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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *TRICHOSANTHES CUCUMERINA* VAR. CUCUMERINA LINN. LEAVES

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ABSTRACT: *Trichosanthes cucumerina* var. cucumerina Linn. is an annual monoecious climber. *Trichosanthes cucumerina* var. cucumerina Linn. mainly distributed in Asian countries like India, Sri Lanka, Bangladesh, Burma, Malaysia, and Australia. *Trichosanthes cucumerina* var. cucumerina is a wild variant (Cucurbitaceae) the major active components are triterpenoids, saponins, and cucurbitacins; chemical constituents like flavonoids, carotenoids and phenolic acids are also present. *Trichosanthes cucumerina* var. cucumerina is used in the treatment of headache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrhea, haematuria, and skin allergy, *etc*. *Trichosanthes cucumerina* is used as an abortifacient, vermifuge, refrigerant, purgative, malaria, laxative, hem agglutinant, emetic, cathartic, bronchitis and anthelmintic. The present study involves the pharmacognostical and phytochemical studies of the plant. The transverse section was taken for the microscopical studies. Powder microscopy shows the presence of annular, spiral, cylindrical, tubular and thick-walled xylem vessels. Different physicochemical evaluation ash value, extractive value, fluorescence analysis, *etc*, were performed. Phytochemical evaluation is performed for the ethanolic extract of leaves presence of alkaloids; flavanoids, glycosides, phenols, *etc*. are confirmed.

INTRODUCTION: Plants plays an important role in the maintenance and support of other biological life. Plants also have a vital role in the treatment of diseases. Pharmacognostical and phytochemical evaluation of plants is important for their identification among other species or verities. *Trichosanthes species* are coming under the family Cucurbitaceae. There are about 110 genera and 640 species in the Cucurbitaceae family.

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The main genera include *Cucurbita*, *Cucumis*, *Ecballium*, *Citrullus*, *Luffa*, *Bryonia*, *Momordica*, *Trichosanthes*, *etc*. [1] *Trichosanthes* species are rich in chemical components like flavonoids, carotenoids, lignin, glycosides, tannins, alkaloids, phenols, and terpenoids. *Trichosanthes cucumerina* Linn. (Cucurbitaceae) is an annual monoecious climber [2]. *Trichosanthes cucumerina* mainly distributed in Asian countries like India, Sri Lanka, Bangladesh, Burma, Malaysia, and Australia.

It has two varieties *Trichosanthes cucumerina* var. anguina L. which is cultivated and used as a vegetable and the second one is *Trichosanthes cucumerina* var. cucumerina L. which is a wild variant with short fruits. There are more than 16

marketed herbal formulations are available which contains *T. cucumerina* var. cucumerina L. as major ingredient [3]. *Trichosanthes cucumerina* is used as antioxidant [4, 13], hepatoprotective [5, 6], gastro-protective [7, 13], anti-inflammatory [8], anti-bacterial [9, 10, 11, 12]. Analgesic [13], anti-diabetic [14], diuretic, anthelmintic [15], anti-fertility [16, 17]. Externally, the leaf juice is rubbed over the liver to relieve liver congestion and used as cathartic and for the treatment of indigestion, bilious fevers, boils, sores, skin eruptions such as eczema, dermatitis, psoriasis, ulcers and in malaria [18].

Plant Profile:

Kingdom: Plantae
Subkingdom: Viridaeplantae
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Order: Cucurbitales
Family: Cucurbitaceae
Genus: Trichosanthes
Species: cucumerina

Common Name: Snake gourd, Tomato gourd, Kattupadavalam, Padval

Useful Parts: Leaves, fruits, roots, *etc*.

MATERIALS AND METHODS:

Collection and Authentication: *Trichosanthes cucumerina* var. cucumerina L. was collected from Kondotty, Malappuram district in Kerala. The plant material collected from September to December. Authentication carried out by A. K. Pradeep, Assistant Professor, Department of Botany, University of Calicut and the voucher specimen number CU 86996, has been submitted in the Calicut University Herbarium, University of Calicut.

Macroscopic Studies: Macroscopic observation of the plant was done. The shape, size, surface characters, texture, color, odor, taste, *etc.* were noticed and recorded.

Microscopic Studies: Microscopic studies of the plant were carried out in fresh leaves of *Trichosanthes cucumerina* var. cucumerina L. Plant sectioning is done in the midrib region of the leaf. The transverse section is taken by the freehand method. Thin sections are collected and treated with phloroglucinol and hydrochloric acid in 1:1 ratio for the staining of the section. Stained sections were mounted in grease free glass slide by using glycerine and observed under a microscope.

Powder Microscopy: Dried leaves are powdered to get fine particles. Fine powder treated with phloroglucinol and hydrochloric acid in equal amount. Stained powder sample mounted with glycerine and covered with a coverslip in a grease-free microscopic slide with the help of a needle.

Determination of Leaf Constants: Leaf constants includes stomatal number, stomatal index, vein islet number, and vein termination number. Leaf constant studies are carried out in the leaves by means of pealing the epidermal layer and mounted with glycerine. The study is carried out with the help of camera Lucida [19].

Determination of Physicochemical Properties:

Determination of Moisture Content: Moisture content determination carried out by means of loss on drying method.

Determination of Ash Values: Total ash value consists of physiological ash which is derived from the plant tissue itself and non-physiological ash derived from the adhering material in the plant surface. Accurately weighted 2.5 gm of dried leaves were placed in a crucible. The leaves spread as a layer and ignited to get constant weight by gradually increasing the heat to 500-600 °C using a muffle furnace.

Acid-insoluble ash indicates the contamination with earth and sand material. 2M HCl (25 ml) was added to a beaker containing the total ash, covered with a watch glass and boiled gently for 5 min. The acid insoluble ash was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the acid insoluble ash was transferred into the original crucible and ignited to get constant weight. Water soluble ash is the water-soluble portion of the ash. 25 ml of water added into a beaker containing the total ash and boiled for 5 min. The water-insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water-insoluble matter was transferred into the original crucible and ignited to get a constant weight. Weight of this residue was subtracted from

the weight of total ash, and the content of water-soluble ash calculated.

Determination of Extractive Values: Alcohol-soluble extractive value amount of plant compounds which get solubilized in ethanol by keeping powdered plant material for 24 h. Water-soluble extractive value percentage of plant compounds which are solubilized in water is calculated by keeping plant material in chloroform-water (5% chloroform in distilled water).

Determination of Fluorescence Analysis: Powdered leaves are observed under visible light, ultraviolet radiation short wavelength and long wavelength after treatment with various reagents.

Pre-liminary Phytochemical Screening: Pre-liminary phytochemical screening is carried out with the ethanolic extract of leaves of *Trichosanthes cucumerina* var. cucumerina Linn. Various chemical tests were performed for the identification of alkaloids, saponins, glycosides, flavonoids, terpenoids, carotenoids, *etc*.

RESULTS AND DISCUSSION:

Macroscopic Studies: Plant is perennial climbing. Flowers are monoecious, axillary; white male flowers occur in racemes with panicles. The female flowers are solitary.

FIG. 1: WHOLE PLANT

FIG. 2: LEAVES

FIG. 3: FRUITS

Leaves are alternate, simple, hairy, 7-15 cm length and 10-15 cm in width. 5-7 lobed, the base is broadly heart shaped. Fruits are cylindrical with the waxy surface, slender and tapering. 5-7 cm in length and 2-3 cm in diameter. The fruits are bitter and used as an emetic. Roots are tuberous with the long and thick tap root system. The thickness of the root is due to the storage of food and water.

Microscopic Studies:

Transverse Section of Leaf: Leaves are dorsiventral.

Lamina: Upper epidermal cells are single layered rectangular cells. Covered with cuticle and contains numerous covering trichomes. The covering trichomes are uniseriate, multi-cellular (3-4 celled) with a stalk and base and having blunt tips.

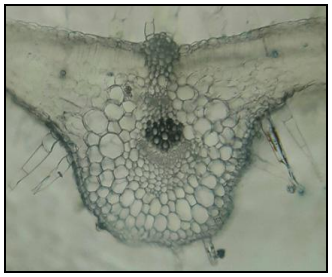

FIG. 4: T. S. OF *TRICHOSANTHES CUCUMERINA* VAR. CUCUMERINA L.

FIG. 5: XYLEM VESSELS

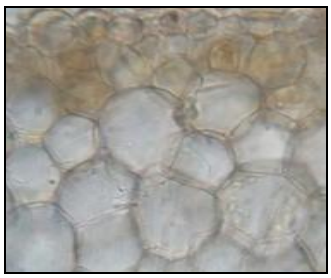

FIG. 6: COLLENCHYMAS

Mesophyll: Mesophyll is divided into palisade and spongy parenchyma. Palisade cells are elongated and single layered and compactly arranged which discontinued over midrib. Spongy parenchyma consists of loosely arranged parenchymatous cells of 4-5 layers. Vascular elements are arranged in 3-4 layer. The lower layer is single-layered with rectangular epidermal cells. Trichomes also present in this layer

Midrib: Dorsal surface is strongly convex and epidermal layer is continuous in lamina and midrib region. Epidermal layer present in both lower and upper epidermis. Arch shaped vascular bundles and are bicollateral (xylem cells covered with phloem cells in both sides) in nature. 4-6 layers of collenchyma cells are present below the upper epidermis and above the lower epidermis.

Powder Microscopy:

FIG. 7: XYLEM VESSELS

FIG. 8: STOMATA

FIG. 9: TRICHOMES

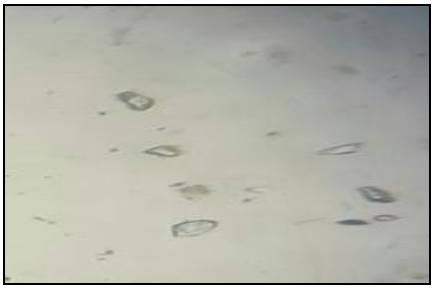

FIG. 10: CAL. OXALATE CRYSTALS

FIG. 11: PHLOEM FIBRE

Xylem Vessels: Annular, spiral, cylindrical, tubular and thick walled.

Stomata: Anomocytic stomata

Trichomes: Uniseriate, multi-cellular, straight and having a blunt tip.

Calcium Oxalate Crystals: In the form of square-shaped prismatic crystals.

Phloem fibers: Are lignified.

Determination of Leaf Constants:

Stomatal number: The average number of stomata per square mm of the epidermis of the leaf is termed as a stomatal number. The ratio of a number of stomata to the total number of epidermal cells in a given area of epidermis is fairly constant for any age of the plant under different climate condition.

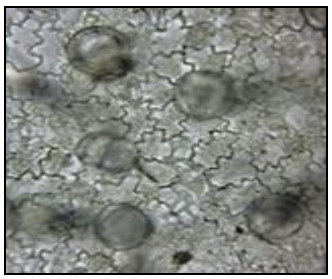

FIG. 12: EPIDERMAL CELLS

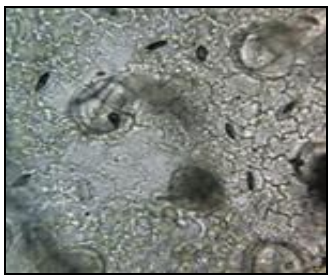

FIG. 13: EPIDERMAL CELLS SHOWING STOMATA

Stomatal Index: Percentage of a number of stomata formed to the total number of epidermal cell. Stomatal index calculated by the following equation:

$$SI = S / S+E$$

SI - Stomatal index; S - Number of stomata per unit area 18 and E - Number of epidermal cell in same unit area 84.

TABLE 1: DETERMINATION OF LEAF CONSTITUENTS

Parameter	Value
Stomatal number	72
Stomatal index	17.64

Determination of Physicochemical Properties:

Determination of Loss on Drying: Moisture content of dried leaves of *Trichosanthes cucumerina* var. cucumerina Linn. was found to be 13.5 ± 0.08.

Determination of Ash Values:

TABLE 2: ASH VALUES OF *T. CUCUMERINA* VAR. CUCUMERINA L. LEAVES

Ash values	Percentage %
Total ash value	24 ± 0.09
Acid insoluble ash values	21 ± 0.05
Water soluble ash values	7 ± 0.067

Determination of Extractive Values:

TABLE 3: EXTRACTIVE VALUES OF *T. CUCUMERINA* VAR. *CUCUMERINA* L. LEAVES

Extract	Percentage %
Water soluble extractive value	28 ± 0.07
Alcohol soluble extractive value	40.40 ± 0.06

Fluorescence Analysis: Fluorescence analysis of leaves of *Trichosanthes cucumerina* var. cucumerina L. was carried out with various reagents.

TABLE 4: FLUORESCENCE ANALYSIS

S. no	Drug + Reagent	Day light	250-270 nm	360-390 nm
1	Powder as such	Green	Dark green	Black
2	50% Sulphuric acid	Dark green	Dark green	Black
3	Con. Hydrochloric acid	Green Light	Green	Black
4	50% Hydrochloric acid	Green	Green	Black
5	10% Sodium hydroxide	Light green	Light green	Black
6	Con. Nitric acid	Orange	Green	Black
7	5% Ferric chloride	Green	Light green	Black
8	Acetic acid	Olive green	Dark green	Black
9	Water	Green	Light green	Black

TABLE 5: PRELIMINARY PHYTOCHEMICAL SCREENING

S. no.	Phytoconstituents	Total ethanolic extract
1 Alkaloids		
a)	Dragendroff's reagent	+
b)	Mayer's reagent	+
c)	Hager's reagent	+
d)	Wagner's reagent	+
e)	Tannic acid	+
f)	Ferric chloride	+
2 Glycosides		
a)	Legal's test	-
b)	Raymond's test	-
c)	Keller killiani test	-
d)	Bromine water test	+
e)	Con. sulphuric acid test	+
f)	Molisch's test	+
3 Phenols		
a)	Ferric chloride test	+
b)	Lead acetate test	+
4 Flavonoids		
a)	Aqueous sodium hydroxide test	+
b)	Shinoda test	+
c)	Lead acetate test	+
d)	Ferric chloride test	+
e)	Zinc -the hydrochloric acid test	+
5 Carbohydrates		
a)	Molisch's test	+
b)	Benedict's test	+
c)	Fehling's test	+
d)	Barfoed's test	+
6 Tannins		
a)	Ferric chloride test	+
b)	Gelatine test	+
c)	Lead acetate test	+
d)	Alkaline reagent test	+
7 Steroids		
a)	Lieberman- bur chard test	+
b)	Salkowaski test	+
8 Saponins		
a)	Foam or forth test	-
9 Lignin		
a)	Labatt test	+
10 Fat and oils		
a)	Stain test	+
b)	Saponification test	+
11 Quinones		
a)	Alcoholic KOH test	-
12 Proteins		
a)	Millions test	-
b)	Xanthoprotein test	-

Extraction of Leaves: 100 gm of coarsely powdered leaves of *Trichosanthes cucumerina* var. cucumerina L. was subjected to continuous hot extraction in Soxhlet apparatus by using ethanol. After completion of the extraction the extract obtained was concentrated under vacuum by using rotary vacuum evaporator.

Preliminary Phytochemical Screening: Preliminary phytochemical screening performed with ethanolic extract of *Trichosanthes cucumerina* var. cucumerina Linn.

CONCLUSION: *Trichosanthes cucumerina* var. *cucumerina* L. which is a wild variant with short fruits of the plant *Trichosanthes cucumerina* under the family Cucurbitaceae. The present work is carried out on the macroscopic, microscopic and phytochemical evaluation of the plant. Pharmacognostical studies on the plant are useful for the identification of the plant from the closely related species and authentication of the plant for the effective use of the plant for the formulation of a pharmaceutical product.

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CONFLICT OF INTEREST: Nil

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