International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 5, May 2015, PP 145-149
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

In vitro Antioxidant Activity of Ethanolic Extract of *Psidium guajava* Leaves

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Abstract: *A study was evaluated the antioxidant potential of ethanolic extract of Psidium guajava leaves in an invitro models. The extract was subjected to DPPH assay, reducing power assay, nitric oxide assay, H2O2 assay and SOD assay. The results of all the methods proved that the ethanolic extract of P. guajava have an antioxidant potential on a concentration dependent manner. The antioxidant activity of the sample was compared to the ascorbic acid as a standard. The study revealed that the ethanolic extract of P. guajava leaves is a good source of antioxidants.*

Keywords: *Psidium guajava, invitro, antioxidant.*

1. Introduction

In our body cells produces the oxidants in normal and/or in pathological conditions. Such oxidants are useful to our body to destroy the microbes. In sometimes the uncontrolled production of oxygen derived free radicals such as reactive oxygen species. This ROS is mediated the oxidative damage to macromolecules and it causes the various diseases such as cardiovascular diseases, cancer, aging, diabetes mellitus, rheumatoid arthritis, cirrhosis (1) etc. Human bodies have a natural protective mechanism to prevent the production of free radicals (2). The protective mechanisms were disturbed at various pathological conditions at that time our body needs antioxidant supplements to prevent the formation of free radicals. The antioxidants are intrusive with the oxidation process through the radical scavenging and chelating effects, it prevents the oxidative damage caused by the free radicals (3). Several synthetic agents such as butylated hydroxylanisole, butylated hydroxyltoluene etc are commercially available, but these agents are causing various side-effects in human and animals (4). The plant derived compounds such as flavonoids, tannins, proanthocyanidins, phenols etc. having the strong antioxidant activity, so researchers are eager to find the antioxidant remedies from the natural sources and without causing any harmful effects.

Psidum guajava is a well known medicinal plant in the family of Myrtaeceae. It is commonly called as guava, goiava, guave (5) etc. The guava plant can grow in all climatic conditions. The leaves are dark and simple elliptic to ovate and extent of about 5-15 centimeters. The *P. guajava* leaves are used as a hypoglycemic agent, cardioprotective, antimicrobial, antifungul and antispasmodic agent (6). Leaves of this plant which contains terpenoids, phenols, tannins etc., (7). The present study was to evaluate the antioxidant activity of ethanolic extract of *P. guajava* Linn. in *invitro* models.

2. Materials and Methods

2.1. Plant Material and Extraction

The fresh leaves of *P. guajava* were collected locally and authentication was obtained from St. Joseph College, Trichy. The shade dried *P. guajava* leaves were powdered mechanically and stored in an air tight container. The plant was extracted by using ethanol. The extraction was carried out by hot percolation method using Soxhlet apparatus. About 100 gm of powder was extracted with 600 ml of

ethanol. The extract was concentrated to dryness under controlled temperature 40- 50°C. The percentage yield was found to be 10.15%. The extract was preserved in refrigerator till further use.

3. DPPH Radical Scavenging Assay

Procedure

The free radical scavenging capacity of the ethanolic extract of *P. guajava* was determined using DPPH. DPPH (200µM) solution was prepared in 95% methanol. From the stock plant extract solution 250, 500, 750, 1000, and 1500µg/ ml were taken in five test tubes. 0.5ml of freshly prepared DPPH solution was incubated with test drug and after 10 minutes, absorbance was taken as 517 nm using spectrophotometer. Standard ascorbic acid was used as reference.

Calculation

% scavenging of the DPPH free radical was measured using following equation

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{Control} - \text{test}}{\text{control}} \times 100$$

4. Reducing Power Assay

1 ml of varying concentrations (1-5 mg/ml) of plant extract was mixed with 2.5 ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Aliquots of 2.5 ml of trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10min. The upper layer of the solution (2.5 ml) was mixed with equal volume of distilled water, to this 0.5ml of freshly prepared ferric chloride solution was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power

Calculation

$$\text{\% increase in reducing power} = \frac{\text{A test}}{\text{A blank}} \times 100$$

A_{test} is the absorbance of test solution: A_{blank} is absorbance of blank. The antioxidant activity of the extract was expressed as IC_{50}.

5. Nitric Oxide Scavenging Activity

Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentrations (250-1500µg/ml) of the ethanolic plant extract dissolved in phosphate buffer (0.025 M, pH 7.4) and tubes were incubated at 25 ^{0}C for 5 hours. Control tube without the plant extract, but with equivalent amount of buffer was maintained in an identical manner. After 5 hours, 0.5ml of the incubated solution was removed and diluted with 0.5ml of Griess reagent (1% sulfanilic acid, 5 % phosphoric acid, and 0.1 % naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite ions with suphanilic acid and its subsequent coupling with Napthylethylenediamine was read at 546 nm. The experiment was repeated in triplicate.

Calculation

$$\text{NO2 Scavenging activity (\%)} = \frac{\text{Control} - \text{test}}{\text{control}} \times 100$$

6. H_2O_2 RADICAL SCAVENGING ACTIVITY

H_2O_2 scavenging ability of etahnolic extract of *P. guajava* leaves was determined according to the method of**.** 0.2-1.0 ml of test sample (10mg/10ml) was taken in different test tubes to which 1ml of H_2O_2 was added. The tubes were incubated for 5 minutes at room temperature. After 5 mins, 2 ml of potassium dichromate:acetic acid reagent was added and the tubes were incubate for 10 minutes at room temperature. The absorbance value of the reaction mixture was recorded at 700 nm. Blank containing the phosphate buffer without the plant extract and a standard was also calculated as

% scavenging [H_2O_2] = [$A_{control:}$ A_{test}/$A_{control}$] ×100

Where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample.

7. SUPEROXIDE ANION (SO) RADICAL SCAVENGING ASSAY

The Superoxide radical scavenging activity of *Psidium guajava* was measured by the method of Fontana et.al., (2001). In this method, the activity is measured by reduction of riboflavin/light/NBT (Nitro blue tetrazolium). The 1 ml of reaction mixture contained Phosphate buffer, NADH, NBT and various Concentrations of sample solution. The method is based on generation of superoxide radical by autooxidation of riboflavin in presence of light. The Superoxide radical reduces NBT to a blue coloured formazon that can be measured at 560 nm.

Table1. *DPPH scavenging activity of Ethanolic extract of P. guajava*

Test	Concentration (μg/ml)	% Inhibition of Extract	% Inhibition of Standard
DPPH (2,2-Diphenyl-1-picryl hydrazyl)	250	23.07	28.16
	500	42.30	46.21
	750	57.69	60.01
	1000	76.92	79.12
	1500	82.16	86.41
IC50		590	550

Table2. *Reducing power assay of Ethanolic extract of P. guajava*

Test	Concentration (μg/ml)	% Inhibition of Extract	% Inhibition of Standard
Reducing Power Assay	250	56.8	58.1
	500	64.2	67.3
	750	77.8	80.3
	1000	85.2	87.1
	1500	91.5	92.4
IC50		230	220

Table3. *Nitric Oxide assay of Ethanolic extract of P. guajava*

Test	Concentration (μg/ml)	% Inhibition of Extract	% Inhibition of Standard
Nitric Oxide	250	22.7	26.3
	500	34.0	38.9
	750	45.4	49.1
	1000	56.8	61.8
	1500	64.1	70.1
IC50		850	780

Table4. *H_2O_2 Assay of Ethanolic extract of P. guajava*

Test	Concentration (μg/ml)	% Inhibition of Extract	% Inhibition of Standard
H2O2 Assay	250	20	24
	500	30	36.7
	750	45	48.2
	1000	60	66
	1500	90	95
IC50		670	640

Table5. *Super Oxide Dismutase assay of Ethanolic extract of P. guajava*

Test	Concentration (μg/ml)	% Inhibition of Extract	% Inhibition of Standard
SOD Assay	250	15.7	16.5
	500	23.46	28.3
	750	43.07	39.01
	1000	54.6	58.6
	1500	62.30	67.43
IC50		920	890

Table 1 showed that the DPPH radical scavenging activity of ethanolic extract of *P. guajava* in a concentration dependent manner. The highest % scavenging activity (82.16%) was recorded at 1500 μg /ml of the fraction of *P. guajava* leaves while the lowest % scavenging activity (23.07%) at 250 μg /ml. Table 2 shows the reducing power of the plant extract. The highest % of reducing power (91.5%) was recorded at 1500 μg/ml and the lower % of reducing power (56.8%) was recorded at the lower concentration of 250μg/ml. Table 3 shows the Nitric Oxide scavenging activity of *P. guajava*. The

Nitric oxide scavenging activity is increased remarkably in the increasing concentration of the extract. The highest activity was noted at the concentration of 1500 µg/ml of the plant extract. Table 4 shows the Hydrogen Peroxide scavenging activity. The scavenging activity is increased when the concentration of the plant extract is increases. The maximum activity was recorded at 1500 µg/ml and the lowest activity was noted at 250 µg/ml. Table 5 shows the results of SOD assay. The higher % of inhibition of the plant extract at 1500 µg/ml (62.30%) and lower % of inhibition at 250 µg/ml (15.7%).

8. Discussion

DPPH scavenging assay is an important assay to determine the antioxidant activity of the plant extracts in *invitro* model. DPPH is free radical which is reacting rapidly with the antioxidant compounds. The antioxidative compounds can donate a hydrogen atom to DPPH and change the color. The intensity of color is measured calorimetrically. The increasing intensity of color is directly proportional to the inhibition of DPPH. The present study shows the increasing concentration of the extract inhibit the activity of DPPH. The maximum inhibition was noticed at 1500 µg/ml.

The reducing power is related to electron transfer ability of the plant extract. In this assay is used to measure the transferring capacity of Fe^{3+} to Fe^{2+} (8). The results showed that the extract possessed antioxidant activity in a concentration dependent manner. Based on the results the *P. guajava* have a ability of transferring the Fe^{3+} in to Fe^{2+} , and it minimize the oxidative damage in the tissues.

Nitric Oxide is a free radical which is formed from sodium nitroprusside and it reacts with oxygen to form nitrite. The antioxidant activity was measured by the inhibition of the nitrite formation, this was done by the plant extracts which directly reacts with oxygen, nitric oxide and other nitrogen compounds (9). The present study proves that the increasing concentration of the extract have a maximum inhibitory activity against the nitric oxide.

Hydrogen peroxide is an important reactive oxygen species because it may be toxic if it is converted into hydroxyl radicals in the cells (10). The antioxidant compounds which donates the electrons to H_2O_2, and neutralize it into water molecule (11). The present study proves the inhibition of hydroxyl radicals from H_2O_2 in the concentration dependent manner.

Superoxide dismutase is an important enzyme in an antioxidant defense system (12). SOD converts the superoxide anion into hydrogen peroxide and thus reduces the toxic effect. The percentage of inhibition of superoxide by SOD may reduce the cellular damages. The present study proves that the increasing concentration of the extract have a maximum inhibitory activity of SOD.

9. Conclusion

The present study revealed that the *in vitro* antioxidant activity of crude ethanol extract of *P. guajva* leaves. The plant showed significant antioxidant activity in an *in vitro* model, so further studies are needed to find the compounds which is responsible for this activity.

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