



## Pharmacognostic and Physicochemical Analysis of the Fruits of *Cucumis trigonus* Roxb.

S. Gopalakrishnan<sup>\*.1</sup>, T. Kalaiarasi<sup>2</sup>

<sup>\*.1</sup> Department of Chemistry, Noorul Islam University, Kumaracoil – 629180, K.K. District, Tamil Nadu, India.

<sup>2</sup> Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli – 627012, Tamil Nadu, India.

\*Corresponding author's E-mail: [sgkmsu@yahoo.co.in](mailto:sgkmsu@yahoo.co.in)

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### ABSTRACT

*Cucumis trigonus* Roxb. a tendrillar herb belongs to the family Cucurbitaceae has been used as a thermogenic, anthelmintic, febrifuge, expectorant, liver tonic, stomachic, appetizer, purgative and as an intellect promoting. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. The characteristic microscopy of the fruits of *Cucumis trigonus* showed the presence of vascular bundles, parenchymatous mesocarp, xylem, phloem elements. Physicochemical characters of the fruit powder of *cucumis trigonus* including ash values, extractive values, and fluorescence analysis have been determined. The percentage of sulphated ash are found to be high than the other ash values. The highest extractive values are found with water and lowest in petroleum ether. The results of the study could be useful in setting some diagnostic indices for the correct identification of the plant drug from adulterants.

**Keywords:** *Cucumis trigonus*, Fruits, Pharmacognosy, Physico-chemical parameters, Fluorescence analysis.

### INTRODUCTION

Plants are an indispensable source of therapeutic preparations, both preventive and curative. With the resurgence in the consumption and demand for medicinal plants, WHO recognized the need for their standardization and quality control<sup>1</sup>. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken<sup>2</sup>. Pharmacognosy is a simple and reliable tool, by which complete informations of the crude drugs can be obtained<sup>3</sup>. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics<sup>4</sup>. Pharmacognostic studies have been done on many important drugs and the resulting observation has been incorporated in various pharmacopeias<sup>5</sup>. In recent years growing demand for herbal products has been increased and plant materials traded within and across the countries activity. Previously the phytochemicals with unknown pharmacological activities have been extensively investigated as source of medicinal agents<sup>6</sup>.

*Cucumis trigonus* Roxb. (Fam. Cucurbitaceae) commonly known as "Thummittikai" in Tamil, "Bitter gourd" in English, "Bislambi" in Hindi and "Vishala" in Sanskrit. It is distributed throughout India and found in areas of Ceylon, Afghanistan, Persia and Northern Australia<sup>7</sup>. It is used for various ailments in Indian Traditional System of Medicine<sup>8</sup> Fruits, roots and seeds are extensively used medicinal parts of the plant. The fruits are used in flatulence, leprosy, fever, jaundice, diabetes, cough, bronchitis, stomachic, ascites, anemia and constipation and acts as a diuretic<sup>9</sup>. Roots are purgative and liver

tonic. In addition, fruit pulp is bitter, acrid, thermogenic, anthelmintic, cardio tonic, appetizer, expectorant and intellect promoting<sup>10</sup>. Seeds have unsaturated lipids as major constituents and acts as a coolant and astringent<sup>11</sup>. Traditionally the Kachri fruit, *Cucumis trigonus* Roxb is used as meat tenderizer in Indian subcontinent. Recently it's proteolytic and serine protease activity has been reported<sup>12</sup>. The pharmacognostic profile of the fruits is helpful in standardization for quality, purity and sample identification. The result of the pharmacognostical standardization of this plant serves as a reference piece and helps in future identification and authentication of this plant specimen.

### MATERIALS AND METHODS

#### Chemicals

All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. (St Louis, MO, USA), Merck (Darmstadt, Germany) and Qualigens (Mumbai, India).

#### Collection of plant materials

The fruits of *Cucumis trigonus* was collected in the month of March from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai- 600 045, Tamil Nadu, India.

A voucher specimen (MSU/PHAR/HER-140) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012, Tamil Nadu, India.

Care was taken to select matured and healthy fruits. The fruits were cut and removed from the plants and fixed in Farmalin: Acetic Acid: 70 % Alcohol (FAA) (5:5:90) v/v/v.



After 24 h of fixing, the specimens were dehydrated with graded series of Tertiary Butyl Alcohol (TBA) as per the schedule<sup>13</sup>. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58<sup>o</sup>–60<sup>o</sup>C) until TBA solution attained supersaturation. The plant materials were cast into paraffin blocks.

## Experimental

### Macroscopic studies

The macroscopic evaluation was carried out for knowing the shape, size, color, and fracture of the drug.

### Microscopic studies

#### Sectioning

The Paraffin embedded fruits of *Cucumis trigonus* Roxb. were sectioned with the help of rotary microtome. The thickness of the section was 10 to 12  $\mu\text{m}$ . Dewaxing of the section was done by customary procedure<sup>14</sup>. The sections were stained with toluidine blue<sup>15</sup>. 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 IKI (for starch).

#### Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 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<sup>16</sup>.

### Extraction of plant material

The collected fruits were shade-dried at room temperature and powdered. The dried powder (500 gm) was successively extracted using petroleum ether (40<sup>o</sup>- 60<sup>o</sup> C), benzene, chloroform, ethanol and water by using a Soxhlet apparatus. The last trace of the solvent was removed under reduced pressure distillation and then vacuum dried. The dried crude extracts were used for the fluorescence analysis.

### Fluorescence Analysis

The fluorescence analysis<sup>17</sup> of the various extracts, petroleum ether, benzene, chloroform, ethanol, water of the fruits of *Cucumis trigonus* were examined under day light and Ultra Violet light (365 nm). These powders were also treated with 1N NaOH (aqueous), 1N NaOH (ethanolic), 1N HCl, 1:1 H<sub>2</sub>SO<sub>4</sub> and 1:1 HNO<sub>3</sub> and the changes in color were recorded in day and UV light.

## Physico-chemical studies

The percentage physico-chemical<sup>18-20</sup> values, viz., loss of weight on drying, total ash, acid insoluble ash, water soluble ash, residue on ignition and extractive values after successive extractions in various solvents such as petroleum ether (40<sup>o</sup>-60<sup>o</sup>C), benzene, chloroform, ethanol and water were determined.

## RESULTS AND DISCUSSION

### Macroscopic characters

*Cucumis trigonus* Roxburghii is an annual or perennial scabrid monoecious tendrillar herb with slender angled stem, leaves deep palmately five lobed, hispid on the nerves beneath and rounded at the apex. Male flowers are small and are found in clusters whereas female flowers are solitary. Fruits are ellipsoid or sub-globose, yellow or yellow with green stripes and seeds are white and ellipsoid<sup>21</sup>. Seeds smooth and compressed, mostly without margin. These macroscopic characters as shown in figure 1a and 1b.



**Figure 1a, 1b:** *Cucumis trigonus* Roxb. fruits (photographic) showing its macroscopic characters.

### Microscopic Characters

#### Fruit

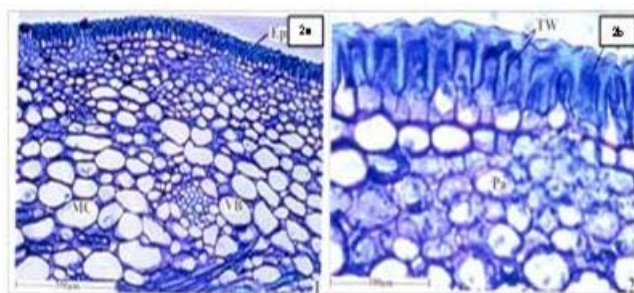
The fruit is a pepo which is a berry type with pericarp comprising epicarp and spongy parenchymatous mesocarp. The epicarp is the epidermis which consists of radially elongated wide compact layer of cells. The cells have outer tangential walls and radial walls thick and liquefied as shown in figure 2a and 2b. The epicarp layer is 50  $\mu\text{m}$  in height and 30  $\mu\text{m}$  thick. The mesocarp consists of thin-walled circular parenchyma cells. They have darkly staining spherical bodies. Vascular strands of varying sizes are frequent in the mesocarp. The vascular bundles have a few wide, thick walled xylem elements and a few phloem elements as shown in figure 3a.

The seed coat has thick sclerotic testa. The testa is thick with sacral short squarish. The sclerenchyma cells are sclerids of brachy sclereid type as shown in figure 3b. The sclerids are compactly arranged.

#### Powder microscopic observation

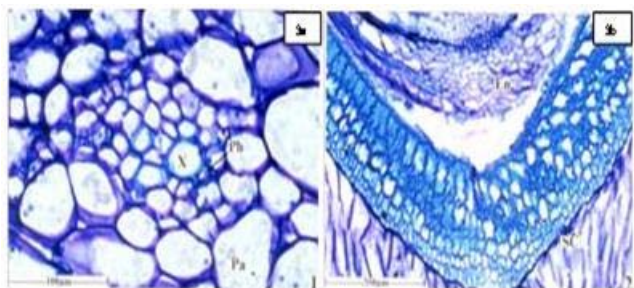
The powder when examined under the microscope exhibits the following inclusions.





**Figure 2:** Anatomy of the fruits of *Cucumis trigonus* Roxb.

Fig. 2a). T.S. of fruit-Outer Pericarp; Fig. 2b). Outer Pericarp showing outer thick-walled cells & inner thin-walled parenchyma cells. (Ep-Epidermis, Mc-Mesocarp, Pa-Parenchyma, TW-Thick Wall, VB-Vascular Bundle).

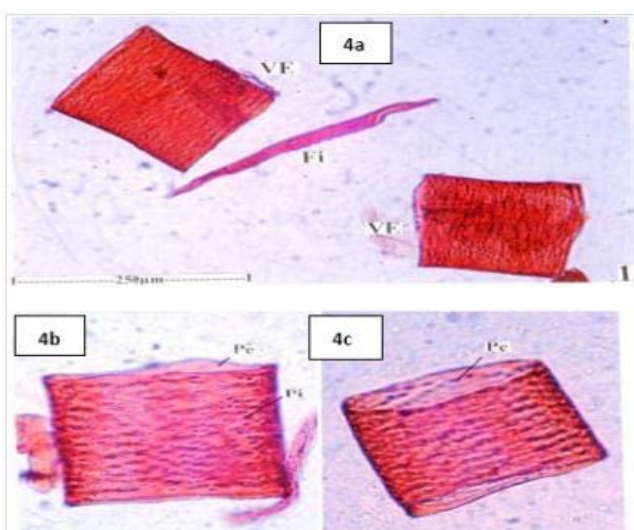


**Figure 3:** Anatomy of the fruit seeds of *Cucumis trigonus* Roxb.

Fig. 3a). T.S. of fruit-A vascular bundle enlarged; Fig. 3b). T.S. of fruit-Seed coat enlarged. (En-Endocarp, Pa-Parenchyma, Ph-Phloem, Sc-Sclerenchyma, X-Xylem).

### Vessel elements

The vessel elements found in the powder are characteristic in shape and size. They are mostly wide, short and barrel shaped. They have wide, circular horizontal perforations as shown in figure 4c. The lateral wall pits are wide, elliptical in shape, alternate and multiseriate as shown in figure 4b. The vessel elements are 180  $\mu$ m long and 160  $\mu$ m wide.



**Figure 4:** Powder microscopy of the fruits of *Cucumis trigonus* Roxb.

4a). Vessel element & fibres; 4b). A vessel element showing perforation plate & pits; 4c). A vessel element-Enlarged. (Fi-Fibre, Scl-Sclereids, SP-Septate Parenchyma).

### Non septate fibres

They are long, narrow, thick walled and no septa as shown in figure 4d and 4f. The lumen is narrow. They are 680  $\mu$ m long and 10  $\mu$ m thick.

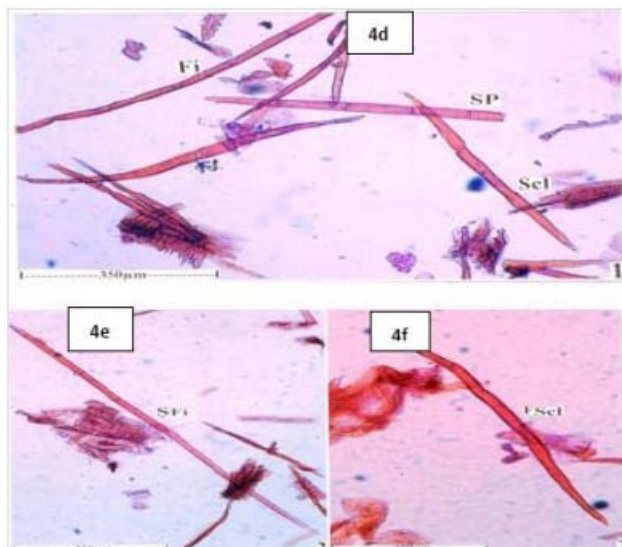


Fig. 4d). Fibres and septate parenchyma showing in the powder

Fig. 4e). A septate fibre enlarged

Fig. 4f). A sclereid in the powder.

### Septate fibres

Septate fibres are longer, narrow, thin-walled and have thin septa or cross walls. The septate fibres are 1.2 mm long and 20  $\mu$ m wide.

### Sclereids

Sclereids are fibre like in size and shape and are called fibre-sclereids. The walls are thick and the lumen is wide. Prominent, simple are abundant in the sclereids.

### Parenchyma cells

Short, squarish or rectangular cells and long, wide fibre like septate parenchyma cells are frequently seen in the powder. It is long and wide.

### Fluorescence Analysis

Fluorescence analysis of powdered drug material and different extracts of the fruits of *Cucumis trigonus* with different reagents as shown in table 1. The fluorescence method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples. The fluorescence color is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent.

**Table 1:** Fluorescent analysis of the fruits of *Cucumis trigonus* Roxb.

| Treatment                              | Under Ordinary light | Under UV light (365 nm)                             |
|--|----------------------|---|
| Powder (P) as such                     | Pale yellow          | Pale yellow   |
| P + 1N NaOH in water                   | Yellowish-green      | Yellowish-green                                     |
| P + 1N NaOH in ethanol                 | Brown                | Pale green  |
| P+ 1N HCl                              | Brown                | Brown   |
| P + 1:1 H <sub>2</sub> SO <sub>4</sub> | Pale green           | Dark green  |
| P+ 1:1 HNO <sub>3</sub>                | Orange               | Fluorescent green at the edge and orange at centre. |
| <b>Extracts:</b>                       |                      |   |
| Petroleum ether (40°-60°C) extract     | Yellowish-green      | Yellowish-green                                     |
| Benzene extract                        | Pale green           | Pale green  |
| Chloroform extract                     | Pale green           | Dark green  |
| Ethanol extract                        | Reddish brown        | Brown   |
| Water extract                          | Dark brown           | Brown   |

### Physicochemical studies

The results of physicochemical characters such as loss of weight on drying, total ash, acid insoluble ash, water soluble ash, residue on ignition and the percentage of extractive values in various solvents such as petroleum ether (40-60°C), benzene, chloroform, ethanol and water of the fruits of *Cucumis trigonus* as shown in table 2. The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs<sup>22</sup>. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica<sup>23</sup>. The acid insoluble ash (2.5 %) is less than that of water insoluble ash (5 %). The water extractive value of the fruits of *Cucumis trigonus* is more than in the other solvents investigated. The results of various types of ashes and extractive values may provide a basis to identify the quality and purity of the drug.

**Table 2:** Physico-chemical characters of the fruits of *Cucumis trigonus* Roxb.

| S. No                     | Particulars               | Percentage (%) |
|---------------------------|---------------------------|----------------|
| <b>Ash Values:</b>        |                           |                |
| 1                         | Loss of weight on drying  | 80.00          |
| 2                         | Total ash                 | 13.00          |
| 3                         | Acid insoluble ash        | 2.50           |
| 4                         | Water soluble ash         | 5.00           |
| 5                         | Sulphated ash             | 15.23          |
| 6                         | Loss on ignition          | 12.50          |
| <b>Extractive Values:</b> |                           |                |
| 7                         | Petroleum ether (40-60°C) | 1.00           |
| 8                         | Benzene                   | 1.12           |
| 9                         | Chloroform                | 1.25           |
| 10                        | Ethanol                   | 1.37           |
| 11                        | Water                     | 1.75           |

### CONCLUSION

The various specific pharmacognostical characters of *Cucumis trigonus* Roxb., can be used as a diagnostic tool for the correct identification of the plant drug and help to differentiate from the closely related other species of *Cucumis* and also to test adulteration if any.

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#### Corresponding Author's Biography: Dr.S.Gopalakrishnan



Dr. S. Gopalakrishnan after completing M. Sc from American College, Madurai - 2, he was awarded Ph. D by Department of Natural Products Chemistry Madurai Kamaraj University, Madurai - 21 in the year 1982. He has 37 years of teaching and research experience since July 1975. Presently he is working as Senior Professor of Chemistry & Controller of Examinations, Noorul Islam University, Kumaracoil – 629 180. K. K. District, Tamil Nadu. He has guided 17 M. Phil scholars and produced 24 Ph. D scholars. He has completed 3 Major and 6 Minor projects. He has published 146 articles in reputed National and International journals. He has published 4 books with ISBN/ reputed Publishers. He has received a No. of National & International awards such as “20<sup>th</sup> Century Achievement Award” from American Biographical Institute, USA in the year 1998, “Gem of Alternative Medicine Award” from Indian Board of Alternative Medicines, Kolkata in the year 1998, “Bharath Jyoti Award” from India International Friendship Society, New Delhi in the year 2003, “Academic Excellence Award” from M.S. University, Tirunelveli-627 012 for the year 2009. He is a Fellow of Indian Chemical Society, Indian Council of Chemists, Fellow and Life Member of Indian Society of Environmental Sciences.