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Research Article

ANTIOXIDANT POTENTIAL OF RHIZOME OF ALOCASIA DECIPIENS SCHOTT

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ABSTRACT

In the present work, we made an attempt to assess the antioxidant potential of the rhizome of plant *Alocasia decipiens* Schott using methanol as solvent. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method and FRAP (ferric reducing antioxidant power) method were used to screen antioxidant activity. Natural antioxidants such as phenol and flavonoid content were also evaluated. It was observed that the DPPH IC_{50} value of the methanol extract was 14.78±0.95µg/ml. The total phenolic content of the methanol extract was calculated as 24.28±0.99mg TAE/g of extract whereas total flavonoid content of the extract was calculated as 38.33±1.05mg RE/g of extract.

Keywords: Antioxidant, *Alocasia decipiens*, flavonoid, phenolic

INTRODUCTION

Reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH^-), peroxyl radical (ROO.) and nitric oxide radical (NO.), attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury. ROS induce peroxidation of lipids (polyunsaturated fatty acids) generating secondary oxidants like heptanol and hexanal which contributes to oxidative rancidity, deteriorating the flavor of the food. These not only cause a loss in food quality but are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity. To overcome these problems a wide range of synthetic antioxidants (butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propyl gallate (PG) and butylatedhydroquinone) have been used as food preservatives. However, these synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, most consumers prefer additive-free foods or a safer approach like the utilization of more effective antioxidants of natural origin [1]. Recently, there have been increasing interests in discovery of natural antioxidants, especially those of plant origin. Natural antioxidants derived from plants, chiefly phenolics are of considerable interest as dietary supplements or food preservatives. Flavonoid is one of the main groups of phenolic compounds and many of them are reported to possess strong antioxidative characteristics [2]. In the present study, we have selected the plant *Alocasia decipiens* Schott, which is a member of family Araceae, to assess the antioxidant potential of its rhizome. We have also attempt to determine the phenolic and flavonoid content of the rhizome, as these are reported to be natural antioxidants present in plant

MATERIALS AND METHODS

Plant materials

The plant, *Alocasia decipiens* was identified by Central National Herbarium, Botanical Survey of India, Shibpur, Howrah-711103, West Bengal, India. The rhizome of the plant was selected for carrying out the experiment.

Extraction

The coarsely powdered shade dried rhizome of *Alocasia decipiens* was extracted in a Soxhlet apparatus with Petroleum ether followed by ethyl acetate and methanol. All the extracts were then concentrated separately by using a rotary evaporator. The methanolic extract was used to screen the antioxidant potential of the plant.

Qualitative Assay

Methanolic extract of the rhizome of selected plant was spotted on a silica gel coated Thin Layer Chromatographic plate and were run on 1:1 petrol-methanol ratio. The TLC plate was air dried and sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the colour changes were noted [3].

Quantitative Assay

DPPH radical scavenging activity

The free radical scavenging activity of the rhizome extract was determined by DPPH (2,2-diphenyl-1 picrylhydrazyl) method [4]. The crude methanolic extract of the rhizome is mixed with methanol to prepare the stock solution (40µg/ml). From this stock solution, five sample solutions were prepared to attain the concentrations 2.5µg/ml, 5µg/ml, 10µg/ml, 20µg/ml and 40µg/ml. Freshly prepared DPPH solution was added in each of this test sample and after 20 minutes absorbance was taken at 550 nm. Ascorbic acid was taken as positive control and the DPPH solution without sample solution was used as control. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. Methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation:

%DPPH Radical Scavenging Activity = [1-(As/Ac)]×100

Ac = absorbance of control, As = absorbance of sample solution

The % DPPH Radical scavenging Activity of the extract was plotted against different concentrations.

Ferric reducing antioxidant potential

The total antioxidant capacity of the rhizome was determined using iron III reduction method [5]. 1ml of sample solutions (2.5µg/ml, 5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml) were mixed with phosphate buffer (0.2M, pH6.6, 2.5ml) and 1% aqueous potassium hexacyanoferrate solution (2.5ml). After 30 minutes of incubation at 50^0c, trichloroacetic acid was added and the mixture was centrifuged at 3000rpm for 10 minutes. Then the upper layer solution was mixed with water and an aqueous ferric chloride (0.1%) solution. The absorbance was read at 700nm and ascorbic acid was used as positive control. The antioxidant content was determined by using a standard curve of ascorbic acid. The total antioxidant contents of rhizome extract was expressed as ascorbic acid equivalent antioxidant content (AEAC/g of extract).

Total phenolic assay

The concentration of phenolics in plant extracts was determined using spectrophotometric method[6]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic

solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of tannic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolic in extracts was expressed in terms of tannic acid equivalent (mg of TAE/g of extract).

Total flavonoid assay

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method [7]. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoid was read (mg/ml) on the calibration line; then, the content of flavonoid in extracts was expressed in terms of rutin equivalent (mg of RE/g of extract).

Statistical analysis

All data presented are means of three determinations along with standard errors. Statistical analysis used the MS Excel software to calculate ascorbic acid, rutin and tannic acid equivalent, to determine inhibition percentage and to establish linear regression equations.

RESULT

Total phenol content

The total phenolic content of the extract of *A. decipiens* was measured spectrophotometrically by the Folin-Ciocalteau method. The result was expressed as tannic acid equivalent (TAE) and the value was calculated as 24.28±0.99 mg TAE/g of extract.

Total flavonoid content

The total flavonoid content of *A. decipiens* was measured by spectrophotometrically by Aluminium chloride method. The result was expressed as rutin equivalent (RE) and the value was calculated as 38.33±1.05 mg RE/g of extract.

Antioxidant potential

The degree of colour change (yellow on purple background) on TLC plate indicates the presence of antioxidant in the rhizome extract of plant (from qualitative assay).

DPPH radical scavenging activity

The results of free radical scavenging potential of rhizome extract of *A. decipiens* at different concentrations tested by DPPH free radical scavenging method are shown in Fig 1. The methanolic extract of the rhizome extract exhibited a logarithmic dose dependent inhibition of DPPH activity with a 50% inhibition (IC_{50}) at a concentration of 14.78±0.95µg/ml. The corresponding IC_{50} value of the standard ascorbic acid was calculated as 9.13±0.89µg/ml.

Ferric reducing antioxidant power

The reducing power of rhizome extract of *A. decipiens* was compared with the standard ascorbic acid. Here, a linear increase in reducing power was observed in both the cases of extract and standard (Fig 2).The total antioxidant content of rhizome extract was determined by using a standard curve of ascorbic acid (y=0.06x+0.188, R^2 = 0.962) and the value was calculated as 23.86±1.45mg AEAC/g of extract.

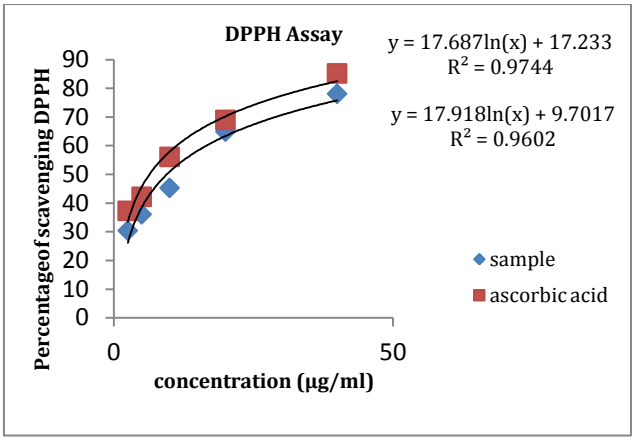

Fig 1: Antioxidant activity estimated by DPPH method.

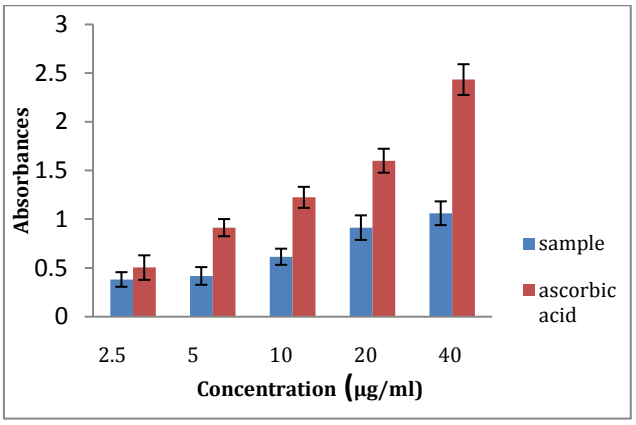

Fig 2: Antioxidant potential of *A. decipiens* estimated by FRAP method.

DISCUSSION

DPPH is a stable nitrogen-centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [5]. DPPH test is a direct and reliable method for determining radical scavenging action. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [8]. The rhizome extract of *A. decipiens* possess free radical scavenging potential as observed in fig 1, from where IC_{50} value has been calculated as 14.78±0.95µg/ml . The reduction ability of the plant extract was evaluated using the Fe^{3+}-Fe^{2+} transformation. The reduction capability of the extract indicated it as potent antioxidant. The outcome of the reducing reaction is to terminate the radical chain reactions that may be very damaging to the tissues. The yellow colour of the reaction mixture changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing ability of the extract can be measured by the direct reduction of $Fe[(CN_6)]_3$ to $Fe[(CN_6)]$, the addition of free Fe^3 to the reduced product leads to the formation of the intense prussian blue complex, $Fe_4[Fe(CN^-)_6]_3$, which show strong absorbance at 700 nm. It indicates the electron donating capacity of the extract. The increasing absorbance of the reaction mixture indicates the reducing capacity due to the increasing blue colour complex formation [9]. In the present study, a linear increase in reducing power (absorbances) has been observed in the methanolic extract with its increasing concentration (fig 2).Phenols have been reported as an active, quenching of oxygen-derived free radicals by donating hydrogen atom or an electron to the free radicals [10]. Total phenol content by Folin Ciocalteau Reagent and invitro antioxidant capacity assays, such as the DPPH and FRAP (which were used in this study), represent convenient methods for the identification of potential

sources of antioxidant compounds [1]. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [11]. As there is evidence of good correlation between total phenolic and DPPH IC_{50} values [12], so the antioxidant potential of plant extract observed, may be due to their phenolic compounds.

CONCLUSION

The result of the present study suggests that the methanol extract of rhizome of *Alocasia decipiens* Schott showed concentration dependent antioxidant activity (as observed in DPPH and FRAP assays). The *in vitro* studies indicate that this plant extract is a significant source of natural antioxidant. Isolation and characterization of compound rich in phenol and flavonoid from the plant may lead to the discovery of new antioxidant compound.

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