



**RESEARCH ARTICLE**

**Phytochemical Screening and Estimation of Total Phenolics and Total Flavonoid  
Content of *Lagenaria Siceraria*, *Praecitrullus Fistulosus* (50:50) Fruit and their  
Mixture**

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**ABSTRACT**

*Cucurbitaceae* family is major source of medicinal agents since ancient time. Various plants parts including fruits of this family have been established for their pharmacological potential. In the series of *Cucurbitaceae* plants, *Praecitrullus fistulosus* and *Lagenaria siceraria* is one of the excellent plants, gifted by the nature having composition of all the essential constituents that are required for normal and good human health. The aim of present study was to investigate the phyto-constituents present within the ethanolic extract of *Praecitrullus fistulosus* 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-ciocalteau reagent. Gallic acid was used as standard compound and the total phenols were expressed as mg/g gallic acid equivalents (Standard curve equation:  $Y = 0.008X + 0.003$ ,  $R^2 = 0.998$ ). The total phenolic content in ethanolic extract of *Lagenaria siceraria*, *Praecitrullus fistulosus* and their mixture was 31.42 mg/g, 25.71 mg/g and 27.71 mg/g respectively. Total flavonoid contents were estimated, using a spectrophotometric technique. The rutin was used as standard compound and the total flavonoids were expressed as mg/g rutin equivalents (Standard curve equation:  $Y = 0.006X + 0.001$ ,  $R^2 = 0.998$ ). The total flavonoid content in ethanolic extract of *Lagenaria siceraria*, *Praecitrullus fistulosus* and their mixture was 26.6 mg/g, 19.8 mg/g and 23.4 mg/g respectively. The maximum phenolic and flavonoid content were found in ethanolic extract of *Lagenaria siceraria* 31.42 and 26.6 mg/g respectively.

**KEYWORDS**

*Praecitrullus Fistulosus*, *Lagenaria Siceraria* Phytoconstituents, Phenols, Flavonoids

**INTRODUCTION**

*Cucurbitaceae* family is commonly known as gourd, melon and pumpkin family. This family is composed of 118 genera and 825 species which are widely distributed in the warmer region of world<sup>1</sup>. Various plants parts including fruits of this family have been established for their pharmacological potential.

In the series of *Cucurbitaceae* plants, *Praecitrullus fistulosus* and *Lagenaria siceraria* is one of the excellent plants, gifted by the nature having composition of all the essential constituents that are required for normal and good human health.

***Lagenaria siceraria***

*Lagenaria siceraria* (Molina) standley (family *Cucurbitaceae*) commonly known as lauki (Hindi) and bottle gourd (English) is a medicinal plant<sup>1</sup>. The plant is widely available

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throughout India. It is a climbing or trailing herb, with bottle- or dumb-bell shaped fruits. Both its aerial parts and fruits are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island, etc. for its cardiogenic, general tonic and diuretic properties<sup>2</sup>. Further, the antidiabetic<sup>3</sup>, antihyperlipidemic<sup>4</sup> antihepatotoxic, analgesic<sup>5</sup>, CNS activity<sup>6</sup>, hypertension<sup>7</sup>, anticancer<sup>8</sup>, CNS depressant<sup>9</sup>, antioxidant<sup>10</sup>, antiinflammatory, antihyperglycemic, immunomodulatory and Cardioprotective<sup>11</sup> 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 properties<sup>12</sup>. 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 mellitus<sup>13</sup>. They also cure pain, ulcers, fever, and are used for pectoral cough, asthma and other bronchial disorders<sup>14</sup>. The fruits are edible and considered as a good source of vitamin C,  $\beta$ -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, H<sup>13</sup> fucosterol, campesterol and flavone C-glycosides<sup>15</sup>.

*L. siceraria* seeds are used in migraine type headache and pain and are reported to contain saponins, essential fixed oils, vitamins<sup>16</sup>.

### ***Praecitrullus fistulosus***

In the series of medicinal plants, *Praecitrullus fistulosus* is one of the excellent plant, gifted by the nature having composition of all the essential constituents that are required for normal and good human health. *Praecitrullus fistulosus* is commonly known as Tendu in Punjabi, Tinda kaaya in Telugu, Kovaikkaai in Tamil and Indian round gourd in English. Leaves are alternate and usually palmately 5-lobed or divided, stipules are absent. Flowers

are actinomorphic and nearly always unisexual. The perianth has a short to prolonged epigenous zone that bears a calyx of 3-6 segments or lobes and 3-6 petals or more frequently a 3-6-lobed sympetalous corolla.

The fruit is a type of berry called a pepo by Gerald Carr. The fruits are approximately spherical, and 5–8 cm in diameter<sup>17</sup>. It is a diffuse annual, creeping or climbing herb with stout stem and rounded fruits of the size of a small turnip, pale or dark green in colour with blackish seeds. *Praecitrullus fistulosus* is cultivated as a vegetable in India, Pakistan and Afghanistan. *Praecitrullus fistulosus* may be a useful source of resistance to whiteflies for the improvement of watermelons<sup>18</sup>.

The seeds of tinda are roasted and consumed in the same way as watermelon or egusi seeds. In India, tinda is used as fodder and in medicine<sup>19</sup>. *Praecitrullus fistulosus* is used for anthelmintic<sup>20</sup> and antioxidant activity<sup>21</sup>. The glucose regulating role of three vegetable of *Cucurbita pepo*, *Cucumis sativus* and *Praecitrullus fistulosus* peels is also study<sup>22</sup>.

## **MATERIALS AND METHOD**

### **Collection and Authentication of plant material**

*Lagenaria siceraria* and *Praecitrullus fistulosus* 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 & 21098) of the plant materials 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<sub>3</sub>, Ethanol, Conc Sulphuric acid, Hydrochloric acid,  $\alpha$ -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**

#### ***Ethanolic Extraction of Lagenaria Siceraria***

The fresh and semi-ripped fruits were sliced using a home slicer and the obtained slice were shade dried, followed by powdering manually using motor and pestle. The dried powdered drug passed through a 20 mesh sieve to remove excessive of mucilaginous hair.

The dried, powdered plant material (60 g) was extracted with 99% ethanol at 60°C for 24 h using a soxhlet apparatus. The collected mass was subjected to drying to evaporate the excess of solvent. The collected brownish colour material was termed as ethanolic extract of *Lagenaria siceraria* fruit.

#### ***Ethanolic Extraction of Praecitrullus Fistulosus***

The fresh and semi-ripped fruits were sliced using a home slicer and the obtained slices were shade dried, followed by powdering manually using motor and pestle. The dried powdered drug passed through a 20 mesh sieve to remove excessive of mucilaginous hair.

The dried, powdered plant material (60 g) was extracted with 99% ethanol at 60°C for 24 h using a soxhlet apparatus. The collected mass was subjected to drying to evaporate the excess of solvent. The collected greenish colour material was termed as ethanolic extract of *Praecitrullus fistulosus* fruit.

#### ***Ethanolic Extraction of Lagenaria Siceraria and Praecitrullus Fistulosus Fruit Soxlation***

The dried, powdered (60 g) of both the plant materials of equal quantity (50:50) was extracted with 99% ethanol of analytical grade at 60°C for 24 hrs using soxhlet apparatus. The collected mass was subjected for drying to evaporate the excess of solvent. The collected

greenish coloured mass was termed as ethanolic extract of mixture of *Lagenaria siceraria* and *Praecitrullus fistulosus* fruit.

### **Phytochemical Screening**

The present phytoconstituents were analysed using freshly prepared reagents. All the used glasswares were thoroughly cleaned before the experimental works. Ethanolic extract of mixture of *Lagenaria siceraria* and *Praecitrullus fistulosus* fruit were analysed for the presence of carbohydrates, alkaloids, glycosides, phenols, flavonoids, tannins, fats, fixed oils, etc<sup>22-23</sup>.

#### ***Estimation of Total Phenolic Content***

The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi<sup>24</sup>. From the stock solution (1 mg/ml) of the LSEE, 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 double 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 *Lagenaria siceraria* and *Praecitrullus fistulosus* fruit extract.

#### ***Estimation of Total Flavonoid Content***

The total flavonoid content was determined with aluminium chloride (AlCl<sub>3</sub>) according to the known method of Zhishen<sup>25</sup> using Rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml NaNO<sub>2</sub> (5%) and incubated for 5 min at 25°C. Later 0.03 ml AlCl<sub>3</sub> (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 *Lagenaria siceraria* and *Praecitrullus fistulosus* fruit extract.

## RESULTS AND DISCUSSION

### Phytochemical Screening of *Lagenaria siceraria*, *Praecitrullus fistulosus* Fruits and their Mixture (50:50 w/w)

The Preliminary phytochemical investigation revealed the presence of various phytoconstituents in ethanolic extracts of *Lagenaria siceraria*, *Praecitrullus fistulosus* fruits and their mixture (50:50 w/w). The results of phytochemical screening were found as given in table below

The ethanolic extracts of *Lagenaria siceraria*, *Praecitrullus fistulosus* fruits and their mixture (50:50 w/w) were analysed for the presence of present phytoconstituents, although the plant material was collected from pollutant free area and hygienic. The phytochemical screening was performed to determine the presence of carbohydrates, alkaloids, glycosides, phenols, fatty material and nitrogenous compounds i.e. proteins and amino acids. The result revealed that all the extracts were rich in terms of alkaloids, carbohydrates, mucilage except non reducing polysaccharides moiety.

The cardiac and saponin glycosides were present while the anthraquinone and cynogenetin glycosides were absent. Yet the extracts were rich with steroidal moiety, the fat and oil related phytochemical test does not confirm their presence. The intensity of phenolic and flavonoid determining inference were high. The amino acids and proteins were absent in all the extracts i.e. *Lagenaria siceraria*, *Praecitrullus fistulosus* fruits and their mixture (50:50 w/w).

### Yield of Extracts

Table 1: % yield of ethanolic extracts of *Lagenaria siceraria*, *Praecitrullus fistulosus* fruits and their mixture (50:50 w/w)

S.No	Plants (ethanolic extract)	Yield of Extract (g)	% yield
1.	<i>Lagenaria siceraria</i>	12	20%
2.	<i>Praecitrullus fistulosus</i>	7.8	13%
3.	Mixture (50:50 w/w)	9.42	15.7%

Table 2: Result of Preliminary phytochemical screening of ethanolic extract of *Lagenaria siceraria*, *Praecitrullus fistulosus* fruits and their mixture (50:50 w/w)

Phytochemicals		<i>Lagenaria siceraria</i>	<i>Praecitrullus fistulosus</i>	Mixture (50:50 w/w)
Alkaloids	General Test	+	+	+
	General Test	+	+	+
Carbohydrates (Monosaccharides, Oligosaccharides and Polysaccharides)	Monosaccharides	+	+	+
	Pentose Sugars	+	+	+
	Hexose Sugars	+	+	+
	Non Reducing Polysaccharides	-	-	-
	Gums	-	-	-
	Mucilage	+	+	+
Proteins and Amino acids	Proteins	-	-	-
	Amino Acids	-	-	-



<b>Glycosides</b>	General Test	+	+	+
	Cardiac Glycosides	+	+	+
	Anthraquinone Glycosides	-	-	-
	Saponin Glycosides	+	+	+
	Cyanogenetic Glycosides	-	-	-
<b>Flavonoids</b>		+	+	+
<b>Tannis and Phenolic Compounds</b>	General Test	+	+	+
<b>Steroids</b>		+	+	+
<b>Volatile Oils</b>		-	-	-
<b>Fats and Oils</b>		-	-	-

Note: + sign indicate the presence; - sign indicate the absence

### Total Phenolic Content

The ethanolic extracts of *Lagenaria siceraria*, *Paecitrullus fistulosus* fruits and their mixture contained high content of phenols. The amount of phenols varied in all the extracts. *Lagenaria siceraria* contained high phenolic content as compared to *Paecitrullus fistulosus* and their mixture.

Table 3: Total Phenolic Content of Standard (Gallic Acid)

S.No	Concentration (µg/ml)	Absorbance of STD (Gallic acid)
1.	10	0.0827±0.01
2.	20	0.1759±0.019
3.	30	0.2617±0.033
4.	40	0.3231±0.023
5.	50	0.4430±0.042
6.	60	0.531±0.058
7.	70	0.5964±0.074
8.	80	0.671±0.088
9.	90	0.7593±0.077
10.	100	0.8583±0.053

Data presented in (Mean ± SD), n=3

Table 4: Total Phenolic Content of Ethanolic Extract of *Lagenaria Siceraria*, *Praecitrullus Fistulosus* Fruits and Their Mixture

S. No	Conc <sup>n</sup> (µg/ml)	Absorbance		
		<i>Lagenaria siceraria</i>	<i>Praecitrullus fistulosus</i>	Mixture
1	50	0.2646 ±0.013	0.2284±0.031	0.2412±0.021
2	100	0.3565 ±0.014	0.323±0.029	0.3545±0.009
3	200	0.4761 ±0.01	0.4697±0.023	0.4959±0.007
4	300	0.6098 ±0.03	0.6215±0.034	0.6385±0.009
5	400	0.7144 ±0.068	0.7307±0.059	0.7584±0.01
6	500	0.8293 ±0.062	0.8489±0.071	0.8992±0.18

Data presented in (Mean ± SD), n=3

*Lagenaria siceraria* contained 31.42 mg/g gallic acid equivalent, *Paecitrullus fistulosus* contained 25.71 mg/g gallic acid equivalent while their mixture contained 27.71 mg/g gallic acid equivalent.

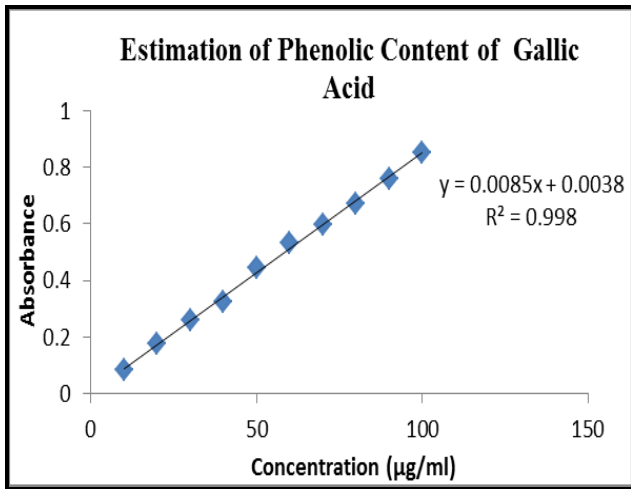


Figure 1: Standard curve of gallic acid

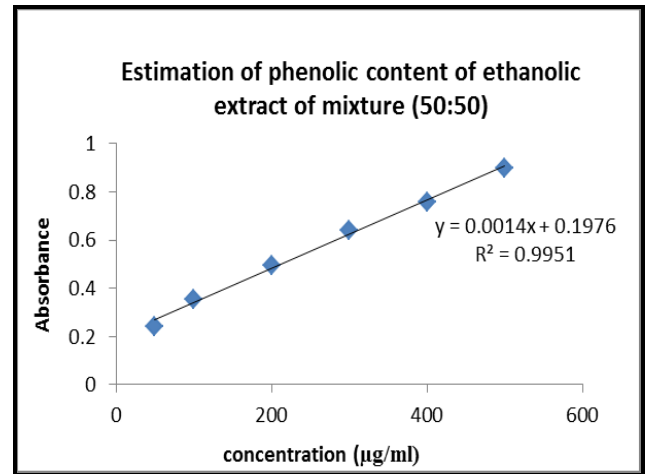


Figure 4: Total phenolic content in ethanolic extract of Mixture (mg/g Gallic acid equivalent)

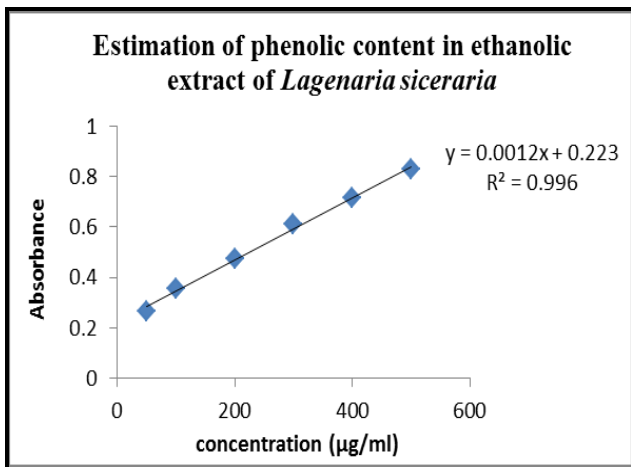


Figure 2: Total phenolic content in ethanolic extract of *Lagenaria siceraria* (mg/g Gallic acid equivalent)

Table 5: Total phenolic content in ethanolic extract of *Lagenaria siceraria*, *Paecitrullus fistulosus* and Mixture (50:50 w/w)

S.No	Ethanolic extract	Total phenolic content (mg /g gallic acid equivalent)
1	<i>Lagenaria siceraria</i>	31.42
2	<i>Paecitrullus fistulosus</i>	25.71
3	Mixture (50:50 w/w)	27.71

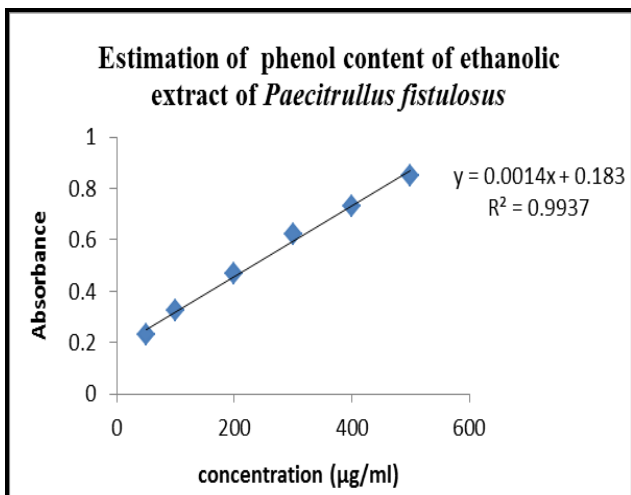


Figure 3: Total phenolic content in ethanolic extract of *Paecitrullus fistulosus* (mg/g Gallic acid equivalent)

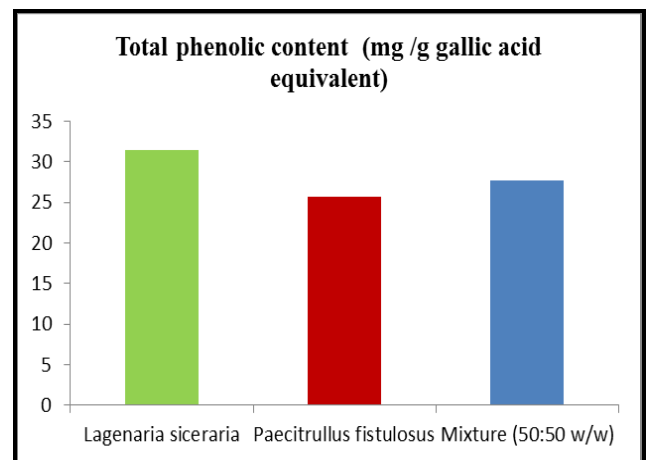


Figure 5: Total phenolic content (mg /g gallic acid equivalent)

### Total Flavonoid Content

The ethanolic extracts of *Lagenaria siceraria*, *Paecitrullus fistulosus* fruits and their mixture contained high flavonoid content. The amount of flavonoid varied in all the extracts. *Lagenaria siceraria* contained high flavonoid content as compared to *Paecitrullus fistulosus* and their mixture. *Lagenaria siceraria* contained 26.6 mg/g Rutin equivalent, *Paecitrullus fistulosus* contained 19.8 mg/g Rutin equivalent while their mixture contained 23.4 mg/g Rutin equivalent.

Table 6: Total flavonoid content of standard (Rutin)

S.No	Concentration (µg/ml)	Absorbance of STD (Rutin)
1.	10	0.0561±0.007
2.	20	0.1217±0.005
3.	30	0.1962±0.021
4.	40	0.2493±0.007
5.	50	0.3199±0.02
6.	60	0.3701±0.01
7.	70	0.4371±0.007
8.	80	0.4921±0.011
9.	90	0.5465±0.014
10.	100	0.6276±0.021

Data presented in (Mean ± SD), n=3

Table 7: Total flavonoid content of ethanolic extract of *Lagenaria siceraria*, *Praecitrullus fistulosus* fruits and their mixture

S. No	Concentration (µg/ml)	Absorbance		
		<i>Lagenaria siceraria</i>	<i>Praecitrullus fistulosus</i>	Mixture
1	50	0.1811±0.01	0.1392±0.01	0.1579±0.01
2	100	0.2531±0.01	0.2341±0.02	0.2403±0.01
3	200	0.3687±0.01	0.3695±0.02	0.3592±0.01

4	300	0.4882±0.01	0.4754±0.01	0.4724±0.01
5	400	0.5845±0.01	0.5967±0.02	0.5729±0.01
6	500	0.7045±0.01	0.7129±0.01	0.6832±0.01

Data Presented in (Mean ± SD), n=3

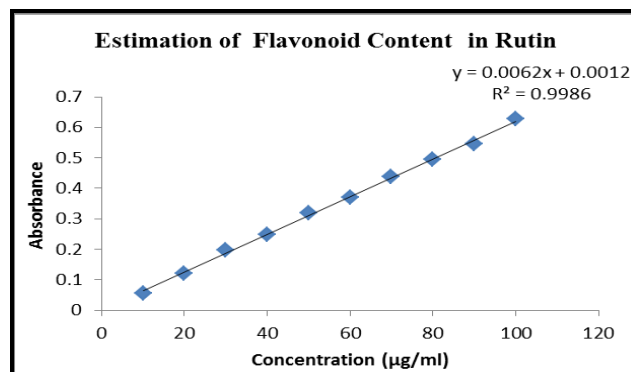


Figure 6: Standard curve of Rutin

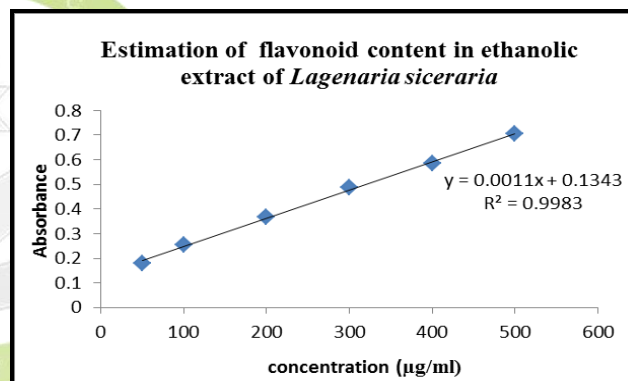


Figure 7: Total flavonoid content in ethanolic extract of *Lagenaria siceraria* (mg/g Rutin equivalent)

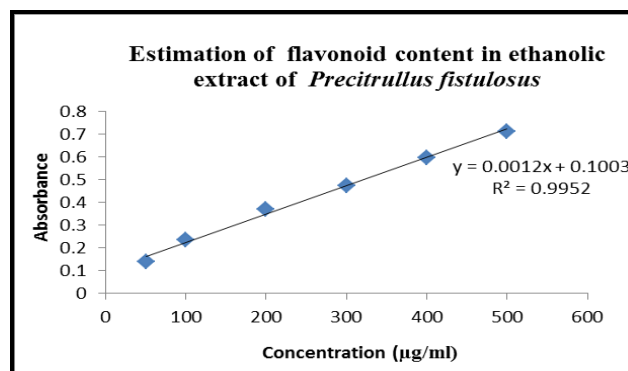


Figure 8: Total flavonoid content in ethanolic extract of *Praecitrullus fistulosus* (mg/g Rutin equivalent)

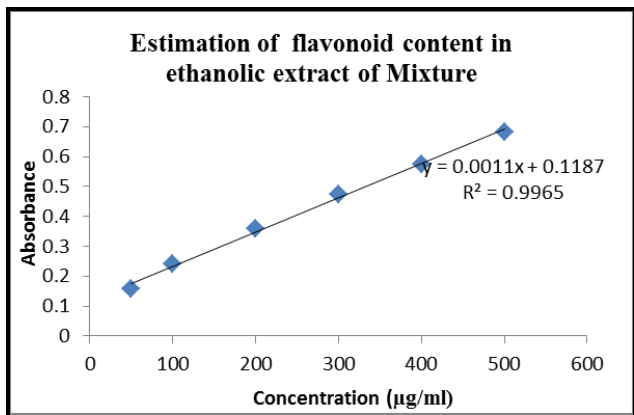


Figure 9: Total flavonoid content in ethanolic extract of Mixture (mg/g Rutin equivalent)

Table 8: Total flavonoid content in ethanolic extract of *Lagenaria siceraria*, *Paecitrullus fistulosus* and Mixture (50:50 w/w)

S.No	Ethanolic extract	Total flavonoid content (mg/g Rutin equivalent)
1	<i>Lagenaria siceraria</i>	26.6
2	<i>Paecitrullus fistulosus</i>	19.8
3	Mixture (50:50 w/w)	23.4

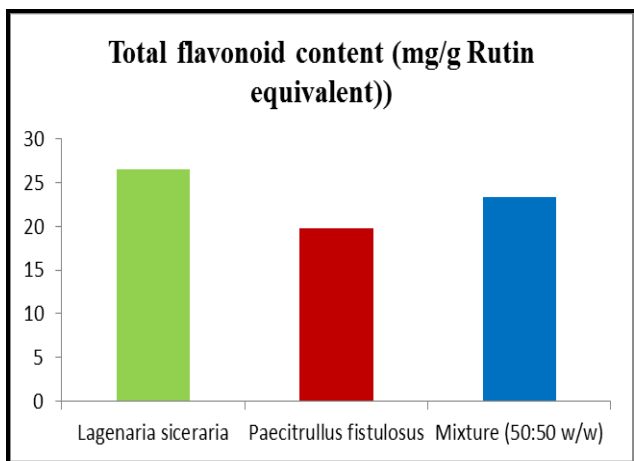


Figure 10: Total Flavonoid Content (mg/g Rutin Equivalent)

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