EVALUATION OF THE PHYTOCHEMICALS, IN VITRO ANTIOXIDANT ACTIVITY, AND TOXICITY OF LAGENARIA SICERARIA SEEDS

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Article History: Received: 10.03.2023 **Revised:** 23.05.2023 **Accepted:** 29.06.2023

Abstract

Lagenaria siceraria (Family: Cucurbitaceae), sometimes referred to as bottle gourd, calabash, doodhi, and lauki, is a plant that is widely utilised as a fruit and vegetable throughout India. The objective of the current study was to assess the toxicity, in vitro antioxidant activity, and Phytochemical content of *Lagenaria siceraria* seeds. The results indicate that the extract lacks polysaccharides, proteins, steroids, tannins, and cardiac glycosides, while it does contain cardiac glycosides, reducing sugar, flavonoids, and carbohydrates. The total flavonoid concentration of the extract, which was measured at 38.64 mg/g, exhibited a robust level of antioxidant activity. The concentration-dependent scavenging of DPPH radicals by the extract was observed, with the highest efficacy being exhibited by the ether+ alcoholic extract. As per the outcomes of the toxicity evaluation, the extract exhibited no toxicity; mice did not manifest any fatality or unfavourable impacts at quantities as high as 5000 mg/kg. It can be inferred that the LD50 value of the extract is greater than 5000mg/kg. The findings suggest that the extract derived from *Lagenaria siceraria* seeds exhibits considerable potential as an antioxidant agent and possesses desirable safety characteristics. Therefore, further investigation into its pharmacological and toxicological properties is warranted.

Keywords: Lagenaria siceraria, seeds, extract, DPHH.

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DOI: 10.31838/ecb/2023.12.s3.565

1. Introduction

Lagenaria siceraria, commonly known as lauki, belongs to the Cucurbitaceae family, which is a type of gourd. The indigenous population commonly refers to it as kado, although it is more widely recognised as a calabash or white flower gourd. There are approximately 825 distinct species that can be identified among the 118 various types. The genus *Lagenaria* encompasses several species, namely *Lagenaria guineensis, Lagenaria sphaerica, Lagenaria breviflora, Lagenaria Rufa, Lagenaria sphaerica,* and *Lagenaria Abyssinia. Lagenaria siceraria* spp. is predominantly cultivated. *Lagenaria siceraria* ssp [1] is an acknowledged botanical classification of the bottle gourd plant. The cultivation of Siceraria and *Lagenaria siceraria* ssp. asiatica commenced in the Americas (new world) during the period ranging from 9 to 10,000 B.P. This was subsequently followed by East Asia, which saw the cultivation of these crops between 6 to 10,000 B.P. Africa also joined in the cultivation of these crops, albeit at a later period, ranging from 4 to 5,000 B.P. There exists a conjecture that this particular flora was among the initial species that humanity cultivated and brought under domestication. The plant in question is an herbaceous annual that exhibits a prostrate growth habit [2]. The *L. siceraria* species exhibits monoecious characteristics, whereby flowers are produced on distinct axes within the same plant. The tendrils are typically observable, while the leaves exhibit alternation and variation. Hence, the promotion of pollination by diverse species is advocated [3].

Figure 1Lagenariasiceraria

The term "wild" or "uncultivated" refers to areas that have not been modified or managed by human intervention. Hermaphrodite flowers of the Bering species may also demonstrate sexual expression of dioecy and andromonoecy. The bottle gourd exhibits a significantly high sex ratio. According to research, the crop yield is significantly influenced by the proportion of male to female flowers [4]. The calabash or bottle gourd is cultivated throughout the year on the plains of Pakistan for its tender and delicate fruits, which are commonly utilised as a household vegetable referred to as Lauki or Kaddu. Formerly identified as L. vulgaris Ser., the plant species is currently recognised as *L. siceraria* [5].

Fruit has the ability to remain afloat in the ocean for extended periods of time without undergoing seed decay for several months. The available evidence indicates that the process of domestication occurred autonomously from wild populations in both the Old and New Worlds. According to a study, land races of subsp. *Siceraria* in Africa and the Americas exhibit distinct physical characteristics in comparison to those found in Asia [6]. The exocarp of decomposed fruits has the potential to be repurposed into a diverse range of practical objects, such as receptacles for liquids, conduits for fluids or gases, musical instruments, and containers for powdered tobacco. Dried clams are a crucial element in the production of stringed musical instruments, such as the sitar and bia. The consumption of pulp is believed to have a potential cooling and antiemetic effect on the human body. The administration of seed oil has been reported as a treatment for external headaches [7][8][9].

Lagenaria siceraria is extensively grown in tropical and subtropical areas, with a distribution range from sea level to approximately 2500 metres above sea level [10]. The observation of fugitives in proximity to riverbanks and other rugged landscapes is a frequent occurrence. The species exhibits a notable capacity for flourishing in diverse ecological niches, encompassing but not limited to alluvial soils with a sandy texture, slopes characterised by mild to medium inclinations, rocky ridges, riverbanks, dry riverbeds, undergrowth in ravines, woodlands, and savannahs [11]. Additionally, it can be observed in secondary forests, areas with disturbed soil near human settlements, and adjacent to thoroughfares. *Lagenaria siceraria*, a plant species, exhibits a preference for tropical regions with high levels of humidity. However, it is also capable of surviving in regions characterised by aridity, such as dry thickets, arid steppes, and deserts, due to its ability to tolerate such conditions [12].

Objective of the study: The goal of this investigation is to assess the potential toxicity, chemical composition, and antioxidant properties of an extract derived from *Lagenaria siceraria* seeds.

2. Methodology

1. Selection, Collection and Authentication of Plant Material

Lagenaria siceraria plant seeds were gathered in the Jaipur District of Rajasthan and verified at the Plant Anatomy Research Centre School of Agricultural Sciences Dr. K.N. Modi University, Newai, Tonk, Rajasthan

2. Phytochemical evaluation

The seed extract was subjected to different qualitative chemical tests for detection of various phytoconstituents present in the extract [13] [14].

The List of test used in qualitative phytochemical analysis is presented in table 1

Table1 List of tests utilized

PHYTOCHEMICAL	TEST
Carbohydrate	• Molisch's test • Fehling's test • Benedict's test
Alkaloid	• Dragondrof's Reagent • Mayer's test • Wagenr's test • Hager's test

Triterpenoid	• Salkowaski test • Liebermann-Burchard test
Glycosides	• Legal's test • Keller-Killiani's test • Baljet test
Steroids And Sterols	• Salkowaski test • Liebermann-Burchard reaction
Phenols	• Ferric chloride test • Lead acetate test
Tannins	• Lead acetate test
Saponins	• Foam Test
Flavonoids	• Alkaline reagent test • Shinodas test [Magnesium hydrochloride reduction test**]**
Proteins &Amino Acids	• Ninhydrine test • Biuret test

Total Flavonoids

The aluminium chloride colorimetric method was used in order to determine the total amount of flavonoids

3. In Vitro Antioxidant Activity

By using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, the fractions' in vitro free radical scavenging activity was determined.Various amounts of the reference substance and sample (5, 10, 15, 20, 25, and 30 g/ml) were added, and the mixture was vigorously shaken before being left in the dark and at room temperature. After 30 minutes, evaluate 515 nm absorbance in comparison to a blank. Ascorbic acid was used as a standard. Control response were given without the use of test material. Each test was carried out three times for the mean. To calculate inhibition, absorbance readings from control and test samples were compared.

Inhibition of free radical scavenging activity (I%) formula:

I% = (Abs Control-Abs sample/Abs control) * 100

4. Toxicity Evaluation

In accordance with the guidelines established by the Organisation for Economic Co-operation and Development (OECD), revised draught guidelines 423, which were obtained from CPCSEA, under the Ministry of Social Justice and Empowerment of the Government of India [15], the study on acute oral toxicity was carried out.

In order to gather sufficient information on the test substance's acute toxicity for the purposes of classification, the methodology used in this study is based on a methodical approach that uses a small number of animals in each phase [16]. Five animals of either gender are used in each phase of the evaluation process for the experimental drug.

3. Result And Discussions

1. Qualitative Assessment of Phytochemicals

Phytochemical analysis of the *Lagenaria siceraria* (cucurbitaceae) plant's seed extract revealed that the sample included carbohydrates, reducing sugar, cardiac glycosides, and flavonoids. Polysaccharides, proteins, steroids, tannins, or steroids were not present in the extract. Table 2 shows the observation table and the conclusion that was made from it.

Table 2 Results of qualitative assessment

PHYTOCHEMICAL	**TEST**	**Interference**
Carbohydrates	Molisch's test	**Positive**
	Fehling's test	**Positive**
	Benedict's test	**Positive**
Alkaloids	Dragondrof's Reagent	Negative
	Mayer's test	Negative
	Wagenr's test	Negative
	Hager's test	Negative
Triterpenoid	Salkowaski test	Negative
	Liebermann-Burchard test	Negative
Glycosides	Legal's test	**Positive**
	Keller-Killiani's test	Negative
	Baljet test	Negative
Steroids And Sterols	Salkowaski test	Negative
	Liebermann-Burchard reaction	Negative
Phenols	Ferric chloride test	Negative
	Lead acetate test	Negative
Tannins	Lead acetate test	Negative
Saponins	Foam Test	Negative
Flavonoids	Alkaline reagent test	**Positive**
	Shinodas test [Magnesium hydrochloride reduction test]	**Positive**
Amino Acids &Proteins	Ninhydrine test	Negative
	Biuret test	Negative

2. Quantification of Flavonoids

Table 3 Quantification of Flavonoids

	Concentration (µg/ml)	**Absorbance**
Standard (1mg/ml)	10	0.032
	20	0.084

	40	0.261
	60	0.501
	80	0.774
	100	1.050
Sample	100	0.703

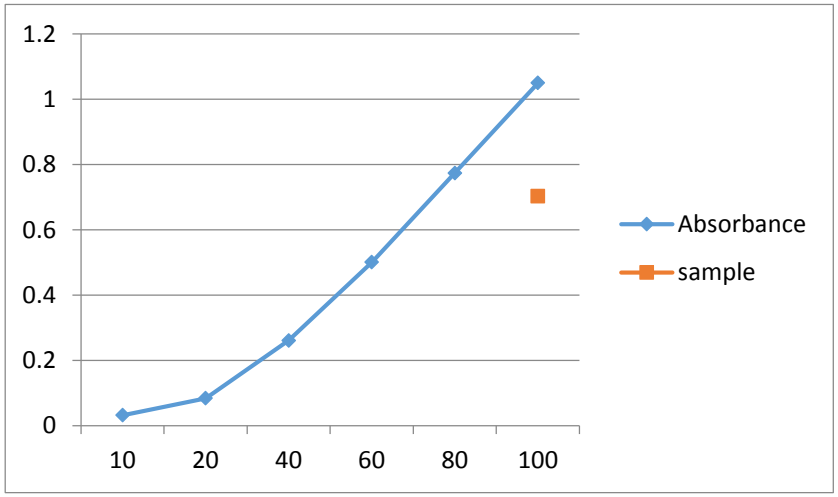

Figure 2 Estimation of total flavonoids content of Lagenaria seeds

The quantification of the total flavonoid concentration was conducted using Quercetin equivalent measurement, resulting in a value of 38.64 mg/g of extract.

3. In Vitro Antioxidant Activity

The presented data exhibits the DPPH radical scavenging potential of the standard, ether+ alcoholic extract, and ether+ acetone extract at different concentrations. The acquisition of an electron or a hydrogen ion may be necessary for the stable free radical DPPH to undergo a transformation into a stable molecule. The antioxidant activity of the sample exhibits a positive correlation with the percentage of DPPH radical inhibition. The concentration of the sample required to neutralize half of the DPPH radicals is commonly referred to as the IC50 value.The tables indicate that the standard exhibited the highest degree of antioxidant activity, as evidenced by its IC50 value of 2.47 µg/ml. The ether+ acetone extract and ether+ alcoholic extract followed suit, with IC50 values of 75 µg/ml. An increase in sample concentration resulted in a corresponding increase in DPPH radical inhibition. The results suggest that the Aqueous *Lagenaria* seed (AQLS) extract exhibits potent antioxidant characteristics and could potentially serve as a natural antioxidant agent.

Table 4 Percentage inhibition and IC50 values of DPPH radical by Ascorbic Acid

S. No	**Standard Concentration(µg/ml)**	**% inhibition**	**IC_{50}(µg/ml)**
1	5	73.2	
2	10	83.8	

3	15	89.8	**2.47**
4	20	92.7	
5	25	93.5	
6	30	94.8	

The percentage inhibition of the standard increased as the concentration increased, peaking at 94.8% when the concentration was 30 µg/ml and 73.2% when the concentration was 5 µg/ml. The IC50 value of 2.47 µg/ml for the standard indicates that a comparatively low concentration of the chemical is sufficient to produce 50% inhibition of the biological process or activity under investigation.

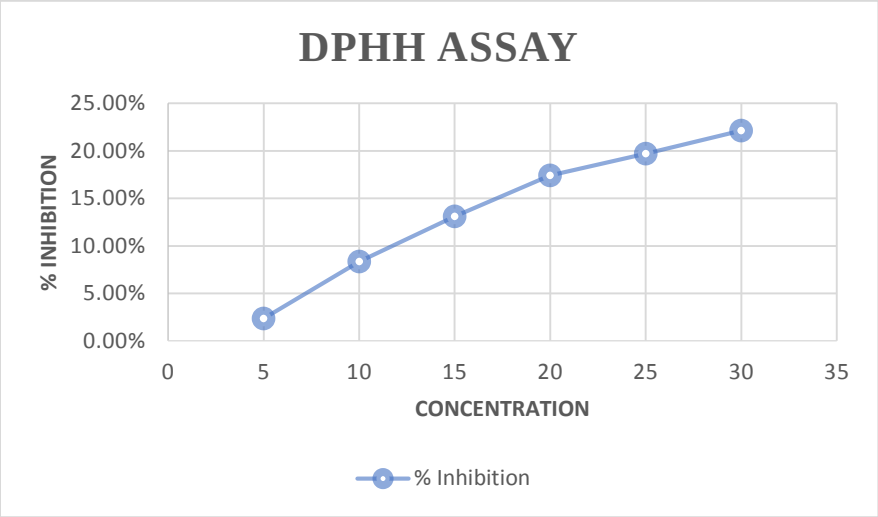

Figure 3 DPPH radical scavenging activity of standard

Table 5 I % and IC50 values of DPPH radical by alcoholic extract

S. No.	**Alcoholic extract Conc. (µg/ml)**	**% Inhibition**	**IC_{50}(µg/ml)**
1	5	8.85%	
2	10	19.1%	
3	15	25.7%	
4	20	30.9%	45.1
5	25	35.0%	
6	30	38.7%	

The percentage of inhibition of free radicals increased proportionally with the concentration of the extract. At a concentration of 30 µg/ml, the extract displayed a high efficacy in free radical inhibition, as evidenced by its lowest IC50 value (45.1 µg/ml) and greatest inhibition percentage (38.7%). The results suggest that the alcoholic extract displays significant antioxidant characteristics and has the potential to function as a feasible source of natural antioxidants.

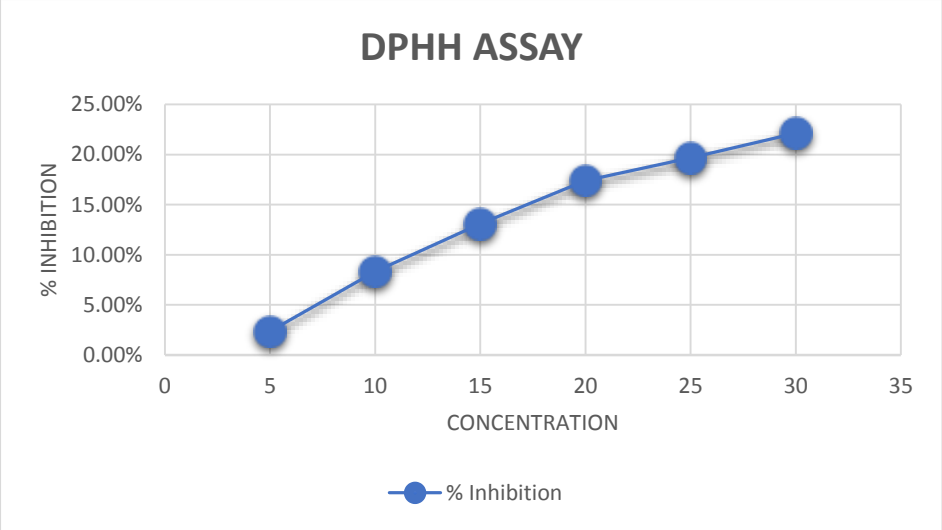

Figure 4 DPPH radical scavenging activity of alcoholic extract

Table 6 I % and IC50 values of DPPH radical by ether+ alcoholic extract

S. No.	Conc. (µg/ml)	ether+ alcoholic extract % Inhibition	IC_{50}(µg/ml)
1	5	1.44%	76.6
2	10	5.76%	
3	15	9.09%	
4	20	12.75%	
5	25	16.04%	
6	30	18.93%	

The findings suggest a direct association between the extract concentration and the percentage inhibition, with the most significant inhibition occurring at a concentration of 30 µg/ml, achieving a maximum value of 18.93%. The study determined the IC50 value to be 76.6 µg/ml, which signifies that a concentration of 76.6 µg/ml of the extract is required to achieve 50% inhibition of its activity.

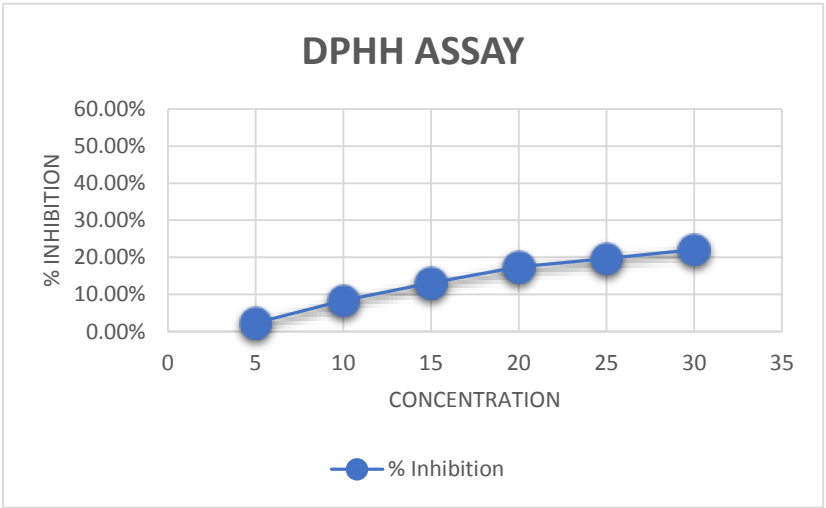

Figure 5 DPPH radical scavenging activity of ether+ alcohol extract

Table 7 I % and IC50 values of DPPH radical by ether+ alcoholic extract

S. No.	Conc. of ether+ acetone extract (µg/ml)	% Inhibition	IC_{50}(µg/ml)
1	5	2.36%	
2	10	8.35%	
3	15	13.10%	
4	20	17.40%	75
5	25	19.69%	
6	30	22.14%	

Table 7 illustrates a positive correlation between the concentration of the extract and the percentage inhibition of the activity. The IC50 value of the extract has been ascertained to be 75 µg/ml, signifying that a concentration of this magnitude can elicit 50% inhibition of the activity.

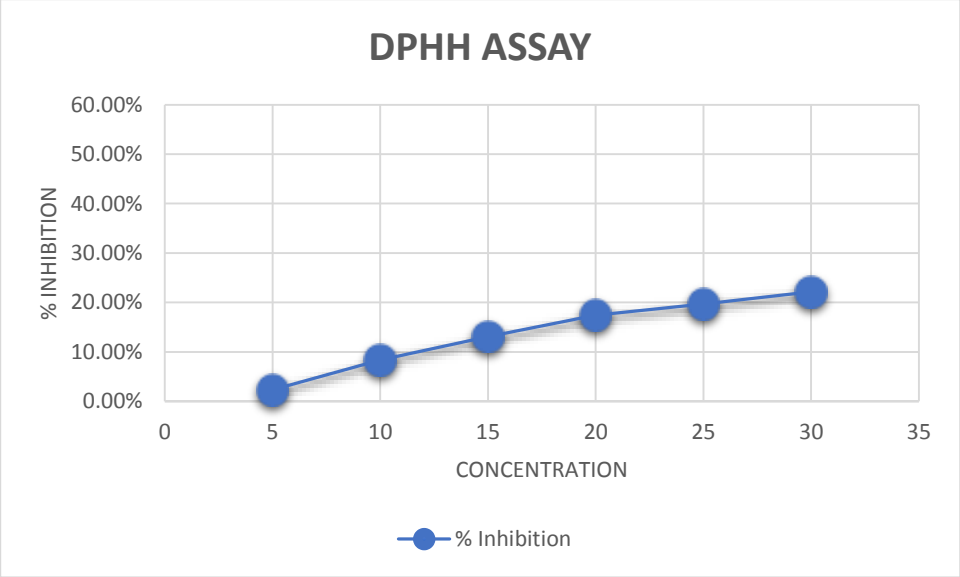

Figure 6 DPPH radical scavenging activity of ether+ acetone

4. Toxicity Evaluation:

The study on acute toxicity entailed the intraperitoneal administration of the Methanol extract of *L. siceraria* seeds extract, with doses ranging from 1000mg/kg to 5000mg/kg.

The administration of *Lagenaria siceraria* seed extract at varying dosages of 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg, and 5000mg/kg did not result in any significant changes in the observed behaviours, respiratory patterns, cutaneous manifestations, sensory reactions, neurological responses, and gastrointestinal effects in mice. No indications of mortality or unfavourable outcomes were detected during the entire course of the study. During a 14-day timeframe, the experimental group did not exhibit any signs of delayed toxicity. The results suggest that the methanolic and aqueous extracts obtained from seeds of *Lagenaria siceraria* exhibited no toxicity when administered at the levels of dosage tested.

Table 8 Toxicity evaluation

Group	Dose	mice	dead	Dose	(a) Mean	(b)

	mg/kg	count	mice count	difference	Mortality	Probit (a×b)
1	DDW	5	0	-	-	-
2	1000	5	0	1000	0	0
3	2000	5	0	1000	0	0
4	3000	5	0	1000	0	0
5	4000	5	0	1000	0	0
6	5000	5	0	1000	0	0

The administration of *L. siceraria* methanolic seed extract at a dosage of 5000 mg/kg body weight resulted in predominantly normal behaviour among all animals. The research findings indicate that no instances of mortality were observed among the mice in the respective groups. The LD50 value of *L. siceraria* seed extract has been determined to be slightly above 5000 mg/kg.

4. Conclusion

The study's findings indicate that *Lagenaria siceraria* seed extract is composed of carbohydrates, reducing sugar, cardiac glycosides, and flavonoids. However, the extract does not contain polysaccharides, proteins, steroids, tannins, or steroids. Therefore, it can be concluded that the seed extract of *Lagenaria siceraria* possesses certain chemical constituents while lacking others. The extract exhibited potent antioxidant activity in vitro, with a total flavonoid content of 38.64 mg/g. The concentration-dependent DPPH radical scavenging activity of the extract was observed, with the ether+ alcoholic extract exhibiting the highest efficacy in free radical inhibition, followed by the ether+ acetone extract. The findings indicate that the extract derived from *Lagenaria siceraria* seeds exhibits promising properties as a natural antioxidant. The results of the toxicity assessment indicate that the *Lagenaria siceraria* seed methanol extract exhibits no toxicity at the given dosage levels, leading to the conclusion that it is non-toxic. No mortality or adverse effects were observed in mice during the study, even at the highest dose of 5000mg/kg. Additionally, there was no evidence of delayed toxicity during a 14-day duration. Thus, it can be inferred that the LD50 value of the extract is higher than 5000mg/kg. The findings indicate that the extract exhibits favourable safety characteristics and warrants consideration for additional investigations in the fields of pharmacology and toxicology.

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