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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* L. 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 flavanoids, carotenoids and phenolic acids are also present. *Trichosanthes cucumerina* var. *cucumerina* is used in the treatment of head ache, alopecia, fever, abdominal tumours, bilious, boils, acute colic, diarrhoea, 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. Transverse section was taken for the microscopical studies. Powder microscopy shows 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 conformed.

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 are important for their identification among other species or varieties. *Trichosanthes* species are comes under the family Cucurbitaceae. There are about 110 genera and 640 species in Cucurbitaceae family. The main genera includes Cucurbita, Cucumis, Ecballium, Citrullus, Luffa, Bryonia, Momordica, *Trichosanthes*, etc ¹.

Trichosanthes species are rich in chemical components like flavanoids, carotenoids, lignin, glycosides, tannins, alkaloids, phenols and terpinoids. *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 have 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 *Trichosanthes cucumerina* var. *cucumerina* L. as major ingredient ³. *Trichosanthes cucumerina* is used as antioxidant ^{4, 13}, hepatoprotective ^{5, 6},



gastro-protective^{7, 13}, anti-inflammatory⁸, anti-bacterial^{9, 10, 11, 12}. Analgesic¹³, anti diabetic¹⁴, diuretic, anthelmintic¹⁵, anti-fertility^{16, 17}. Externally, the leaf juice is rubbed over the liver to relieve liver congestion. Used as cathartic and for the treatment of indigestion, bilious fevers, boils, sores, skin eruptions such as eczema, dermatitis, psoriasis, ulcers and in malaria¹⁸.

Plant Profile:

Kingdom: Plantae
 Subkingdom: Viridaplantae
 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 in the time period of 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, colour, odour, 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. Transverse section is taken by free hand 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 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 cover slip 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 peeling the epidermal layer and mounted with glycerine. The study is carried out with the help of camera Lucida¹⁹.

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 g 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 ash less 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 it is the water soluble portion of the ash. 25 ml of water added in to a beaker containing the total ash and boiled for 5 min. The water insoluble matter was collected on an ash less 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 solubilised in ethanol by keeping powdered plant material for 24 h. Water soluble extractive value percentage of plant compounds which are solubilised in water are 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 wave length and long wave length after treatment with various reagents.

Preliminary Phytochemical Screening: Preliminary 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, flavanoids, terpinoids, carotenoids, etc.

RESULTS AND DISCUSSION:

Macroscopic Studies: Plant is perennial climbing. Flowers are monoecious, axillary, white male flowers occurs in racemes with panicles, The female flowers are solitary. 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 waxy surface, slender and tapering. 5 - 7 cm in length and 2 - 3 cm in diameter. The fruits are bitter in taste and used as emetic. Roots are tuberous with long and thick tap root system. Thickness of the root is due to the storage of food and water.



FIG. 1: WHOLE PLANT



FIG. 2: LEAVES

FIG. 3: FRUITS

Microscopic Studies:

Transverse Section of Leaf: Leaves are dorsi-ventral in nature.

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.

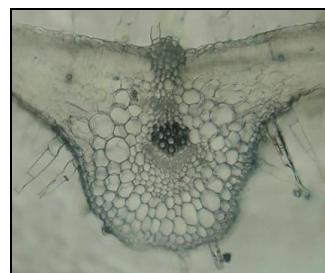


FIG. 4: T. S. OF *TRICHOSANTHES CUCUMERINA* VAR. *CUCUMERINA* L.

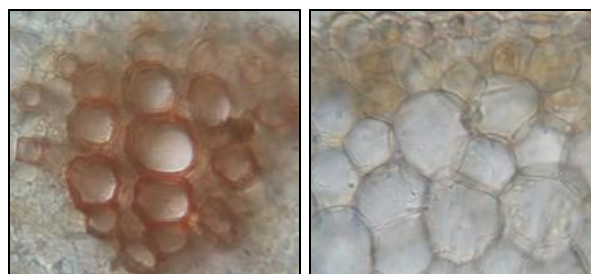


FIG. 5: XYLEM VESSELS FIG. 6: COLLENCHYMAS

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 layer. Vascular elements are arranged in 3 - 4 layer. 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 upper epidermis and above lower epidermis.

Powder Microscopy:

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

Stomata: Anomocytic stomata

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

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

Phloem fibres: Are lignified



FIG. 7: XYLEM VESSELS



FIG. 8: STOMATA

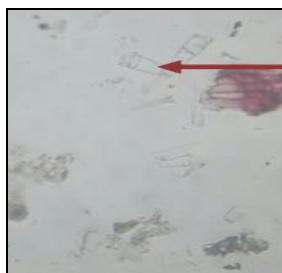


FIG. 9: TRICHOMES



FIG. 10: CALCIUM OXALATE CRYSTALS



FIG. 11: PHLOEM FIBRE

Determination of Leaf Constants:

Stomatal number: The average number of stomata per square mm of epidermis of the leaf is termed as stomatal number. The ratio of 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.

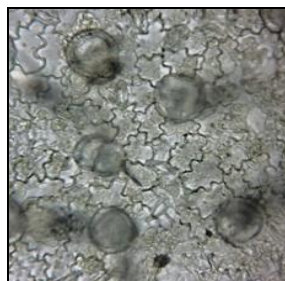


FIG. 12: EPIDERMAL CELLS

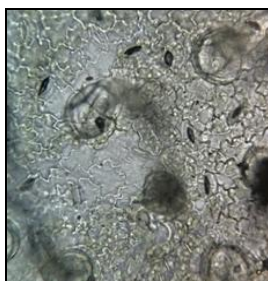


FIG. 13: EPIDERMAL CELLS SHOWING STOMATA

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

$$SI = \frac{S}{S+E}$$

SI - Stomatal index

S - Number of stomata per unit area

E - Number of epidermal cell in same unit area

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 *TRICHOSANTHES 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 *TRICHOSANTHES 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	Green	Black
4	50% Hydrochloric acid	Light	Green	Black
5	10% Sodium hydroxide	Green	Green	Black
6	Con. Nitric acid	Light green	Light green	Black
7	5% Ferric chloride	Orange	Green	Black
8	Acetic acid	Green	Light Green	Black
9	Water	Olive green	Dark green	Black
		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	Flavanoids	
	a) Aqueous sodium hydroxide test	+
	b) Shinoda test	+
	c) Lead acetate test	+
	d) Ferric chloride test	+
	e) Zinc -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 is 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.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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