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Research Article

ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *Maranta arundinacea* .L TUBEROUS RHIZOMES

NISHAA.S[1], VISHNUPRIYA.M[1], SASIKUMAR.J.M[1], HEPHZIBAH P CHRISTABEL[1],GOPALAKRISHNAN.V.K[2]*

[1]**Department of Biotechnology, Karpagam University, Coimbatore,Tamilnadu, India – 641021,[2]Department of Biochemistry and Bioinformatics, Karpagam University, Coimbatore Tamilnadu, India – 641021.Email:** vkgopalakrishnan@gmail.com

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ABSTRACT

Free radicals contribute to more than one hundred disorders in humans. The ethanolic extract of *M.arundinacea* .L (Marantacea) was screened for antioxidant activity using 1,1-diphenyl-2-picryl hydroxyl (DPPH) quenching assay, 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) cation decolorization test, Reducing power, scavenging capacity towards hydrogen peroxide (H_2O_2) radical, nitric oxide (NO) radical inhibition activity using established assay procedures and Ferric Reducing Antioxidant Power (FRAP) Assay. The extract exhibited high antiradical activity against DPPH, ABTS, hydrogen peroxide and nitric oxide radicals with IC_{50} value of 293.4, 297.4, 336.1, and 258.7 µg/ml respectively. The Reducing power and Ferric Reducing Antioxidant Power (FRAP) increased with increasing concentration of the sample. The antioxidant activity of the sample was comparable with that of the standard butylated hydroxyl toluene (BHT).

Keywords: *Maranta arundinacea*, Antioxidant activity, DPPH, ABTS, FRAP, Nitric oxide scavenging activity.

INTRODUCTION

There is our days, an increasing interest in the measurement and use of plant antioxidants for scientific research as well as industrial (dietary, pharmaceutical and cosmetic) purposes. This is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis[1]. Therefore, the need exits for safe, economic, powerful, and natural antioxidants to replace these synthetic ones[2]. Many plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. Epidemiological studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases[3,4,5]. Therefore, in recent years it is considered to be an important task in evaluating plant antioxidant activity and their free radical quenching ability.

Arrowroot (*Maranta arundinacea* L.) is an herbaceous rhizomatous plant cultivated widely in tropical countries for its starchy roots. The West Indian Arrowroot (*Maranta arundinacea* L.), a native of tropical America, is widely cultivated in countries like India, Srilanka, Indonesia, Australia and Philippines. The edible tuberous rhizomes are rich in starch and are also a commercial source of fine grade starch used often in weaned foods and biscuits. The starch also is reported to have medicinal uses and is an important ingredient in the preparation of barium meals and tablets. The plant which grows under shade is resistant to many insects and pathogen attack. Traditionally the tuberous rhizomes are used in the treatment of diarrhea. The objective of this research was to evaluate the antioxidant activity of ethanolic extract of arrowroot extracts *in vitro*.

MATERIALS AND METHOD

Plant material

The tuberous rhizomes with leaves and flowers of *M. arundinacea* L. were collected in December from Malampuzha, Palakkad District, Kerala and identified by Dr.G.V.S.Murthy. A voucher specimen was deposited in the herbarium of the Botanical Survey of India, Coimbatore with herbarium code number No.BSI/SRC/5/23/10-11/Tech. Plant materials were washed with distilled water and dried at room temperature. The dried rhizomes were manually ground to a fine powder.

Preparation of extracts

The coarsely powdered tuberous rhizomes of *M.arundinacea* L. were extracted sequentially with petroleum ether, ethyl acetate and ethanol by cold percolation method. Based on the yield the ethanolic extract was used for the study.

Assessment of antioxidant activity

DPPH Radical Scavenging Activity

This test was measured as described by Blois[6]. One milliliter of the fraction solutions (100, 200,300,400 and 500 µg/ml in ethanol) was added to 1ml of a DPPH solution (0.2mM in ethanol). After a 30 min of reaction at room temperature, the absorbance of the solution was measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

ABTS radical scavenging activity

ABTS radical-scavenging activity of the extract was determined according to Re *et al*[7]. The $ABTS^{\cdot+}$cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The plant extract at various concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 µl of $ABTS^{\cdot+}$ solution and 50 µl of BHT. As for the antiradical activity, ABTS scavenging ability was expressed as IC_{50} (µg/ml). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Reducing power assay

Reducing power was determined by the method prescribed by Oyaizu *et al*[8]. The sample in 1ml of methanol at various concentrations was mixed with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%), and the mixture was incubated at 50°C for 20 min. Next, 5ml of trichloroaceticacid (10%) were added to the reaction mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (5 ml) was

mixed with distilled water (5ml) and ferricchloride (1 ml, 1%), and the absorbance was measured at 700 nm. A stronger absorbance will indicate increased reducing power.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch *et al*[9]. A solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The extract at different concentrations in 3.4 ml phosphate buffer was added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm.

$$H_2O_2 \text{ scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions, which can be estimated using Griess Illosvosy reaction[10]. Scavengers of NO compete with oxygen, leading to reduced production of NO and a pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated as

$$NO \text{ scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain [11]. The FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) TPTZ solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations (100,200,300,400 and 500 µg/ml) was then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample (DW).

RESULT AND DISCUSSION:

Polyphenols are a large and diverse class of compounds, many of which occur naturally in a wide range of food and plants. The flavonoids are the largest and best studied group among polyphenols. A range of plant polyphenols is either being actively developed or already currently sold as dietary supplements and/or herbal, derived medicines. Although these compounds play an unknown role in nutrition (non-nutrients), many of them have properties including antioxidant,anti-mutagenic,anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome[12]. Antioxidant quality is a measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture[13].The percentage scavenging and IC_{50} values were calculated for all models.

DPPH Radical Scavenging Activity

The reactivity of ethanolic extract of *M.arundinacea* extract was analyzed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stechiometrically related to the number of electrons gained[14].The DPPH radical scavenging (%) activity is shown in the Fig 1, *M.arundinacea* extract exerted an inhibition of 74.06% and that of BHT was 89% at 500µg/ml and the IC_{50} of the extract was 293.4µg/ml, while that of BHT was 226.7µg/ml. In the previous study the rhizomes of *Costus pictus* D. Don ethanolic extract showed an inhibition of 64% at 400µg/ml[15].

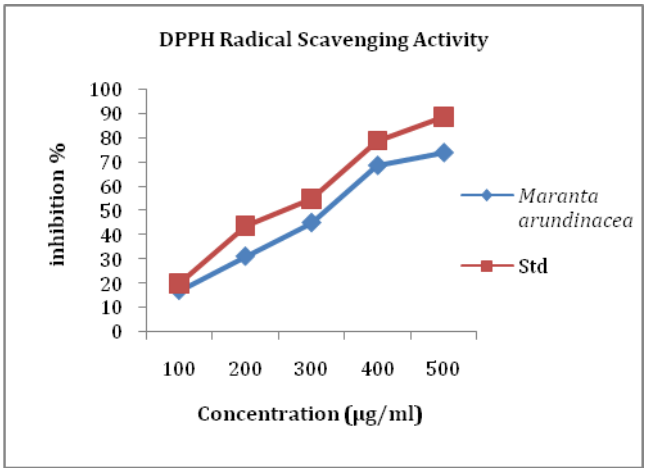

Fig 1:DPPH Radical Scavenging Activity

ABTS radical scavenging activity

The ABTS·+ scavenging assay, which employs a specific absorbance (734 nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of the test samples[16]. In Fig. 2, *M.arundinacea* extract was found to be effective in scavenging radicals and the increase was concentration-dependent. At 500µg/ml, the inhibition of the extract was 69.45% and that of BHT was 72.10%. The IC_{50} of BHT was 255.7µg/ml while the plant extract was 297.4µg/ml. This shows that *M.arundinacea* extract presents a good ability to scavenge the ABTS radical. The flowers of *Tamarix* methanolic extracts showed the ABTS quenching activity with IC_{50} value 316.7 µg/ml[17]. The antioxidant activities against ABTS or DPPH were correlated with the concentration, chemical structures, and polymerization degrees of organ antioxidants[18].

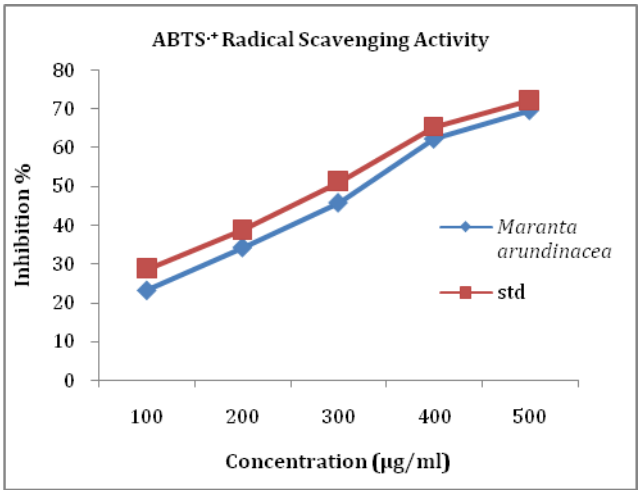

Fig 2:ABTS radical scavenging activity

Reducing power assay

The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of $Fe^{3+}(CN^-)_6$–$Fe^{2+}(CN^-)_6$ [19]. The product was visualized by forming the intense Prussian blue color complex and then measured at λ700nm. As shown in Fig. 3, a higher absorbance value indicates a stronger reducing power of the samples. *M.arundinacea* extract showed concentration-dependent reducing power. However, its reducing power was weaker than that of BHT, which exhibited the strongest reducing power. The reducing power activity of 70% ethanolic extract tubers of *Momordica tuberosa* at 50 and 100 µg were significantly higher than the standard,sodium metabisulphate[20]. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species[21].

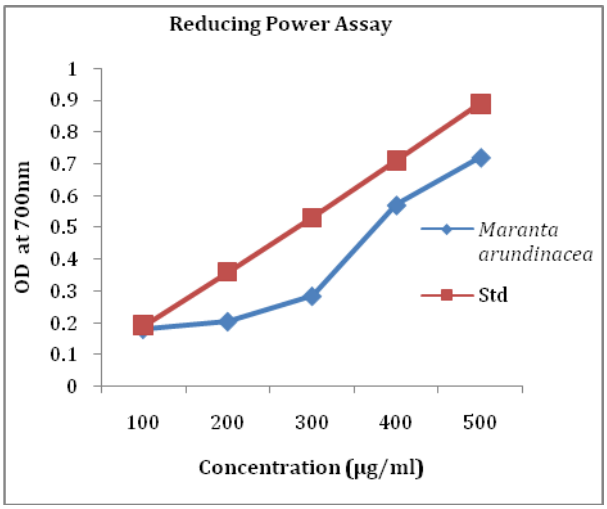

Fig 3:Reducing power assay

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects[22]. Hydrogen peroxide scavenging activity of the extract is presented in Fig. 4; the extract exerted a concentration dependent scavenging. *M.arundinacea* extract showed a maximum activity of 69.49 % inhibition very much comparable to that of BHT with an activity of 71% at the same concentration of 500µg/ml. The 30 mg/ml of Monodesmosides and Crude extract of *Leontice smirnowii* tubers exhibited 85% and 79% scavenging activity on hydrogenperoxide, respectively. The IC_{50} value of the extract was 258.7µg/ml, whereas the standard exerted an IC_{50} of 169.5µg/ml[23].

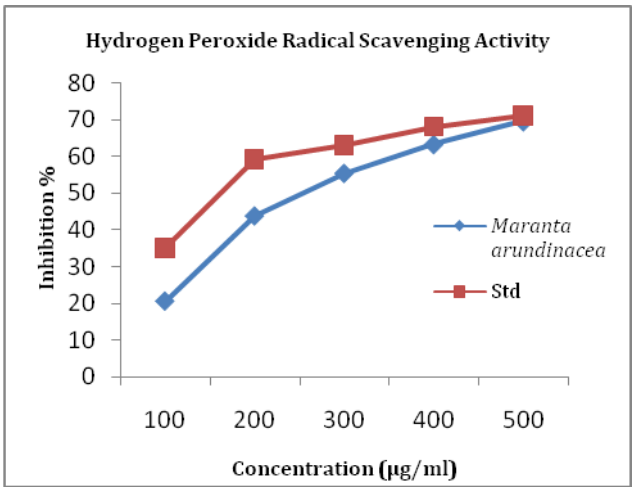

Fig 4:Hydrogen peroxide scavenging activity

Nitric oxide scavenging activity

Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumour activities[24]. Suppression of released NO may be partially attributed to direct NO scavenging, as the *M.arundinacea* extract decreased the amount of nitrite generated from the decomposition of SNP *in vitro* .The scavenging of NO by the extract was increased in concentration dependent manner. Fig 5 illustrates a significant decrease in the NO radical due to the scavenging ability of extract and BHT. The ethanolic extract of *M.arundinacea* showed maximum activity of 62.96% at 500µg/ml, whereas BHT at the same concentration exhibited 87% inhibition. The IC_{50} values were found to be 248.4 and 336.1 for BHT and the extract respectively. The inhibition % and IC_{50} value of aqueous extract and methanol extract, of *Amorphophallus campanulatus* tubers were 66.94% and 62.97% and 70.20 2g/ml and 77.02 2g/ml respectively[25].

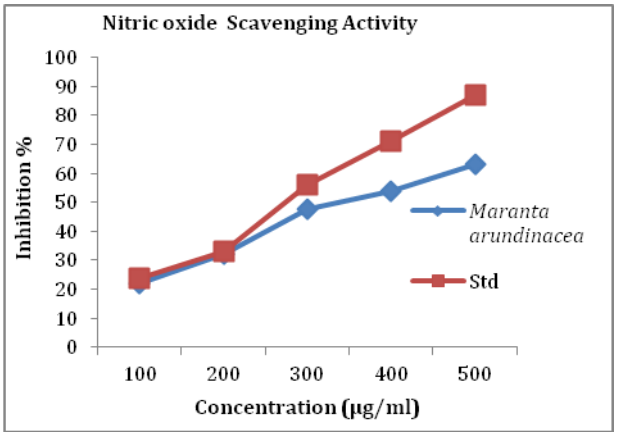

Fig 5: Nitric oxide scavenging activity

FRAP Assay

Frap assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe^{3+}-TPTZ] complex and producing a coloured ferrous tripyridyltriazine [Fe^{2+}-TPTZ][11]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom[26].Frap assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction[27]. In the present study, the trend for ferric ion reducing activities of *M.arundinacea* and BHT are shown in Fig 6. The absorbance of *M.arundinacea* clearly increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. The water and ethanol extracts of sumac (Rhus.coriaria L.) showed increased ferric reducing power with the increased concentration as standard antioxidants[28]. Hence they should be able to donate electrons to free radicals stable in the actual biological and food system.

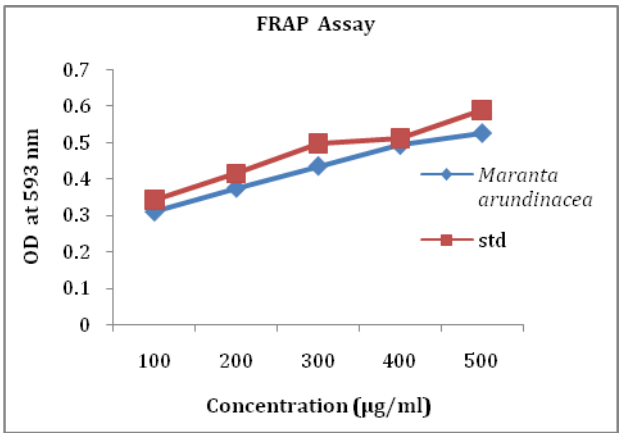

Fig 6:FRAP Assay

The ethanolic extract of *M.arundinacea* was found to be an effective scavenger of ABTS, DPPH, H_2O_2, and NO and also possessed a good reducing power and Frap activity. Earlier reports on the antioxidant activity of *M.arundinacea* are very rare in the literature. Therefore, it is very difficult to compare our results with that of previous studies. The high antioxidant activity of *M.arundinaacea* enhanced the potential interest in these under-exploited tubers for improving the efficacy of different products as nutraceutical and pharmacological agents. The consumption of the arrowroot may play a role in

preventing human diseases in which free radicals are involved, such as cancer, cardiovascular disease, and aging.

We conclude that, the results presented indicate that *M.arundinacea* extract attenuated oxidative stress via its antioxidant properties. However, further investigations on phenols, flavonoids, active principle, their *in vivo* antioxidant activity, and the different antioxidant mechanism are warranted.

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