



IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF RAW PEPINO FRUIT (*SOLANUM MURICATUM* AITON)

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ABSTRACT

The aim of this research was to investigate the antioxidant activity of exotic fruit pepino. The content of total phenols, flavonoid, the level of antioxidant potential by DPPH, ABTS, OH radical, reducing power, chelation, FRAP and total antioxidant in ethyl acetate extract were determined. In all the methods the extract exhibited good scavenging activity. The EC₅₀ values of raw ethyl acetate extract on DPPH radical, reducing power, ferrous ion chelation, ABTS radical, FRAP and hydroxyl radical were found to be 0.44, 0.48, 32.51, 10.03, 1.51 and 0.23 mg/ml, respectively. Substantial amount of phenol and flavonoids was noticed. The free radical scavenging and antioxidant characteristics of the extract may be due to the presence of polyphenols in the fruit extract. This study showed the potential of using fresh fruits to develop functional foods with high antioxidant activity

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

INTRODUCTION

Reactive oxygen species (ROS) production occurs during normal cell metabolism, both in animals and plants. Excess of ROS leads to oxidative stress, resulting in oxidative DNA damage which is implicated in the pathogenesis of numerous disorders, e.g. cardiovascular, atherosclerosis, reperfusion injury, cataractogenesis, rheumatoid arthritis, inflammatory disorders and cancer^{1,2}.

Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used to retard the oxidation process; however, the use of synthetic antioxidants must be under strict regulation due to potential health hazards³. The search for natural antioxidants as alternatives is therefore of great interest among researchers.

The consumption of a diet rich in fresh fruits and vegetables has been associated with a number of health benefits including the prevention of chronic diseases. This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body⁴.

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 reactions⁵.

In the present study, the ethyl acetate extract of raw pepino fruit was determined for its antioxidant activities by *in vitro* methods, including DPPH, ABTS, OH radical scavenging assay, reducing power, chelating activity, FRAP and total molybdenum assay and for its total phenolic and flavonoid contents.

MATERIAL AND METHODS

Fruit samples

The raw pepino fruits was 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 sulphate, 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 by⁶. 0.25 ml of various extracts was diluted with 1.25 ml of distilled water. 75 µl of a 5% NaNO₂ solution were added and after 6 min 150 µl of a 10% AlCl₃.H₂O 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 from⁷ 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 Na₂CO₃ (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 of⁸. 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 were 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 of⁹. 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: $(A_0 - A_1)/A_0 \times 100$, 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 of¹⁰. 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 of¹¹. Briefly, 2 ml of various concentrations of the extracts in methanol were added to a solution of 2 mM FeCl₂ (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-Fe²⁺ complex formation was calculated as $[(A_0 - A_1)/A_0] \times 100$, 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 by¹². The ABTS⁺ 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: $[(A_0 - A_1)/A_0] \times 100$, 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 of¹³. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM FeCl₃.6H₂O 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 of¹⁴. The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM FeSO₄, 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: $[1 - (A_1 - A_2) / A_0] \times 100$, 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 \pm 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 13.48%.

It was known that plant phenolic compounds are responsible for effective free radical scavenging and antioxidant activities¹⁵. The total phenol and flavonoid content of pepino extract were found to be 24.68 mg GAE/g dry weight, 53.60 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 303.23 nM GAE/g respectively (Table 1).

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

Pepino	Extraction yield (%)	Total phenols (mg GAE/g) ^A	Total flavonoids (mg RE/g) ^B	Phosphomolybdenum assay (nM GAE/g) ^A
Raw	13.48 \pm 0.39	24.68 \pm 0.71	53.60 \pm 1.50	303.23 \pm 8.79

[#] Values are expressed as mean \pm SD (n =3); ^AGAE - Gallic acid equivalents; ^BRE- Rutin equivalents

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

Being a stable free radical, DPPH· is frequently used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form, DPPH-H¹⁶. Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. Figure 1 shows free radical scavenging

activity of the raw pepino extract at different concentrations. The radical scavenging activity of pepino extract increased with increasing concentrations, with 12.00%, 43.68%, 67.61%, 90.54% and 91.53% scavenging activity for 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml extract, respectively (Figure 1). The IC₅₀ values was found to be 0.44 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.

Reducing power

The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential

antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of test specimen. Greater absorbance at 700 nm indicated greater reducing power. Figure 2 presents the reductive capabilities of the ethyl acetate extract of raw pepino fruit. In the concentration range investigated, all the extracts demonstrated reducing power that increased linearly with concentration. At 0.4, 0.8, 1.2, 1.6, 2.0 mg/ml, reducing power of pepino extract were found to be 0.433, 0.788, 1.124, 1.161, 1.820 respectively. The IC_{50} values was found to be 0.48 mg/ml. The reducing power of the extract might be due to their hydrogen-donating ability. Possibly, pepino fruit contain high amounts of reductone, which could react with radicals to stabilize and terminate radical chain reactions.

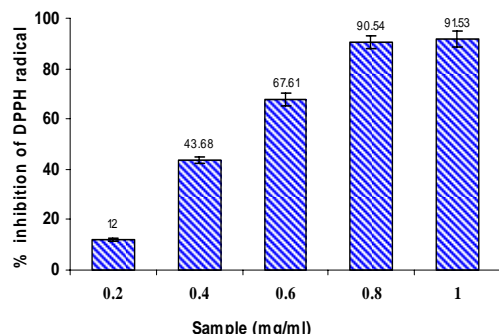


Fig. 1: DPPH radical scavenging activity of ethyl acetate extract of raw pepino fruit

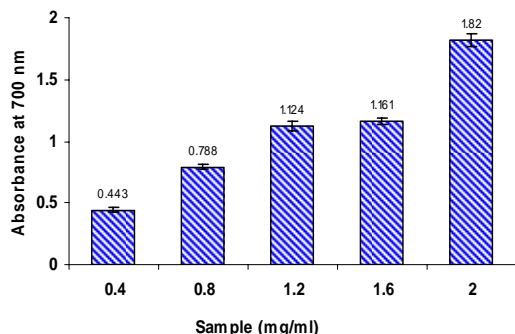


Fig. 2: Reducing power of ethyl acetate extract of raw pepino fruit

Metal chelating activity

Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage. Metal ion chelating capacity plays a significant role in antioxidant mechanisms, since it reduces the concentration of the catalysing transition metal in LPO¹⁷. 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 19.00% and 69.68%, respectively (Figure 3). The IC_{50} values was found to be 32.50 mg/ml. The results of the present study suggest that an ethyl acetate extract of pepino fruit exhibits good chelating activity on ferrous ions.

ABTS radical scavenging activity

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants¹⁸. 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 50.00% to 98.03% at a concentration

of 10-50 mg/ml. The extract exhibited an IC_{50} value of 10.03 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.

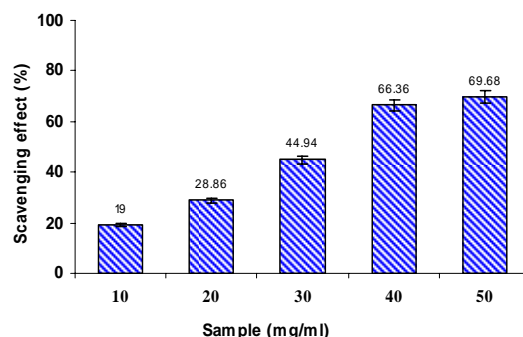


Fig. 3: Chelating activity of ethyl acetate extract of raw pepino fruit

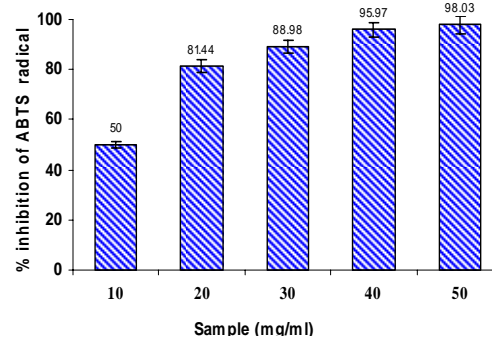


Fig. 4: ABTS radical scavenging activity of ethyl acetate extract of raw pepino fruit

Ferric reducing antioxidant power (FRAP)

FRAP assay is based on the ability of an antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm. The absorbance decrease is proportional to the antioxidant content¹³. The trend for ferric ions reducing activities of pepino extract at different concentrations are shown in Figure 5. The IC_{50} values was found to be 1.51 mg/ml. Our results showed significant ferric reducing power which indicated the hydrogen donating ability of the extract.

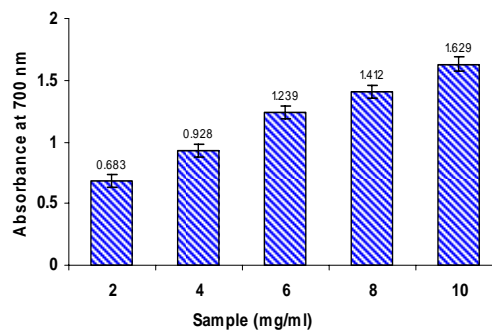


Fig. 5: FRAP assay of ethyl acetate extract of raw pepino fruit

OH radical scavenging activity

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules¹⁹. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins²⁰. 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 46.46% and in a concentration of 1.0 mg/ml, was found to be 89.63%. The IC₅₀ value was found to be 0.23 mg/ml. Hence, the pepino extract can be considered as a good scavenger of hydroxyl radicals.

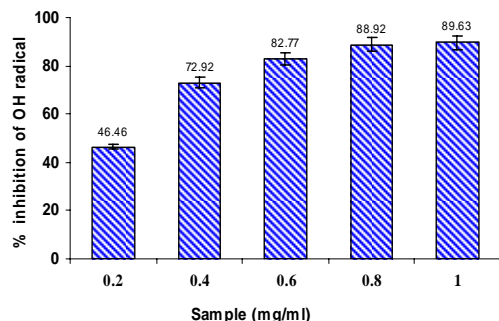


Fig. 6: Hydroxy radical scavenging activity of ethyl acetate extract of raw pepino fruit

CONCLUSION

In present study, antioxidant activities of the ethyl acetate extract obtained from raw pepino were investigated. The extracts were found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the DPPH, ABTS, OH radical, reducing power, chelating effect on ferrous ions, FRAP and total antioxidant activity. Generally, EC₅₀ values of lower than 10 mg/ml indicated that the extracts were effective in antioxidant properties. In the present study it is found that the ethyl extract of pepino fruit contains substantial amount of phenolics and flavonoids and it is the extent of phenolics present in this extract being responsible for its marked antioxidant activity as assayed through various *in vitro* models. Thus, it can be concluded that ethyl acetate extract of pepino fruit can be used as an accessible source of natural antioxidants with consequent health benefits.

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