Determination of Polyphenolic Content and In-vitro Antioxidant Capacity of the Leaves of *Lagenaria siceraria* (mol.) standl

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**Abstract**

The leaves of *Lagenaria siceraria* (bottle gourd), belonging to the family cucurbitaceae being used for the treatment of jaundice and also claimed to be effective in many other diseases. The present research work was undertaken to investigate the in-vitro antioxidant activity of aqueous and ethanolic extracts. The free radicals are capable of independent existence and cause oxidative tissue damage. The therapeutic effects of tannins and flavonoids can be largely attributed to their antioxidant properties. So that the quantitative determinations were undertaken, the total Phenolic content of aqueous and ethanolic extracts showed the content values of 3.75 ± 0.22% w/w and 22.12±1.23%w/w respectively and total flavonoids estimation of aqueous and ethanolic extract showed the content values of 1.33±0.08%w/w and 3.61± 0.32%w/w respectively . Further investigation were carried out for In-vitro antioxidant activity and Radical scavenging assay by calculating its %inhibition by means of IC₅₀ values (it is nothing but inhibition concentration to obtain 50% of maximum scavenging capacity), all the extracts concentration has been adjusted to come under the linearity range and here many reference standards like Tannic acid, Gallic acid, Quercetin, Ascorbic acid have been taken for the method suitability. The results revealed that the leaves of this plant have antioxidant potential . Among these results ethanolic extract has more potent than traditionally claiming aqueous decoction. In conclusion that *Lagenaria siceraria* leaves possesses the antioxidant substance which may be potential responsible for the treatment of jaundice and other oxidative stress related diseases.

**Keywords:** *Lagenaria siceraria* leaves (L.S), Total Phenolic Content (TPC), Ferric reducing antioxidant power (FRAP), radical scavenging assay (DPPH – RSA).

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**INTRODUCTION**

*Lagenaria siceraria* commonly known as Bottle gourd called as Doodhi in Gujarati, Lauki (Hindi), Kadoo (Marathi) which is official in Ayurvedic Pharmacopoeia. It is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health(1). The archaeological evidences suggest that *Lagenaria* is not a monotypic genus and has an ancient pan tropical distribution. Two varieties of this fruit drug sweet and bitter are mentioned. Botanically, both belongs to the same genus, the sweet varieties is generally used as a vegetable, while the wild Variety bitter, preferred for the medicinal use. The difficulty in procuring and loosing interest in cultivation of wild variety, the sweet and edible variety is now being used in medicine as well(2). Leaves of *Lagenaria siceraria* are taken as emetic in the form of leaf juice or decoction. This by adding sugar also used in Jaundice. Crushed leaves are used for baldness and applied on the head for the headache. Leaves are also used as alternative purgative (3-4). The edible portion of fruits is fair source of ascorbic acid, beta carotene and good source of vitamin B complex, pectin dietary soluble fibers and contains highest source of choline level-anisotropic factor, a healer of mental disorders, along with required metabolic and metabolite precursors for brain function, amongst any other vegetable known to man till date. It is also good source of minerals and amino acids (5-6). The fruit is reported to contain the triterpeniode cucurbitacins B, D, G, H and 22-deoxycucurbitacin “the bitter principle of cucurbitaceae”. The fruit juice contains
betaglycosidedase-elasterase enzyme. Two sterols were identified and isolated from petroleum ether fractions of ethanol extract of dried fruit pulp of L.S namely Fucosterol and campesterol(7). HPLC analysis of extract of flowering plant of Lagenaria siceraria shows presence of flavone-C glycosides (8). The effect of semi purified dietary fibers isolated from the fruit of L.Seffects on fecal steroid excretion was reported(9). Although the L.S plant leaves carries high potential uses, especially for the treatment of jaundice, but the proper scientific studies have not been much reported for the leaves of this plant especially for an antioxidant perspective, an antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols(10).

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics (11). So that we got an interest to fulfill the paucity of studies by means of preliminary In-vitro antioxidant work which we have carried out in leaves portion of Lagenaria siceraria, here is our initiation for the future drug.

**MATERIAL AND METHODS**

**Chemicals and reagents**

Chemicals used in this study were 1, 1-diphenyl-2-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, U.S.A, Phosphomolybdic acid, sodium tungstate, potassium ferricyanide and sodium nitroprusside, naphthylethlenediamine dihydrochloride, sodium nitrite, trichloroacetic acid, ascobic acid, ethylenediamine tetraacetic acid, phosphoric acid, nitro bluetetrazolium, phenazine methosulfate, ferrous ammonium sulfate are obtained from Sd Fine Chemicals Ltd, India. All other reagents and solvents used in the study were of analytical grade.

**Plant material**

The leaves of Lagenaria siceraria (Family: cucurbitaceae) were collected with flowering top during the month of October from local cultivating field area of Mehsana district, Gujarat, India. The plant material was authenticated at the Department of Botany, Govt. Arts and science College Himatnagar, Gujarat. A voucher specimen as a herbarium (LS/RES/SSPC-05/2007) has been kept in our museum for future reference. The leaf parts were chopped and dried at room temperature for 10 days and used as raw material. The dried leaves of the drug have powdered using mechanical method and resulting powder was passed through the 40 # sieve and stored in the airtight container.

**PREPARATION OF RAW MATERIAL**

**Preparation of crude aqueous extract**

Then weighed accurately 100 gm of powder was taken in stainless steel vessel and mixed with 2000 ml (1:20) of distilled water. Then the mixture was boiled for about 2 hours using gas burner. After that, the mixture was filtered through cotton bag and then using vacuum filter assembly (or) fixed standard Whatman filter paper size No.1, here the filtrate must be poured in a borosilicate 500ml beaker. Then the filtrate was evaporated on hot plate until it reaches the concentrated quantity (do not be in viscous state)

**To prepare dry powder form of extract**

The dry powder of this extract was prepared by using the simple saloon water sprayer by spraying the extract on stainless steel evaporating plate, after the predetermined flow conditioned consistency thick solution was poured into the sprayer (here the above concentrated extract solution varies to nature of plant material), by which it was heated on hot plate at constant temperature of about 60°C. The clumpy dry powder obtained was scraped by the knife and made into fine powder form by using preheated mortar and pestle glass type from the plate and packed in air tight plastic container every steps must be carried out at above the room temperature (demerit of this method can be overcome by using hot air hair dryer) and stored in the freezer or in vacuum desiccator’s as such or in the form of stock solution prepared by the same solvent until further use. The preconditioned set method can be optimized by
evaluating the quantitative test of any existed constituents like tannins, flavonoids or any existed markers by suitable validated methods. This present study was undertaken by the spectrophotometrical method

**Preparation of ethanolic extracts**

Then weighed loading limit amount of 45.20 gm of powder of drug was packed in thimble flask and 550ml of ethanol (70%) was added in 1 liter round bottom flask. Then the Soxhlet assembly was set up to complete 10 to 15 cycles. After that the extract was filtered and filtrate was concentrated up to 50 ml using water bath. From the concentrated 10ml of extract was taken in evaporating dish (Borosilicate glass) which is previously weighed. The total weight of evaporating dish containing 10ml extract was recorded and the extract was evaporated till thick liquor was obtained. After then calculate the difference in weight was noted at every 10 min until the constant weigh was obtained. The residue at the constant weigh (it can be obtained from the graph %L.O.D) is used as dry extractives (12), which can be used to prepare the stock solutions (w/v). The weight of dry extract was 0.94 gm and the total yield was (4.5 gm) and the % yield would be 9.96% w/w and stored in the freezer until further use.

**QUALITATIVE PHYTOCHEMICAL SCREENING**

The shade dried leaves was coarsely powdered and extracted with different solvents like n-Hexane, Chloroform, Ethyl acetate, 70% Ethanol and Water successively. All the extracts were concentrated under reduced temperature and pressure to get dry residue. The different qualitative chemical tests were performed (13) for establishing profile of extracts and to detect the various phytoconstituents present in them. But the alcoholic and aqueous extracts showed much of the antioxidant potential constituents (14). So that our studies as carried out the extracts made by using these solvents only.

**ESTIMATION OF TPC BY SPECTROPHOTOMETER**

**By Folin – Denis Method**

The method is based on the oxidation of molecule containing --OH groups. The tannin and tannin like compound reduce Phosphotungustomolybdic acid in alkaline solution to produce a highly blue colored solution (15-16). 1ml of the aqueous and ethanolic extract that has adjusted to come under the linearity range i.e. (50μg/ml) of both the drugs was withdrawn in 10ml volumetric flask separately. To each flask 0.5ml of Folin-Denis reagent and 1ml of Sodium carbonate was added and volume is made up to 10ml with distill water. The absorbance was measured at absorption maxima 700nm within 30 minute of reaction against the blank. The total phenolic content was determined by using calibration curve (5 to 30μg/ml).Three readings were taken for each and every solution for checking the reproducibility and to get accurate result. Results are provided in (Table 2 and Figure 1).The intensity of the solution is proportional to the amount of tannins and can be estimated against standard tannic acid, the total phenolic content, expressed as mg tannic acid equivalents per 100 g dry weight of sample.

**TOTAL FLAVONOID CONTENT BY SPECTROPHOTOMETER**

**Aluminum chloride colorimetric assay method**

Total flavonoid contents were measured with the aluminum chloride colorimetric assay. (17).Aqueous and ethanolic extracts that has been adjusted to come under the linearity range i.e. (400μg/ml) and different dilution of standard solution of Quercetin (10-100μg/ml) were added to 10ml volumetric flask containing 4ml of water. To the above mixture, 0.3ml of 5% NaNO2 was added. After 5 minutes, 0.3ml of 10% AlCl3 was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distill water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Results are provided in (Table 3 and Figure 2).Total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per 100 g dry weight of sample.

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**Figure 1:** Results of Totalphenollic content. 
$R^2$ values represented mean data set of n+3

**Table 2:** Results of Totalphenolic content

<table>
<thead>
<tr>
<th>No</th>
<th>conc. of extracts</th>
<th>%w/w of total tannin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L.S Aqueous. 50μg/ml</td>
<td>13.75 ± 0.22</td>
</tr>
<tr>
<td>2</td>
<td>L.S Ethanolic. 50μg/ml</td>
<td>22.12 ± 1.23</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=3
Determination of Polyphenolic Content and In-vitro Antioxidant Capacity of the Leaves of *Lagenaria siceraria* (mol. standl)

**FRAP method**

The ferric reducing property of the extract was determined by (18) taking 1ml of different dilutions of standard solutions of Gallic acid (10 -100 μg/ml) or aqueous and ethanolic extract that has adjusted to come under the linearity range (500μg/ml) was taken in 10ml volumetric flasks and mixed with 2.5ml of potassium buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min. Then 2.5ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. To the 2.5ml of above solution 2.5ml of distill water is added and then 0.5ml of 0.1% of FeCl₃ was added and allowed to stand for 30min before measuring the absorbance at 593 nm. Results are provided in (Table 4 and Figure 3). The absorbance obtained was converted to Gallic acid equivalent in mg per gm of dry material (GAE/gm) using Gallic acid standard curve.

**SCAVENGING ACTIVITY ASSAYS**

**Nitric oxide scavenging assay**

Nitric oxide radical inhibition was estimated by the use of Griess Illosvory reaction (19-20). In this investigation, Griess Illosvory reagent was generally modified by using Naphthyl ethylene diamine dihydrochloride (0.1%w/v) instead of the use of 1-napthylamine (5%). The reaction mixture (3ml) containing 2ml of 10 mM sodium nitroprusside, 0.5ml saline phosphate buffer and 0.5ml of standard solution or aqueous and ethanolic extract of (500 -1000μg/ml) were incubated at 25°C for 150min. After incubation, 0.5ml of the reaction mixture was mixed with 1ml Sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5min for the completion of the reaction of diazotization. After that further 1ml of the Naphthyl ethylene diamine dihydrochloride was added, mixed and was allowed to stand for 30min at 25°C. The concentration of nitrite was assayed at 546nm and was calculated with the control absorbance of the standard nitrite solution (without extracts or standards, but the same condition should be followed). Here the blank is taken as the buffer and make up solvents and the Ascorbic acid and Quercetin (10 -50 μg/ml) was taken as standard. Results are provided in (Figure 4–7) . The percentage inhibition was calculated using the formula:

\[
%\text{Scavenging Activity} = \frac{A_{\text{control}} - A_{\text{test or A Std}}}{A_{\text{control}}} \times 100
\]

Where, \( A_{\text{control}} \) + absorbance of control  \\
\( A_{\text{test or A Std}} \) + absorbance of test or std

**Hydrogen Peroxide scavenging Assay**

The ability of extracts to scavenge hydrogen peroxide was determined (21) by little modification here the solution of hydrogen peroxide (30mm) was prepared instead of 40mM in phosphate buffer saline of (PH 7.4), at various concentration of aqueous and ethanolic extract (100 -1000 μg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was...
determination after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. In case of control takes absorbance of hydrogen peroxide at 230 nm without sample extracts. Results are provided in (Figure 8–10). The percentage inhibition activity was calculated from \[
\left(\frac{A_0 - A_1}{A_0}\right) \times 100,
\]
where \(A_0\) is the absorbance of the control and \(A_1\) is the absorbance of extract/standard taken as Gallic acid (10 - 100 μg/ml).
**DPPH –RSA method**

The free radical scavenging activity of aqueous and ethanolic extracts and the standard L-Ascorbic Acid (Vitamin C) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (22-23). Here, 0.1 mM solution of DPPH in alcohol was prepared and it must be protected from light influence by maintaining the dark condition and also fold by aluminum foil and 3 ml of this solution was added to 1 ml various conc. (100-2000 μg/ml) of extracts or standard solution of (10-100 μg/ml). Absorbance was taken after 30 min at 517 nm. Results are provided in (Figure 11–13).

![Figure 11: Results of DPPH radical scavenging assay](image1)

**Table 1: Results of Qualitative Phytochemical screening:**

<table>
<thead>
<tr>
<th>Tests</th>
<th>n-Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>70% ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycosides</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tannins</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Indicators:

+: Positive; ++: More Positive (intensity of color or ppt)

–: Negative

The percentage inhibition activity was calculated from \( \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \), where \( A_0 \) is the absorbance of the control and \( A_1 \) is the absorbance of extract/standard taken as Ascorbic acid.

**STATISTICAL ANALYSIS**

Values were represented as mean ± S.E.M of three parallel data’s.

**RESULTS AND DISCUSSION:**

**Results of Qualitative Phytochemical screening**

The qualitative analysis of the extracts was carried out and the results obtained were given in (Table 1). The results revealed that the presence of carbohydrates, glycosides, phytosterols, tannins and phenolic compounds, flavonoids.


**Effect of TPC & Flavonoid content**

The quantitative determination of the total phenolic content, expressed as mg tannic acid equivalents and per 100 g dry weight of sample TPC of L.S aqueous and ethanolic extracts showed the content values of 13.75±0.22%w/w and 22.12±1.23%w/w and total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per 100 g dry weight of sample the total flavonoids estimation of aqueous and ethanolic extracts showed the content values of 1.33 ± 0.08%w/w and 3.61 ± 0.32%w/w. The above results showed that aqueous contain less tannins and flavonoid content than the alcoholic extract. It may due to the solubility of principle contents presence be higher incase of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents.

**Capacity of FRAP method**

At low pH, measuring the change in absorption at 593 nm can monitor reduction of a ferric complex to the ferrous form, which has an intense bluish green color. The change in absorbance is directly related to the combined or “total” reducing power of the electron-donating antioxidants present in the reaction mixture. Here the FRAP showed the results of aqueous and ethanolic extracts that of 53.76 ± 0.28 mg equivalent to Gallic acid(GAE)/gm of sample and 66.53 ± 2.54 mg GAE/gm of sample respectively.

**Capacity of Nitric oxide scavenging assay**

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O\textsubscript{2} to produce the stable product nitrates and nitrite through intermediates through NO\textsubscript{2}, N\textsubscript{2}O\textsubscript{4} and N\textsubscript{3}O\textsubscript{4}. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, the %inhibition of aqueous and ethanolic extract of three parallel readings of (r\textsuperscript{2}+0.9796) showed that IC\textsubscript{50} values 938.92μg/ml and 805.85μg/ml (r\textsuperscript{2} + 0.9766) respectively as compared to the standard of Ascorbic acid and Quercetin of 43.71μg/ml (r\textsuperscript{2} + 0.9908) and 28.73μg/ml (r\textsuperscript{2} + 0.9954) respectively.

**Capacity of Hydrogen Peroxide scavenging**

H\textsubscript{2}O\textsubscript{2} itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H\textsubscript{2}O\textsubscript{2} is very important for protection of food systems. Scavenging of Hydrogen peroxide and its %inhibition of aqueous and ethanolic extract showed that IC\textsubscript{50} values 653.28μg/ml (r\textsuperscript{2} + 0.9932) and 341.55μg/ml (r\textsuperscript{2} + 0.9911) respectively. Gallic acid has taken as reference which showed 62.28μg/ml. (r\textsuperscript{2} + 0.9922)

**Capacity of DPPH –RSA**

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- Diphenyl-2- Picryl Hydrazine. The ability to scavenge the free radical, DPPH was measured at an absorbance of 517 nm. So the DPPH – RSA and its %inhibition of aqueous and ethanolic extract showed that IC\textsubscript{50} values 831.36μg/ml (r\textsuperscript{2} + 0.9957) and 561.18μg/ml (r\textsuperscript{2} + 0.9946) respectively. Ascorbic acid has taken as reference which showed 58.96μg/ml. (r\textsuperscript{2} + 0.9852) among these results ethanolic extract has more potent than traditionally claiming decoction.

The overall results of % inhibition as shown in the (Table 5) respective to IC\textsubscript{50} values and regression r\textsuperscript{2} is the mean value of (n+3).

<table>
<thead>
<tr>
<th>Table 5: Results and discussion of all the % Inhibition studies</th>
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<tr>
<td><strong>Results of % Inhibition</strong></td>
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<tr>
<td>% Inhibition by Nitric oxide assay</td>
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<td>% Inhibition by Hydrogen Peroxide assay</td>
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<tr>
<td>% Inhibition by DPPH-RSA</td>
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*Data set of n=3 and mean r\textsuperscript{2} values obtained from the graphs.
CONCLUSION

In conclusion that *Lagenaria siceraria* leaves possesses the antioxidant substance which may be potential responsible for the treatment of jaundice. So there are many scopes are there in leaves portion and more number of studies can be undertaken like oxidative stress hepatoprotective, anticancer activities and etc. In future we look forward to check the potency of the leaves by means of In-vivo antioxidant studies.

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