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PHARMACOGNOSTICAL STUCIES ON THE SEEDS OF MULAM CITRULLUS LANATUS (THUNB.) MATS & NAKAI (CUCURBITACAE)

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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 (Aphrodisiac). 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 Anmaiperukki (Aphrodisiac) (Mudaliar 1988) In the Ayurvedic system of medicine, the seeds are known as chayapula Krishna beeja/Rakta beeja and used as Sheetala (Cooling) mootrala (Diuretic), Madhura (Sweet), Vrshya (Aphrodisiac) and Balakara (tonic) (Bhavaprakash 1969). In Mysore district seeds are used to treat impotency (Rao 1977). Seed oil is used as a substitute for almond oil (Chopra et al. 1956). Milky juice of the seeds is used in high blood pressure, scanty urination, burning micturation, haemoptysis in tuberculosis and gonorrhoea. Regular use of the seed milk extract protects the arterial lumen and reduce the blood cholesterol. Seed contain glucoside cucurbotrine (Aman 1985). Seeds contain protein citrullin and aminoacids and are also a rich source of an enzyme called Ureas (Anonymous 1950). Defatted seeds contain 66.2% of protein. A new aminoacid β-(1 Pyrazol) alanine has been isolated from the seeds (Chopra et al. 1956).

Since literature review reveled that no Pharmacognostical studies have been carried out on the seeds of C.lanatus the present work in under taken (Iyengar 1950., Roma Mitra 1986).

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, lmp. 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, F1.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, monecious. Male flowers; calyx tube campanulate, lobes5, stamens 3, short, anthers scarcely covering 1 celled, 2 celled cells conduplicate, connective not produced. Female flowers; clayx 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 usually margined. Plate I (1,2,3,&4) (Nair & Henry 1983; Hooker 1879).

VERNACULARNAMES:

Tamil: Mulam, Pullum, Pitcha, Dharbushini.
Sanskrit: Rakta beeja, Chayapula, Chayaphala, chitravallika, Krishnabeeja
Kannada: Kallangadi hannu, Kallangadi balli
Telugu: Kallangadi pandu, Kharbujadosa and Puchha Kaya.
English: Water melon
Hindi: Halinda karbuj, Tarbuj
Malayalam : Kharbuj

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. Coty-ledons 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 cotyledon 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 parcenchmatous 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.

Section	Reagents	Change in colour	Test for	Result
1	Iodine Solution	Blue	starch	++

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^{o}C, benzene, chloroform and alcohol successively and the percentage of each extract was determined. The physicochemical parameters are given in Table3. The qualitative tests for the organic constitutents with the above different extracts indicate the presence of steroids, phenolics, aminoacids, sponins, tannins, sugar and alkaloids.

THIN LEYER 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.

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

1. % loss on drying at 110^{o}C	1.98
2. % Ash content	3.29
3. % Acid insoluble ash	0.11
4. % 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, car bonate, phosphate, iron, calcium and magnesium.

7. Extractive values

a. % in petroleum ether 60-80^{o}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 60-80oC	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. HNO_3	Light grey	Dark purple	Dark violet
Powder +Dil. H_2SO_4	Light grey	Dark grey	Violet grey
Powder +40% NaOH In methanol	Violet grey	Dark purple	Bluish brown
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.

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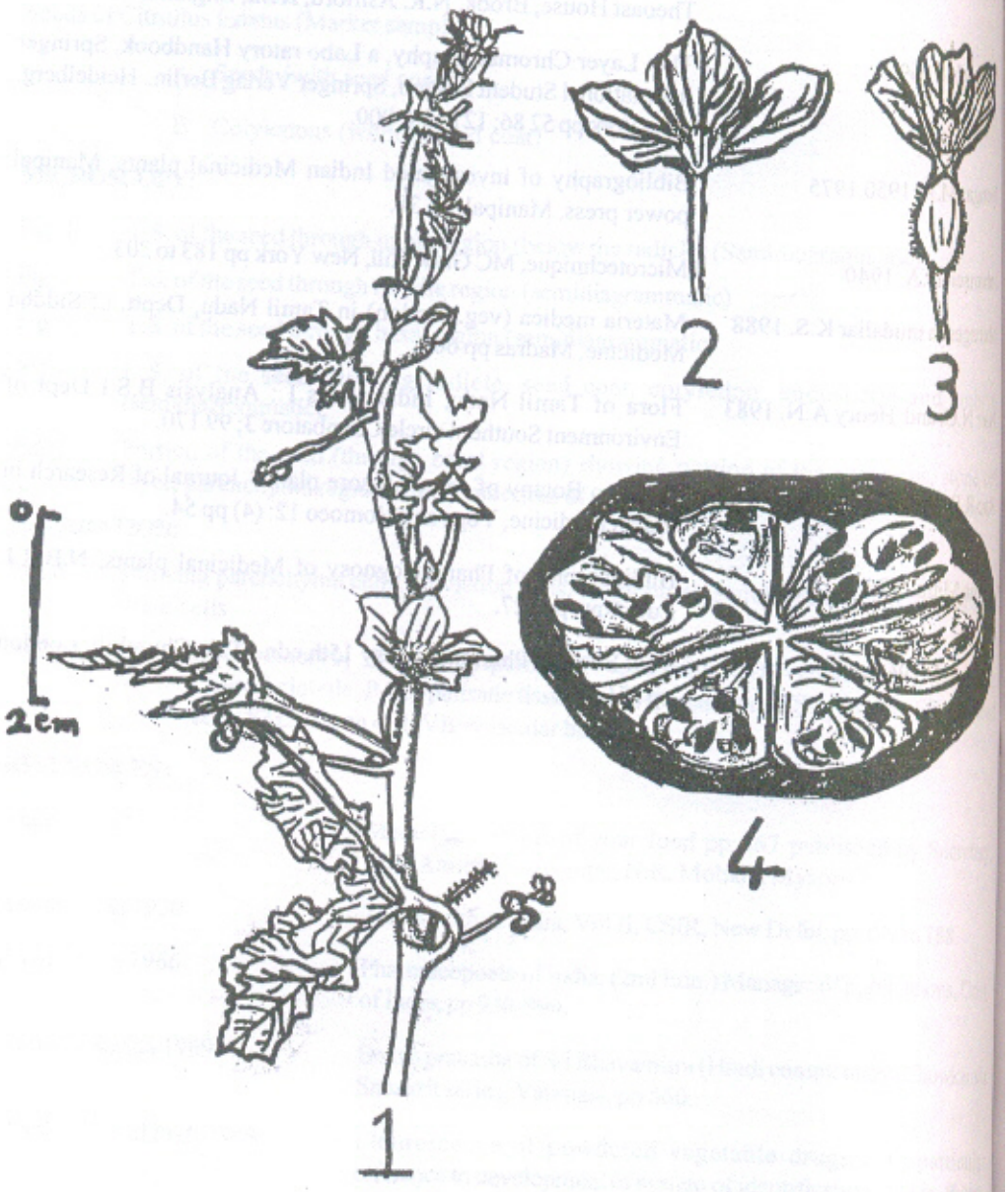
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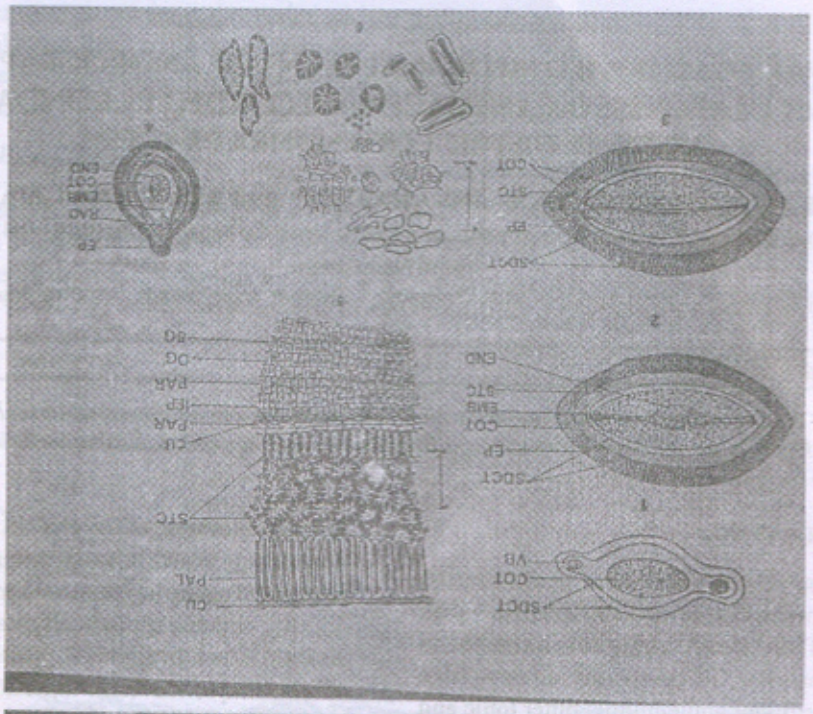

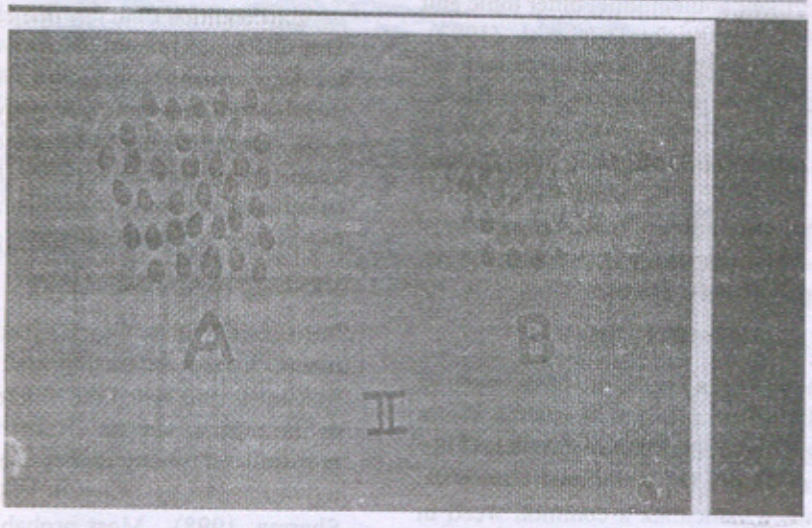
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