



Phytochemical Screening And Estimation Of Total Phenolics And Total Flavonoid Content Of *Lagenaria Siceraria* Fruit

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Abstract

Lagenaria siceraria (Molina) Standley (Family: Cucurbitaceae) is commonly known as Bottle gourd, an excellent fruit in the nature having a composition of all the essential constituents that are required for normal and good health of humans. The aim of present study was to investigate the phyto-constituents present within the various extract of *Lagenaria siceraria* fruit and to estimate the total phenolic and total flavonoid contents. The amount of total phenols, were analyzed using a spectrophotometric technique, based on Folin-ciocalteu reagent. Gallic acid was used as standard compound and the total phenols were expressed as mg/g gallic acid equivalents (Standard curve equation: $y = 0.009x + 0.0092$ $R^2 = 0.9982$). The Rutin was used as standard compound and the total flavonoids were expressed as mg/g rutin equivalents (Standard curve equation: $y = 0.0061x + 0.0085$ $R^2 = 0.9986$).

Keywords: *Lagenaria siceraria*, phytoconstituents, phenols, flavonoids.

Introduction:

Lagenaria siceraria (Molina) standley (family *Cucurbitaceae*) commonly known as lauki (Hindi) and bottle gourd (English) is a medicinal plant¹. The plant is widely available throughout India. It is a climbing or trailing herb, with bottle- or dumb-bell shaped fruits. Both its aerial part and fruit are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island, etc. for its cardioprotective, general tonic and diuretic properties². Further, the antidiabetic³, antihyperlipidemic⁴, antihepatotoxic, analgesic⁵, CNS activity⁶, hypertension⁷, anticancer⁸, CNS depressant⁹, antioxidant¹⁰, antiinflammatory, antihyperglycemic, immunomodulatory and cardioprotective¹¹ activities of its fruit extract have been evaluated. A novel protein, lagenin, has also been isolated from its seeds and it possesses antitumor, immunoprotective and antiproliferative properties¹².

Although extensive studies have been carried out on its fruits and seeds, the pharmacology of the aerial parts of *L. siceraria* has not been studied yet. In many countries, this plant has been used traditionally as a single treatment for diabetes mellitus¹³. They also cure pain, ulcers, fever, and are used for pectoral cough, asthma and other bronchial disorders¹⁴. The fruits are edible and considered as a good source of vitamin C, β -carotene, vitamin B-complex, pectin and also contain highest choline level – a lipotropic factor. Modern phytochemical screening methods showed the presence of triterpenoid cucurbitacins B,D,G,H¹³ fucosterol, campesterol and flavone C-glycosides¹⁵. *L. siceraria* seeds are used in migraine type headache and pain and are reported to contain saponins, essential fixed oils, vitamins¹⁶.

Materials and Method

Collection and Authentication of plant material

Lagenaria siceraria fruits were collected from local market of Jaipur (Rajasthan, India). Before the processing of experimental work the plant part was authenticated by Mr. Vinod Sharma, Herbarium Head, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (viz. no. RUBL 21097) of the plant material was preserved in the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and one set was preserved in our laboratory for future reference.

Chemicals and Reagents

Folin-Ciocalteu's phenol reagent, Gallic acid, Rutin, $AlCl_3$, Ethanol, Conc Sulphuric acid, Hydrochloric acid, α -naphthol, Picric acid, Potassium iodide, Ethyl acetate, Sodium Nitroprusside, Glacial acetic acid, Ninhydrin, Sodium nitrate, Sodium hydroxide, Chloroform, Sodium chloride, Sodium lauryl sulphate, Pyridine, Ferric chloride, Calcium chloride, Copper sulphate, Sodium carbonate, Ammonia and Sodium nitroprusside were procured from the standard companies.

Preparation of extracts

Lagenaria siceraria ethanolic extract

The fresh and semi-ripped fruits were sliced using a home slicer and the obtained slice were shade dried, followed by powdering manually using mortar and pestle. The dried powdered drug passed through a 20 mesh sieve. The dried, powdered plant material was extracted with ethanol, petroleum ether, acetone, ethyl acetate at 60°C for 24 hours using an Soxhlet apparatus. The collected mass was subjected to drying to evaporate the excess of solvent. The collected brownish colour material was termed as extract of *Lagenaria siceraria* fruit.

Phytochemical Screening

The present phytoconstituents were analysed using freshly prepared reagents. All the used glasswares were thoroughly cleaned before the experimental works. Various extracts of *Lagenaria siceraria* were analysed for the presence of carbohydrates, alkaloids, glycosides, phenols, flavonoids, tannins, fats, fixed oils, etc.¹⁷⁻¹⁸.

Estimation of total phenolic content

The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi¹⁹. From the stock solution (1 mg/ml) of the various extracts of *Lagenaria siceraria*, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin Ciocalteu's reagent. After 5 min, 4 ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with distilled water. The absorbance was recorded at 765 nm, after 30 min. The total phenolic content is expressed as milligrams of gallic acid equivalent (GAE) to per gram of dry LS Extract.

Estimation of total flavonoid content

The total flavonoid content was determined with aluminium chloride (AlCl₃) according to the known method of Zhishen²⁰, using Rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml NaNO₂ (5%) and incubated for 5 min at 25°C. Later 0.03 ml AlCl₃ (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The total flavonoid content is expressed as milligrams of rutin equivalent (RE) to per gram of dry LSE extract.

Results

Phytochemical screening

Phytochemicals	TEST	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Ethanol
Alkaloids	General Test	+	+	+	+	+
Carbohydrates (Monosaccharides, Oligosaccharides & Polysaccharides)	General Test	+	+	+	+	+
	Monosaccharides	+	+	+	+	+
	Pentose Sugars	+	+	+	+	+
	Hexose Sugars	+	+	+	+	+
	Non Reducing Polysaccharides	-	-	-	-	-
	Gums	-	-	-	-	-
	Mucilage	+	+	+	+	+
Proteins & Amino acids	Proteins	-	-	-	-	-
	Amino Acids	-	-	-	-	-
Glycosides	General Test	+	+	+	+	+
	Cardiac Glycosides	+	+	+	+	+
	Anthraquinone Glycosides	-	-	-	-	-
	Saponin Glycosides	+	+	+	+	+
	Cyanogenetic Glycosides	-	-	-	-	-
Flavonoids		+	+	+	+	+
Tannins & Phenolic Compounds	General Test	+	+	+	+	+

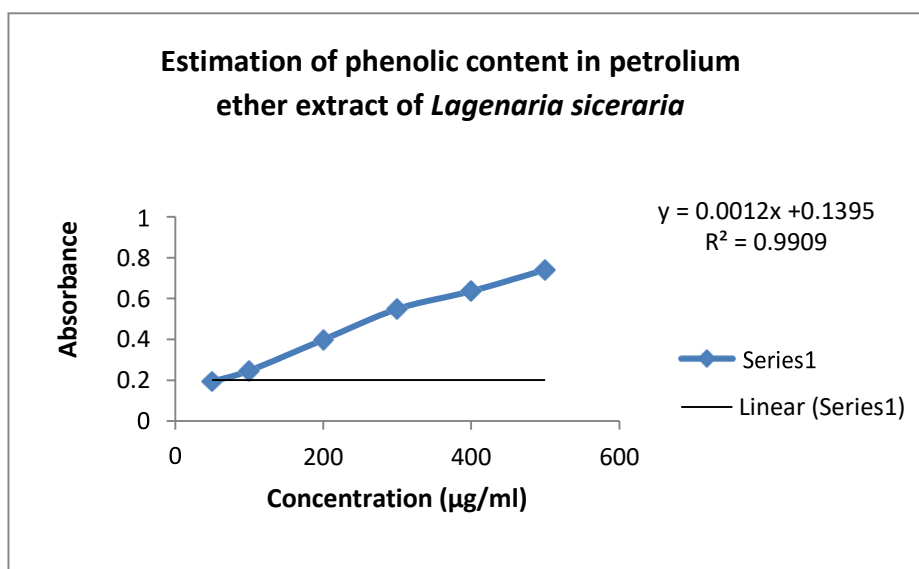
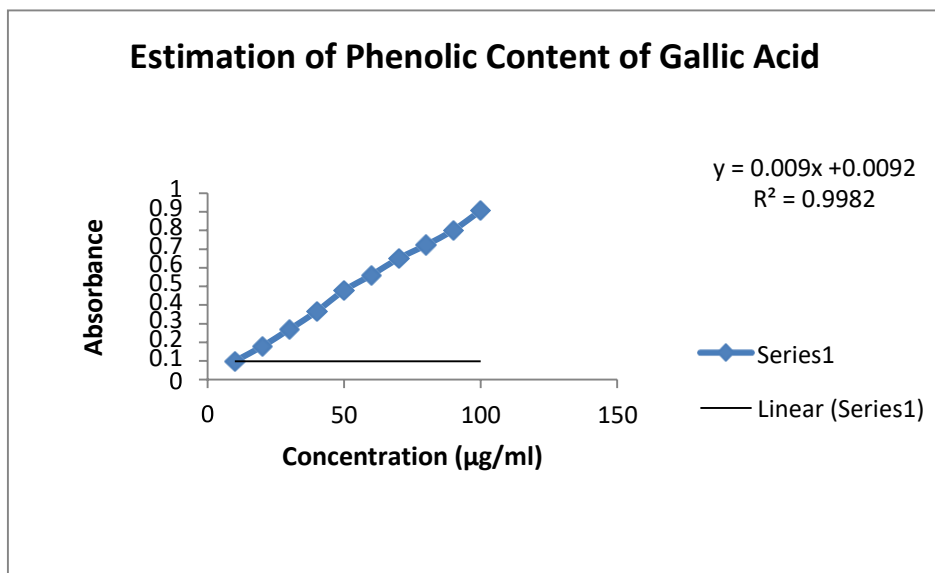
Steroids		+	+	+	+	+
Volatile Oils		-	-	-	-	-
Fats & Oils		-	-	-	-	-

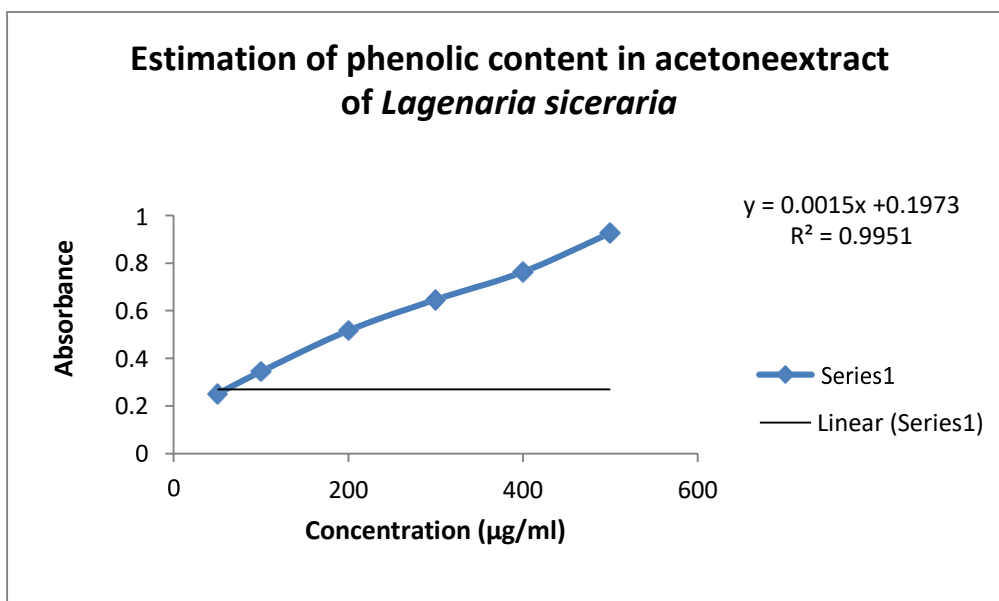
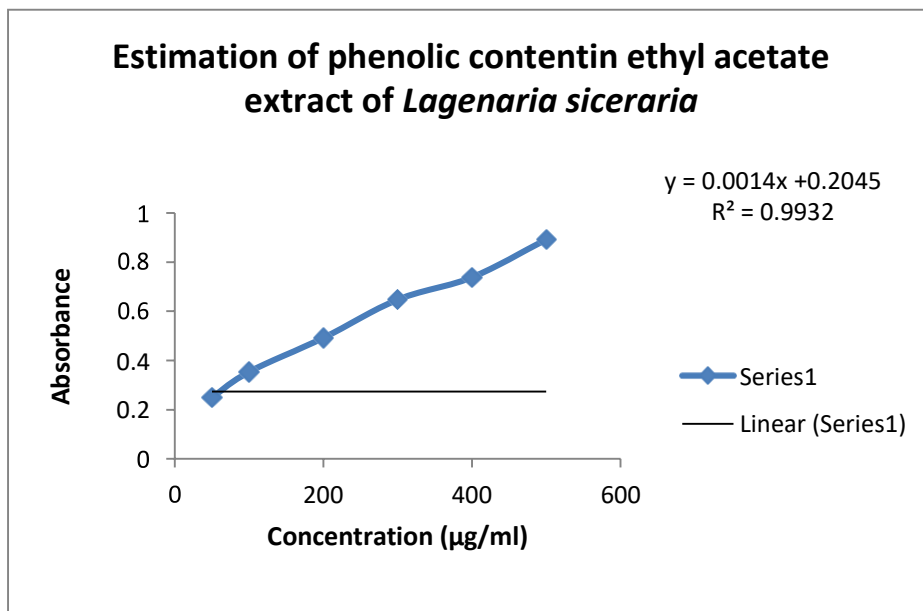
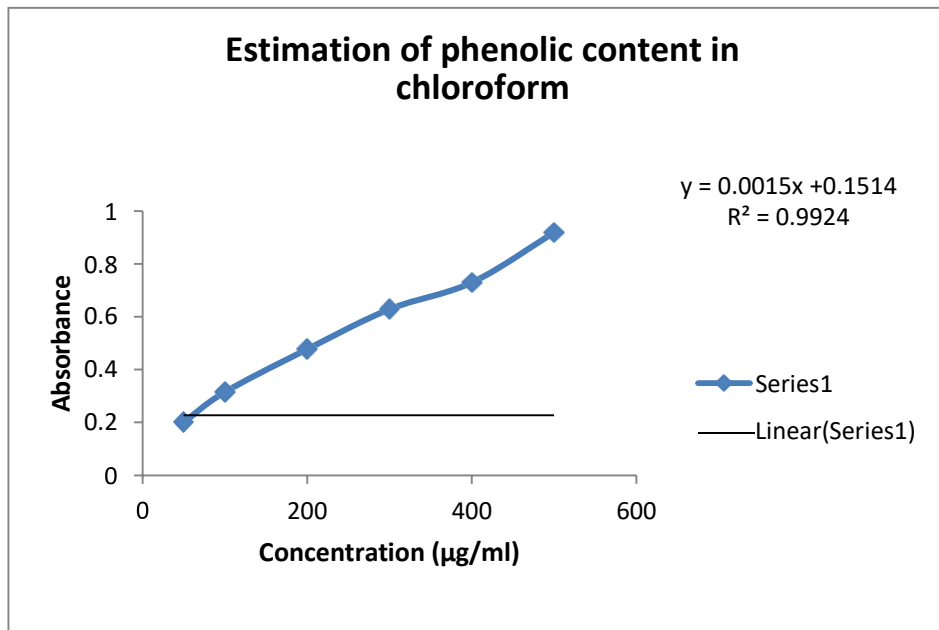
Total Phenolic content

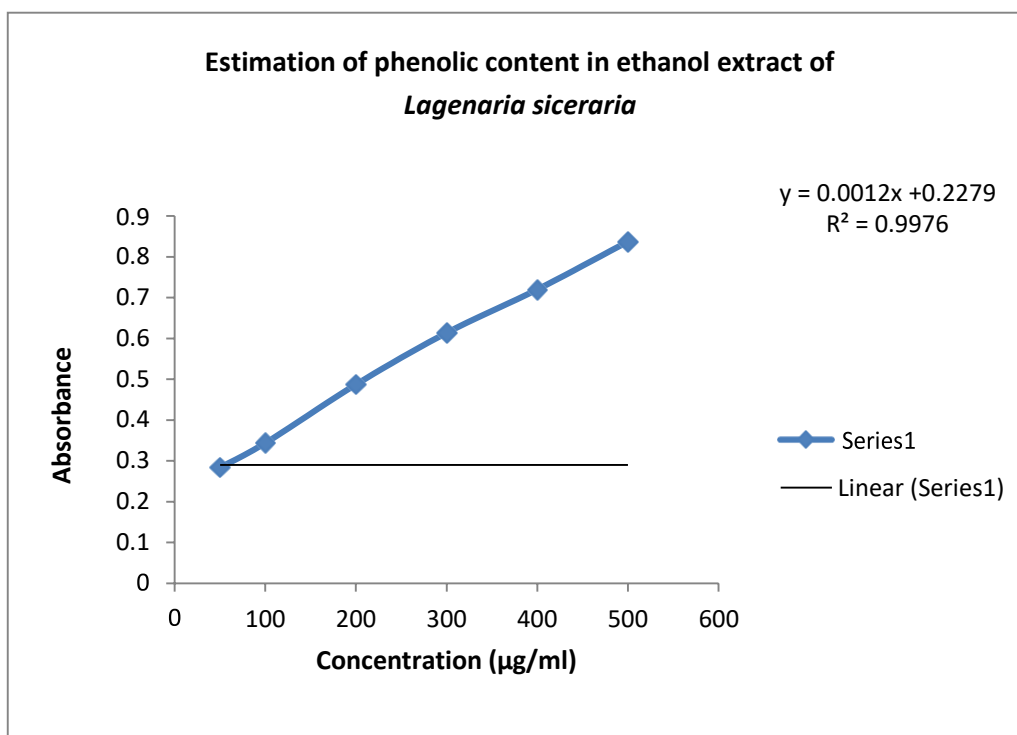
Data presented in (Mean ± SD), n=3

S.No	Concentration (µg/ml)	Absorbance				
		<i>Petroleum ether</i>	<i>Chloroform</i>	<i>Ethyl acetate</i>	<i>Acetone</i>	<i>Ethanol</i>
1	50	0.1942±0.01	0.2013±0.02	0.2495±0.01	0.2504±0.01	0.2833±0.01
2	100	0.2471±0.02	0.3154±0.01	0.3534±0.01	0.3445±0.01	0.3433±0.02
3	200	0.3985±0.01	0.4776±0.01	0.4918±0.01	0.5167±0.02	0.4867±0.01
4	300	0.5491±0.01	0.6289±0.02	0.6474±0.01	0.6467±0.01	0.6133±0.01
5	400	0.6364±0.02	0.7299±0.01	0.7383±0.01	0.7633±0.01	0.7194±0.01
6	500	0.7413±0.01	0.9189±0.01	0.8918±0.02	0.9267±0.02	0.8367±0.02

Data presented in (Mean ± SD), n=3







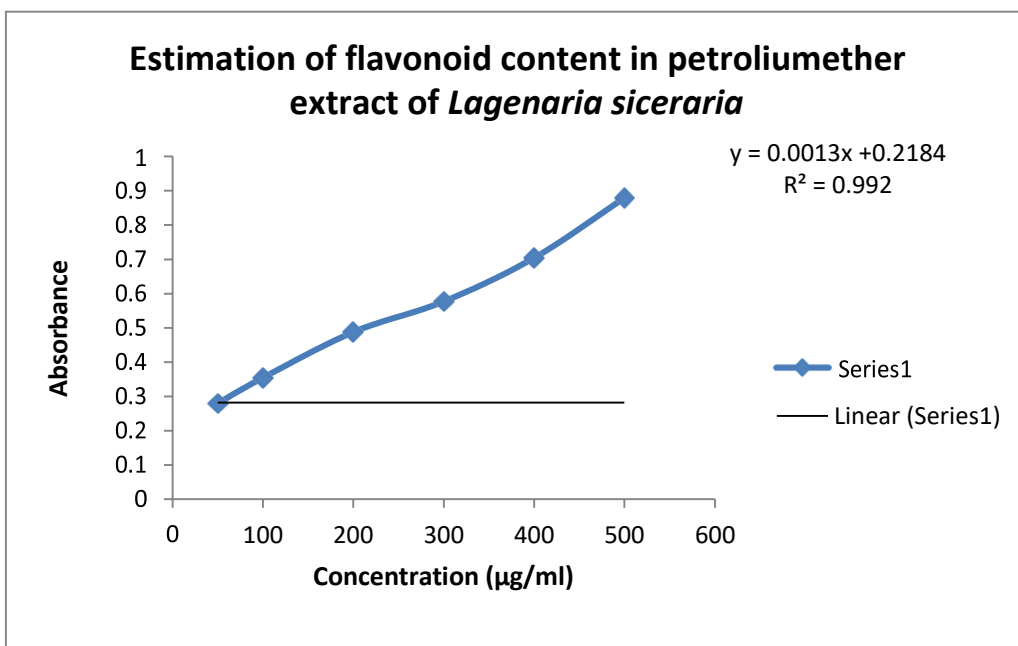
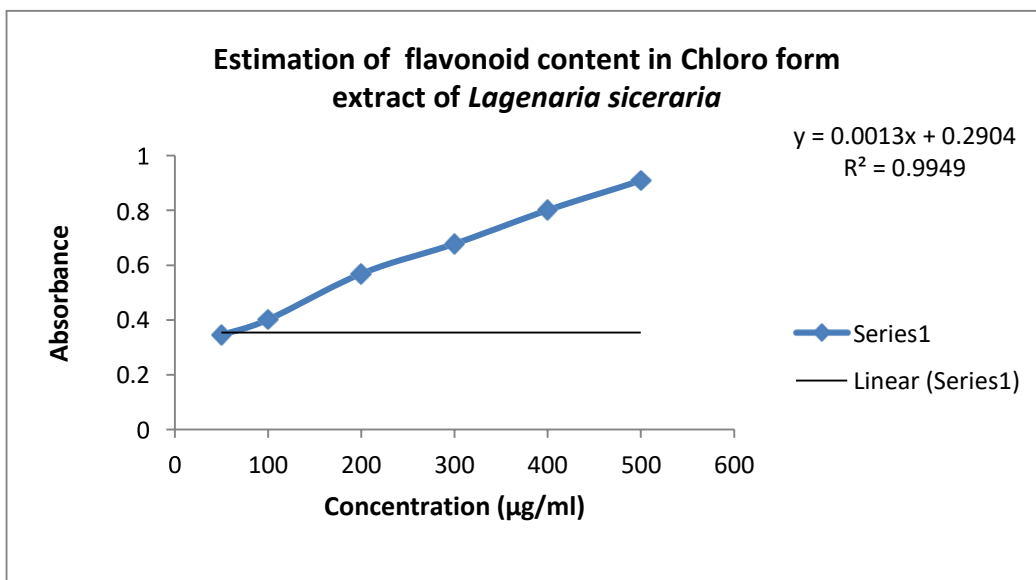
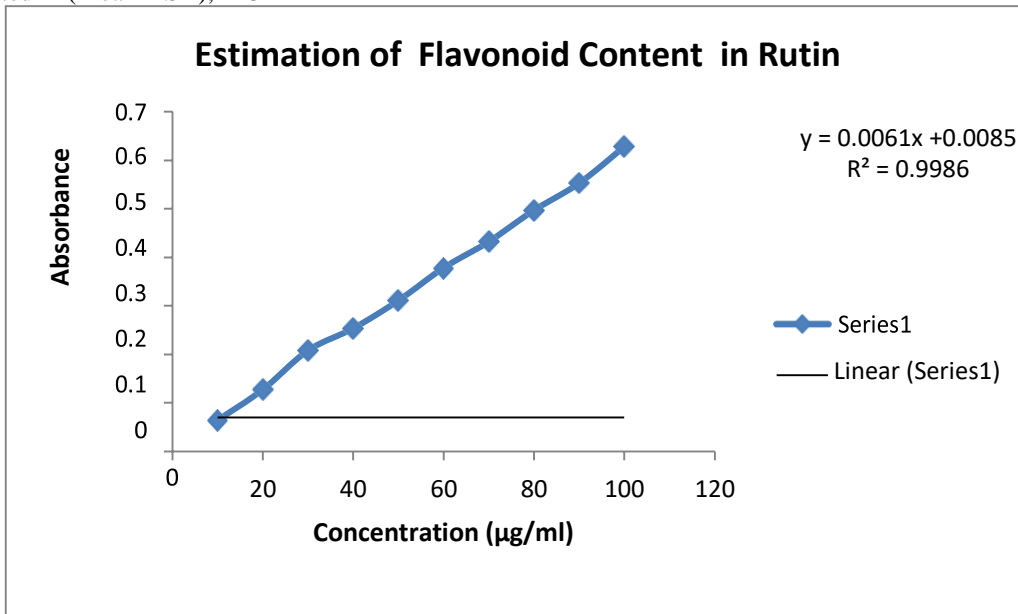
Total Flavonoid Content

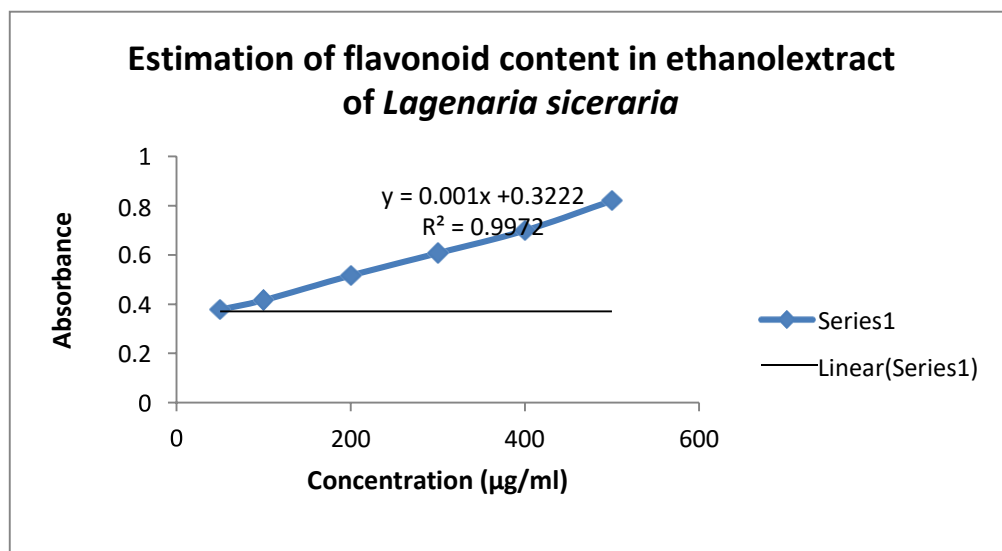
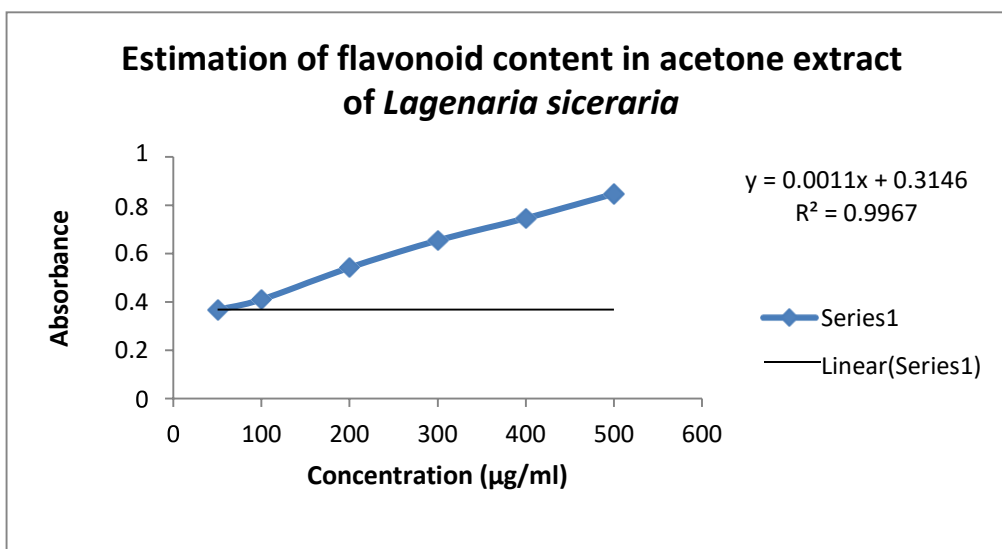
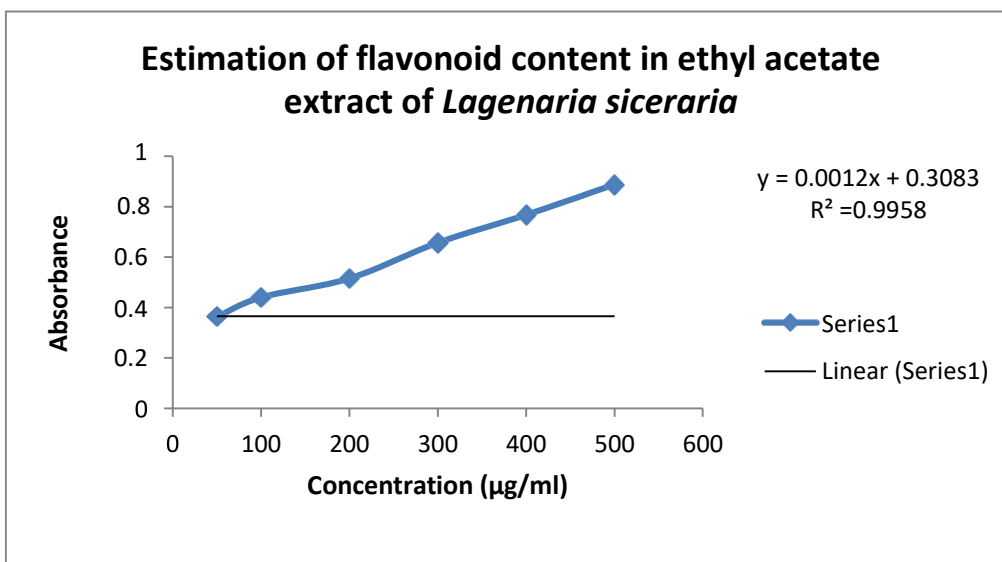
Data presented in (Mean ± SD), n=3

<i>Total flavonoid content of standard (Rutin)</i>		
S.No	Concentration (µg/ml)	Absorbance of STD (Rutin)
1.	10	0.0643±0.021
2.	20	0.1274±0.024
3.	30	0.2081±0.013
4.	40	0.2537±0.004
5.	50	0.3111±0.018
6.	60	0.3784±0.014
7.	70	0.4324±0.009
8.	80	0.4973±0.034
9.	90	0.5532±0.031
10.	100	0.6285±0.014

<i>Total flavonoid content of various extract of Lagenaria siceraria</i>						
S.No	Concentration (µg/ml)	Absorbance				
		<i>Petroleum ether</i>	<i>Chloroform</i>	<i>Ethyl acetate</i>	<i>Acetone</i>	<i>Ethanol</i>
1	50	0.2798 ±0.032	0.3457 ±0.019	0.3641 ±0.031	0.3675 ±0.004	0.3786 ±0.019
2	100	0.3543 ±0.016	0.4017 ±0.043	0.4407 ±0.019	0.4097 ±0.015	0.4167 ±0.018
3	200	0.4876 ±0.13	0.5692 ±0.010	0.5157 ±0.018	0.5421 ±0.020	0.5167 ±0.042
4	300	0.5767 ±0.009	0.6783 ±0.017	0.6568 ±0.015	0.6547 ±0.039	0.6078 ±0.056
5	400	0.7041 ±0.034	0.8011 ±0.029	0.7676 ±0.043	0.7468 ±0.026	0.6997 ±0.015
6	500	0.8791 ±0.028	0.9089 ±0.12	0.8876 ±0.028	0.8479 ±0.037	0.8211 ±0.032

Data presented in (Mean ± SD), n=3





Conclusion

The phytochemical screening of the ethanolic extract of fresh fruits of *Lagenaria siceraria* showed the presence of the following: tannins, alkaloids, saponins, flavonoids, and cardiac glycosides. The results however showed that anthraquinone and cyanogenetic glycosides were absent (Table 2). The results thus showed that the ethanolic extract of fresh fruits of *Lagenaria siceraria* is rich in cardiac glycosides, alkaloids, saponins, tannins, and flavonoids. Alkaloids usually have marked physiological action on animals. Saponins on the other hand are of great pharmaceutical importance Because of their relationship to compounds such as the sexhormones, cortisones, diureticsteroids, vitamin Dand cardiac

glycosides. Tannins like alkaloids are substances which show protein precipitation and are related to the physiological effects of herbal medicines. Flavonoid containing plants have influence on arachidonic acid metabolism, thus could have anti-inflammatory, antiallergic, antithrombotic or vasoprotective effects. Cardiac glycosides are steroidal glycosides which exert a slowing and strengthen effect on the failing heart. The implication of all these is that this plant is of great medicinal importance. The presence of cardiac glycosides in this plant has further confirmed its medicinal use as antihyperlipidemic agent. The presence of flavonoid in this plant is therefore evidenced of its anti-inflammatory properties. Estimation of total flavonoid and phenolic content show that the ethanol extract of the plant possesses antidiabetic activity.

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