Invitro Antioxidant and Antibacterial analysis of *Lagenaria siceraria* peel

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ABSTRACT

The plant kingdom is a diverse kingdom in the world and most of the plants contain medicinal and pharmaceutical properties and wide range of uses in medicine, food and more. The unused parts of vegetable are the source of important phytochemicals like polyphenols, flavonoids, alkaloids, sugars, vitamins, minerals etc and have numerous pharmacological properties. The present study was carried out to screen the phytochemicals, free radical scavenging activity by DPPH method and antibacterial activity of 60% ethanolic extract of Lagenaria siceraria peel. All the analysis were carried out according to the standard methods. The 60% ethanolic extract of Lagenaria siceraria peel were screened for phytochemicals by standard procedures which revealed the presence of majority of secondary metabolites in higher concentration. The DPPH analysis of same extract shows that peel have more antioxidant activity at its highest concentration against standard L-ascorbic acid. The antibacterial activity by using well diffusion method shows a significant activity against the standard.

Keywords: *Lagenaria siceraria*, Ethanolic extract, Antioxidant, Antibacterial, Phytochemicals, L-ascorbic acid.

1.Introduction

The plant kingdom is a diverse kingdom in the world. There are about 320,000 plant species are known and most of the plants contain medicinal and pharmaceutical properties and wide range of uses in medicine, food and more. About 1,097 varieties of vegetables are grown for various purposes.

Vegetables are significant source of nutrients, minerals, dietary fibre, antioxidants and other phytochemicals.[1] In recent studies, numerous scientific examinations reported that the unused parts example peel of vegetable are the source of important phytochemicals like polyphenols, flavonoids, alkaloids, sugars, vitamins, minerals etc. These unused parts like peel have numerous pharmacological properties like antimicrobial, antifungal, antibacterial, anti-mutagenic, cardioprotective, antioxidant and neuroprotective properties.[2]

Lagenaria siceraria is a semi tropical plant but grown all over the world. It belongs to Cucurbitaceae family. [7] *Lagenaria siceraria* is commonly known as calabash gourd or bottle ground. Fruit appear in variety of different shapes and has a smooth, light green skin when young, but matures to yellow or light brown.[8]

Figure 1 (*Lagenaria siceraria* vegetable)

The objective of this study was to carry out the preliminary phytochemical analysis, Antioxidant and Antibacterial analysis. The phytochemical analysis of plants is very important in production of new drugs and commercial products.

2. Materials and Methods

2.1 Collection of Plant - Healthy fresh vegetable of *Lagenaria siceraria* were collected from the local market of Coimbatore district**.**

2.2 Preparation of Extract -The vegetable is washed with distilled water and the peel is collected. The collected peel is homogenised in a mixture with 60% of ethanol. The above extract is stored in refrigerator for further analysis.

2.3 Phytochemical Screening

The following phytochemicals were tested using 60% ethanolic extract of *Lagenaria siceraria* are listed in the table 1

PHYTOCHEMICALS	METHOD
Alkaloids	About 1 ml of extract was treated with 4-5 drops of Wagner's reagent. The formation of reddish brown precipitate confirms the presence of Alkaloids. [3]
Flavonoids	To about 1ml of extract, few drops of NAOH solution was added. formation of intense yellow color which turns to colorless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.[3]
Phenol	About 1ml of the extract was treated with 10% ferric chloride solution and observed for the formation of deep blue / black colour.[3]
Protein	To a 1 ml of extract added small amount of Ninhydrin reagent. A purple or violet colour formed indicates the presence of amino acids and proteins.[3]
Tannins	To about 1ml of extract added few drops of dilute ferric chloride solution. The presence of tannin is confirmed by the formation of dark green or blue colour.[3]
Glycosides	To 1ml of extract added few ml of concentrated sulphuric acid. Formation of red colour indicates the presence of glycoside.[3]
Phytosterol	To 1 ml of the extract was treated with 2 ml of chloroform and few drops of acetic anhydride were added. To that mixture added equal amount of concentrated sulphuric acid was added. The formation of bluish green colour indicates the presence of phytosterols [5]
Reducing sugar	To 1 ml of the extract added few drops of Fehling's reagent and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of blue colour.[3]
Steroids	About 2 ml of chloroform and 0.2 ml of concentrated sulphuric acid was added to 1ml of extract. The formation of red colour precipitate indicates the presence of steroids.[4]
Saponins	To 1 ml of the extract added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for few seconds. The presence of foam confirms the presence of saponins.[4]

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Table 1- phytochemicals tested and method used

2.4 *Invitro* Antioxidant Activity

2.4.1 Determination of Antioxidant Activity by DPPH Assay

DPPH (2,2-diphenyl-2-picrylhydrazyl) free radical scavenging assay of 60% ethanolic extracts of *Lagenaria siceraria* peel was determined according to the method by **[6]** with slight modifications. The reaction mixture consists of plant extract with the varying concentration (20,40,60,80and 100µl) and 1.0ml of DPPH was added and made upto 3.0ml with distilled water. The tubes were shaken well and incubated in the dark at room temperature for 30 minutes. A blue colour formed, and the absorbance was measured Spectrophotometrically at 518nm. Ascorbic acid was used as a standard for DPPH activity. The ability to scavenge DPPH radical was calculated using the formula

$$\% \text{ of Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5 *In-Vitro* Antibacterial Activity

The antibacterial activity of crude extract was determined by Well Diffusion method **[9]**. The stock culture of bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*) were received by inoculating in nutrient broth media and grown at 37 % for 18 hours. The agar plates of the above media were prepared. Cut the 5 wells Pour the extract in ratio 25 µl, 50 µl 75 µl 100 µl and 8 µl of standard. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm.

3. Result and Discussion

3.1 Phytochemical Analysis

PHYTOCHEMICALS	RESULT
Alkaloids	+++
Flavonoids	+++
Phenol	+++
Protein	++
Tannins	+++
Glycosides	+++
Phytosterol	+++
Reducing sugar	++
Steroids	++
Saponins	++

Table 2 (Phytochemical analysis of 60% ethanolic extract of *Lagenaria siceraria* peel)
"+++'' indicates Strongly Positive, "++" indicates Positive

The result of phytochemical analysis of 60% ethanolic extract of *Lagenaria siceraria* peel is presented in table 2. The result revels that all phytochemicals are present in the using 60% ethanolic extract of *Lagenaria siceraria.* Alkaloids, flavonoids, phenol, tannin, glycosides, phytosterol are present in higher amount.

3.2 Determination of *In Vitro* Antioxidant Activity

3.2.1 Evaluation 0f DPPH Analysis

Figure 2 shows that the free radical scavenging activity by DPPH analysis of 60% ethanolic extract of *Lagenaria siceraria* peel and standard ascorbic acid. The percentage of inhibition increases as the concentration of sample increases. The plant has significant antioxidant activity when compared with the standard L- ascorbic acid

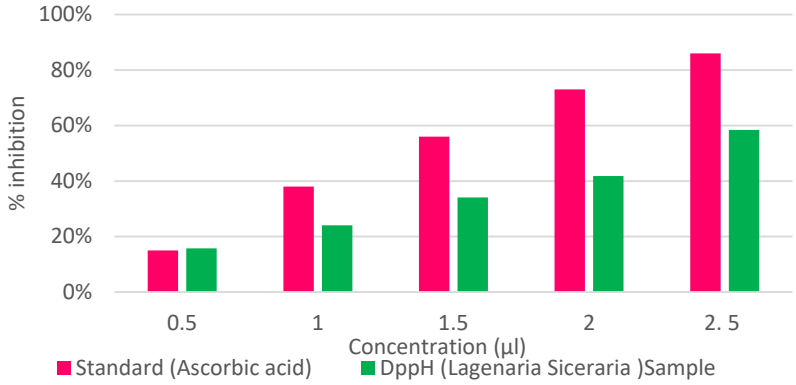

Figure.2 (DPPH radical scavenging activity of 60% ethanolic extract of *Lagenaria siceraria* peel)

3.3 Antibacterial Activity

The antibacterial activity of 60% ethanolic extract of *Lagenaria siceraria* peel is found good against gram positive bacteria and shows the zone of inhibition against both *Staphylococcus aureus*, *Streptococcus pyogene*. The concentration of the sample was taken as 25 µl, 50 µl, 75 µl, 100 µl respectively. Chloramphenicol, an antibiotic is used as standard. The activity that is zone of inhibition increases with the increase in concentration of sample, shows that ethanolic extract of *Lagenaria siceraria* peel shows significant antibacterial activity when compared with standard.

Table 3 (Anti-bacterial effect (zone of inhibition) of *Lagenaria siceraria* peel)

Name of Organism	Zone of Inhibition				
	Standard (mm)	25 µl (mm)	50 µl (mm)	75 µl (mm)	100 µl (mm)
Streptococcus pyogenes	**13**	**9**	**10**	**11**	**12**
Staphylococcus aureus	**10**	**3**	**5**	**6**	**9**

Figure 6
(Standard and *Lagenaria siceraria* peel Extract against *Streptococcus pyogenes*)

Figure 7
(Standard and *Lagenaria siceraria* peel extract against *Staphylococcus aureus*)

Conclusion

The findings of the current study are summarized as follows: Ethanolic extract of *Lagenaria siceraria* peel were found to contain Alkaloids, flavonoids, phenol, tannin, glycosides, phytosterol are in higher amount and protein, reducing sugar, steroid, saponin are in significant amount.Antioxidant activity by the DPPH, shows that Ethanolic extract of *Lagenaria siceraria* peel have significant antioxidant activity.Antibacterial activity by well diffusion method shows that Ethanolic extract of *Lagenaria siceraria* peel shows high antioxidant activity against gram positive organisms.

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