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RESEARCH ARTICLE

Pharmacognostical and Phytochemical investigation of aerial parts of *Lagenaria siceraria* (Mol.) Standley

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ABSTRACT

Lagenaria siceraria (Mol.) Standley is a large pubescent, climbing or tailing herb with stout 5- angled stems and bifid tendrils belonging to the family Cucurbitaceae. Various parts of *Lagenaria siceraria* (Mol.) Standley has traditional and folklore claims in the treatment of diabetes, obesity, cardiovascular diseases, urolithiasis, adenopathy, cancer, hypercholesterolemia, dyskinesia, hepatosis, jaundice, diarrhoea, dysuria, fever, rheumatism, myalgia, ophthalmia, uterosis, scabies, ulcer. In the present investigation, the detailed microscopic study of *Lagenaria siceraria* fruit and seed, have been carried out. Physiochemical, phytochemical studies for the aerial parts have been carried out to lay down the standards which could be useful in future experimental studies.

KEYWORDS

Lagenaria siceraria, Sclereids, Unitratose epidermis, Stomata, Oleo-resin, Phytochemical, Estimation.

INTRODUCTION

Herbal medicine refers to the practice of herbal medicine-a holistic outlook on health-which uses herbs and herbal preparations in support of a holistic healing strategy. Cucurbits are a well-recognized source of secondary metabolites. Therefore, cucurbits are among the largest and the most diverse plant families and are cultivated worldwide in a variety of environmental conditions. *Lagenaria siceraria* is one such plant which is found throughout India, either wild or cultivated. It is generally accepted that *Lagenaria siceraria* is indigenous to Africa and it reached the temperate and tropical areas in Asia and Americas about 10,000 years ago. It is widely cultivated in tropical and pan-tropical regions of the world.

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In India, it is cultivated in Tamil Nadu, Delhi, Punjab, Haryana, Gujarat, Assam, Meghalaya, Maharashtra, Karnataka and Rajasthan.

MATERIALS AND METHODS

Plant Material

The plant specimens for the proposed study were collected from the local farms of Dheeran Nagar of Thirichirapalli district, Tamilnadu and authenticated by Prof. P. Jayaraman, Institute of Herbal botany, Plant Anatomy Research Centre, Tambaram, Chennai. The plant material was certified as *Lagenaria siceraria* of family Cucurbitaceae and the specimen no. is PARC/2012/1344.

Chemicals

All the chemicals used were of analytical grade procured from Supra chemicals, Chennai, India.

Pharmacognostical Studies

Evaluation of a drug is performed to confirm the identity and to determine the quality of crude drug by seeking the presence of adulterants mixed with it which leads to degradation of quality.

Microscopy[6-11]

The required parts of the plant were removed and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). The specimens were then dehydrated with tertiary butyl alcohol and in filterated by addition of paraffin wax. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The sections was stained with toluidine blue (polychromatic stain) The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed.

Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid and glycerine and observed through microscope. Lignified cells can be identified by their pink stain and presence of calcium oxalate crystals can be identified by using polarised light microscope.

Proximate Analysis of Crude Leaves Powder[12-21]

The powdered aerial parts of the plant were subjected to various physicochemical properties, which include foreign organic matter, ash values, loss on drying, extractive values etc., (Table 1).

Ash Values

Ash values are used for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter. The important indices to illustrate the quality as well as purity of herbal medicine are total ash and acid insoluble ash.

Extractive Value

Extractive value is the parameter used to quantify the extraction potential of a vegetable matter. It gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction.

Loss on Drying

The loss on drying test is designed to measure the amount of water and volatile matters present in a sample when the sample is dried under specified conditions.

Crude Fibre Content (Dutch Method)

Crude fibre content is the residue of resistant tissues which can be obtained after giving treatment to the vegetable powder with dilute acid and alkali. It is useful in distinguishing between similar drugs or in the detection of adulteration. The method helps to remove the more resistant parts of plant organs which can be used for microscopical examination and removes starch and other cell contents and destroys lignin.

Qualitative Estimation of Heavy Metals and Inorganic Elements [19]

Essential inorganic elements in plants have functional roles like charge balance and electrolytic conductivity (Na, K, Cl), structure and templating (Ca, Zn, Si, S), signalling (Ca, B, NO), Bronstead Acid-Base Buffering(P, Si, C), Lewis Acid-Base Catalysis (Zn Fe Ni Mn Zn, Fe, Ni, Mn), electron Transfer (Fe, Cu), group transfer (e.g, Fe, Co, Ni, Cu, Mo), redox catalysis (Mn, Fe, Co, Ni, Cu, S, Se), energy storage(P, S, Na, K, Fe), bio mineralization (Ca, Mg, Fe, Si, Sr, Cu, P) which may be beneficial or harmful to humans. Presence of heavy metals like lead, arsenic, Cadmium, Cyanide, Chromium etc.., definitely pose toxic effects when present beyond a limit. Hence, qualitative estimation of inorganic elements in

the plant *Lagenaria* was carried out and were expressed in (Table 2 & 3).

Phytochemical Investigation

Phytochemical evaluation is used to determine the nature of phytoconstituents present in the plant by using suitable chemical tests. It can be done by qualitative analysis using specific reagents. Therefore a complete investigation is required to characterize the phytoconstituents qualitatively and quantitatively.

Preparation of Extracts[21]

Continuous Hot Percolation Method

The coarsely powdered shade dried plant material was successively extracted in a Soxhlet apparatus by continuous hot percolation method with petroleum ether, chloroform, ethyl acetate and ethanol for 48 hrs for each solvent. All the extracts were filtered and concentrated by distillation using Rotary vacuum evaporator and the solvents were recovered. The final solution was evaporated to dryness at room temperature. The extracts were stored in the desiccator and were used for further studies. The Percentage yield of different extracts were given in (Table 4)

Phytochemical Screening [15, 19, 22, 23]

The powdered aerial parts of *Lagenaria siceraria* were subjected to qualitative chemical tests to check the presence of different primary and secondary metabolites. The presence of constituents are quoted in (Table 5)

Fluorescence Analysis [24, 25]

The plant powders and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light and results were tabulated in (Table 6 & 7)

Quantitative Estimation of Phytoconstituents [26-30]

The Phytoconstituents present in different extracts were estimated and the standard calibration graph was represented in (Figures 18-22) and the corresponding concentration was determined.

RESULTS AND DISCUSSION

Pharmacognostical Studies

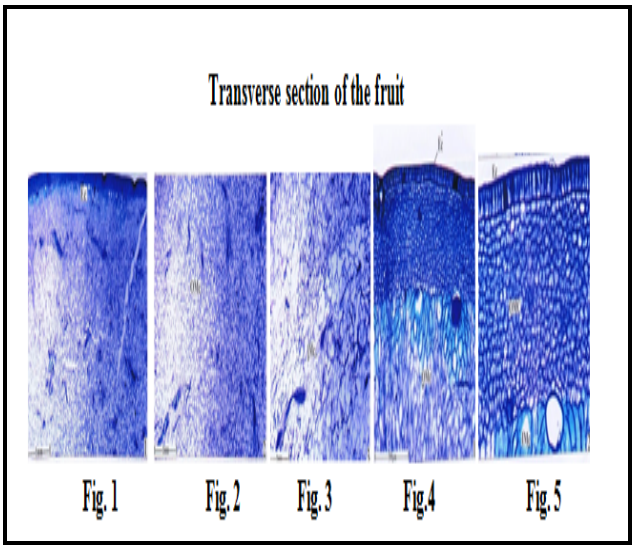
Morphological Characters

Fruit-The fruit was pale green in colour with characteristic odour and bitter taste. It was globular, bottle shaped with smooth surface.

Seed- The seed was dull white or pale brown in colour with characteristic odour and bitter taste. It was simple, oblong in shape, upto 2cm in length, smooth surface with a base emarginated with 2 flat facial ridges.

Microscopical Characters

The fruit is a pepo which develops from an inferior tricapillary ovary with fleshy parietal placentum. The fruit consists of a thin unistratose epidermis or epicarp and wide parenchymatous mesocarp (Fig. 1, 2, 3). Epidermis consists of vertically elongated columnar cells. The cells are fairly thick walled and have prominent cuticle (Fig. 4, 5). The mesocarp portion is differentiated into outer zone and inner zone. The outer mesocarp is a thick zone of small angular compact parenchyma cells with thick walls and lignified. (Fig.5).The inner mesocarp is thicker than the outer zone. The outer boundary of the inner mesocarp includes slightly radially elongated thick walled cells which are slightly lignified. The remaining portion of the inner mesocarp is thin walled, the cells being larger, polygonal and compact (Fig. 4)



The seed is narrowly elliptical and cylindrical in sectioning view (Fig.6). It consists of a thick seed coat (testa) and embryo located within the endospermous tissue. The seed coat is differentiated into outer layer of compact columnar cells with thick lignified walls (Fig.6, 7). The palisade zone of outer seed coat is 250 µm thick. The inner seed coat is multilayered comprising of compact circular parenchyma cells. Vascular strands are seen in the inner seed coat. The vascular bundle is collateral with conical mass of xylem circular mass of phloem (Fig.8).Embryo is located within a small chamber surrounded by densely stained endosperm tissue (Fig.8).Thick and prominent vascular strands are seen on the upper end (Fig.9) and the lower end (Fig.10) of the seed coat. The vascular strands are bicollateral with central mass of xylem and phloem units on both ends.

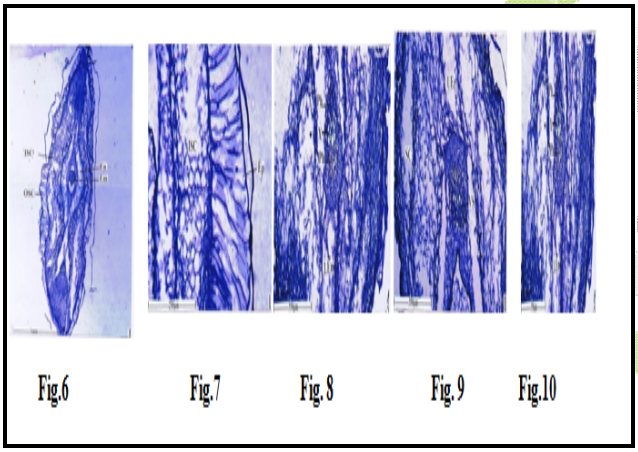


Fig.6 Fig.7 Fig.8 Fig.9 Fig.10

Powder Microscopy

1. Epicarp: (epidermis) - Thin peelings of the fruit seen in surface view show angular, compact, lignified and thick walled sclerenchyma cells. Stomata are frequently seen in the epidermis. The stomata are circular with prominent elliptical pore and the guard cells are 15 µm in diameter. (Fig.11).

2. Large, spherical reddish oleo-resin bodies are seen scattered in large numbers in the powder (Fig.12).

3. Pericarp of the fruit is also seen in sectional view. The sectional segments exhibit the outer sclerotic epicarp where the cells are vertically rectangular with wide lumen (Fig.12). Inner to the epicarp a wide zone of polygonal thick walled and lignified outer sclerenchyma zone are seen (Fig.13). At the inner border of sclerenchyma zone, isolated wide circular annular sclereids are observed (Fig. 13).The

inner mesocarp consists of heterogenous cell type. These are elongated thick walled, densely pitted sclereids, thick walled parenchyma cells and xylem elements. All these cells have thick lignified walls (Fig.14).

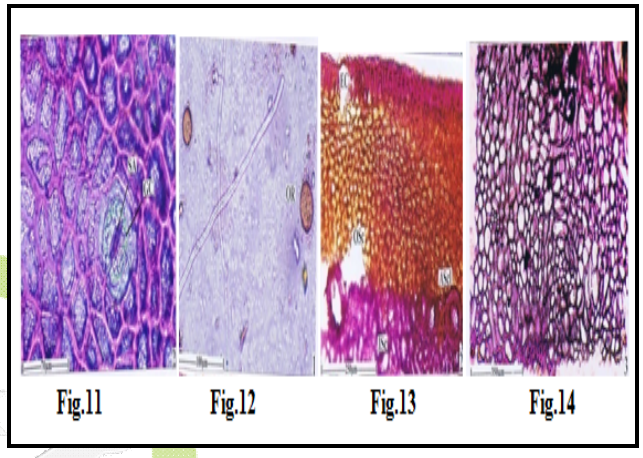


Fig.11 Fig.12 Fig.13 Fig.14

Seed consists of

1. Clusters of trichomes are found scattered in the powder. Each cluster has a thick basal cell from which originates thin long fibrillar trichomes (Fig 15).

2. There are long, cylindrical sclereids of irregular shape and size (Fig 16). The sclereids have thick secondary walls with dense pits (Fig.17). The sclereids are 450µm long and 60µ wide.

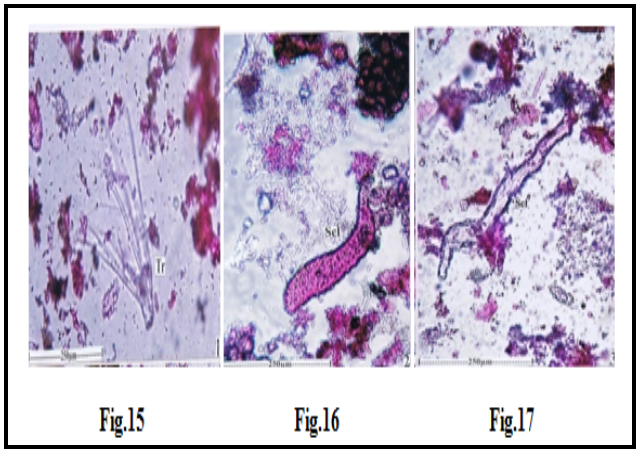


Fig.15 Fig.16 Fig.17

Physiochemical Parameters

Table 1: Physico-chemical constants of the aerial parts of *lagenaria siceraria (mol.)* standley

S.No	Parameters	Percentage(%W/W)
I	**Ash Values**	
1.	Total Ash	10.2±0.08
2.	Acid insoluble Ash	3.8±0.03
3.	Water soluble Ash	5.3±0.32
4.	Sulphated Ash	12.4±0.06
II	**Extractive values**	
1.	Water soluble extractive	10.5±0.13
2.	Ethanol soluble extractive	9.5±0.15
3.	Ether soluble (non-volatile) extractive	4.44±0.02
4.	Ether soluble (volatile) extractive	3.89±0.03
III	**Loss on Drying**	3.67±0.3
IV	**Crude Fibre Content**	14.67±1.2
V	**Foaming Index**	<100
VI	**Swelling Index**	3ml/gm

Values are expressed as a mean ± SD

The swelling index was found to be 3ml/gm and the crude fibre content was found to be 14.67%w/w.

Table 2: Qualitative analysis of inorganic elements

S.No	Inorganic elements	Observation
1	Calcium	+
2	Magnesium	+
3	Sodium	+
4	Potassium	+
5	Iron	+
6	Sulphate	_
7	Phosphate	_
8	Chloride	_
9	Nitrate	_
10	Aluminium	_
11	Arsenic	+
12	Borate	_
13	Copper	+
14	Lead	-
15	Mercury	-
16	Manganese	+
17	Silver	-

Note: +ve indicates presence, -ve indicates absence

Table 3: Qualitative analysis of heavy metals

Plant	Specification	Result
***Lagenaria siceraria* (Mol.) Standley**	Less than 20ppm	Complies

Phytochemical Studies

Table 4: Percentage yield of successive solvent extracts of the aerial parts of *lagenaria siceraria (mol.)* standley

S. No	Extract	Method of extraction	Physical nature	Colour	Yield % W/W
1.	Petroleum ether	Soxhlet Extraction	Sticky	Blackish green	6.2
2.	Chloroform		Sticky	Blackish green	5.3
3.	Ethyl acetate		Semisolid	Dark green	8
4.	Ethanol		Semisolid	Brownish Green	10.2

The Percentage yield of ethanolic extract was high with 10.2%w/w followed by ethyl acetate extract with 8%w/w, pet. ether extract 6.2%w/w and chloroform extract 5.3%w/w.

Table 5: Qualitative phytochemical analysis of the powder and extracts of the aerial parts of *lagenaria siceraria (mol.)* standley

S.No	Chemical Constituents	Powder Drug	Pet. Ether	$CHCl_3$	Ethyl acetate	Ethanol
1	Carbohydrates	+	+	+	+	+
2	Alkaloids	-	-	-	+	+
3	Steroids	+	+	+	+	+
4	Glycosides	+	+	-	+	+
5	Saponins	+	-	-	-	+
6	Flavanoid	+	-	-	+	+
7	Tannins	+	-	-	-	+
8	Phenolic Compound	+	-	+	-	-
9	Proteins	+	-	-	-	+
10	Amino acids	+	-	-	+	+
11	Gums and Mucilage	+	-	-	-	+
12	Terpenoids	+	+	+	+	+
13	Resins	-	-	-	-	-
14	Chlorogenic acid	-	-	-	-	-
15	Fats and Oils	-	-	-	-	-

Note: +ve indicates presence, -ve indicates absence

Most of the secondary metabolites are present in ethanolic extract followed by ethyl acetate, petroleum ether and chloroform extracts

Table 6: Fluorescence analysis of the powder of aerial parts of *lagenaria siceraria (mol.)* standley

S.No	Treatment	Day Light	Short UV (254 nm)	Long UV(366 nm)
1.	Powder	Green	Green	Blackish green
2.	Powder+ Water	Brown	Light green	Greenish brown
3.	Powder + 1 N Hcl	Brown	Green	Brown
4.	Powder+1 N H_2SO_4	Yellowish brown	Green	Light green
5.	Powder + 1N HNO_3	Pale brown	Green	Green
6.	Powder + Acetic acid	Brown	Light green	Light green
7.	Powder + 1N NaOH	Reddish brown	Reddish brown	Brown
8.	Powder + 1N Alc. NaOH	Yellowish brown	Light green	Green
9.	Powder + 1N KOH	Pale brown	Yellow	Pale green
10.	Powder + 1N Alc.KOH	Brownish yellow	Green	Dark green
11.	Powder + Ammonia	Yellowish brown	Green	Greenish yellow
12.	Powder + Iodine	Blackish brown	Green	Blackish brown
13.	Powder + $FeCl_3$	Brownish black	Black	Black
14.	Powder + Ethanol	Greenish brown	Green	Fluorescence green

Table 7: Fluorescence analysis of various extracts of the aerial parts of *lagenaria siceraria (*Mol*.)* standley

Sr No	Extracts	Daylight	Short 254nm	Long 365nm
1	Petroleum Ether	Dark Green	Green	Green
2	Chloroform	Light Brown	Light brown	Brown
3	Ethyl acetate	Brown	Dark Brown	Dark Brown
4	Ethanol	Brown	Dark Brown	Brownish Black

Quantitative Estimation of Phytoconstituents in Various Extracts of *Lagenaria Siceraria* (mol.) Standley

The phytoconstituents like Phenols, Alkaloids, Flavanoids, Total sugars and Tannins were estimated quantitatively by UV absorption techniques

Total Alkaloid content [26]

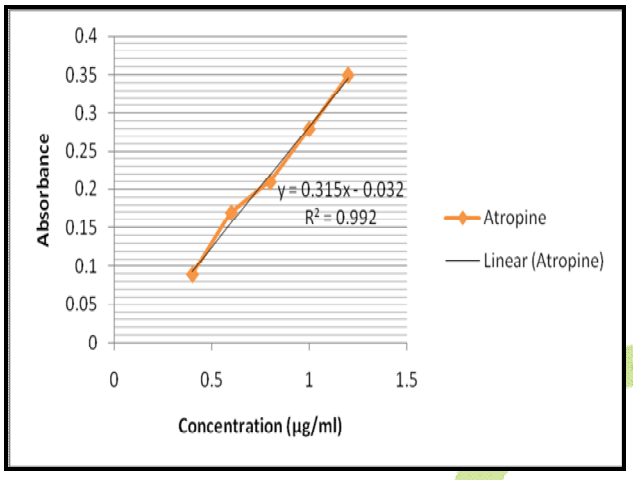


Figure 18: Calibration curve of standard Atropine sulphate

From the calibration curve, the concentration of alkaloid in ethyl acetate and ethanol extracts was found to be 0.09 and 0.97 µg/ml respectively.

Total Flavanoid content [27]

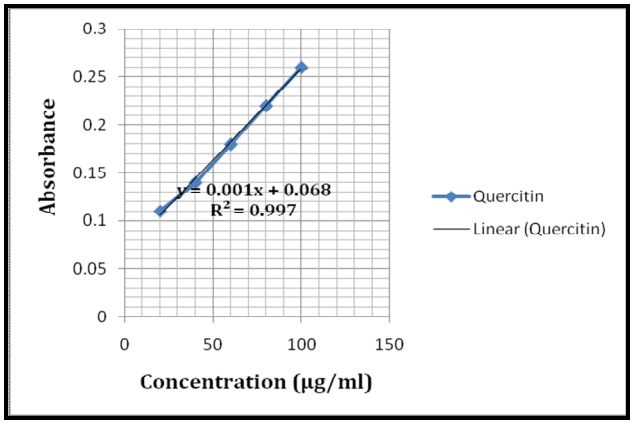


Figure 19: Calibration curve of standard Quercitin

From the calibration curve, the concentration of alkaloid in ethyl acetate and ethanol extracts the concentration of phenol was found to be 2.98 and 172 µg/ml respectively.

Total Sugar content [28]

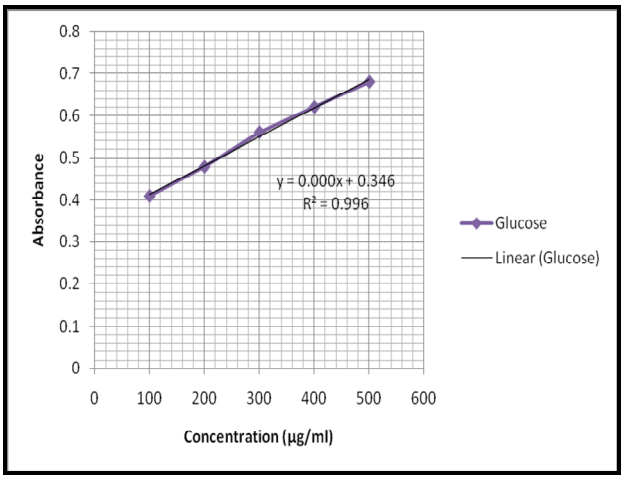


Figure 20: Calibration curve of standard Glucose

From the calibration curve, the concentration of total sugar in the petroleum ether, chloroform, ethyl acetate and ethanol extracts showed absorbance in the standard range and the concentrations were found to be **291.03, 94.48, 438.39 and 495.73 µg/ml** respectively.

Total Tannin content [29]

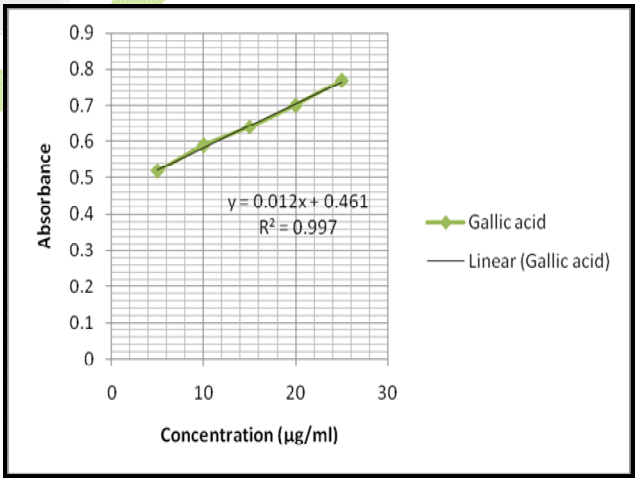


Figure 21: Calibration curve of standard Gallic acid

From the calibration curve, the concentration of total tannin in ethanol extract showed absorbance of 0.53 and the corresponding concentration was found to be **24.85 µg/ml.**

Total Phenolic content [30]

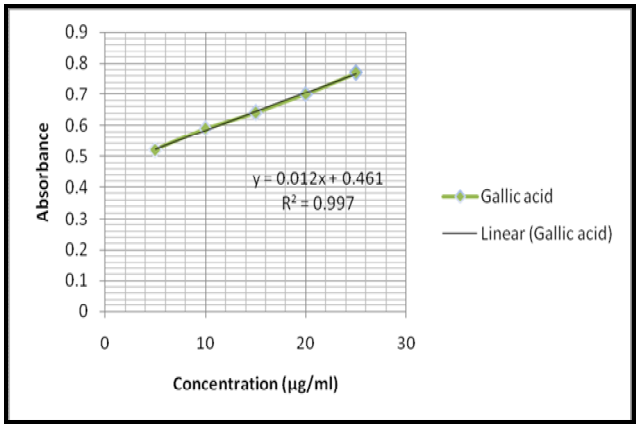


Figure 22: Calibration curve of standard Gallic acid

From the calibration curve, the concentration of total phenolic content in chloroform extract showed the absorbance of 0.13 and the corresponding concentration calculated was **1.67 µg/ml.**

CONCLUSION

From the above mentioned results, it is observed that the plant *Lagenaria siceraria* (Mol.) Standley is found with varied microscopic features and found to have a number of active constituents which have been previously proved to be effective in treating various diseases. Hence the data obtained from this study may provide valuable information for identification as well as predicting the possible pharmacological activities of the plant.

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