

# In Vitro Antioxidant Efficacy and Phenolic Content of Aqueous and Ethanolic Extracts from *Lagenaria Siceraria* Fruit.

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## Abstract

This investigation assesses the in vitro antioxidant capacity of ethanolic and aqueous extracts derived from the fruits of *Lagenaria siceraria* (Cucurbitaceae), a plant commonly used in ethnomedical traditions. Fresh fruits were coarsely ground, dried and extracted with water and ethanol via the Soxhlet method. Antioxidant activity was measured using three separate assays: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, nitric oxide radical scavenging, and a reducing power assay. Phytochemical screening verified the presence of phenolic compounds in the extracts. The findings indicated that the ethanolic extract (LSEE) produced greater antioxidant activity than the aqueous extract (LSAE) at all concentrations tested. In the DPPH assay, the IC<sub>50</sub> values were 139.43 µg/ml for the ethanolic extract and 190.59 µg/ml for the aqueous extract. In the nitric oxide scavenging assay, the ethanolic extract again demonstrated stronger activity, with an IC<sub>50</sub> of 104.62 µg/ml relative to 146.09 µg/ml for the aqueous extract. The reducing power of both extracts increased in a dose-dependent manner, and the ethanolic extract yielded higher absorbance values. Overall, these results imply that fruits of *Lagenaria siceraria* constitute an important source of natural antioxidants with potential for pharmacological application.

Keywords: - *Lagenaria siceraria*; Antioxidant activity; Ethanolic extract; DPPH radical scavenging; Nitric oxide scavenging.

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## Introduction

Medicinal plants play a crucial role in preserving human health [1]. Their long-standing historical use in managing disease spanning centuries has helped enable the discovery of more than half of today's modern pharmaceuticals [2]. Although there are an estimated 500,000 plant species worldwide, only 1% have been subjected to phytochemical investigation, indicating a major opportunity for identifying new bioactive compounds [3]. The World Health Organization states that between 65% and 80% of people in developing countries depend on medicinal plants for treatment [4,5]. This underscores the continued global growth of traditional medicine. These plants are valuable sources for the development of novel therapeutic agents. Owing to their rich content of strong bioactive secondary metabolites, they are widely applied in both ethnomedical practice and modern herbal use to manage a wide range of health conditions, providing safe and effective options compared with synthetic chemicals [6,7,8]. Furthermore, herbs have contributed some of the most important life-saving drugs included in the therapeutic arsenal. Because these agents are less damaging to the human body than synthetic drugs, research laboratories around the world are conducting screening studies of plants for biological activities with therapeutic potential. The market for Ayurvedic medicines is projected to expand at an annual rate of 20%. Over the past ten years (1987–96), sales of medicinal plants have

increased by nearly 25% in India, representing the highest growth rate worldwide. However, India's per capita spending on medicines per year remains among the lowest globally. In other developing countries as well, plants serve as a primary source of medicine. Two of the largest consumers of medicinal plants are China and India. Traditional Chinese Medicine relies on more than 5000 plant species, whereas India uses about 7000 [9]. Medicinal plants are essential for pharmacological research and drug development, whether plant constituents are applied directly as therapeutic agents or used as starting materials for drug synthesis, or as models for pharmacologically active compounds. Derivatives of medicinal plants are non-narcotic and have little or no side effects [10].

## Material and Method

### Plant collection and preparation of extracts

*Lagenaria siceraria*, belonging to the family Cucurbitaceae [11], was selected. Fresh fruits of *L. siceraria* were collected from local farms in Dhule District and authenticated by the Department of Botany, S.S.V.P. S's Dr. P.R Ghogrey Science College, Dhule. The fruits were coarsely powdered and shade dried for exsiccation. The coarsely powdered plant material was extracted separately with two solvents, water and ethanol, using the Soxhlet extraction method. The resulting extract was then evaporated and dried under vacuum to yield a thick, sticky extract. Preliminary phytochemical

screening was performed to determine the presence of phytochemical classes in both extracts.

#### Chemicals

Sodium carbonate, Folin-Ciocalteu reagent, deionized water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, Sodium nitroprusside, Sulphanilamide, phosphate buffer saline, Potassium ferricyanide, Trichloroacetic acid, Ascorbic Acid, Ferric chloride, N-(1-naphthyl) ethylenediamine dihydrochloride, and all other reagents were of analytical grade. Instrument: UV spectrophotometer (Systronic double-beam UV-2201).

#### Total Phenolic Content.

The total phenolic contents of the two extracts were assessed using the Folin-Ciocalteu assay. Briefly, 0.6 mg samples of each of the two extracts were dissolved in methanol (1 mL), deionized water (1 mL), and 95% ethanol (1 mL), respectively, and then 11.4 µL aliquots of each solution were combined with sodium carbonate (2%, 227.32 µL). The resulting mixtures were kept at room temperature for 2 min, after which the Folin-Ciocalteu reagent (50%, 11.4 µL) was added to each sample mixture. Following incubation at room temperature for 30 min, the absorbance of the reaction mixtures was determined at 750 nm using a tunable microplate reader (VersaMax, Molecular Devices Corporation, Sunnyvale, Calif, U.S.A.). Gallic acid (0.2–1.0 mg/mL in methanol) served as the standard, and the total phenolic contents of the two extracts were reported as milligrams of gallic acid equivalents per gram of extract (mg gallic acid/g extract). [12]

#### Radical-scavenging activity of extracts against DPPH radicals

Determination of DPPH (1,1-diphenyl 2-picrylhydrazyl) radical-scavenging activity. The free radical-scavenging activity of the ethanolic and aqueous extracts of dried fruit of the plant *L. siceraria* was evaluated in terms of hydrogen-donating or radical-scavenging capacity using the stable radical DPPH (Bliss, 1958). A 0.1 mM DPPH solution in ethanol was prepared, and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (20–200 µg/mL). After 30 min, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture corresponded to higher free radical-scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following equation [13,14,15]:

$$\text{Inhibition percentage} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} \times 100$$

#### Determination of nitric oxide radical-scavenging activity

Nitric oxide was generated from sodium nitroprusside and quantified using the Greiss reaction. In aqueous solution at physiological pH, sodium nitroprusside spontaneously generates nitric oxide (Marcocci et al., 1994), which reacts with oxygen to form nitric ions that can be measured using the Greiss reagent. Nitric oxide scavengers compete with oxygen, thereby reducing nitric oxide production. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was combined with 3.0 mL of different concentrations of the ethanolic and aqueous extract (200 - 1000 µg/mL) and incubated at 25 °C for 150 min. The samples were then added to the Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and the subsequent coupling with naphthyl ethylenediamine was read at 546 nm, and it was compared with the absorbance of standard solutions of ascorbic acid treated in the same manner with Greiss reagent as a positive control. The percentage of inhibition was determined using the following equation [16]:

$$\text{Inhibition percentage} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} \times 100$$

#### Reducing power assay

The reducing power was assessed using the procedure described by Berker et al [17]. The ethanolic and aqueous extracts (100–500 µg/mL, 2.5 mL) were combined with 2.5 mL of 200 mM sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide, and the resulting mixture was incubated at 50°C for 20 min. Following the addition of 2.5 mL of 10% trichloroacetic acid, the reaction mixture was centrifuged at 3000 rpm for 10 min. Approximately 5 mL of the upper layer was then mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance corresponded to a higher reducing power.

#### Result

##### Phytochemical screening

Phytochemical screening of the crude ethanolic and aqueous extract of fruits of *Lagenaria siceraria* revealed the presence of flavonoids, saponins, glycosides and phenolic compounds. Total phenolic content found to be 5.3%.

##### DPPH radical scavenging assay

The DPPH radical is treated as a model for a lipophilic radical. In lipophilic radicals, a chain reaction is initiated by lipid autoxidation. DPPH is a

stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule [18]. The reduction ability of DPPH was evaluated from the reduction in its absorbance at 517 nm, which is caused by antioxidants. A positive DPPH test indicates that the samples act as free radical scavengers. At concentrations of 25, 100 and 200 µg/ml of the ethanolic extract, the percentage inhibition was 12.22, 36.55 and 71.22%, respectively, whereas the aqueous extract showed percentage inhibition values of 9.48, 25.45 and 57.22%, respectively. However, the scavenging activity of ascorbic acid at the same concentrations was 33.12, 71.12 and 97.79%. The IC<sub>50</sub> values of Standard ascorbic acid, ethanolic and aqueous extract of *Lagenaria siceraria* was found at the concentration of 85.61µg/ml, 139.43µg/ml and 190.59 µg/ml.

Table 1: Results of in-vitro antioxidant activity by DPPH method

| Conc.<br>(µg/ml) | % Inhibition  |                   |                 |
|------------------|---------------|-------------------|-----------------|
|                  | Ascorbic Acid | Ethanolic extract | Aqueous extract |
| 25               | 33.12         | 12.22             | 9.48            |
| 50               | 49.28         | 19.23             | 14.49           |
| 75               | 63.36         | 26.63             | 19.13           |
| 100              | 71.12         | 36.55             | 25.45           |
| 125              | 79.13         | 43.67             | 29.46           |
| 150              | 90.63         | 54.43             | 37.42           |
| 175              | 93.38         | 62.97             | 45.22           |
| 200              | 97.79         | 71.22             | 57.22           |

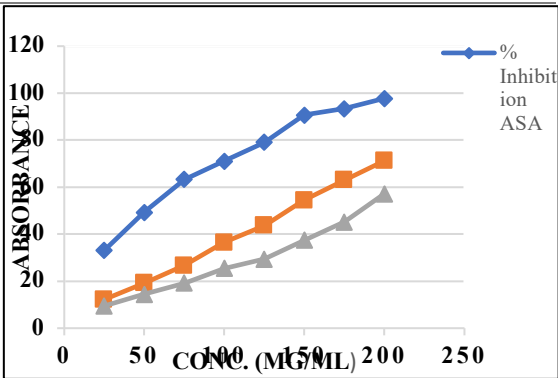


Fig 1: DPPH Radical Scavenging Activity of Extracts

#### Nitric oxide radical scavenging

Nitric oxide plays an important role in various types of inflammatory processes in the animal body. In the present study, the crude ethanolic extract of the *Lagenaria siceraria* was evaluated for its inhibitory effect on nitric oxide production. Nitric oxide generated from sodium nitroprusside at physiological pH was found to be inhibited. The *Lagenaria siceraria* extract at different concentrations exhibited a marked inhibitory effect on nitric oxide radical scavenging activity. The

aqueous extract of *Lagenaria siceraria* effectively reduced the generation of nitric oxide from sodium nitroprusside. The absorbance of the chromophore was measured at 546 nm in the presence of the extract. At concentrations of 25, 100 and 200 µg/ml of the ethanolic extract, the percentage inhibition was 17.29, 49.18 and 83.42%, respectively, while the aqueous extract showed percentage inhibition values of 10.93, 35.22 and 67.12%, respectively. In contrast, the scavenging activity of ascorbic acid at the same concentrations was 33.03, 69.23 and 95.55%. The IC<sub>50</sub> values for standard ascorbic acid, the ethanolic extract, and the aqueous extract of *Lagenaria siceraria* were found to be 88.81 µg/ml, 104.62 µg/ml and 146.09 µg/ml, respectively.

Table 2: Results of in-vitro antioxidant activity by Nitric oxide method

| Conc.<br>(µg/ml) | % Inhibition  |                   |                 |
|------------------|---------------|-------------------|-----------------|
|                  | Ascorbic acid | Ethanolic extract | Aqueous extract |
| 25               | 33.03         | 17.29             | 10.93           |
| 50               | 47.33         | 30.36             | 18.17           |
| 75               | 59.89         | 39.21             | 27.25           |
| 100              | 69.23         | 49.18             | 35.22           |
| 125              | 74.26         | 59.17             | 43.12           |
| 150              | 85.63         | 68.12             | 52.22           |
| 175              | 91.56         | 77.27             | 59.13           |
| 200              | 95.55         | 83.42             | 67.12           |

Fig 2: Nitric Oxide Scavenging Activity of Extracts

Table 3: Results of in-vitro antioxidant activity by reducing power method

| Conc.<br>(µg/ml) | Absorbance    |                   |                 |
|------------------|---------------|-------------------|-----------------|
|                  | Ascorbic acid | Ethanolic extract | Aqueous extract |
| 25               | 0.028         | 0.029             | 0.026           |
| 50               | 0.037         | 0.038             | 0.031           |
| 75               | 0.058         | 0.052             | 0.039           |
| 100              | 0.075         | 0.061             | 0.047           |
| 125              | 0.089         | 0.078             | 0.059           |
| 150              | 0.098         | 0.089             | 0.065           |
| 175              | 0.135         | 0.104             | 0.072           |
| 200              | 0.169         | 0.124             | 0.078           |

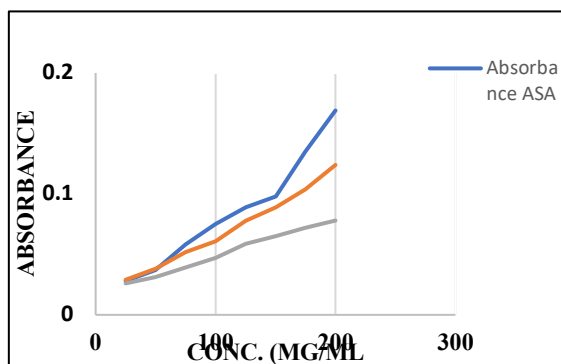


Fig 3: Reducing Power Capacity of Extracts

#### Reducing power assay

To identify the active species capable of donating hydrogen and thereby contributing to reducing power activity, the reducing power activity was determined. The observed high reducing power suggests the hydrogen-donating ability of the active species present in the extract. The antioxidant potential of the ethanolic and aqueous extracts of *Lagenaria siceraria* was assessed using the potassium ferric cyanide reduction method. In the present study, the reducing power of the ethanolic extract of *Lagenaria siceraria* was found to be excellent and increased steadily in direct proportion to rising extract concentrations. The absorbance values for the reducing power of the ethanolic extract at concentrations of 25, 100 and 200 µg/ml were 0.029, 0.061 and 0.124, respectively, which were relatively higher than those of the aqueous extract, where corresponding values at 25, 100 and 200 µg/ml were 0.026, 0.047 and 0.078, respectively. Compared with the standard ascorbic acid at concentrations of 25, 100, and 200 µg/ml, the values were 0.028, 0.075 and 0.169 respectively.

#### Conclusion

In conclusion, the study confirms that both ethanolic and aqueous extracts of *Lagenaria siceraria* fruits possess significant antioxidant activity. The ethanolic extract consistently demonstrated higher efficacy in scavenging free radicals and exhibited stronger reducing power than the aqueous extract. This antioxidant potential is likely linked to the presence of phenolic compounds and other bioactive secondary metabolites. These results provide scientific evidence supporting the traditional medicinal use of the plant and highlight its potential as a natural, safe alternative to synthetic antioxidants for managing oxidative stress-related conditions. Further studies are required to isolate the specific bioactive molecules responsible for these effects.

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