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IN VITRO ANTIOXIDANT ACTIVITY AND QUANTITATIVE ESTIMATION OF PHENOLIC CONTENT OF LAGENARIA SICERARIA

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ABSTRACT

The plant, Lagenaria siceraria (Family: Cucurbitaceae), known as bottle gourd, Calabash, Doodhi, and Lauki, is a common fruit vegetable used throughout the India. The antioxidant activities of different concentrations of ethanol extracts of fruits of Lagenaria siceraria the were determined by the four assay techniques i.e. DPPH radical scavenging assay, Reducing power ability, Hydrogen peroxide scavenging assay and thiocyanate method. Ethanol extract of fruits of Lagenaria siceraria has shown effective antioxidant activity in all assay techniques. The results obtained in the present study indicate that the fruits of Lagenaria siceraria are a potential source of natural antioxidants.

Key Words: Antioxidant activity, DPPH, reducing power, Thiocyanate, Lagenaria siceraria

INTRODUCTION

The plant, *Lagenaria siceraria* (Family: Cucurbitaceae), known as bottle gourd, Calabash, Doodhi, and Lauki, is a common fruit vegetable used throughout the India. *Lagenaria siceraria* is official in Ayurvedic Pharmacopoeia of India. *L. siceraria* fruits are traditionally used as a nutritive agent having cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion stings, alternative purgative, and cooling effects. It cures pain, ulcers, and fever and is used for pectoral-cough, asthma and other bronchial disorders.[1, 2, 3, 4]

The commercial development of plants as sources of antioxidants to enhance health is of current interest. I t has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease. Role of reactive oxygen species in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer is well known. Therefore, research for the determination of the natural antioxidants source is important. These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others (Rice-Evans et al. 1996).

EXPERIMENTAL

Extraction of Plant Material

The fresh fruits of *L. siceraria* were collected in the months of July-August from the local market of Amaravati, Maharashtra state, India, and authenticated by the authority of the botany department, VMV, Amaravati. A voucher specimen was submitted at Institute's herbarium department for future reference. Dried fruits were ground to coarse powder. Powder was first defatted with pet. Ether and then extracted with ethanol.

DPPH radical scavenging assay [5]

The free radical scavenging activity of the fractions was measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Mensor et al., 2001). About 0.3 mM solution of DPPH in 100% ethanol

was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a shimandzu spectrophotometer. The percentage scavenging activity at different concentrations was determined and the IC_{50} value of the fractions was compared with that of ascorbic acid (vit. C), which was used as the standard.

Reducing power ability [5]

The reducing power was assayed as described in kuda et al.2005 with some modifications. Different concentrations of ethanolic extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ^{o}C for 20 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power.

Hydrogen peroxide scavenging assays [6]

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the extracts was determined and the IC_{50} values were compared with the standard, vit. C.

Thiocyanate method [6]

The peroxy radical scavenging activity was determined by thiocyanate method using vit. C (50-800 µg/ml) as standard. Increasing concentration of the fractions (50-800 µg/ml) in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04M, pH 7) in a test tube and incubated in darkness at 37^{o}C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentage scavenging activity was calculated and the IC_{50} values of the fractions were compared with the standard, Ascorbic Acid. A control was also prepared replacing water with plant extract

Estimation of total phenolic content [5]

The assay used for the determination of total phenolics content employs Folin and Ciocalteu's phenol reagent which response depending on the chemical structure of phenolics (i.e. the higher the number of functional –OH group the higher the total phenolics content). Total soluble phenolic compounds in the ethanolic extracts were measured according to the method of Singleton et al. 1965 and expressed as gallic acid equivalents. A sample of the ethanolic extract was added to distilled water for a final volume of 2 ml. After, it was mixed with 0.3 ml of a saturated sodium carbonate (Na_2CO_3) solution and 0.1 ml of 1 N Folin–Ciocalteu's phenol reagent. The mixture was placed for 1 h at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of gallic acid equivalents.

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at three different concentrations of the fractions. Percentage inhibition (I %) was calculated using the formula,

$$I\% = \frac{(Ac-As)}{Ac} \times 100$$

Where, Ac is the absorbance of the control and as is the absorbance of the sample.

RESULTS AND DISCUSSION

Phytochemical screening:

Phytochemical screening of the crude ethanolic extract of fruits of *Lagenaria siceraria* revealed the presence of flavonoids, saponins glycosides and phenolic compounds. Total phenolic content found to be 79.43%.

DPPH radical scavenging method:

ROS produced *in vivo* include superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidising species, the hydroxyl radical. The antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1,1-diphenyl-2-picryl hydrazine with decoloration. The scavenging effects of extract increased with their concentrations to similar extents. The percentage inhibition of concentration 20, 40, 60 mg/ml are about 79.12, 87.34 and 91.23 % respectively as shown in table no. 1. The standard vit. C presented a scavenging effect of 96.12 % at the concentration of 60 mg/ml.

Reducing power method:

Table no. 2 shows the reducing power of the LS ethanolic extracts as a function of their concentration. In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl's Prussian blue at 700 nm, it is possible to determine the Fe^{2+} concentration. The reducing power of the LS ethanolic extracts increased with their concentrations. At 20, 40 and 60 mg/ml, reducing powers of both extracts were around 0.12, 0.17 and 0.22, respectively, while a solution of 40 mg/ml of vit. C, the positive control used in this test, had a reducing power value of 0.3.

Hydrogen Peroxide:

Extracts of fruits of *Lagenaria siceraria* scavenged hydrogen peroxide in a concentration-dependent manner. The ethanol extracts of fruits of *Lagenaria siceraria* showed strong H2O2 scavenging activity with 0.219 mg/ml IC_{50} value whereas that of the standard, vit. C was 0.152 mg/ml.

Thiocyanate method:

Results shown in table no. 3 obtained from FTC assay revealed that extracts of *Lagenaria siceraria* carry the antioxidative potential for chain-breaking inhibition of lipid peroxidation and for free radical scavenging as extract has shown 72, 81 and 93% inhibition.

Table-1:Results of DPPH radical scavenging assay

Compounds	Concentration	Absorbance	% Inhibition
EE	20	0.451	79.12
	40	0.332	83.34
	60	0.227	91.03
AA	20	0.305	85.90
	40	0.225	91.92
	60	0.122	98.12

Table-2: Results of reducing power ability assay

Drug	Concentration	Absorbance
EE	20	0.1247
	40	0.1723
	60	0.2289
AA	20	0. 23508
	40	0.28879
	60	0.33213

Table-3:Results of Ferric Thiocyanate assay:

Drug	Concentration	Absorbance	% Inhibition
EE	10	0.5475	73.45
	20	0.4130	81.12
	30	0.3020	93.95
AA	10	0.5524	72.10
	20	0.3598	87.34
	30	0.2791	96.78

Where, AA: ascorbic acid and EE: Ethanolic extract

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"There are two ways to live: you can live as if nothing is a miracle; you can live as if everything is a miracle."

- **Albert Einstein**