytochemical analysis results

The preliminary phytochemical screening shows the positive results shown in the table 2.

Table 2: Phytochemical screening results

Phytochemicals	Positive results
Alkaloids	Prominent yellow precipitate. Reddish brown precipitate. Turbid extract is obtained.
Flavanoids	Yellow color. Yellow color turns colorless
Tannins	Blue black or blue coloration
Phenolic compounds	White precipitate
Saponins	Bulky white precipitate. Presence of froth
Terpenoids	Reddish brown coloration at the interface
Carbohydrates	Violet ring Yellow and brick red precipitate.
Proteins	Pink to red color
Glycosides	Bluish green color

Discussion

The anatomical works of the *Cucumis dipsaceous* plant is first time reported. All the species of cucurbitaceae is drought resistant (Ooman and grubben, 1977). The presence of cuticle, schlerenchymatous sheath in the leaf and increased number of glandular hairs in stem and leave. The medullary rays alternate with the bundles linking with the cortex instead of forming a continous cylinder. This then explains why the plants are creeping/trailng and also a climber in *C. dipsaceous*. The diagnostic features of all the species therefore as belonging to the family cucurbitaceae includes the presence of bicollateral vascular, arrangements of the vascular bundles in two rows coincides with the five ridges and forrows also presence of siphonosteles. The observed anatomical features of the plant studies indicated that phylogenetic analysis related taxa. These anatomical differences observed and showed evolution, conferring heritable variations that could be exploited for taxonomic purposes. The present study is an attempt to provide anatomical data on the species of cucurbit earlier described by Hutchinson and Dalzeil (1954).

The result showed that the methanolic and aqueous leaf extract of *Cucumis dipsaceous* contains saponins, tannins, glycosides, alkaloids, flavonoids and reducing sugars. These phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract. Flavonoid has also been reported to have greater potential benefit to human health.

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is`` taxonomically distinct (Parekh*et al.,* 2005). It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of natural medicines. This investigation is very useful for the anatomy, forensic science where identification and authentication of plant specimens are necessary.

Conclusion

This study helps in the pharmacological and anatomical knowledge of the plant. The secondary metabolites are very useful in the preparation of the medicines. Anatomical studies show that the variations of the xylem and phloem arrangements which is used for further research of the structural and functional analysis of this plant.

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