PHARMACOGNOSTICAL STUDIES ON THE SEEDS OF MULAM CITRULLUS LANATUS (THUNB.) MATS & NAKAI (CUCURBITACEAE)

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ABSTRACT: In the Siddha system of Medicine cotyledons of the seeds of C. Lanatus are known as Mulam/Pullum/Pitcha (Tamil) and used as Pulukolli (Vermifuge), Karpa moolikai (General tonic) and as Aanmeiperukki (Aphrodisia). In the Ayurvedic system of Medicine seeds are said to have properties like Sheeta (cooling), Mootrala (Diuretic) and Vrshya (Aphrodisiac). The present paper deals with macro and microscopical studies, maceration, histochemical tests, solubility, physical contents, extractive values, tests for inorganic and organic constituents, U.V. and thin layer chromatographic studies.

INTRODUCTION:

Citrullus lanatus (Thunb.) Mats & Nakai (=C. vulgaris Schard.) belongs to the family Cucurbitaceae. In Tamil it is known as Mulam/Pullum. In the Siddha system of Medicine, seeds of Mulam are dried and only cotyledons are used as a pulukolli (Vermifuge), Karpa moolikai (General tonic) and as Aanmeiperukki (Aphrodisiac). In the Ayurvedic system of Medicine seeds are known as chayapula Krishna beeja/Rakta beeja and used as Sheetala (Cooling), Mootrala (Diuretic) and Vrshya (Aphrodisiac). The present work deals with macro and microscopical studies, maceration, histochemical tests, solubility, physical contents, extractive values, tests for inorganic and organic constituents, U.V. and thin layer chromatographic studies.

MATERIALS AND METHODS:

Seeds were collected from the fresh fruit and also from the local market, where it is sold under the common name kallangadi Hannu/Beeja (Kannada). Seeds were soaked in 70% alcohol, free hand sections were taken following Johansen (1940) and Wallis (1967). Transverse section of the seeds were taken for detailed microscopical observations. Dry powdered seeds were used for chemicals analysis. Physico
chemical analysis were carried out as per standard procedure (Anonymous 1966). All reagents used for chemical analysis were of G.P.R. grade. TLC studies were carried out following Logon stahl (1969). The fluorescence analysis of the powdered drug under ultraviolet light was done according to the methods described by Chase and Pratt (1949).

**TAXONOMY:**

Citrullus lanatus (Thunb.) Matsumara & Nakai in Cat. Sem. Hort Bot, Univ. Imp. Tokyo, 1916; 30 1916. Momordica lanata Thunb. Prodr. Pl. Cap. 13. 1974. Citrullus vulgaris Schrader ex. Eckl. & Zeyher, Enum. 279. 1836; Clarke in Hook. F., Fl. Brit. India, 2: 621. 1879; Gamble, Fl. pres. Madras 1: 379. 1957 (repr. ed).

**BOTANICAL SESCRIPTION:**

Climbing annual large climber, leaves deeply divided or moderately lobed, glabrous, hairy, tendrils, bifid, stout, pubescent. Flowers large, yellow, monocious. Male flowers; calyx tube campanulate, lobes 5, stamens 3, short, anthers scarcely covering 1 celled, 2 celled cells conduplicate, connective not produced. Female flowers; calyx and corolla as in the male, ovary ovoid, style short, stigmas 3, reniform, ovules many, horizontal, placenta 3. Fruit subglobose or ellipsoid, smooth greenish, flesh juicy, red or yellowish white. Seeds numerous, small, compressed, chocolate brown to black, variable in shape and colour embedded in soft and spongy reddish or pick pulp. Surface smooth, seed coat thick brown to black, usually margined with black streaks or spots. Cotyledons white with prominent radicle. Seeds measures ½ to ¾ cms I length. Tastes sweet with pleasing aroma.

**VERNACULARNAMES:**

Tamil: Mulam, Pullum, Pitcha, Dharbushini. Sanskrit: Rakta beja, Chayapula, Chayaphala, chitravallika, Krishnabeeja Kannada: Kallangadi hannu, Kallangadi balli

**DISTRIBUTION:**

Throughout India, cultivated and distributed in all warmer countries of the world on the sandy river beds for its fruits (Hooker 1879).

Macroscopical Characters: (Plate II A & B)

Seeds numerous, small, compressed, chocolate brown to black, variable in shape and colour embedded in soft and spongy reddish or pick pulp. Surface smooth, seed coat thick brown to black, usually margined with black streaks or spots. Cotyledons white with prominent radicle. Seeds measures ½ to ¾ cms I length. Tastes sweet with pleasing aroma.

Microscopical character: (fig. 1,2,3,4, and 5) T.S of the seed, below the radicle region (upper region) shows outer integument (seed coat), cotyledons and 2 small vascular bundles on either side with xylem and phloem. (Fig 1)

T.S. of the middle region of the seed shows outer integument, cotyledons, endosperm and a; small embryo. Endosperm region is made up of many layered, thin walled polygonal parenchymatous cells (Fig.2)

T.S. of the basal region of the seed shows outer integument and cotyledon region (fig.3).

L.S. of the seed shows radicle, seed shows outer integument and cotyledon region (fig 3).
T.S. of the basal region of the seed coat shows, outer integument (outer testa) made up of single outer epidermal layer consisting of elongated palisade cells with brown thickening in the centre. Epidermis is followed by 5 to 8 layered, compactly arranged polygonal stone cells with heavily lignified walls with pits inside the lumen, stone cell region is followed by single layer of elongated stone cell layer with large lumen with pits and 2 to 3 layers of crushed parenchymatous layer. Parenchymatous region is followed by inner epidermis of outer integument consisting of single layer of rectangular parenchyma cells covered by thin cuticle. Inner epidermis is followed by many layered thin walled, polygonal parenchymatous cells filled with abundant simple starch grains and oil globules constituting the cotyledonary portion of the seed (Fig 3&5).

MACERATION: (Fig.6)

Maceration of the seed shows fragments of elongated palisade cells with brown thickening in the centre. Polygonal parenchymatous cells loaded with simple starch grains and oil globules polygonal to elongated stone cells with lignified walls, large lumen with pits, thin walled crushed parenchyma cells.

DIAGNOSTIC CHARACTERS:

1. Presence of elongated palisade cells with brown thickening in the centre.

2. Presence of polygonal parenchymatous cells with abundant simple starch grains and oil globules in the cotyledonary region.

3. Presence of thick, balck to chocolate brown seed coat the brown streaks 7 spots.

4. Presence of polygonal and elongates stone cells with pits inside the lumen.

5. Absence of inner integument (inner seed coat) and seed coat is derived form the outer integument.

The measurements of different cells and tissues are tabulated in Table-1.

Histo chemical tests of the sections were also carried out and tabulated in Table 2. Table Measurements of different cells and tissues in micron.

TRANSVERSE SECTION:

1. Palisade Cells: 20-28-40x5-8-12
2. Stone cells: 10-15-20-x5-9-14
3. Parenchyma: 5-8-12x5-7-9
4. Cotyledon: 10-20-30x 8-18-25
5. Oil globules: 10-15-20 (Diameter)
6. Starch grains: 8-10-12 (Diameter)
7. Epidermis: 6-8-12x4-5-8

MACERATE:

1. Palisade cells: 18-20-35 x5-10-14
2. Parenchyma: 5-9-13 x5-10-12
3. Cotyledon: 10-25-30 x8-12-28
4. Oil globules: 10-18-20 (Diameter)
5. Starch grains: 5-15-20 (Diameter)
6. Epidermis: 6-7-10 x 4-6-9

Table 1:
Table 2: Histochemical tests are tabulated in Table 2.
2 Ferric chloride soln No change Tannin -
   (Aqueous) No change Tannin -
3 Ferric chloride soln
4 Sudan II soln. Pink colour Oilglobules ++
5 Con.HCI Majenta Lignin ++

++=present  ---= absent

PHYSICOCHEMICAL STUDIES:
The properties like ash content, solubilities, fibre content etc. of the powdered seeds were determined. The ash was analysed for inorganic constituents. The air dried drug was subjected to soxhlet extractions using petroleum ether 60 to 80°C, benzene, chloroform and alcohol successively and the percentage of each extract was determined. The physicochemical parameters are given in Table 3. The qualitative tests for the organic constituents with the above different extracts indicate the presence of steroids, phenolics, aminoacids, sponins, tannins, sugar and alkaloids.

THIN LAYER CHROMATOGRAPHIC STUDIES
T.L.C. studies of the above four extracts were carried out in various solvent systems at 300C, using silica gel G as adsorbent. The Rf values are recorded in Table 4.

FLUORESCENCE ANALYSIS:
The fluorescence behaviour of the powdered drug in different solutions towards ordinary light and ultraviolet light (both long and short wave lengths) were observed and the results are recorded in Table 5.

The seeds yield a fixed oil with specific gravity 0.92, iodine value 1.23, saponification value 1.92, acid value 3 and unsaponifiable matter 1.6% respectively.

Table 3: Physico-chemical parameters

|   | % loss on drying at 110°C | 1.98 |
|---|--------------------------|------|
| 1 | % Ash content            | 3.29 |
| 2 | % Acid insoluble ash     | 0.11 |
| 3 | % Crude fibre            | 32.0 |
5. Solubility

a. % ethyl alcohol 12.91
b. % in Water 19.62

6. Qualitative inorganic analysis of the ash
Presence of chloride, sulphate, carbonate, phosphate, iron, calcium and magnesium.

7. Extractive values

a. % in petroleum ether 60-80°C 22.3
b. % in benzene 2.62
c. % in chloroform 2.45
d. % in alcohol 10.85

Table 4: Thin layer chromatography – Rf values

| Extracts | Solvent system                  | Developer/spray Rf values |
|----------|---------------------------------|---------------------------|
| Petroleum Ether 60-80°C | Benzene Methanol | 0%H2So4 in 0.21,0.30,0.35,0.42,0.52,0.71,0.93 |
| Benzene | Benzene, 50:50 Chloroform | “ 0.21,0.29,0.37,0.25,0.63,0.77,0.92 |
| Chloroform | Chloroform Methanol 40:5 | “ 0.21,0.37,0.57,0.64,0.8,0.9 |
| Alcohol | Chloroform, Acetone 60:40 | “ 0.1,0.2,0.49,0.53,0.6,0.73,0.78,0.9,0.95 |

Table 5. Fluorescence studies of Citrullus lanatus seed powder

| Treatment | Ordinary light | UV light long wave 365 mµ | Short wave 245 mµ |
|-----------|----------------|---------------------------|-------------------|
| Powder as such | Greenish violet | Dark grey | Grey |
| Powder + water | Light grey | Dark brown | Violet grey |
| Powder +Dil.Hcl | Greyish violet | Dark purple | Bluish violet |
| Powder +Dill. HNO₃ | Light grey | Dark purple | Dark violet |
| Powder +Dil. H₂SO₄ | Light grey | Dark grey | Violet grey |
| Powder +40% NaOH | Violet grey | Dark purple | Bluish brown |
| In methanol | | | |
| Powder +acetic acid | Light grey | Dark grey | Violet grey |
| Powder +iodine soln. | Light grey | Olive green | Violet grey |
SUMMARY:

The cotyledons of Citrullus lanatus constitutes the drug ‘Mulam’. The cotyledons (without seed coat) is sold in the local market under the name ‘Kallangadi beeja’ (Kannada). In the present investigation the detailed macro and microscopical structure of the seed along with physico chemical details, U.V. and T.L.C. studied and therapeutic uses in both siddha and ayurvedic system of medicine are presented.

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Plate I: Line drawing of Citrullus lanatus

(1,2,3&4): 1 Flowering twig
2 Male flower
3 Female flower
4 T.S. of the fruit with seeds

PLARE II: (A & B) MACROSCOPY

Seeds of Citrullus lanatus (Marker sample)

A Seeds (with seed coat)
B Cotyledons (without seed coat)

MICROSCOPY:

Fig 1 T.S. of the seed through upper region (below the radicle) (Semidiagrammatic)

Fig 2 T.S. of the seed through middle region (semidiagrammatic)

Fig 3 T.S. of the seed through basal region (semidiagrammatic)

Fig 4 L.S. of the seed showing radicle, seed coat, cotyledon, endosperm and embryo.
(semidiagrammatic)

Fig 5 Portion of the seed (through basal region) showing portion of the epidermis, stone cell layer, parenchyma region, inner epidermis of outer integument and cotyledon portion.

NACERATION:

Fig 6 Showing parenchyma cells, cotyledon cells, oil globules, starch grains, palisade cells and stone cells.

Abbreviations: EMB=embryo; END=endosperm; EP=epidermis;
COT=cotyledon;CU=cuticle;OG=oil globule; PAL= Palisade tissue;
PAR=parenchyma; SG= Starch; SDCT=seed coat; STC=Stone cell; VB=vascular bundle.

REFERENCES:

Aman o 1985 Medicinal secrets of your food pp 267 published by Secretary,
Indo American Hospital, N.R. Mohalla Mysore7.
Anonymous 1950  The wealth OF India, Vol II, CSIR, New Delhi, pp 187 to 188.

Anonymous 1966  Pharmacopoeia of India, (2nd Edn.) Manager of publications, Govt. of India, pp 930-990

Bhava prakash 1969  Bhava prakash of Sri Bhavamisra (Hindi commentary) Chowkamba Sanskrit series, Varanasi, pp 560.

Chase C.R. and Pratt 1969  Flourescence of powdered vegetable drugs with particular reference to development of system of identification. J. Am. Pharm Assoc. (Scied.) 38: 324 to 331.

Chopra, R.N. Nayar S.L. and  Glossary of Indian medicinal plants

Chopra I.R. 1956  C.S.I.R., New Delhi pp 67

Hooker J.D. 1879  The Flora of British India, Vol II pp 621, L. Reeve & Co., Ltd., Theoast House, Brook, N.R. Ashford, Kent, England.

Igon stahl 1969  Thin Layer Chromatography, a Laboratory handbook, Springer International Student edition, Springer Verlag Berlin. Heidelberg. New York PP 52 86; 127 128, 900.

Iyengar M.A. 1950 19745 Boolography of investigated Indian Medicinal plants, manipal power press, mainpal, pp 38.

Johansen D.A. 1940  Microtechnique, MCGraw Hill, New York pp 183 to 203.

Murugesha mudaliar K.S 1988  Materia medica (veg. section) in Tamil Nadu, Deptt. Of Siddha medicine, madras pp 607.

Nair N.C. and Henry A.N. 1983  Flora of Tamil Nadu, India series I: Analysis B.S.I Dept of Environment southern circle Coimbatore 3; 99 170.

Rao R.R. 19977  ‘Medico Botany of some Mysore plants’Journal of Research in India medicine, Yoga and Homoeo 12: (4) pp 54.

Roma Mitra 1986  Bibliography of Pharmacognosy of medicinal plants, N.B.R.I. Lucknow, pp 127.

Wallis T.E. 1967  Text Book of Pharmacognosy 15th edn., T.A. Churchill, London pp 571 – 582.
