Pharmacognostic studies of *Lagenaria siceraria* (Molina) standley fruits

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ABSTRACT

Background: *Lagenaria siceraria* (LS) fruits belonging to Cucurbitaceae family is widely used in Indian traditional medicine for its various medicinal values. As per best of our knowledge there were no pharmacognostical reports, specifically to determine anatomical and other physicochemical standards required for its standardization. **Material and Methods:** In this study various standardization parameters like macroscopic and microscopic studies, physicochemical constants, extractive values and preliminary phytochemical screening were studied and reported. **Results:** Different standardization parameters were reported, which would be of immense use to identify and establish the authenticity of the plant. **Conclusion:** Preliminary pharmacognostic evaluation of *Lagenaria siceraria* fruits can give some useful information, which will be further used for standardization.

Keywords: Pharmacognostic, Bhopla, Cucurbitaceae, standardization, Lagenaria siceraria.

INTRODUCTION

Lagenaria siceraria (LS) locally called as 'Bhopla' is a large softly pubescent annual climber distributed in Asia, America and tropical Africa, wild or cultivated in all warmer regions as a vegetable. The fruit is reported as diuretic and antipyretic. Traditionally a decoction of leaves mixed with sugar is given in jaundice. The seeds are being used to cure cough, fever, earache, as brain tonic and anti-inflammatory.[1–4] The fruit contains saponins and some researchers have already reported saponins plays a vital role as hepatoprotective.[4–8,15]

In the present study, various standardization parameters of LS fruits like macroscopical and microscopical characters, extractive values, ash values and preliminary phytochemical screening were carried out to identify the major phytoconstituents.

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MATERIALS AND METHODS

Plant material

The fruits of LS were collected in the month of October, 2005 from local areas of Pune region and authenticated by Botanical Survey of India, Pune and voucher specimen number, deposited as PCV 1. *Lagenaria siceraria* (Mol.) Standl., belonging to family Cucurbitaceae

Chemicals and instuments

All the chemicals were used of analytical grade. Compound microscope, watch glass, cover slip, glass slide and other common glass wares were used in this experiment. Paraffin embedded blocks were sectioned with help of rotary microtome. Photographs were taken with Nikon Labphot 2 microscopic unit. Various solvents used mainly Ethyl alcohol (70%), tertiary butyl alcohol and reagents used for staining different sections like formalin, acetic acid, toluidine blue, safranin, fast-green and IKI were procured from CDH, Mumbai, India.

Macroscopic and microscopic studies

Macroscopic study of fruit such as size, shape, color, odor and taste was performed.

Microscopic study: The fruit of LS was taken and cut into small pieces. It was fixed in FAA (formalin – 5mL + Acetic acid – 5mL + 70% Ethyl alcohol – 90mL). After 24 hrs. of fixing, the specimen were dehydrated with graded series of tertiary- butyl alcohol as per the schedule given.[9] Infiltration of specimen was carried out by gradual addition of paraffin wax until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

The paraffin embedded blocks were sectioned with help of rotary microtome. The thickness of the section was 1–12µm. Dewaxing of the sections was by customary procedures.[10] The sections were stained with Toluidine blue as per the standard method.[11] Since Toluidine blue is a polychromatic stain, the staining was remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells and protein bodies, dark green to suberin, violet to the mucilage etc., wherever necessary sections were stained with safranin and fast-green and IKI (for starch).

Photomicrographs

Photomicrographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For the study of lignified cells polarized light was employed. Magnification of the figures is indicated by the scale bars. Descriptive terms of the anatomical features are as given in standard anatomy books.[12]

Determination of physicochemical constants

Ash value[1,13]

Total ash

The total ash is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Method

About 2.003g of powdered material was accurately weighed and taken separately in a silica crucible, which was previously ignited and weighed. The powder was spread as a fine, even layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried drug.

Acid-insoluble ash

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This ash value particularly indicates contamination with silicious material such as earth and sand.

Method

The ash obtained as described above was boiled with 25mL of 2N HCl for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a silica crucible, ignited, and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water-soluble ash

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Method

The ash obtained as described in the determination of total ash was boiled for 5 minutes with 25mL of water. The insoluble matter was collected on ashless filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 minutes, and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Extractive values[1,13]

Alcohol soluble extractive value

About 5g each of coarsely powdered material was accurately weighed and macerated with 100mL of alcohol of specified strength, separately in a closed flask for 24 hours. Shaking was done frequently during first 6 hours and then allowed to stand for 24 hours. This was filtered rapidly taking precaution against loss of alcohol. Then, 25mL each of these alcoholic extracts were evaporated to dryness in a tared flat bottom shallow dish and were dried at 105°C and weighed. Percentage of alcohol

soluble extractive was calculated with reference to the air-dried drug.

Water soluble extractive value

About 5g each of coarsely powdered material was accurately weighed and macerated with 1000mL of chloroform water, separately in a closed flask for 24 hours. Shaking was done frequently during first 6 hours and then allowed to stand for 24 hours. This was filtered and then, 25mL each of these chloroform water extracts were evaporated to dryness in a tared flat bottom shallow dish and were dried at 105°C and weighed. Percentage of water soluble extractive was calculated with reference to the air-dried drug.

Preliminary phytochemical screening[1,13–15]

Preliminary phytochemical screening for different constituents was carried out in petroleum ether extract and ethanolic extract.

RESULTS

Macroscopic study:

Macroscopic and sensory characters of fruits are as shown below

Length: 25–35 cm.

Shape: Bottle, dumbbell, cylindrical shaped.

Width: 5–8 cm.

Surface: Densely hairy to glabrous.

Weight: 400–800 gm.

Colour: Faint green.

Odour: Pleasant.

Taste: Sweet.

Microscopic study

The histological findings of LS fruits are given as follows:

Fruit

The pericarp is the rind of the fruit. The pericarp has epicarp and mesocarp. Epicarp is unistrstose forming the epidermal layer of the fruit. The epidermis is 20mm thick; the cells are radically oblong and wide. The outer and inner tangential walls are thin; stomata are sporadically seen in the epidermis.

The mesocarp is differentiated into outer zone and inner zone (fig. 1.1). The outer zone is 120mm wide and 10 to 15 layered. The cells are small, tangentially elliptic, compact and darkly staining. As the fruit matures, the epicarp and the outer mesocarp combined together develop into the hard rind. Inner and outer mesocarp is the inner mesocarp, which fills the entire pulp of the fruit. This tissue has large, polygonal compact cells. A few layers of the cells in the peripheral region are thick walled and lignified. The lignified cell zone is gradually transformed into thin walled soft parenchyma cells. Sporadically, one or two cells in between the two zones of mesocarp have dark amorphous contents.

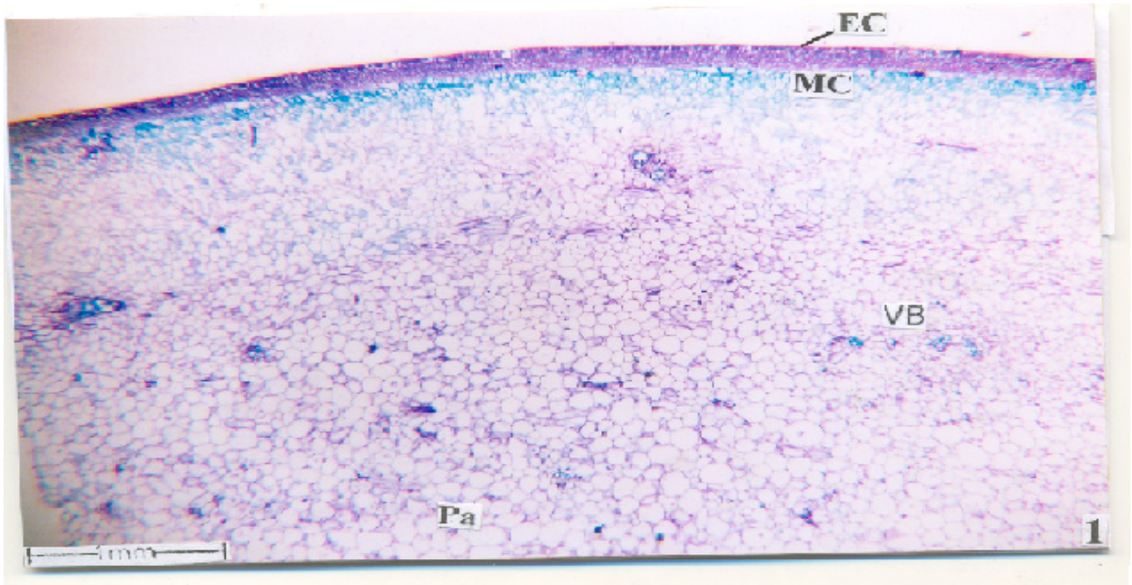

Figure 1.1 T.S. of fruit under low magnification Where: VB – Vascular bundle, MC – Mesocarp, EC – Epicarp, Pa – Palisade cells.

Vascular strands are scattered within the thin walled parenchymatous tissue. The vascular strands consist of one or two wide, thick walled meta xylem elements and proto xylem elements. Small groups of phloem elements occur on the outer part of the meta xylem elements (fig. 1.2). The meta xylem elements are 70µm wide.

When the section of the fruit wall is viewed under the polarized light microscope the lignified cells of the inner mesocarp tissue exhibit scalariform piths, their walls also appear bright denoting the lignin content of the cellwalls.

Physicochemical constants

Table 1.1 Various Ash values of crude drug.

Sr. No.	Particulars	Dried fruit pulp (% w/w)
1.	Total Ash	4.40*
2.	Acid-insoluble Ash	0.70*
3.	Water soluble ash	2.30*

* = Average of three values

Table 1.2 Various Extractive values of crude drug.

Sr. No.	Particulars	Dried fruit pulp (% w/w)
1.	Alcohol soluble extractive	6.42*
2.	Water soluble extractive	18.59*

* = Average of three values

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to know the presence of various phytochemical constituents. Sterols, flavonoids, phenolics and tannins found present in Petroleum ether (PE) and Ethanolic (ETH) extract, saponins present was noted in ETH extract.

DISCUSSION

Macroscopic, microscopic characters and various physicochemical constants such as total ash, acid insoluble ash, water soluble ash, water and alcoholic extractive value of LS fruits were studied and it may be important tools for the identification, authentication and standardization of the plant. Ethanolic extract of LS fruits contain saponins, which may be responsible for various biological activities right from anti-inflammatory to anticancer.

CONCLUSION

Preliminary pharmacognostic evaluation of *Lagenaria siceraria* fruits reveals the presence of flavonoids, saponins and steroids which play vital role in the manifestation of different disease conditions. The further study will be done for isolation and characterization of different phytoconstituents and its probable biological activities.

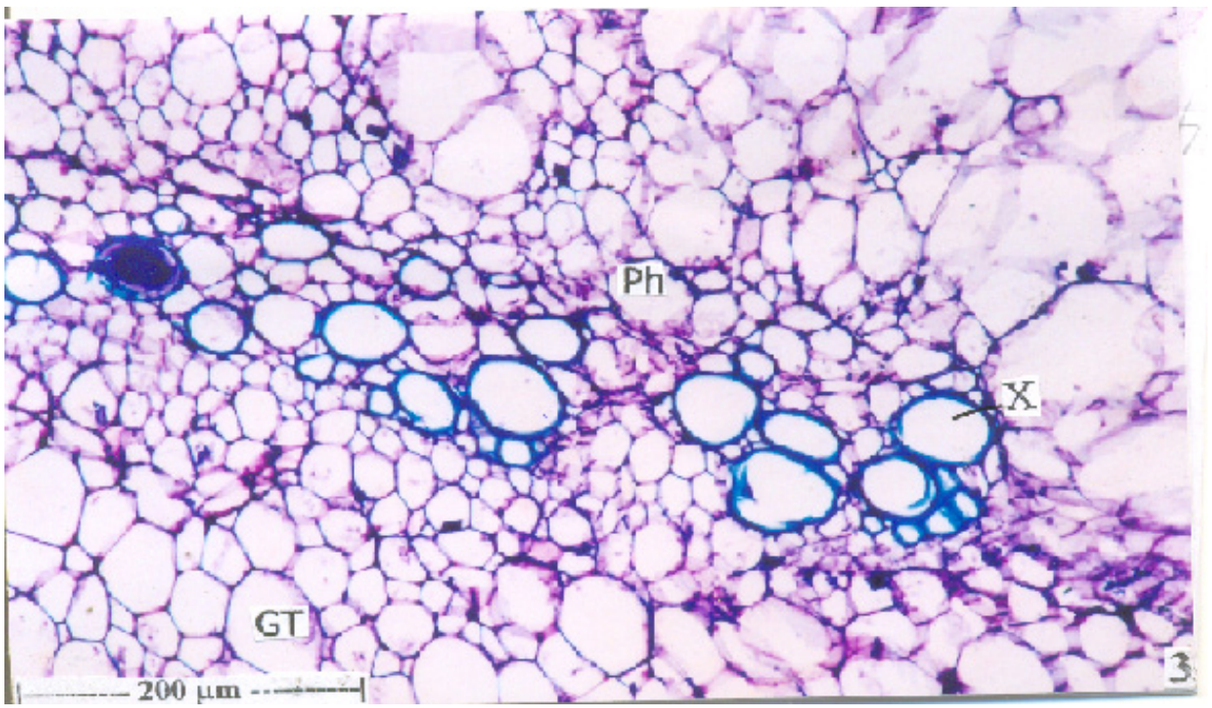

Figure 1.2 T.S. Showing Vascular strands in the parenchymatous tissue in mesocarp Where: Ph – Phloem, X – Xylem, GT – ground tissue.

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