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Pharmacognostic and Phytochemical Investigation of *Luffa acutangula* var. *amara* Fruits

Kalaskar Mohan G.* ,Surana Sanjay J.

R C Patel Institute of Pharmaceutical Education & Research,

Shirpur, Dhule, MS,India

Corres.author: mohan.kalaskar@rediffmail.com
Phone No: +919860302190

Abstract: The *Luffa acutangula* Linn. Var. *amara* Roxb. is medicinal climber found in western, central and southern India, the fruits used in vata, kapha anemia, asthma, leucoderma, tumors, also useful as diuretic and in splenic enlargement. Scientifically it is proved as CNS depressant. In view of its medicinal importance and taxonomic confusion, pharmacognostic studies, microscopical structure, morphological characters, chemical analysis were carried out. The macromorphological, micromorphological and chemomicromorphological indicated the presence of marked ten acute projection on fruits running along the length, presence of lignified fibers below the epicarp, and scattered lignified fibers throughout mesocarp, partially lignified fiber covered vascular bundle, lignified endocarp andstarch grains. The present study on pharmacognostical characters of *Luffa acutangula var. amara* Roxb. fruits will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species of *Luffa* and varieties *Luffa acutangula.*
Key words: *Luffa acutangula* Linn. Var. *amara,* microscopy, morphology, pharmacognostic studies.

Introduction

The *Luffa acutangula* Linn. Var. *amara* Roxb. is a fairly large climber found in western, central and southern India, and regarded as wild variety of cultivated species. It is resembles to *Luffa acutangula* in every aspects, except the leaves, flower, fruits amd seeds are smaller. It is commonly in Marathi as Kadudodak, Ranturai, in Sanskrit Katukoshataki, while in Hindi karviturai. All parts of plant are exceedingly bitter. A crystalline bitter principle identical with cucurbitacin B, luffin, and colocynthin is present.[1] While seeds shows presence of saturated and unsaturated fatty acid palmatic, stearic, oleic, linoleic and traces of lignoceric acid. The plant possesses laxative and purgative property. It shows presence of oleanane type triterpene saponins – acutoside A, B, C, D, E, F, and G. Fruit shows presence of cucurbetacin B and E and oleanalic acid.[2] The plant possesses laxative and purgative property. It is tonic to intestine. Cures vata, kapha anemia, asthma, jaundice, leucoderma, tumors. Also useful as diuretic and in splenic enlargement. The dried fruit powder of fruit used in the form of snuff in jaundice. The seeds possesses emetic, expectorant, and demulcent property.[3] Scientifically it is proved as CNS depressant, it is used traditionally in insect bites by tribes of Western Maharashtra.[4] However, available literature revealed that no pharmacognostic study has been carried out on the fruit; hence the present investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of fruit; ash values, extractive values, microscopical characteristics of powdered fruit and preliminary phytochemical analysis of *Luffa acutangula* Linn. Var. *amara* Roxb. fruits.

Material and Methods

Fresh fruits.of *Luffa acutangula* Linn. Var. *amara* Roxb. were collected in Toranmal hills, Nandurbar, India in October and authenticated by Dr. D. A. Patil Taxonomist, SSVPS college, Dhule, Maharashtra, India. A herbarium is maintained in R. C. Patel College of Pharmacy, Shirpur, India. The fresh fruits were separated and used for the study of macroscopic and microscopical characters, whereas dried fruits powder material was used for the determination of ash values, extractive values, fluorescence analysis and phytochemical constituents. All the reagents used were of analytical grade obtained from Sigma Chemical Co, St. Louis, USA or Fine Chemicals Ltd., Mumbai, India.

Results and Discussion

Macroscopical characters (Figure 1)

Color: Mature fruit are dark green to yellowish green

Size: 2.5 to 8cm (l) and 1.3 to 2.5 cm.

Characteristic odour and intense bitter taste.

Extra feature: the fruit is obovate, having ten acute lines on it, which is from base to apex of the fruit. Fruit are narrow toward bottom and broader toward apex of fruit.

Microscopical characteristic (Figure 2)

The free hand thin transverse sections of fruit were treated with different staining agent and mounted on a glass slide. Transverse section of a fruit shows all the general microscopic characteristic of fruit i.e. presence of epicarp, mesocarp and endocarp, which are well differentiated.

Epicarp: It is the outermost layer of fruit made up of thin rectangular cells with a thick cuticle (Fig -1, a) and Stomata are seen at regular intervals. In mature fruits and there are layers of sub epidermal collenchyma.

Mesocarp*:* It is made up of many layers of thin compactly arranged parenchymatous cells, shows lignified fibers and scleranchymatous cells and layer of lignified cells below epicarp (Fig 1. b,c, and d) are present in parenchyma; vascular tissue (Fig 1, e and f.) is also observed in parenchymatous region which is partially covered with lignified fiber. Starch grains are also present. The lignified fibers are scattered in mesocarp.

Endocarp: It is made of simple large polygonal parenchymatous cell. Which envelops the seeds. These are partially lignified and sclerenchyamtous cells are scattered. (Fig 1, i)

Powder analysis (Figure 3)

1. Stone cell as solitary or in groups are observed. (fig 2, i)
2. Numerous actinocytic stomata meaning thereby that the cells surrounding the stomatal pores are uniformly arranged along the redii of circle. (fig 2, ii)
3. Fragments of mesocarp tissue containing spiral vascular strands measures 25 – 48 micron in diameter. Sclerenchymatous cells are seen many in number measures 80-130 micron in length 36-53 micron in breadth. (fig 2,iii)
4. Fibers are more, lignified well developed sclerenchymatous fibers from the vascular bundle region, thin, and isolated fibers measure 500 - 660 microns in length and 30 - 40 microns in breadth. (fig 2, iv)
5. The rounded starch grains of 7 – 10 microns in diameter are abundant and observed as free or in fragments of parenchymatous cells. (fig 2, v)

Histochemical color reactions

The different histochemical color reactions were performed on the fruit transverse sections to differentiate the different cell compositions and identification[5] and results were given in Table 1.

Behavior of powder with chemical reagents

Behavior of fruit powder with different chemical reagents was studied to detect the presence of phytoconstituents with color changes under daylight by reported method[6] and the results were shown in Table 2.

Ash values

Total ash, acid-insoluble ash, water-soluble ash, and sulphated ash values of the fruit powder were done as per the reported methods[7] and the results are tabulated in Table 3.

Extractive values

Extracts were prepared with various solvents by reported method.[8] Percentages of the extractive values were calculated with reference to air-dried drug (Table 4). Color and consistency of extracts[6] are given in Table 5.

Qualitative phytochemical screening

Freshly prepared fruit extracts were tested for the presence of phytoconstituents using reported methods[9] and the results are given in Table 6.

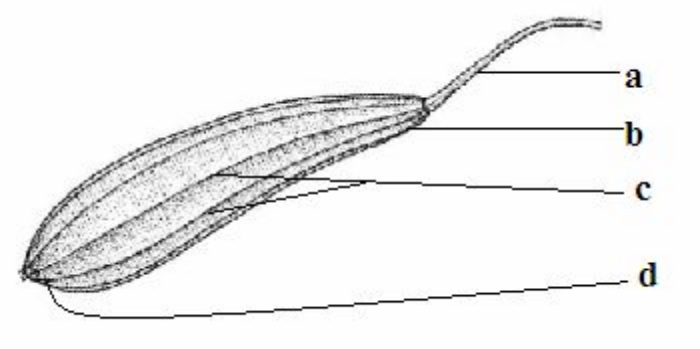
Fluorescence analysis of extracts

All the fruit extracts are examined in daylight, short and long UV to detect the fluorescent compounds by the reported method.[8] The observations are given in Table 7.

Conclusion

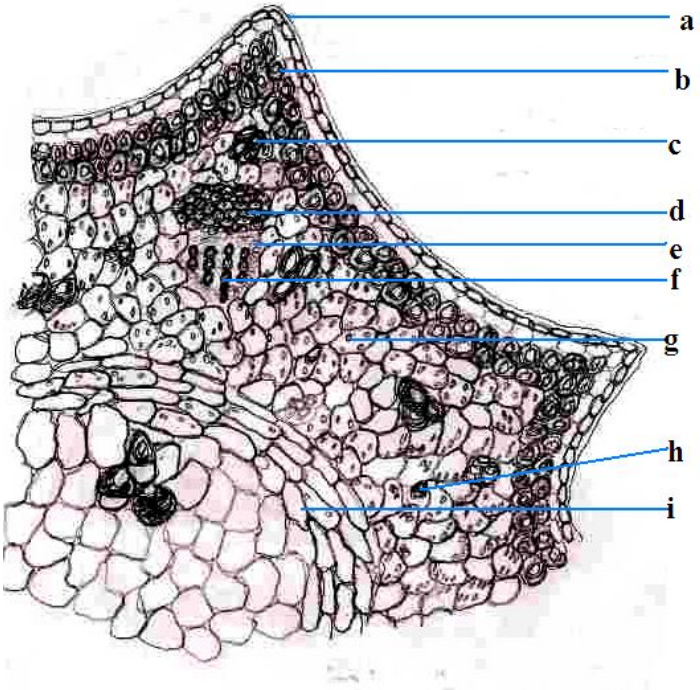
In conclusion, the present study on pharmacognostical characters of *Luffa Acutangula var. amara* (L). Jacq fruits will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species and varieties of *Luffa Acutangula.* Around the vascular tissues sclerenchymatous fibers occurs, as bundle sheath is a characteristic feature of *Luffa Acutangula var. amara* (L). Jacq. The presence of isolated lignified sclerenchymatous fibers, spiral vascular strands, starch grains and numerous actinocytic stomata is important observation in powder form of fruit. The other parameters observed may be useful for the future identification of the plant.

Fig 1. The *L.* acutangula var. amara fruit



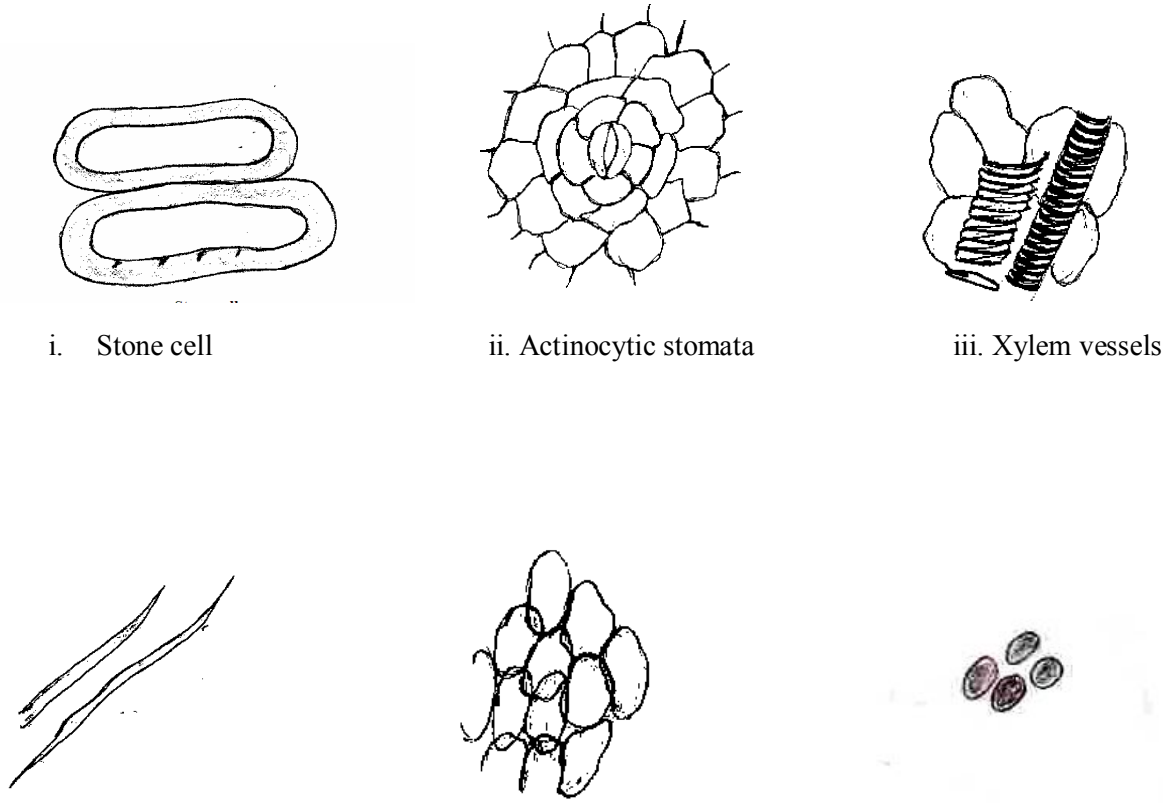
a. Pedicle b. base c. ridges d. apex

Fig 2. T.S of *L. acutangula* var. amara fruit



a. Cuticulized epidermis b. lignified cells c. sclerenchymatous stone cells d. lignified pericyclic fibres e. phloem f. xylem g. starch grain h. lignified fibres i. endocarp.

Fig. 3. Powder analysis of *L . acutangula* var. amara fruit



i. Stone cell ii. Actinocytic stomata iii. Xylem vessels

iv. Lignified fiber v. Liginfied paranchyama vi. Starch grians

Table 1. Histochemical color reactions of *Luffa acutangula* Var. *amara*

Reagents	Constituent	Color	Histological zone	Degree of intensity
Aniline So4 + H2SO4	Lignin	Yellow	Xylem,	++
Phloroglucinol + HCl	Lignin	Pink	Xylem, Sclerenchyma	+++
Conc. H2SO4	Cellulose	Green	mesocarp	+
Weak Iodine solution	Starch	--	--	--
Millons reagent	Proteins	--	--	--
Dragendorffs reagent	Alkaloids	---	--	--
Caustic alkali + HCl	Ca. Oxalate	--	--	--
Keddy reagent	Glycosides	--	--	--
SbCl3	Steroids/Triterpenoids	Reddish pink	mesocarp	+++
5% Aq. KOH	Anthraquinone glycosides	--	--	--

+++ High, ++ Moderate, + Slight, - Negative.

Table 2. Behavior of *Luffa acutangula* var. *amara* powder with different chemical reagents

Regents	Color/ppt	Constituents
Picric acid	No precipitations	Alkaloids absent
Conc. H_2SO_4	Reddish brown	Steroids/triterpenoids present
Aq. $Fecl_3$	Greenish black	Tannins and Flavonoids present
Iodine solution	Purple to black	Starch present
Ammonia present	No change	Antroquinone glycosides absent
5% Aq. KOH	No change	Antroquinone glycosides absent
Mayer's reagent	No perception	Alkaloids absent
Spot test	Stains observed	Fixed oils present
Aq. $AgNo_3$	No precipitation	Proteins absent
Aq. NaoH	Yellow	Flavonoids present
Mg – Hcl	Magenta	Flavonoids present
Dragendroff's reagent	No ppt	Alkaloids absent
Aq. Lead acetate	White precipitations	Tannins present
Liberman Burcherd's test	Reddish green	Steroids and tannins are present

Table 3. Ash value *Luffa acutangula* var. *amara* fruit

Types of ash value	% w/w
Total ash	9.0
Acid insoluble ash	1.0
Water soluble ash	7.6
Sulphated ash	6.4

Table4. Extractive value of *Luffa acutangula* var. *amara* fruit.

Type of solvent	%w/w
Petroleum ether 60-80^0	1.03
Ethyl acetate	0.97
Alcohol	1.56
Water	9.3

Table 5. A consistency, color, and fluorescence character of successive extract of *Luffa acutangula* var. *amara* fruit.

Parameter	extracts			
	Pet. Ether	***Ethyl acetate***	***Ethanol***	***Aqueous***
Consistency	Viscous	Viscous	Sticky	Free flowing powder
Color (day light)	Green	Greenish	Reddish	Brown
Short light	Yellowish green	Greenish	Yellowish red	Yellowish brown
Long light	Green	Greenish	Reddish	Brown

Table 6. Qualitative phytochemical analysis of *Luffa acutangula* var. *amara* fruit.

Constituents	*Pet. Ether*	*Ethyl acetate*	*Ethanol*	*Aqueous*
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	+
Cumarines	-	-	-	-
Flavonoids	-	+	+	-
Fixed oils	+	-	-	-
Glycosides	-	-	-	-
gums and resins	-	-	-	-
Mucillages	-	-	-	-
Proteins and amino acids	-	-	-	+
Saponins	-	-	-	-
Steroids and sterols	+	+	-	-
Tannins	-	-	+	+
triterpenoids	+	+	-	-

+ present - absent

Table 7: Fluorescence analysis of *Luffa acutangula* var. *amara* fruit.

Color reaction	Day light	Uv light 365nm
Powder + NaOH	Yellow color	Yellow fluorescence
Powder + Methanol + nitrocellulose	Reddish brown	Yellowish green fluorescence
Powder + nitrocellulose	Reddish brown	Strong yellow fluorescence
Powder + NaOH in water	Yellow	Faint yellow fluorescence
Powder + nitrocellulose +Hcl	Reddish grayish	Faint green color
Powder + Hcl	Yellowish grey	Dark brown with faint yellow fluorescence
Powder + H_2SO_4	Blackish	Black
Powder + HNO_3	Reddish	Black
Powder	Buff	Yellow florescence

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