



Research Article

ANTIOXIDANT AND ANTICANCER ACTIVITY OF *LAGENARIA SICERARIA* FRUIT EXTRACTS ON MCF-7 CANCER CELL LINES

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ABSTRACT

Object: To evaluate the anticancer and antioxidant activity of *Lagenaria siceraria*. Materials and methods: The different extracts of *Lagenaria siceraria* were studied for anticancer activity against MCF-7. The inhibitory properties of these extracts are compared with standard 5-Fluoro Uracil for MCF-7 cell line. The Percentage cancer cell inhibition profiles were found to be concentration dependent. The maximum concentration ($\mu\text{g/ml}$) used in the study was 80 $\mu\text{g/ml}$ and the extracts were also studied for antioxidant activity by DPPH radical scavenging activity. Results: The ethanolic extract of *Lagenaria siceraria* shows the better results as compared to other extracts and in phenol and Flavonoid content estimation ethanolic extract showed the better results. The Phytochemical investigation showed the presence of phenol, flavonoid, glycosides, alkaloids Saponins. Conclusion: The existence of phenolic and flavonoid compounds in the extract may be accountable for the anticancer and antioxidant activity. Thus this activity can be contributed to the phytochemicals present in it. The ethanolic extract of *Lagenaria siceraria* can be concluded to possess highest amounts of phenolic, flavonoid and DPPH free radical scavenging activities from the present studies.

Keywords: *Lagenaria siceraria*, Anticancer, anti oxidant

INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. If left untreated, tumours can grow and spread into the surrounding normal tissue, or to other parts of the body via the bloodstream and lymphatic systems, and can affect the digestive, nervous and circulatory systems¹.

Approximately 60 % of anticancer agents are derived from medicinal plants and other natural resources; however, there are still a number of plants that have an anticancer potential but they have not yet been fully investigated. Thus, the alternate solution for the harmful effects of synthetic drugs is the use of complementary alternative medicines as very few studies have been reported on the use of herbal medicine in treatment of cancer.

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 world². Among all the plants of Cucurbitaceae family *Lagenaria* species is the most popular. The bottle gourd belongs to the genus *Lagenaria* that is derived from the word lagena, meaning the bottle. In the older literature it is often referred as *Lagenaria vulgaris* (common) or *Lagenaria leucantha* (white flowered gourd), but now it is known as *Lagenaria siceraria* (Mol.). *Lagenaria siceraria* (Molina) standley (family Cucurbitaceae) commonly known as lauki (Hindi) and bottle gourd (English) is a medicinal plant³. The plant is widely available throughout the

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 vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island, etc. for its cardiogenic, general tonic and diuretic properties⁴. The cultivated form of *Lagenaria siceraria* (Mol.) is considered to be of African and Asian origin. *Lagenaria siceraria* (Mol.) is a popular vegetable, grown almost all the year round, particularly in frost free areas. It can be cultivate in all kinds of soil, but thrives best in heavily manured loams. It requires warm humid climate or plenty of water when grown during dry weather. Seeds may be sown in nursery beds and seedlings transplanted when they have put forth 2-3 leaves. They may be also shown directly, 4-5 seeds together in manured beds or pits 5-6 ft. Apart; the strongest among the seedlings is retained, while others are removed and transplanted. Seedling transplantation is done where an early crop is desired. Generally two crops raised in India; the summer crop is sown from the middle of October to the middle of march and the later crop, from the beginning of march to the middle of July. Round fruit types are usually sown for the early crop and bottle-shaped types for the second crop. Vines are allowed to trail on the ground or trained over walls; trees or other support trailing over to give high yield of fruit⁵. It is also known as alabu, tumbi ishavaaku, katutumbi, tiktaalaabu and alaabu in sanskrit, laus and lokitumbi in bengali, bottle gourd in english, dudi and tumbadi in gujrati, lauki and ghia in hindi, isugumbala and tumbi in kannad, chorakka, churan, choraikka, piccura, tumburini and cura in malyalam, phopla in marathi, tumbi and dani in punjabi, shorakkai, surai and suraikkai in tamil, sorakaya and anapakaya in telugu and ghiya and lauki in urdu.

MATERIALS AND METHODS

Collection of Plant Material

The fresh fruits of *Lagenaria siceraria* were procured from the local market of Agra in month of September –October (2017).

Identification and Authentication

The collected plant parts were identified and authenticated from the department of botany, University of Rajasthan, Rajasthan. A voucher specimen [RUBL 21097] (*Lagenaria siceraria*)

Extraction of *Lagenaria siceraria*

Powdering

The fresh and semi –ripped fruits were sliced using a home slicer and the obtained slices were shade dried, followed by powdering manually using mortar and pestle.

Sieving

The dried powdered plant material was passed through a 20 mesh sieve to remove excessive mucilaginous hair.

Soxhlation

The dried, powder plant material were extracted with different solvents at 60°C for 24 h using a soxhlet apparatus. The collected mass was subject to drying to evaporate the excess of solvent. The collected material was termed as extract of *Lagenaria siceraria* fruit.

The extraction was carried out with following solvents successively.

1. Petroleum ether
2. Chloroform
3. Ethyl acetate
4. Acetone
5. Ethanol

Phytochemical investigation

Chemical test was carried out on all extracts for the qualitative determination of phytochemical constituents⁶.

Estimation of total phenolic content in various extracts of *Lagenaria siceraria* fruit

The total phenolic content of the extracts was estimated according to the method described by Singleton and Rossi⁷. From the stock solution (1 mg/ml) of the various extracts of plant, 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. After 30 min, the absorbance was recorded at 765 nm. Percentage of total phenolic was calculated from calibration curve of gallic acid (50-500 µg) plotted by using same procedure and total phenolics were expressed as % equivalent to gallic acid.

Estimation of total flavonoid content in various extracts of *Lagenaria siceraria* fruits

The total flavonoid content was determined with aluminium chloride (AlCl₃) according to the method of Zhishen et al, 1999⁸ using rutin as a standard. The plant extracts (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 (1 mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The flavonoid content was calculated from a rutin standard curve.

Assessment of *in vitro* antioxidant activity

DPPH free radical scavenging activity

Principle

To determine the antioxidant activity, the stable 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical is extensively used. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptive of DPPH radical at 517 nm reduces as the odd electron of DPPH radical undergoes pairing with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is absorbance produced at 517 nm.⁹

Preparation of Reagents

DPPH Solution

DPPH solution (100 mM) was prepared by dissolving 33 mg of DPPH in 100 ml of methanol. From this stock solution, 10 ml was taken and diluted to 100 ml using methanol to obtain 100 mM DPPH solution and kept in a test tube covered with the aluminum foil to protect from sunlight.

Ascorbic acid standard solution

The stock solution (100 µg /ml) was prepared by dissolving 10 mg of ascorbic acid in 100 ml of distilled water. From this different dilution containing 10, 20, 40, 60, 80, 100 µg /ml of ascorbic acid solution was prepared.

Preparation of test solutions

A stock solution of 1 mg/ml concentration was prepared by adding 10 mg of extracts in 10 ml methanol and solutions of different dilution of 50, 100, 200, 400, 800, 1000 µg /ml were prepared.

Procedure

100 µl of various concentrations (50-1000 µg /ml) of different extracts and 100 µl solution of DPPH (100 mM in methanol) were incubated at 37°C for 30 minutes and change in absorbance of reaction mixture was read at 517 nm. An equal amount of methanol and DPPH was served as control. The experiment was performed in triplicate and percentage radical scavenging activity was calculated by formula given below:

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Anticancer activity (in vitro)

Human Breast Cancer Cell Line MCF-7

Experimental Procedure for SRB Assay

The cell lines were developed in RPMI 1640 medium containing 10% fetal ox-like serum and 2 mM L-glutamine. For present test, cells were vaccinated into 96 well micro titer plates. After cell immunization, the micro titer plates were hatched at 37°C, 5 % CO₂, 95 % air and 100 % relative moistness for 24 h preceding expansion of exploratory medications.

Trial drugs were at first solubilized in dimethyl sulfoxide at 100 mg/ml and weakened to 1 mg/ml utilizing water and put away solidified preceding use. At the season of medication expansion, an aliquote of solidified concentrate (1 mg/ml) was defrosted and weakened to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these diverse medication weakening were added to the fitting micro titer wells previously containing 90 µl of medium, bringing about the required last medication fixations i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound expansion, plates were brooded at standard conditions for 48 hours and examine was ended by the expansion

of cold TCA. Cells were fixed in situ by the delicate expansion of 50 µl of cold 30 % (w/v) TCA (last focus, 10 % TCA) and hatched for an hour at 4°C. The supernatant was disposed of; the plates were washed multiple times with faucet water and air dried. Sulforhodamine B (SRB) arrangement (50 µl) at 0.4 % (w/v) in 1 % acidic corrosive was added to every one of the wells, and plates were hatched for 20 minutes at room temperature. Subsequent to recolouring, unbound color was recuperated and the lingering color was expelled by washing multiple times with 1 % acidic corrosive. The plates were air dried. Bound stain was therefore eluted with 10 mM trizma base, and the absorbance was perused on a plate peruser at a wavelength of 540 nm with 690 nm reference wavelength.

Percent development was determined on a plate-by-plate reason for test wells in respect to control wells. Percent Growth was communicated as the proportion of normal absorbance of the test well to the normal absorbance of the control wells × 100.

The rate development was determined at every one of the medication focus levels. Utilizing the six absorbance estimations [time zero (Tz), control development (C), and test development within the sight of medication at the four focus levels (Ti)]. Rate development hindrance was determined as: $[Ti/C] \times 100 \%$ ¹⁰

RESULTS AND DISCUSSION

Yield of extracts

Table 1: % yield of various extracts of *Lagenaria siceraria* fruits

S. No.	<i>Lagenaria siceraria</i> (Extracts)	Yield of Extracts (g)	% yield
1.	Petroleum Ether	2.4	4%
2.	Chloroform	3.8	6.3%
3.	Ethyl Acetate	5.1	8.5%
4.	Acetone	7.4	12.3%
5.	Ethanol	10.7	17.8%

Result of Phytochemical screening of *Lagenaria siceraria* fruit

The Preliminary phytochemical investigation revealed the presence of various phytoconstituents in various extracts of *Lagenaria siceraria* fruits. The results of phytochemical screening were found as given in table below.

Table 2: Result of Preliminary phytochemical screening of various extracts of *Lagenaria siceraria*, fruits

Phytochemicals		Petroleum Ether	Chloroform	Ethyl Acetate	Acetone	Ethanol
Alkaloids	Mayer's Reagent test	+	+	+	+	+
Carbohydrates (Monosaccharides, Oligosaccharides and Polysaccharides)	General Test	+	+	+	+	+
	Monosaccharides	+	+	+	+	+
	Disaccharides	+	+	+	+	+
	Non Reducing Polysaccharides	-	-	-	-	-
	Gums					
Proteins and Amino acids	Mucilage	+	+	+	+	+
	Proteins					
Glycosides	Amino Acids					
	General Test	+	+	+	+	+
	Cardiac Glycosides	+	+	+	+	+
	Anthraquinone Glycosides					
	Saponins Glycosides	+	+	+	+	+
Flavonoids	Cyanogenetic Glycosides					
	Alkaline reagent test	+	+	+	+	+
Tannins and Phenolic Compounds	Ferric Chloride Test	+	+	+	+	+
	Steroids	+	+	+	+	+
Volatile Oils						
Fats and Oils						

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

Total phenolic content

The various extracts of *Lagenaria siceraria* fruits contained high content of phenols. The amount of phenols varied in all the extracts. The total phenols varied from 13.375 mg/g, 15.75 mg/g, 21.375 mg/g, and 23.625 mg/g to 26.625 mg/g in petroleum ether, Chloroform ethyl acetate, acetone and ethanol extracts of *Lagenaria siceraria* respectively. The maximum phenolic content was found in ethanolic extract of *Lagenaria siceraria* was 26.625 mg/g.

Table 3: Absorbance of standard (Gallic acid)

S. No.	Concentration (µg/ml)	Absorbance of STD (Gallic acid)
1.	10	0.137±0.011
2.	20	0.228±0.012
3.	30	0.316±0.010
4.	40	0.436±0.008
5.	50	0.544±0.009
6.	60	0.633±0.009
7.	70	0.716±0.012
8.	80	0.804±0.011
9.	90	0.896±0.011
10.	100	0.994±0.006

Data presented in (Mean ± SD), n = 3

Table 4: Total phenolic content of various extracts of *Lagenaria siceraria*

S. No.	Conc. (µg/ml)	Absorbance				
		Petroleum Ether	Chloroform	Ethyl acetate	Acetone	Ethanol
1.	50	0.216±0.006	0.246±0.010	0.277±0.008	0.297±0.011	0.298±0.011
2.	100	0.327±0.010	0.316±0.016	0.376±0.009	0.375±0.009	0.400±0.055
3.	200	0.403±0.012	0.454±0.010	0.466±0.008	0.507±0.007	0.525±0.011
4.	300	0.544±0.011	0.545±0.011	0.596±0.007	0.617±0.008	0.645±0.008
5.	400	0.688±0.008	0.725±0.009	0.736±0.007	0.745±0.012	0.794±0.010
6.	500	0.846±0.011	0.876±0.010	0.895±0.008	0.914±0.011	0.945±0.009

Data presented in (Mean ± SD), n=3

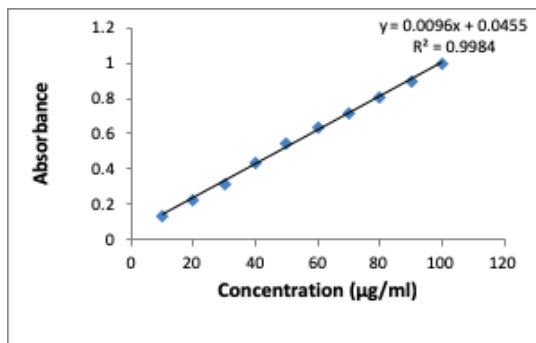


Figure 1: Standard curve of gallic acid

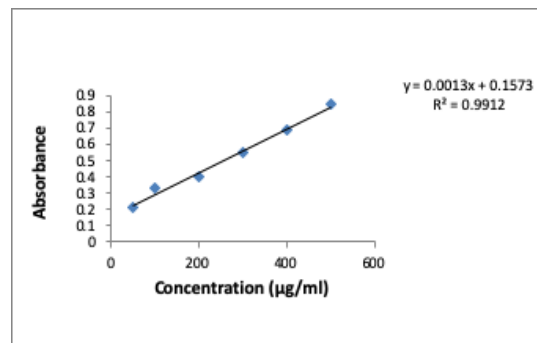


Figure 2: Total phenolic content in petroleum ether extract of *Lagenaria siceraria* (mg/g Gallic acid equivalent)

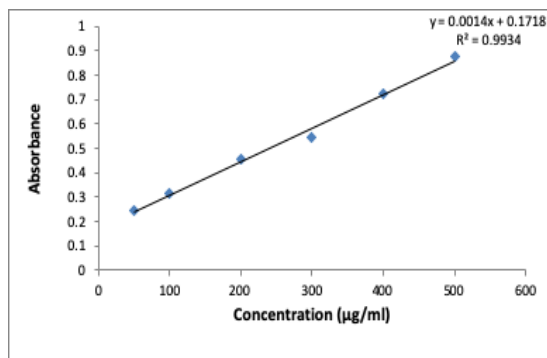


Figure 3: Total phenolic content in chloroform extract of *Lagenaria siceraria* (mg/g Gallic acid equivalent)

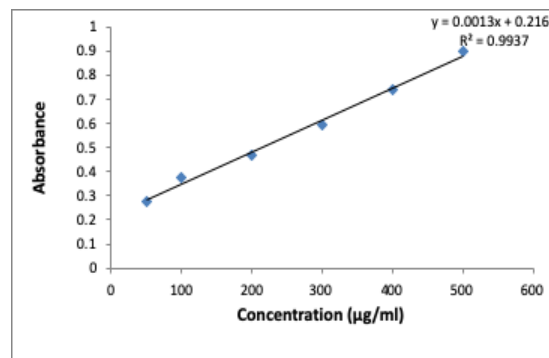


Figure 4: Total phenolic content in ethyl acetate extract of *Lagenaria siceraria* (mg/g Gallic acid equivalent)

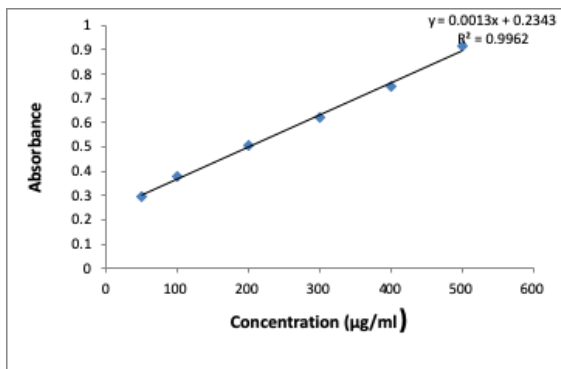


Figure 5: Total phenolic content in acetone extract of *Lagenaria siceraria* (mg/g Gallic acid equivalent)

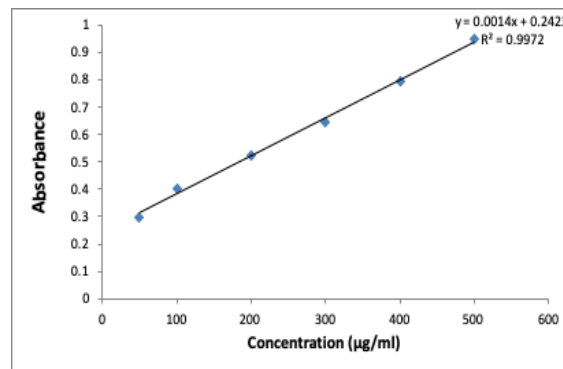


Figure 6: Total phenolic content in ethanolic extract of *Lagenaria siceraria* (mg/g Gallic acid equivalent)

Total flavonoid content

The various extracts of *Lagenaria siceraria* fruits contained high flavonoid content. The amount of flavonoid varied in all the extracts. The total flavonoid varied from 26.43 mg/g, 28 mg/g, 35.85 mg/g and 45.85 mg/g to 47.14 mg/g in petroleum ether, Chloroform ethyl acetate, acetone and ethanol extracts of *Lagenaria siceraria*. The maximum flavonoid content was found in ethanolic extract of *Lagenaria siceraria* 47.14 mg/g.

Table 5: Absorbance of standard (Rutin)

S. No.	Concentration (µg/ml)	Absorbance of STD (Rutin)
1.	10	0.197±0.011
2.	20	0.288±0.009
3.	30	0.385±0.009
4.	40	0.483±0.010
5.	50	0.566±0.012
6.	60	0.664±0.009
7.	70	0.747±0.010
8.	80	0.811±0.014
9.	90	0.899±0.011
10.	100	0.987±0.009

Data presented in (Mean ± SD), n = 3

Table 6: Total flavonoid content of various extracts of Lagenaria siceraria

S. No.	Conc. (µg/ml)	Absorbance				
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Ethanol
1.	50	0.347±0.011	0.367±0.018	0.405±0.021	0.485±0.010	0.500±0.017
2.	100	0.457±0.010	0.426±0.021	0.493±0.015	0.547±0.010	0.554±0.009
3.	200	0.528±0.008	0.564±0.009	0.607±0.010	0.656±0.009	0.679±0.010
4.	300	0.685±0.009	0.687±0.012	0.704±0.011	0.770±0.008	0.784±0.008
5.	400	0.795±0.011	0.753±0.012	0.796±0.019	0.873±0.010	0.874±0.013
6.	500	0.896±0.010	0.895±0.013	0.908±0.011	0.947±0.011	0.984±0.008

Data presented in (Mean ± SD), n = 3

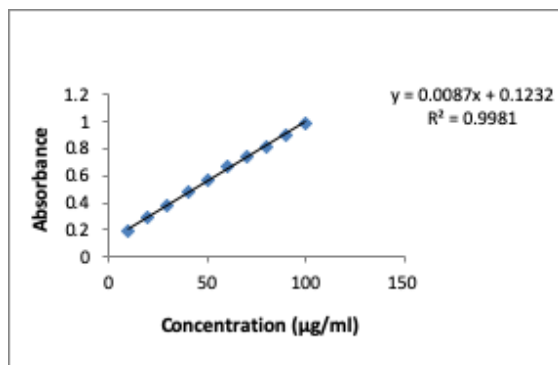
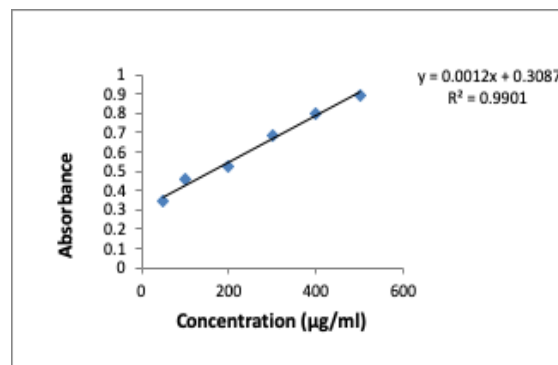


Figure 7: Standard curve of Rutin *Lagenaria siceraria* (mg/g Rutin equivalent)



Total flavonoid content in petroleum ether extract of *Lagenaria siceraria* (mg/g Rutin equivalent)

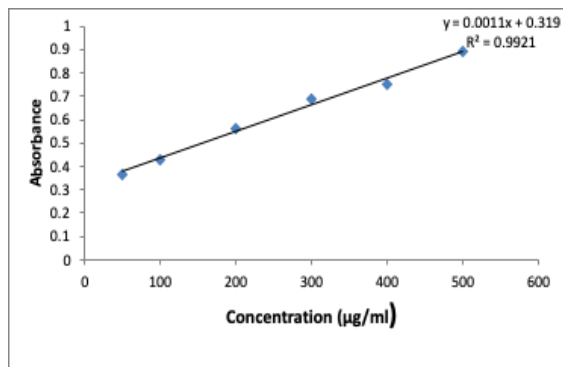


Figure 8: Total flavonoid content in chloroform extract of *Lagenaria siceraria* (mg/g Rutin equivalent)

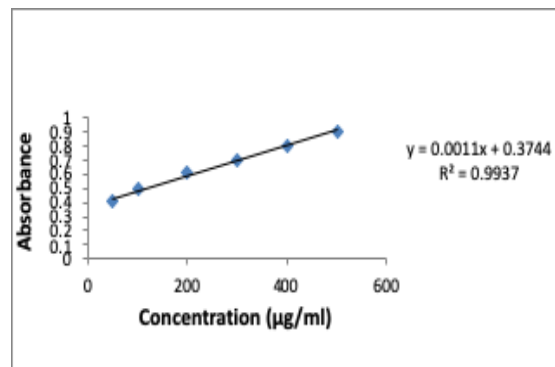


Figure 9: Total flavonoid content in ethyl acetate extract of *Lagenaria siceraria* (mg/g Rutin equivalent)

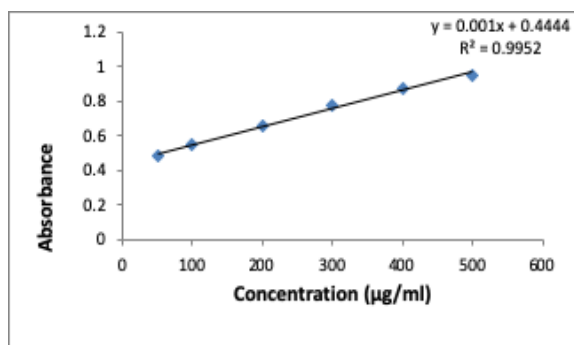


Figure 10: Total flavonoid content in acetone extract of *Lagenaria siceraria* (mg/g Rutin equivalent)

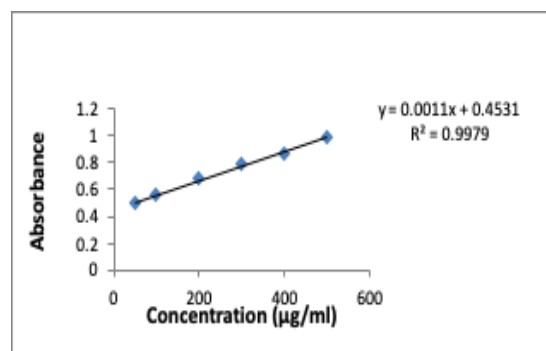


Figure 11: Total flavonoid content in ethanol extract of *Lagenaria siceraria* (mg/g Rutin equivalent)

Anti oxidant activity

Free radicals scavenging activity of DPPH has been widely used to evaluate the antioxidant activity of natural products obtained from plant and microbial sources. In DPPH scavenging activity model it was observed that extracts significantly scavenged DPPH in a concentration dependent manner. However extract showed weak scavenging activity in lower concentrations; the higher concentrations exhibited promising DPPH scavenging activity ranging from 49.521 %, 52.631 %, 62.200 %, and 67.703 % to 73.205 % in petroleum ether, Chloroform ethyl acetate, acetone and ethanol extracts of *Lagenaria siceraria*. DPPH is a relatively stable free radical and the assay determines the ability

of extracts to reduce DPPH to the corresponding hydrazine by converting the unpaired electrons to form pairs. This conversion is the action of the antioxidant.

Table 7: Absorbance of standard (ascorbic acid)

S. No.	Concentration (µg/ml)	Absorbance
1.	10	28.468 ±0.006
2.	20	42.105 ±0.014
3.	30	51.913 ±0.005
4.	40	65.311 ±0.008
5.	50	76.555 ±0.007

Data presented in (Mean ± SD), n = 3

Table 8: Total Antioxidant content of various extracts of *Lagenaria siceraria*

S. No.	Conc. (µg/ml)	Absorbance				
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Ethanol
1	10	13.397±0.032	14.593±0.432	17.464±0.231	22.248±0.437	24.881±0.324
2	20	20.813±0.016	22.966±0.187	25.358±0.329	30.861±0.276	33.493±0.221
3	30	28.468±0.13	32.057±0.324	35.645±0.164	47.129±0.365	47.60±0.168
4	40	35.645±0.009	38.516±0.176	48.086±0.194	57.655±0.198	60.526±0.134
5	50	49.521±0.034	52.631±0.187	62.200±0.254	67.703±0.654	73.205±0.245

Data presented in (Mean ± SD), n = 3

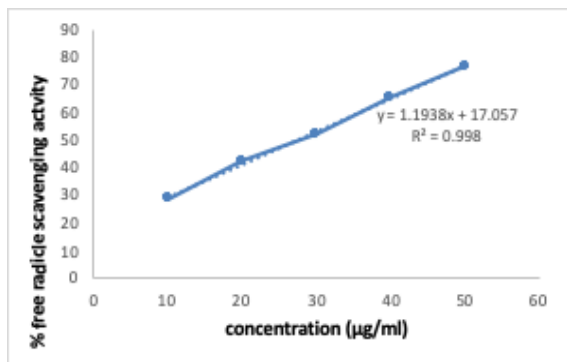


Figure 12: Standard curve of ascorbic acid

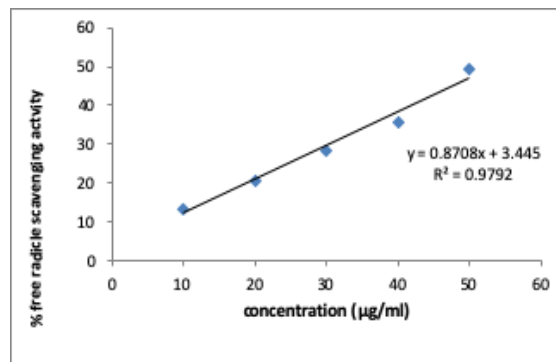


Figure 13: Total antioxidant content of petroleum ether extract of *Lagenaria siceraria*

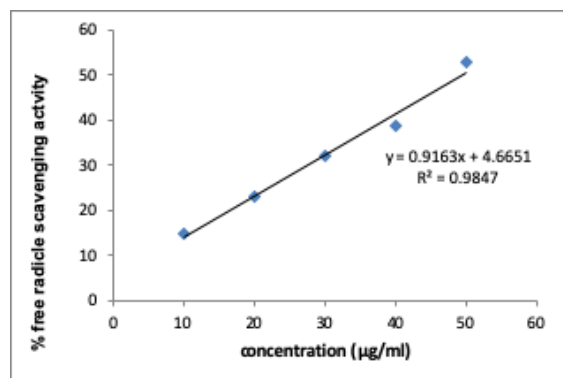


Figure 14: Total antioxidant content of chloroform extract of *Lagenaria siceraria*

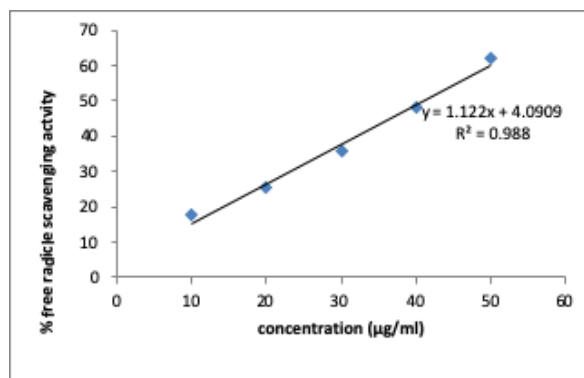


Figure 15: Total antioxidant content of ethyl acetate extract of *Lagenaria siceraria*

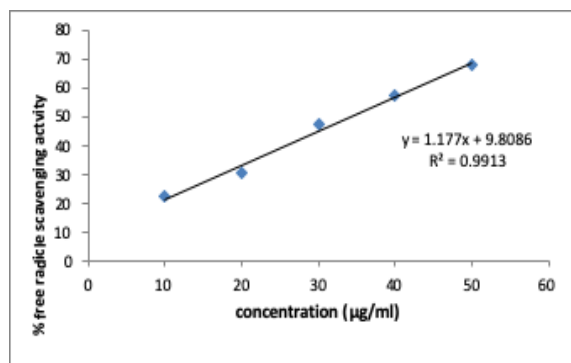


Figure 16: Total antioxidant content of acetone extract of *Lagenaria siceraria*

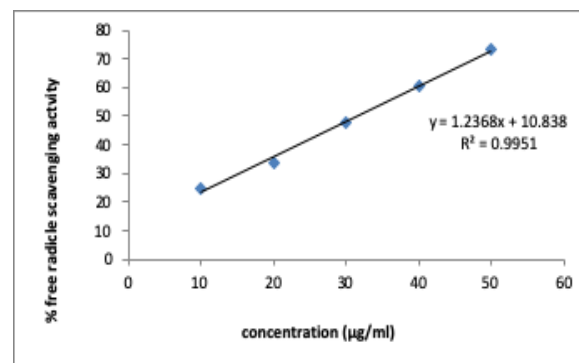


Figure 17: Total antioxidant content of ethanol extract of *Lagenaria siceraria*

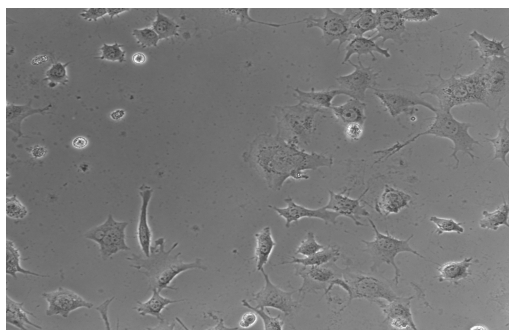
Anti cancer activity

Screening of Various extract of *Lagenaria siceraria* resulted in moderate anticancer activities against MCF-7. The inhibitory properties of these extracts are compared with standard 5-Fluoro Uracil for MCF-7 cell line. The Percentage cancer cell inhibition profiles were found to be concentration dependent. The maximum concentration (µg/ml) used in the study was 80 µg/ml. Following results were obtained when anticancer activities of plant extracts were studied.

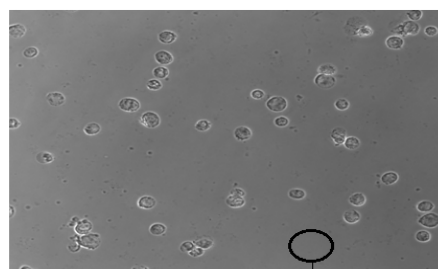
Table 9: Human Breast Cancer Cell Line MCF-7 (SRB Assay)

Group	% Control Growth			
	Conc. 10(µg/ml)	Conc. 20 (µg/ml)	Conc. 40 (µg/ml)	Conc. 80 (µg/ml)
LS 1	10.87±0.13	18.45±0.19	24.87±0.13	34.98±0.17*
LS 2	14.87±0.43*	21.76±0.17*	31.42±0.27*	39.45±0.19*
LS 3	19.76±0.15*	26.67±0.54*	38.78±0.34*	41.89±0.43*
LS 4	21.76±0.28*	30.56±0.28*	42.56±0.55*	47.56±0.28*
LS 5	26.37±0.19**	35.86±0.38**	56.69±0.28**	68.42±0.26**
5flourourcil (20 mg/kg)	28.46±0.19**	38.27±0.17**	65.31±0.42**	76.55±0.42**

Values are expressed as mean ± SEM; n = 6; *P < 0.01, **P < 0.001; one way ANOVA followed by Dunnett's multiple comparisons test

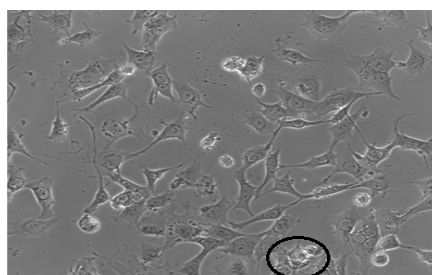


MCF-7 Control



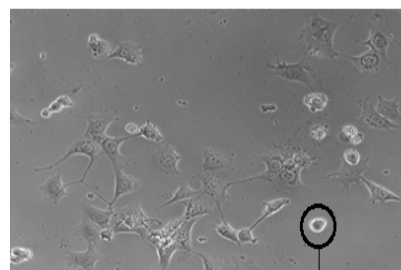
→ No DNA Fragmentation and nuclear condensation

MCF-7 Positive control



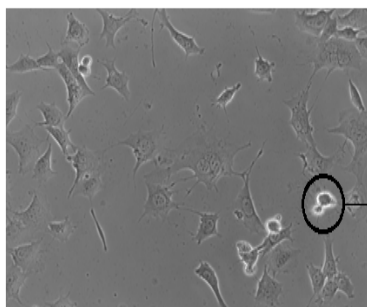
→ DNA Fragmentation and nuclear condensation

LS1 (petroleum ether extract of *Lagenaria siceraria*)



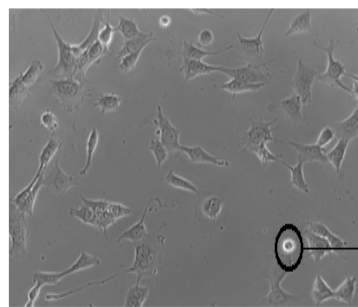
→ DNA Fragmentation and nuclear condensation

LS2 (Chloroform extract of *Lagenaria siceraria*)



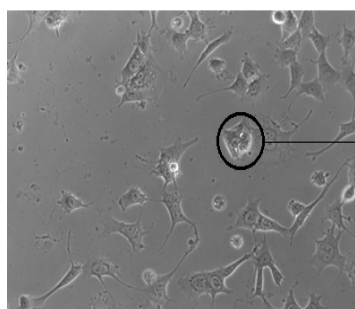
→ DNA Fragmentation and nuclear condensation

LS3 (Ethyl acetate extract of *Lagenaria siceraria*)



→ DNA Fragmentation and nuclear condensation

LS4 (Acetone extract of *Lagenaria siceraria*)



→ DNA Fragmentation and nuclear condensation

LS5 (Ethyl alcohol extract of *Lagenaria siceraria*)

CONCLUSION

The existence of phenolic and flavonoid compounds in the extract may be accountable for the anticancer activity and antioxidant activity. Thus this activity can be contributed to the phytochemical present in the extracts like alkaloids, glycosides, phenol, steroid, flavonoids and tannins. The ethanolic extract

Lagenaria siceraria can be concluded to possess highest amounts of phenolic, flavonoid and DPPH free radical scavenging activities from the present studies.

Therefore the anticancer and antioxidant activity of the extracts of *Lagenaria siceraria* can be attributed to its phytochemical compound present in the extracts.

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