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#### **Research Article**

# PHYSICOCHEMICAL STANDARDISATION AND AN OVERVIEW OF *LAGENARIA SICERARIA* (MOLINA) STANDLEY-A SIDDHA HERBAL DRUG

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## **ABSTRACT**

Lagenaria siceraria (Molina) Standley (Family- Cucurbitaceae) is commonly used plant in Siddha, and other Indian traditional systems of medicine and is considered as an important remedy for the treatment of several diseases. In traditional system of medicine, different parts (leaves, stem, flower, roots seeds and whole plant) of L. siceraria have been used for ailment of various diseases throughout India. The fruits of cultivated L. siceraria are a good source for nutrients like protein, fat, fibre, carbohydrates, calcium and magnesium. The aim of the present work is the standardization and HPTLC fingerprinting of L. siceraria whole plant. Physico-chemical parameters such as total ash, acid insoluble ash, moisture content, water and alcohol soluble extractives were carried out according to standard methods. HPTLC fingerprinting studies of L. siceraria (whole plant) was carried out at 254 nm, 366 nm and 575 nm after derivatization using vanillin-sulphuric acid. The solvent system used for the development of plate was toluene:ethyl acetate (5:1). The physic-chemical parameters were documented. HPTLC fingerprinting revealed the presence of different bands at 254 nm, 366 nm and after derivatization with vanillin sulphuric acid at 575 nm, corresponding to various constituents. The study will help in developing the pharmacopoeial standards for L. siceraria.

KEYWORDS: Lagenaria siceraria, Cucurbitaceae, phytochemistry, pharmacological activity.

## INTRODUCTION

Herbal remedies are safer and less damaging to the human body than synthetic drugs. Indian traditional medicinal systems have emphasized the importance of green medicines in the management of diseases. Even practitioner of modern system has realized the significance of herbals, in the form of nutraceutical elements, in the treatment of chronic diseases [1]. They grow in tropical, subtropical, arid deserts and temperate locations and are usually called as gourds. The gourd family consists of a number of bioactive plants which have been used extensively from ancient time for their therapeutic values.

The plant, Lagenaria siceraria (Molina) standley (Family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout India. The plant was originated in tropical Africa and now has sub-tropical distribution. The fruit is used as immune suppressant, diuretic, cardio-tonic, cardio-protective and nutritive agent. The fruit is also reported to have good source of vitamin-B complex and choline along with fair source of vitamin-C and  $\beta$ -carotene  $^{[2]}$ . It was also reported to contain Cucurbitacins, fibres, and polyphenol, sterols namely campesterol.

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The current study is intended to perform the physicochemical standardization and HPTLC fingerprinting of whole plant of L. siceraria along with the general overview of the plant.

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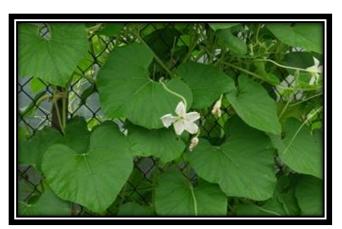


Fig.1 Fresh whole plant of L. Siceraria

## Macroscopical characters of L. Siceraria:

It is an annual climber, stems prostrate, climbing, angular, ribbed. Stems are, thick, brittle, and softly hairy, up to 5 m long. Cut stems exude no sap. Leaves simple, up to 400 mm long and 400 mm broad, shortly and softly hairy, broadly egg-, kidney- or heart-shaped in outline, undivided angular or faintly 3-7-lobed, lobes rounded, margins shallowly toothed, leaves non-aromatic. Leaf stalks up to 300 mm long, thick, often hollow, densely, with hairy two small, lateral glands inserted

at the leaf base. Tendrils split in two (Fig. 1). They are cultivated throughout the country. August is the flowering season.

#### Microscopical characters of L. siceraria:

L. siceraria leaf is 7.9-15.5 cm long, elliptical shaped having entire margin and parallel venation. The apex of the leaf is acute having leathery surface with firm texture, dark green colour, bitter taste and characteristic odour. The transverse section of L. siceraria leaf showed upper epidermis consists of elongated parenchymatous cells, covered by cuticle. The upper epidermis shows few stomata, which are of anisocytic type. Lower epidermis contains elongated wavy walled parenchymatous cells covered by cuticle. Collapsed trichomes are present, while very few glandular trichomes are also present. Palisade cells are present at upper and lower epidermis. Mesophyll is made up of 3-4 layered chloroplasts containing, compactly arranged, oval to circular cells. It is interrupted by vascular bundles of various sizes. Vascular bundles are surrounded by 2-3 layered sclerenchyma; they are conjoint, collateral and closed. Xylem is placed towards upper epidermis and phloem towards lower epidermis [3].

#### Traditional uses:

L. siceraria was used as medicine in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. The fruit pulp is used as an emetic, sedative, purgative, cooling, diuretic and pectoral [4]. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Plant extracts show antibiotic activity. Leaf juice is widely used for baldness. Decoctions containing a combination of L. siceraria and Rivina humilis are given for gas in pregnancy. In combination with garlic, a decoction is taken for gas pain in the heart area. Leaves with salt or coconut oil are often used as poultices for mange, skin irritation. A poultice of the crushed leaves has been applied to the head to treat headaches. Taken with Achyranthes sp., the seed is used to treat aching teeth and gums, boils, etc. Pulverized seed kernels are taken to expel intestinal worms [5,6] reported the hepatoprotective activity of L. siceraria fruit extracts against carbon tetra chloride induced hepatotoxicity which revealed potent activity against hepatic injury.

## MATERIALS AND METHODS

## Collection and authentication of plant materials:

The whole plant of *L. siceraria* was collected from SMPG, Mettur dam. The plant material was chopped, crushed and shade dried. The dried plant specimens were kept in airtight containers and used for all experimental purposes.

### Physico-chemical analysis:

Physico-chemical analysis such as determination of ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive and loss on drying at  $105 \, ^{\circ}$ C were carried out by standard methods  $^{\circ}$ 7, 8].

#### **HPTLC** analysis:

#### Preparation of extracts:

Extract of the plant material was prepared by boiling 4 g of the drug in 40 ml chloroform. The filtrates were concentrated on a water bath to1 ml. This extract was used for chromatographic studies.

## High performance thin layer chromatography (HPTLC):

Instrumentation and chromatographic conditions: HPTLC was performed on 10 x 10 cm aluminium backed plates coated with silica gel 60 F<sub>254</sub> (Merck, Germany). Samples were applied to the plates as bands 10 mm wide and 8 mm from bottom edge of the same chromatographic plate by use of a Camag (Switzerland) sample applicator equipped with a 100  $\mu$ L Hamilton (USA) Syringe. Ascending development to a distance of 80 mm was performed at room temperature with Toluene: Ethyl acetate (5:1) as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapour. After development, the plate was air dried visulaized and scanned at 254 nm and 366 nm. For derivatization, the plate was dipped in Vanillin Sulphuric acid reagent and was dried in hot plate. Then the plate was visualized and scanned under visible light (575 nm) [9, 10].

## RESULTS AND DISCUSSION

#### Physico-Chemical analysis:

The physico-chemical parameters of the whole plant of the L. Siceraria are given in Table1. The total ash is particularly important in the evaluation of purity of drugs. It indicated the amount of minerals and earthy material attached to the plant material. Acid insoluble ash usually represents the amount of silica present as sand and dust. Loss on drying at  $105^{\circ}\mathrm{C}$  shows the presence of moisture content and volatile oil (if any) present in the drug. The water soluble extractive value indicates the presence of polar constituents such as tannin, sugar, plant acid, mucilage and glycosides. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc.

The loss on drying of whole plant of L. Siceraria is 13.22 % and total ash value is 11.45%. The acid insoluble ash of whole plant is 2.95% and the water soluble extractive value is 19.51%. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc. The alcohol soluble extractive value is 7.6% which signifies that the fewer amounts of constituents of the drug were soluble in alcohol.

Table No. 1: Physicochemical parameters of L. siceraria

Parameters	Results (%w/w)
Loss on drying	13.22
Total ash	11.45
Acid insoluble ash	2.95
Water soluble extractive	19.51
Alcohol soluble extractive	7.6

## High performance thin layer chromatographic analysis (HPTLC):

HPTLC is the simplest separation technique for phytochemical studies. This method describes the identification and quantification of active constituents in the plant material, which may be useful for proper standardization of herbals and its formulations. HPTLC also facilitates repeated detection of chromatogram with same or different parameters.

## Development of high performance thin layer chromatographic (HPTLC) profile:

The HPTLC fingerprinting patterns of chloroform extract of whole plant of *L. siceraria* was developed at 254nm, 366nm and after derivatisation with vanillin-sulphuric acid at 575nm (Fig.2).

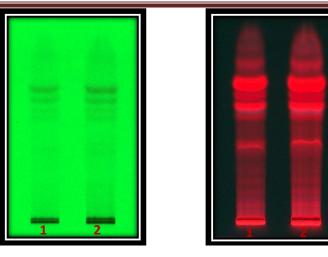
 $R_{\rm f}$  values and colour of spots obtained under UV 254, UV 366 and 575 nm were represented in the Table 2, denotes the total number of bands obtained with their characteristics colours. The present study showed the development of dark green, purple, red and light yellow bands for the chloroform extract of the whole plant of *L. siceria* suggesting the presence of various secondary metabolites.

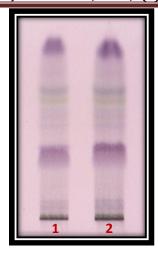
The HPTLC finger printing patterns of chloroform extract of the whole plant of L. siceraria was developed at 254nm, 366nm and after derivatisation with vanillin – sulphuric acid at 575nm. The solvent system, Toluene: Ethyl acetate (5:1) efficiently resolved the components present in the crude extract. HPTLC photo documentation profile of the chloroform extract of L. siceraria at 254nm 366nm and after derivatisation is given in Fig 3,4 and 5.

The HPTLC finger profile print at UV 254 nm showed nine peaks among which the peak at  $R_{\rm f}$  0.74 is the major peak with an area of 16.80% followed by the peak at 0.68 and 0.59 with area of 8.36% and 4.17% respectively (Fig.3).

At 366nm showed nine peaks among which the peak at  $R_f$  0.63 is the major peak with an area of 20.64% followed by the peak at0.76, and 0.73 with area of 17.21%, 10.94% respectively. Other peaks appeared at 0.86, 0.68, 0.51, 0.44, 0.28, 0.22 with area of 4.28%, 4.49%, 0.98%, 9.49%, 0.59% and 1.31% (Fig: 4).

At 575nm after derivatization with vanillin-sulphuric acid (Fig. 5) showed nine peaks among which the peak at  $R_f$  0.41 (43.88 %) is the major peak. Other peaks appeared at  $R_f$  are 0.74 (9.03%), 0.96 (4.91%), 0.6 (4.99%), 0.10 (2.74%), 0.19 (0.41%), 0.54 (4.47%), 0.60 (4.20%), 0.68 (3.55%) respectively (Fig. 5).





**Under UV short** 

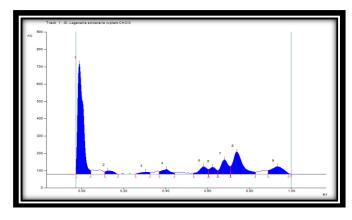
**Under UV long** 

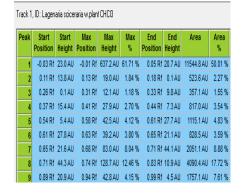
Under white light after derivatisation

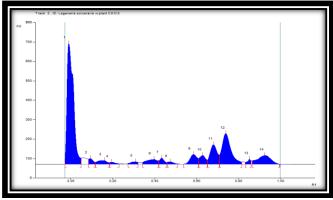
Fig. 2: HPTLC Photodocumentation of the chloroform extract of L. Siceraria

Table No. 2:  $R_f$  values and colour of spots of chloroform extract of L. Siceraria

At 254 nm	l	At 366	nm	Post derivatization		
R <sub>f</sub> value Area (%)		R <sub>f</sub> value Area (%)		R <sub>f</sub> value	Area (%)	
0.74 (Dark green)	16.80	0.86 (Red)	4.28	0.06 (Light yellow)	4.99	
0.68 (Dark green)	8.36	0.76 (Red)	17.21	0.10 (Dark green)	2.74	
0.63 (Dark green)	3.11	0.73 -Red	10.94	0.19 (Dark green)	0.41	
0.59 (Light green)	4.17	0.68 (Red)	4.49	0.41 (Dark green)	43.88	
0.48 (Litght green)	0.90	0.63 (Red)	20.64	0.54 (Dark green)	4.47	
0.44 (Light green)	1.69	0.51 (Red)	0.98	0.60 (Light yellow)	4.20	
0.40 (Light green)	3.25	0.44 (Red)	9.49	0.68 (Light yellow)	3.55	
0.31 (Light green)	1.02	0.28 (Purple)	0.59	0.74 (Light yellow	9.03	
0.20 (Light green)	0.66	0.22 (Purple)	1.31	0.96 (light yellow	4.91	







Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
- 1	-0.03 Rf	16.1 AU	-0.01 Rf	621.3 AU	52.43 %	0.05 Rf	31.3 AU	13372.8 AU	47.99 %
2	0.09 Rf	24.8 AU	0.09 Rf	29.0 AU	2.44 %	0.12 Rf	9.7 AU	431.8 AU	1.55 %
3	0.12 Rf	10.0 AU	0.16 Rf	19.3 AU	1.63 %	0.19 Rf	7.5 AU	611.6 AU	2.19 %
4	0.19 Rf	7.7 AU	0.20 Rf	10.0 AU	0.84 %	0.23 Rf	2.1 AU	184.7 AU	0.66 %
5	0.28 Rf	4.1 AU	0.31 Rf	12.5 AU	1.05 %	0.32 Rf	9.6 AU	284.1 AU	1.02 %
6	0.34 Rf	10.2 AU	0.40 Rf	24.9 AU	2.10 %	0.42 Rf	18.0 AU	904.9 AU	3.25 %
7	0.42 Rf	18.2 AU	0.44 Rf	32.0 AU	2.70 %	0.46 Rf	9.0 AU	471.4 AU	1.69 %
8	0.46 Rf	9.7 AU	0.48 Rf	12.4 AU	1.04 %	0.51 Rf	1.4 AU	251.7 AU	0.90 %
9	0.54 Rf	0.0 AU	0.59 Rf	50.3 AU	4.24 %	0.61 Rf	29.7 AU	1161.8 AU	4.17 %
10	0.61 Rf	29.7 AU	0.63 Rf	44.4 AU	3.74 %	0.65 Rf	21.6 AU	866.9 AU	3.11 %
11	0.65 Rf	22.8 AU	0.68 Rf	100.0 AU	8.44 %	0.71 Rf	47.6 AU	2329.0 AU	8.36 %
12	0.71 Rf	48.2 AU	0.74 Rf	157.7 AU	13.31 %	0.82 Rf	11.2 AU	4680.3 AU	16.80 9
13	0.83 Rf	10.0 AU	0.85 Rf	26.4 AU	2.23 %	0.86 Rf	17.9 AU	300.2 AU	1.08 9
14	0.87 Rf	18.1 AU	0.93 Rf	44.9 AU	3.79 %	1.00 Rf	0.2 AU	2015.4 AU	7.23 %

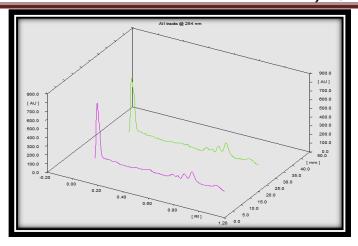
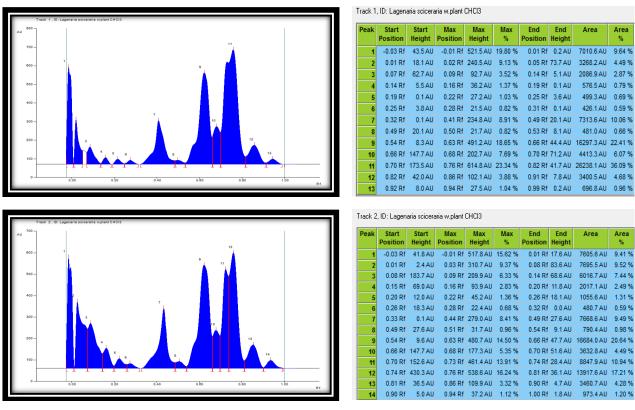


Fig. 3: HPTLC fingerprinting profile of whole plant of L. Siceraria at 254nm



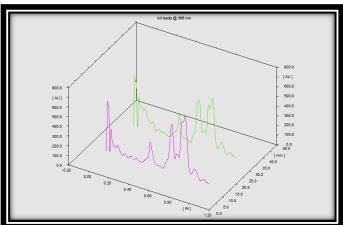
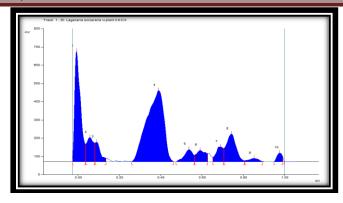
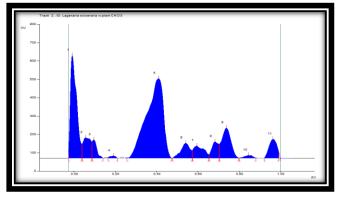


Fig. 4: HPTLC fingerprinting profile of whole plant of L. Siceraria at 366 nm





Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
- 1	-0.03 Rf	18.0 AU	-0.00 Rf	605.4 AU	36.34 %	0.04 Rf	96.9 AU	11972.5 AU	22.96 %
2	0.04 Rf	97.3 AU	0.06 Rf	132.7 AU	7.96 %	0.08 Rf	02.6 AU	3003.7 AU	5.76 %
3	0.08 Rf	102.7 AU	0.09 Rf	106.7 AU	6.40 %	0.14 Rf	19.9 AU	1950.7 AU	3.74 %
4	0.26 Rf	0.4 AU	0.39 Rf	390.5 AU	23.44 %	0.46 Rf	0.6 AU	22851.8 AU	43.82 %
5	0.47 Rf	0.0 AU	0.54 Rf	67.2 AU	4.03 %	0.56 Rf	36.2 AU	1927.1 AU	3.70 %
6	0.57 Rf	36.9 AU	0.59 Rf	62.8 AU	3.77 %	0.63 Rf	44.4 AU	1999.8 AU	3.84 %
7	0.65 Rf	24.4 AU	0.69 Rf	81.7 AU	4.90 %	0.71 Rf	72.9 AU	2030.4 AU	3.89 %
8	0.71 Rf	73.8 AU	0.75 Rf	152.5 AU	9.15 %	0.81 Rf	6.2 AU	4973.8 AU	9.54 %
9	0.81 Rf	6.2 AU	0.85 Rf	18.8 AU	1.13 %	0.89 Rf	1.2 AU	605.2 AU	1.16 %
10	0.95 Rf	0.2 AU	0.98 Rf	47.8 AU	2.87 %	0.99 Rf	27.5 AU	830.0 AU	1.59 %



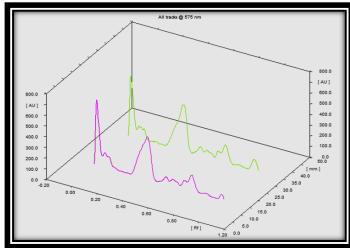


Fig. 5: HPTLC fingerprinting profile of whole plant of L. sicerariaat 575 nm after derivatization with vanillin sulphuric acid

## CONCLUSION

The HPTLC fingerprinting profile developed along with the physico- chemical parameters can be used as a diagnostic tool to determine the quality and purity of the whole plant of *L. sicearria* (Molina) Standley. Also, the present study provides an overview of this plant material with respect to its macroscopical, microscopical characters and traditional uses.

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