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Pharmacognostical study on the leaf of *Tricosanthes Cucumerina* Linn

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ABSTRACT

Tricosanthes cucumerina belongs to the family cucurbetaceae is a monoecious annual herb climbing by 2–3-branched tendrils upto 5 to 6 meters high or less. The genus Trichosanthes is native to Southern and Eastern Asia, Australia and Islands of the western Pacific. Trichosanthes cucumerina is found wild throughout these areas. It was probably domesticated in ancient times in India. It is consumed as vegetable. It has gained scientific importance for its nutritional value and cytotoxic activity, anti-diabetic, hypoglycaemic, anti-inflammatory, hepatoprotective, anti-fertility and gastroprotective activites. Apart from its pharmacological standardization, the plant needs pharmacognostic standardization. In the present work the leaf part of the plant was subjected to various microscopical and physical evaluations. In the microscopical studies, the cytomorphological parameters were studied. The powder microscopy showed the presence of annular, spiral and scalariform xylem vessels. Various leaf constants were obtained. In physic chemical evaluation the ash values and extractive values were studied. Fluorescence analysis performed showed the wide range of fluorescence colours for the crude powder as well as the extracts. The powdered leaf was extracted with petroleum ether, chloroform, ethanol and water. The extract obtained were concentrated and subjected to chemical tests. From the preliminary chemical tests presence of carbohydrates, alkaloids and saponins were observed.

Keywords: *Trichosanthes cucumerina*, nutritional value, microscopy, xylem vessels, preliminary chemical tests

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INTRODUCTION

Tricosanthes cucumerina (cucurbetaceae) is a monoecious annual herb climbing by 2–3-branched tendrils. The stems are slender, green, 4-angled, somewhat hairy, and faintly disagreeable in odor. The roots are somewhat tuberous and whitish. The leaves are alternate, simple with no stipules. The staminate inflorescences are long-peduncle and axillary, with six to fifteen flowers. Flowers are unisexual, regular, and white in colour with green and hairy calyx.

Corolla is tubular in with lobes fringed and hair like outgrowths. The male flowers are many-flowered with axillary racemes on 10– 30 cm long peduncles. They are with 3 stamens but the female flowers are solitary and sessile with inferior, single celled ovary, long and with hairy stigmas. Fruits are very slender, long and cylindrical berry, often twisted, greenish-white when immature, dark red when mature. The seeds are half-ellipsoid, somewhat compressed, undulate, hard, rugose, nearly one centimeter long, greyish-brown, sculptured, margin undulate and imbedded in a soft foetid with red pulp[1,2,3,4].*Tricosanthes cucumerina* is a rich source of nutrition. It is highly constituted with proteins, fat, fibre, carbohydrates, vitamin A and E. The total phenolics and flavonoids content are 46.8% and 78.0% respectively. The fruit is rich in Vitamin C and E. The crude protein content is 30.18%. The predominant mineral elements were potassium (121.60mg 100-1g) and phosphorus (135.0mg 100-1g). The chemical constituents present in *T.cucumerina* are cucurbitacin B, cucurbitacin E, isocucurbitacin B, 23, 24-dihydroisocucurbitacin B, 23, 24-dihydrocucurbitacin E, sterols 2 *β*-sitosterol stigmasterol. A novel isoflavone glucoside, 5,6,6'-trimethoxy-3',4'- methylenedioxyisoflavone7-O-beta-D-(2"-O-p-coumaroylglucopyranoside) has been characterized from the seeds of *Trichosanthes*20. The positive effects of the plant are due to the carotenoids, flavonoids, lycopene, phenolics and ß-carotene present in it.

To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbals. Hence, in the present work the pharmacognostical standardization has been performed for the leaf of the plant.

MATERIALS AND METHODS

Collection and authenticaton

The plant species *Tricosanthes cucumerina* were collected in regions of nalgonda district. The plant material is collected in the months of November to January. The plant material is authenticated by Mr. A. Lakshma Reddy, lecturer, Dept. of Botony, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant was identified as *Tricosanthes cucmerina* and was certified under Voucher No: NCOP-NLG/ph'cog/2009-10/002.

Microscopical studies

Transverse section of leaf [5, 6]

Free hand sectioning was done for fresh leaf to obtain a thin section. Phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope.

Powder microscopy [7]

Shade dried leaves were powdered with the help of an electric grinder till a fine powder was obtained .This fine powder was subjected to powder microscopy, as per standard procedures mentioned.

Measurement of cell structure and content [7]
The length and width of phloem fibres and diameter of the starch grains were measured using stage micrometer and the eyepiece micrometer by standard methods.

Determination of leaf constants [7]
leaf constants include stomatal number, stomatal index, vein terminations, vein islets. The measurements of leaf constants help to differentiate between species of a genus.

Determination of physico chemical properties [7]
Total ash, acid insoluble ash and water soluble ash of *Tricosanthes cucmerina* leaf was determined by standard method and the results are tabulated in table. The crude fibre content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and petroleum ether soluble extractive values of *Tricosanthes cucmerina* leaf were determined by standard method and the results obtained are tabulated in table.

Determination of Fluorescence analysis [8, 9, 10]
Powdered root was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents.

Extraction method
100 gm coarse powder of air dried leaves of *Tricosanthes cucumerina linn* were packed and subjected to soxhlet extraction for continuous hot extraction with petroleum ether, chloroform, ethanol, distilled water. Then the each extracts were filtered and filtrate was concentrated under vacuum using rotary vacuum evaporater.

Preliminary chemical screening [6, 11, 12]
The extract obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books.

RESULTS AND DISCUSSION

Transverse section of leaf
The transverse section of the leaf showed dorsiventral nature.

Lamina:
Upper epidermis was single layered with rectangular epidermal cells with distinct cuticle, abundant covering trichomes were observed. The covering trichomes were uniseriate, multi-cellular (3- 4 celled) with a stalk and base and with blunt tips. Mesophyll was differentiated into palisade and spongy parenchyma. Palisade cells were elongated arranged compactly in single layer and were discontinued over midrib. Spongy parenchyma consists of loosely arranged parenchymatous cells of 4-5 layers. Lower epidermis was single layered with rectangular cells. The number of trichomes found in this layer was more. Vascular elements were arranged in 3-4 layers (Fig 1).

Midrib
The dorsal surface was strongly convex and epidermal layer of lamina continued along midrib below the upper epidermis and above the lower epidermis. Vascular bundles were arch shaped, collateral in nature. 4-6 layers of collenchyma cells are present below upper epidermis and above lower epidermis (Fig 1).

Powder microscopy
Xylem vessels have annular, spiral and scalariform thickening vessels (Fig 2, 3, 4). Starch grains are in form of small and large spherical granules (Fig 5). Calcium oxalates are in form of square shaped prismatic crystals (Fig 6). Epidermal cells are in irregular shape and compactly arranged (Fig 7). Anomocytic stomata are present (Fig 8). Trichomes are multicellular, uniseriate, covering trichomes(Fig 9). Phloem fibres are long, lignified and slender (Fig 10).

Measurement of cell structure and content
This helps in identification of adulteration. The results obtained are tabulated in Table2.

Determination of leaf constants
The stomatal number, stomatal index, vein islet and vein termination numbers obtained are tabulated in Table 1.

Determination of physico chemical properties
The physico chemical properties help to estimate the amount of impurities like soil and particle present in the drug. It also helps to assess the calculi salts present in the drug sample. The results obtained for the proximate analysis are tabulated in Table3.

Determination of Fluorescence analysis
Fluorescence analysis is a tool to determine the kind of chemical nature of the drug. The fluorescence obtained in short wavelength, long wave length and day light after treatment with different chemicals and reagents are tabulated in Table4.

Preliminary chemical screening
Chemical test helps in the confirmation of the chemical nature of the active principles present in the plant when extracted with different solvents. The results of the chemical tests are tabulated in Table5.

Table 1: Leaf constants

Parameter		***Result %w/w***
Stomatal number	Lower epidermis	7
	Upper epidermis	6
Stomatal index	Lower epidermis	26.92
	Upper epidermis	24
Vein terminations		14
Vein islets		6

Table 2: Measurements of *Tricosanthes cucumerina*

Measurement of leaf constants	**Length**
Length of Trichomes	6µ-16-26µ
Diameter of Starch	1µ
width of xylem vessels	6.4µ--13.7--21 µ

Table 3: Physico chemical properties

Parameter	Result %w/w
Total ash value	22.5%
Acid insoluble ash	7%
Water soluble ash	8.5%
Loss on drying	90.2%
Crude fibre content	33%
Pet. ether extractive value	7.2%
Chloroform extractive value	23.2%
Ethanol extractive value	14.4%
Water extractive value	24.8%

Table 4: Fluorescence analysis

	Day light	UV Short wave length	UV Long wave length
Drug + 50%H_2SO_4	Light yellow	Bright green	Yellow
Drug +50%HNO_3	Reddish brown	Green	Fluorescent yellow
Drug +5%NaOH	Yellow	Bright green	Yellow
Drug+1NMethanolic NaOH	Green	Bright green	Yellow
Drug +1N KOH	Yellow	Bright green	Yellow
Drug +5%KOH	Yellow	Green	Green
Drug +5% $FeCl_3$	Brown	Green	Yellow
Drug +Methanol	Light green	Bottle green	Yellow
Drug +Conc. HCl	Yellow	Dark green	Green
Drug+Conc. H_2SO_4	Brown	Bottle green	Florescent yellow
Drug +Ammonia	Yellow	Green	Greenish yellow
Drug +Conc .HNO_3	Reddish brown	Green	Green

Table 5: Qualitative Preliminary phytochemical studies

	Pet. ether extract	Chloroform extract	Alcohol extract	Water extract
Carbohydrates	Absent	Absent	Present	Present
Flavonoids	Absent	Absent	Absent	Present
Alkaloids	Absent	Present	Present	Absent
Proteins	Absent	Present	Absent	Absent
Phenolic compounds	Absent	Absent	Present	Absent

Steroids	Absent	Absent	Absent	Absent
Tannins	Absent	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent	Absent
Saponins	Absent	Present	Present	Present
Glycosides	Absent	Absent	Absent	Absent

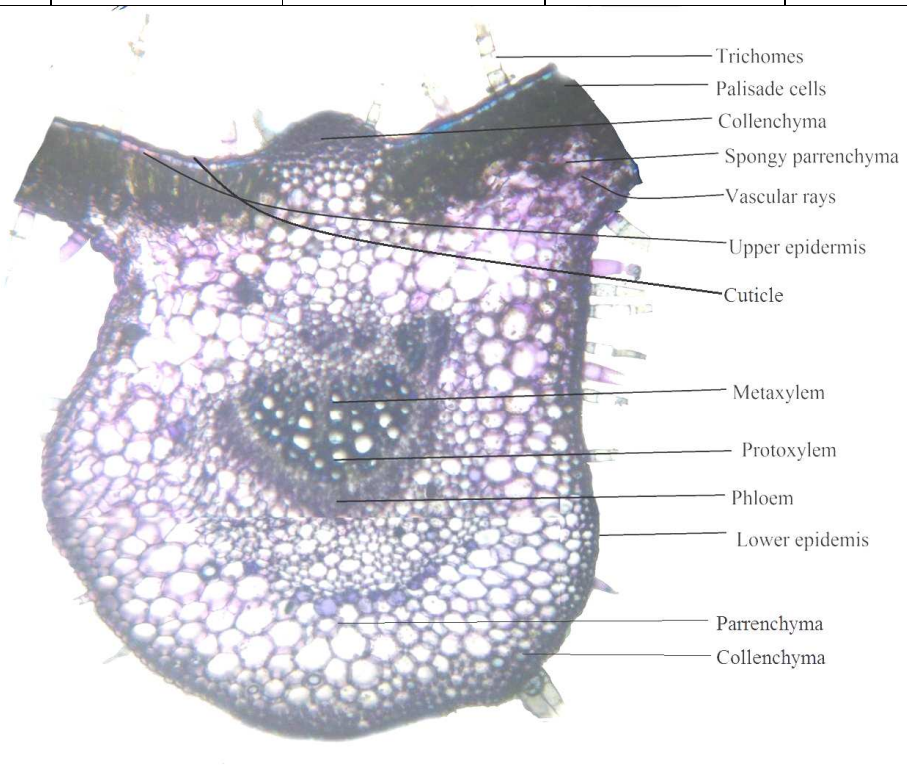

Figure 1: Leaf T.S of *Tricosanthes cucumerina*

Figure2:Spiral xylem vessels

Figure3:Scalariform vessels

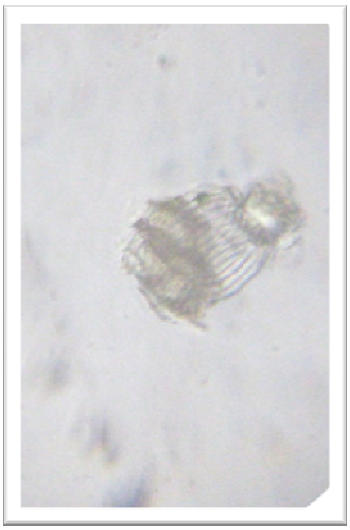

Figure4:Annular xylem vessels

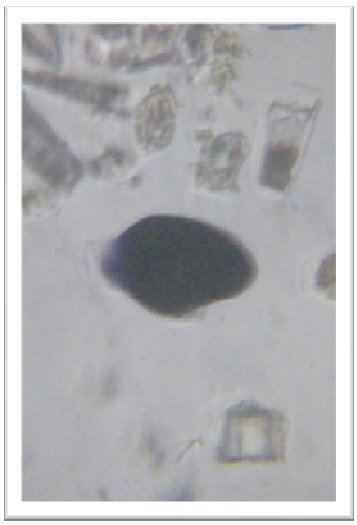

Fig no:5- Starch

Fig no:6- Calcium oxalate

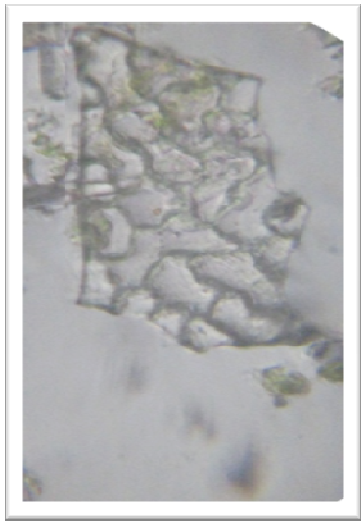

Fig no:7- Epidemal cells

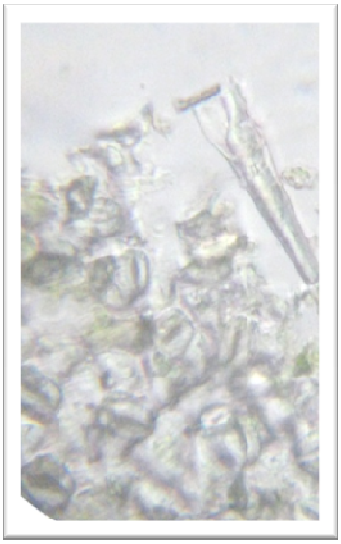

Fig no:8- Stomata

Fig no:9- Trichomes

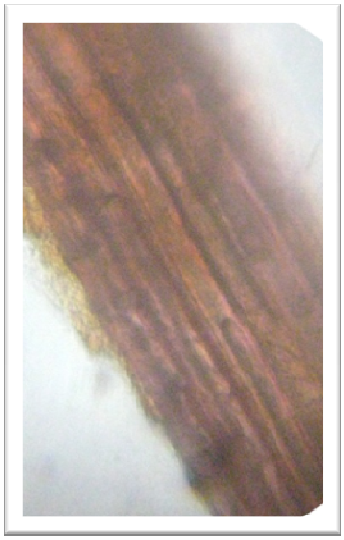

Fig no:10- Phloem fibres

CONCLUSION

The cytomorphological evaluations performed for the leaf of Trichosanthes cucumerina will help in its proper identification. Proximate analysis, fluorescence analysis and the preliminary chemical tests data's performed will be beneficial in the development of a suitable plant profile. The diagnostic features reported enable its easy identification and distinction from other species *Trichosanthes*.

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