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Research Article

ANTIOXIDANT ACTIVITY OF RIPE PEPINO FRUIT (*SOLANUM MURICATUM* AITON)

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ABSTRACT

The aim of the study was to evaluate the antioxidant activity, phenol and flavonoid content of ethyl acetate extract of ripe pepino fruit. The antioxidant activities were examined by seven different methods, DPPH, reducing power, iron chelation, ABTS, OH, phosphomolybdenum, and FRAP assays. In all the methods the extract exhibited good scavenging activity. The EC_{50} values of ripe ethyl acetate extract on DPPH radical, reducing power, ferrous ion chelation, ABTS radical, FRAP and hydroxyl radical were obtained to be 0.16, 0.82, 39.51, 9.53, 1.06 and 0.26 mg/ml, respectively. Considerable amount of phenol and flavonoids was noticed. This study suggests that possible mechanism of the biological activities may be due to free radical scavenging and antioxidant characteristics which may be due to the presence of polyphenols in the fruit extracts.

Keywords: Pepino fruit, Total phenols, Flavonoids, Antioxidant, Scavenging activity

INTRODUCTION

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, such as, cancer, cardiovascular disease, Alzheimers, autoimmune disease, diabetes, multiple sclerosis and arthritis[1]. Free radicals are highly reactive particles with an unpaired electron and are produced by radiation or as by-products of metabolic processes. They initiate chain reactions, which lead to disintegration of cell membranes and cell compounds, including lipids, proteins and nucleic acids[2]. A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress[3]. Oxidative stress is related to the aging process and some chronic diseases such as cancer, cardiovascular diseases and diabetes[4]. Dietary antioxidants protect the body against free radicals. Epidemiologic studies have indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of a number of human diseases[5]. However, some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene need to be replaced with natural antioxidants due to their potential health risks and toxicity[6].

The protection offered by fruits and vegetables against oxidative stress in several diseases has been attributed to various antioxidants and vitamins. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, and anticarcinogenic activities[7]. Therefore search for natural antioxidants of plant origin gained momentum in recent years.

The pepino fruit (*Solanum muricatum* Ait.), which is an exotic fruit, is also known as melon pear and sweet cucumber. Although it is native to South America, it is also grown in Australia, New Zealand and USA. It contains a high percentage of their fresh weight as water (92%), it is low in calories, very rich in minerals and contains vitamins like thiamine, niacin, riboflavin and ascorbic acid (vitamin C), ideal for a number of metabolic and antioxidant reactions[8]. The aim of this study was to investigate the $DPPH^{\cdot}$ radical scavenging, $ABTS^{\cdot+}$ radical, OH radical scavenging, reducing power, ferrous ions chelating activity, phosphomolybdenum assay and FRAP assay of ethyl acetate extract of pepino fruit. The phenol and flavonoid content were also determined.

MATERIAL AND METHODS

Fruit samples

The ripe pepino fruits were obtained from a farm in The Nilgris. The fruits were carefully selected in order to obtain a uniform batch in relation to size and degree of maturity.

Sample extraction

The fruits were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 50°C. All samples, after drying, had water contents below 10%. They were ground to a fine powder in a mechanical blender and kept at room temperature prior to extraction. 10 g of the sample were extracted by using a Soxhlet extractor for 3 h with 100 ml of ethyl acetate under reflux conditions. The extract was then rotary evaporated at 40°C to dryness. The extract was stored at 4°C for further use. Analyses were carried out in triplicate.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and rutin were obtained from Sigma Co. (St. Louis, MO, USA). 2,2-azino-bis-(3-ethylbenzothiazoline- 6-sulphonic acid) (ABTS), ferrozine, gallic acid, 2, 4, 6-tripyridyl-s-triazine (TPTZ) and ascorbic acid were obtained from Himedia, Mumbai. Potassium ferricyanide, ferric chloride, trichloroacetic acid, aluminium chloride, potassium persulphate, ammonium persulphate, ferrous suphate, sodium salicylate, ammonium molybdate, sodium carbonate, aluminium chloride, sodium nitrate, sodium hydroxide, Folin-Ciocalteu's phenol reagent, ferrous chloride, sodium hydroxide and solvents were obtained from Merck, Mumbai.

Phytochemicals

Estimation of total flavonoid content

Total flavonoid content was determined as described by[9]. 0.25 ml of various extracts was diluted with 1.25 ml of distilled water. 75 µl of a 5% $NaNO_2$ solution were added and after 6 min 150 µl of a 10% $AlCl_3.H_2O$ were added and mixed. After 5 min, 0.5 ml of 1 M NaOH was added. The absorbance was measured immediately against the prepared blank at 510 nm. Rutin was used as a standard and the results were expressed as mg of rutin equivalents (RE) per g of dry extract.

Determination of total phenolic content

Total phenol content was determined by the method adapted from[10] with some modifications using the Folin-Ciocalteu reagent. 1 ml of the extract was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated Na_2CO_3 (35%) was added to the mixture and it was made up to 10 ml by adding deionised distilled water. The mixture was kept for 90 min at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content is expressed as mg of gallic acid equivalents (GAE) per gram of dry extract.

Antioxidant capacity

Phosphomolybdenum assay

The antioxidant activity of the sample was evaluated by the phosphomolybdenum method according to the procedure of [11]. An aliquot of 0.1 ml of sample solution was mixed with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped with silver foil and incubated at 95°C for 90 min. The tubes were cooled to room temperature and the absorbance of aqueous solution was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed nM gallic acid equivalents (GAE) per gram of dry extract.

DPPH radical scavenging activity

The scavenging effect of fruit extracts on DPPH radicals was determined according to the method of [12]. Various concentrations of sample (4 ml) were mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. The percentage inhibition was calculated according to the formula : $(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

Determination of reducing power

The reducing power of fruit extracts was determined according to the method of [13]. 2.5 ml of various concentrations of the extract, 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml and 2.5 ml of 1% potassium ferricyanide were mixed and incubated at 50°C for 20 min and centrifuged for 10 min at 5000 g after addition of 2.5 ml of 10% trichloroacetic acid. 2.5 ml aliquot of supernatant was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1% ferric chloride. After 10 min of incubation, the absorbance was measured at 700 nm against a blank.

Chelating effects on ferrous ions

The ability of the fruit extracts to chelate ferrous ions was estimated by the method of [14]. Briefly, 2 ml of various concentrations of the extracts in methanol were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). The mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control, and A_1 of the mixture containing the extract or the absorbance of a standard solution.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was performed with slight modifications described by[15]. The $ABTS^{\cdot+}$ cation radicals were produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.700 ± 0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 10 µl of test sample with 1.0 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly after 6 min. The percentage inhibition was calculated according to the formula : $[(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control, and A_1 was the absorbance of the sample.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to estimate the reducing capacity of fruit extracts, according to the method of [16]. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37°C. 900 µl FRAP reagent was mixed with 90 µl water and 30 µl of the extract. The reaction mixture was incubated at 37°C for 30 minutes and the absorbance was measured at 593 nm.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of fruit extracts was assayed by the method of [17]. The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM $FeSO_4$, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and varied concentrations of the extracts. After incubation for 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The scavenging activity of hydroxyl radical effect was calculated as follows : $[1-(A_1-A_2) / A_0]$ x 100, where A_0 is absorbance of the control (without extract) and A_1 is the absorbance in the presence of the extract, A_2 is the absorbance without sodium salicylate.

Statistical analysis

All assays were carried out in triplicates and results are expressed as mean ± SD. The data were subjected to one way analysis of variance (ANOVA) and the difference between various concentrations were determined by DMRT test using SPSS software. The P values of <0.05 were considered significant.

RESULTS AND DISCUSSION

The extraction yield, total phenolic content, total flavonoid content and total antioxidant activity of ripe pepino fruit extract is presented in Table 1. Percent yield of ripe ethyl acetate extract of pepino fruit was found to be 11.12%.

It was known that plant phenolic compounds are responsible for effective free radical scavenging and antioxidant activities[18]. The total phenol and flavonoid content of pepino extract were found to be 20.43 mg GAE/g dry weight, 53.85 mg RE/g dry weight respectively.

The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex, which has an absorption at 695 nm. The total antioxidant capacity observed in the ripe ethyl acetate extract of pepino fruit was 238.27 nM GAE/g respectively (Table 1).

Table 1: Extraction yield, total phenols, flavonoid contents and phosphomolybdenum assay of pepino fruit extract[#]

Pepino	Extraction yield (%)	Total phenols (mg GAE/g)[A]	Total flavonoids (mg RE/g)[B]	Phosphomolybdenum assay (nM GAE/g)[A]
Ripe	11.12 ± 0.32	20.43 ± 0.59	53.85 ± 1.56	238.27 ± 6.60

[#] Values are expressed as mean ± SD (n =3); [A]GAE - Gallic acid equivalents; [B]RE- Rutin equivalents

In this work, the antioxidant properties of pepino fruit were evaluated by different *in vitro* antioxidant assays such as reducing power, DPPH / OH / ABTS radical scavenging activity, FRAP and chelation activity.

DPPH radical scavenging activity

DPPH, a stable free radical, decreases significantly on exposure to proton radical scavengers. This assay was used to evaluate the free radical scavenging activity in foods and biological systems[19]. It has also been used to evaluate the free radical scavenging activity of natural antioxidants[20]. The radical scavenging activity of pepino extract increased with increasing concentrations, with 57.30%, 62.85%, 84.56%, 91.92% and 93.61% scavenging activity for 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml extract, respectively (Figure 1). The IC_{50} value was found to be 0.16 mg/ml. These results indicated that pepino extract exhibited the ability to quench the DPPH radical, which indicated that extract was good antioxidant with radical scavenging activity.

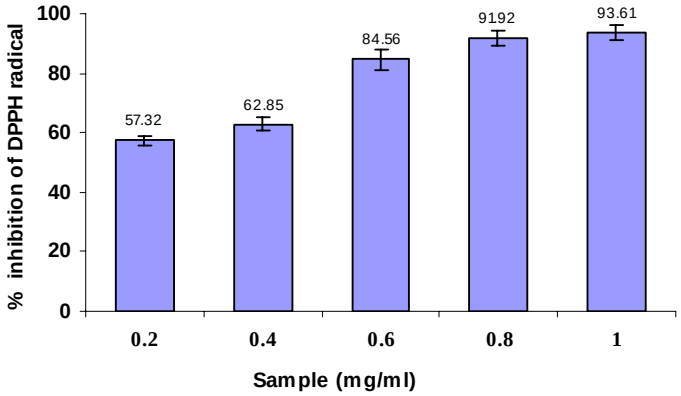

Fig. 1: DPPH radical scavenging activity of ethyl acetate extract of pepino fruit

Reducing power

The reducing power of the extract, which may serve as a significant reflection of antioxidant activity, was determined using a modified Fe^{3+} to Fe^{2+} reduction assay, whereby the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of the samples. The presence of antioxidants in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form, and Fe^{2+} can be monitored by measurement of the formation of Perl's Prussian blue at 700 nm[21]. At 0.4, 0.8, 1.2, 1.6, 2.0 mg/ml, reducing power of pepino extract were found to be 0.335, 0.476, 0.817, 1.051, 1.511 respectively (Figure 2). The IC_{50} value was found to be 0.82 mg/ml. The reducing power of the extract might be due to their hydrogen-donating ability, as described by[12]. Possibly, pepino fruit contain high amounts of reductone, which could react with radicals to stabilize and terminate radical chain reactions.

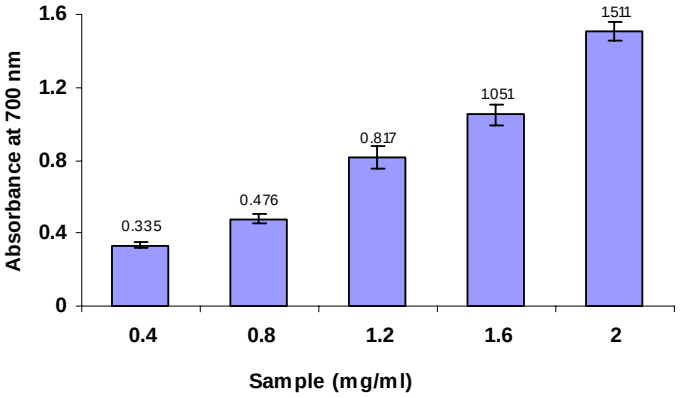

Fig. 2: Reducing power of ethyl acetate extract of pepino fruit

Metal chelating activity

Transition metals have been proposed as the catalysts for the initial formation of radicals. Chelating agents may stabilize transition metals in living systems and inhibit generation of radicals, consequently reducing free radical-induced damage. To better estimate the antioxidant potential of the pepino extract, its chelating activity was evaluated against Fe^{2+}. The chelating effects of pepino extract on ferrous ions increased with increasing concentrations (Figure 3). At concentrations of 10 and 50 mg/ml, the pepino extract exhibited chelating effects of 9.80% and 67.26%, respectively (Figure 3). The IC_{50} value was found to be 39.51 mg/ml. The results of the present study suggest that an ethyl acetate extract of pepino fruit exhibits good chelating activity on ferrous ions.

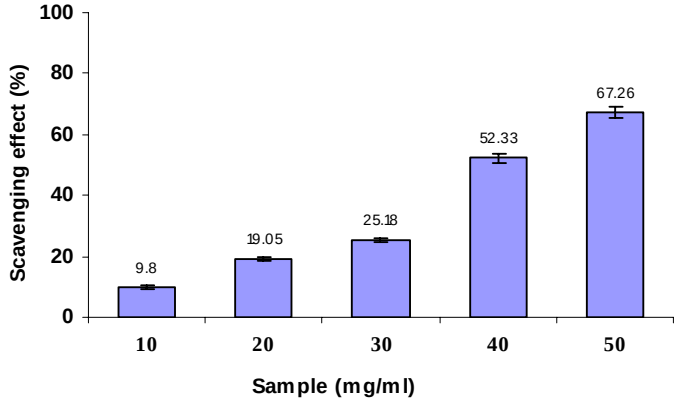

Fig. 3: Chelating activity of ethyl acetate extract of pepino fruit

ABTS radical scavenging activity

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants. The extract efficiently scavenged ABTS radicals generated by the reaction between 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammonium persulfate (Figure 4). The activity was found to be increased in a dose-dependent manner from 57.60% to 97.73% at a concentration of 10-50 mg/ml. The extract exhibited an IC_{50} value of 9.56 mg/mL. Therefore, the ABTS radical scavenging activity of ethyl acetate extract of pepino fruit indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

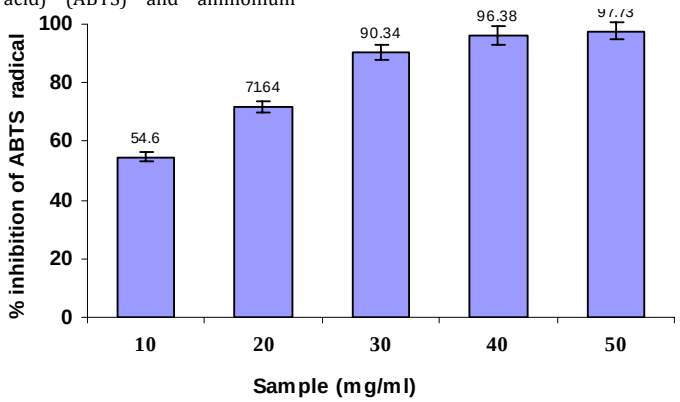

Fig. 4: ABTS radical scavenging activity of ethyl acetate extract of pepino fruit

Ferric reducing antioxidant power (FRAP)

FRAP assay is based on the ability of compounds to reduce $Fe^{(3+)}$ to $Fe^{(2+)}$. When this occurs in the presence of 2,4,6-trypyridyl-s-triazine, the reduction is accompanied by the formation of a colored complex with $Fe^{(2+)}$ and provides the information of total antioxidant or reductant[16]. Ferric reducing power of pepino fruit extract increased from 0.963 to 1.438 at 2-10 mg/ml (Figure 5). The IC_{50} value was found to be 1.06 mg/ml. Our results showed significant ferric reducing power which indicated the hydrogen donating ability of the extract.

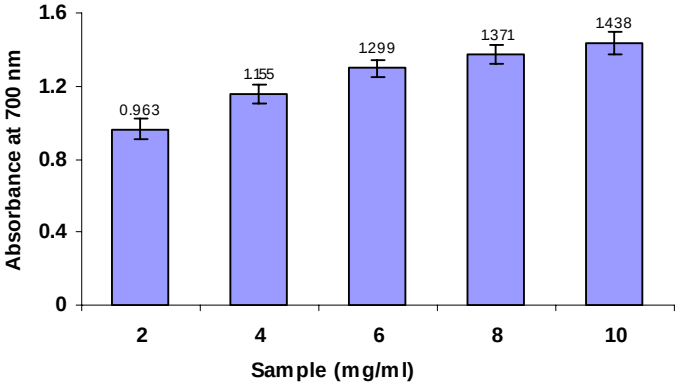

Fig. 5: FRAP assay of ethyl acetate extract of pepino fruit

OH radical scavenging activity

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules[22]. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins[23]. The •OH scavenging` activity of mushroom extracts was assessed by its ability to compete with salicylic acid for •OH radicals in the •OH generating/detecting system. In the present study, the hydroxyl radical-scavenging effect of the pepino extract, in a concentration of 0.2 mg/ml, was found to be 39.08% and in a concentration of 1.0 mg/ml, was found to be 78.00%. The IC_{50} value was found to be 0.26 mg/ml. Hence, the pepino extract can be considered as a good scavenger of hydroxyl radicals.

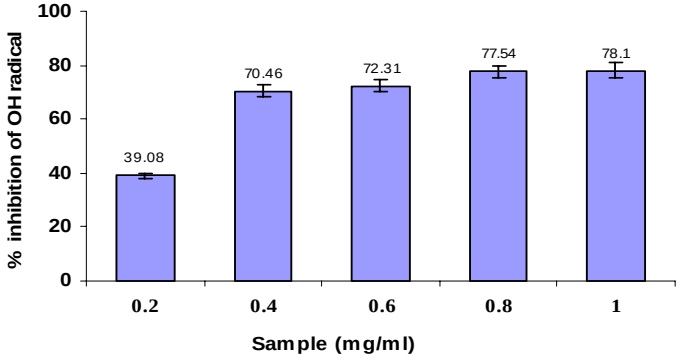

Fig. 6: Hydroxy radical scavenging activity of ethyl acetate extract of pepino fruit

CONCLUSION

The antioxidant capacities, total phenolic and total flavonoid contents of pepino extract were evaluated, by using several established biochemical assays in vitro: DPPH, ABTS, OH radical scavenging assay, reducing power assay, ferrous iron chelaing ability assay, FRAP and phosphomolybdenum assay. Generally, EC_{50} values of lower than 10 mg/ml indicated that the extracts were effective in antioxidant properties. It was found that ethyl acetate extracts of pepino fruit possessed antioxidant and free radical scavenging properties with considerable total phenolic and flavonoid content. In conclusion, our results suggested that pepino fruit could be a promising source of natural antioxidants.

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