Research Article

In Vitro Antioxidant Activity of *Psidium Guajava* Leaves

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ABSTRACT
A study was undertaken to evaluate the antioxidant potential of Leaves of *Psidium guajava* Linn (Myrtaceae). Aqueous and ethanolic extracts of the leaves were subjected to *in vitro* antioxidant activity screening models such as ABTS, DPPH, nitric oxide and superoxide radical scavenging activity, inhibition of lipid peroxidation, reduction of ferric ions and total antioxidant capacity. Ascorbic acid was used as the standard. In all the models studied, the extracts showed potent antioxidant activity, thereby augmenting it into the present day system of medicine.

Keywords: Free radicals, Antioxidant, *Psidium guajava* Linn, lipid peroxidation.

INTRODUCTION

The effects of free radicals on human beings are closely related to toxicity, disease and aging (Maxwell .,1995). Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS) (Sato *et al* .,1996). Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis and the aging process (Stajner et al .,1995**;** Sanchez-Moreno *et al*., 1999; Malencic *et al* .,2000). The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers.

Many plants contain substantial amounts of antioxidants like vitamin C and E, carotenoids, flavonoids, tannins, etc. that can be used to scavenge the excess free radicals from human body. The intake in the human diet of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanism can prevent and in some cases, help in the treatment of some oxidative related disorders (Gupta *et al* .,2007).

In India, numerous medicinal plants are used for the treatment of various disorders. *P. Guajava* is a common shade tree or shrub in door-yard gardens in the tropics. It belongs to family Myrtaceae ,genus: Psidium ,species: guajava and common names of the plant are Guava, goiaba, guayaba etc. Plant parts which are used are fruits, leaves and barks. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Guava fruit is higher in vitamin C than citrus (80 mg of vitamin C in 100 g of fruit) and contains appreciable amounts of vitamin A as well. Guava fruits are also a good source of pectin – a dietary fiber. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have monstrated antibacterial activity. Quercetin is thought

to contribute to the antidiarrhea effect of guava; it is able to relax intestinal smooth muscle and inhibit bowel contractions. In addition, other flavonoids and triterpenes in guava leaves show antispasmodic activity. (Kritikar *et al* .,2000; Rastogi *et al* .,1985).

MATERIALS AND METHODS

All chemicals used were of analytical grade. DPPH (1,1 di phenyl 2 picryl hydrazyl) and ABTS i.e., 2,2 azino bis (3 ethyl benzo thiazoline 6 sulphonic acid) were obtained from Sigma Chemicals, USA. Sodium do decyl sulphate , nitro blue tetrazolium chloride, phenanthroline, naphthyl ethylene di amine di hydrochloride, potassium per sulphate, dimethyl sulphoxide, hydroxylamine hydrochloride, ammonium molybdate, sulphanilamide, ortho phosphoric acid, sodium nitroprusside, riboflavin, EDTA and sodium phosphate were obtained from Loba Chemie Private. Limited, Mumbai, India

Plant material

The leaves of *Psidium guajava was* collected from the Thanjavur ,Tamilnadu, during the month of Augest- September The voucher specimen has been maintained in our department for further reference.

Preparation of Extracts

The collected leaves was dried and extracted successively with 95% ethanol using soxhlet apparatus and aqueous extract was prepared by cold maceration method (Kokate et al .,2001). The alcoholic and aqueous extracts was concentrated in vacuum and kept in a vaccum dessicator for complete removal of solvent. Both the extracts were used for the antioxidant study.

DPPH radical scavenging activity (Anandjiwala ., 2007)

15 mg of DPPH was dissolved in 10 ml of methanol. 75µl of this solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75µl of DPPH was added to a mixture of methanol and 50 µl of extract. The final volume was adjusted to 3 ml. Decrease in absorbance of the DPPH was measured 517 nm.

ABTS radical scavenging activity (Shirwaikar *et al* .,2006)

ABTS 2mM and Potassium per sulphate 70mM were prepared in distilled water (0.0548g in 50 ml and 0.0189g in 1ml respectively). 200ml of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. To 0.5 ml of various concentrations of the extracts, 0.3 ml of ABTS radical cation and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract, methanol for alcoholic extract and water for aqueous extract were taken. The absorbance was measured at 734 nm.

DMSO radical scavenging activity (Nanjan *et al*., 2007)

To 0.5 ml of different concentration of the extracts, 1 ml alkaline DMSO and 0.2 ml NBT 20mM (50 mg in 10ml phosphate buffer pH 7.4) were added. The absorbance was measured at 560 nm.

Nitric oxide radical scavenging activity (Shirwaikar *et al* .,2004)

Griess reagent was prepared accordingly: Solution A: 1% Sulphanilamide in 5% ortho Phosphoric acid or 25% v/v Hydrochloric acid. Solution B: 0.01% Napthyl ethylene diamine in distilled water. Solution A and Solution B were in mixed equal volumes within 12 hrs of use. Sodium nitroprusside 5mM was prepared in phosphate buffer PH 7.4 (0.0373g in 25 ml). To 1ml of various concentrations of the extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25 ^{o}C for 5hr. 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm.

Superoxide dismutase scavenging activity(Anandjiwala .2007)

To 1.3 ml of different concentrations of the extract was added a mixture containing

0.2 ml EDTA 60mM (4.47 mgin 10ml water), 0.25 ml Riboflavin 53_m (31.92 mg in 100ml distilled water), 0.25 ml Hydroxylamine HCl 10mM (0.114g in 100ml distilled water) and 2 ml phosphate buffer pH 7.4. Riboflavin was added at the end after the tubes had been brought to a standard temperature of 20-22_C. The above solutions were incubated for 30 min in room temperature. Then, 1ml of Griess reagent was added to all test tubes. After 20 minutes, the absorbance was measured at 540 nm.

Reduction of ferric ions by ortho-phenanthroline color method (Hukkeri *et al* .,2008**)**

A reaction mixture containing 1ml ortho-Phenanthroline (0.005g in 10 ml methanol), 2 ml ferric chloride 200 _ M (3.24 mg in 100 ml distilled water) and 2 ml of various concentrations of the extracts was incubated at ambient temperature for 10 min, then the absorbance was measured at 510 nm.

Total antioxidant capacity (Shirwaikar *et al* .,2003**)**

To 0.1 ml of the extract, 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate combined in eppendorf tube) was added .The tubes were capped and incubated at 35^0 C for 90 min. After cooling to room temperature the absorbance was measured at 695 nm against blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Lipid peroxidation assay (Unni *et al* .,2004**)**

15% w/v Trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25N Hydrochloric acid were mixed to form the stock Thio-barbituric acid (TBA) – Tri-choloro acetic acid (TCA) – HCl reagent. This solution was mildly heated to assist the dissolution of TBA]. Albino rats (180-200g) of either sex were used for the study. After decapitation, the brain was removed carefully. The tissue was immediately weighed and homogenated with cold 1.15%w/v KCl to make 10%v/v homogenate. The homogenate (0.5ml) was added to 1 ml of various concentrations of the extracts. Then the mixture was incubated for 30 min. The per-oxidation was terminated by the addition of 2 ml of TBA-TCA –HCl reagent. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm.

The % inhibition of various radicals was calculated by comparing the results of the test with those of control using the formula.

$$\% \text{ inhibition} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

All experiments were performed in triplicate and the results averaged. Linear regression analysis was used to calculate the IC_{50} values(Madhujit *et al* .,2004)**.**

RESULTS AND DISCUSSION

Several concentrations ranging from 50 - 150 µg/ml of the ethanolic and aqueous extracts of were compared for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner up to the given concentration in all the models. On a comparative basis, both the extracts showed almost near values (Table – 1).

DPPH is a stable free radical. The *in vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stochiometrically depending on the number of electrons taken up (Kaleem *et al* .,2006). From the present results, it may be concluded that the extracts reduce the radical to the corresponding hydrazine

when they react with the hydrogen donors in the antioxidant principles.

In the present study, the nitrite produced by the incubation of solution of sodium nitrprusside in phosphate buffer was reduced by both the extracts of both species. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with nitric oxide and thus inhibit the generation of nitrite (Khlifi *et al* .,2005).

Ortho – substituted phenolic compounds may exert pro-oxidant effects by interacting with iron. O – phenanthroline quantitatively forms complexes with ferric ion which get disrupted in the presence of chelating agents. The extracts interfered with the formation of ferrous – O – phenanthroline complex, thereby suggesting that the extract has metal chelating activity.

The total antioxidant activity of the extracts was calculated based on the formation of phosphomolybdenum complex which was measured spectrophotometrically (Kharya *et al* .,2007).

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like liver and brain. In this study, lipid peroxidation was induced *in vitro* and the extracts showed concentration dependent prevention towards generation of lipid peroxides. Preliminary phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins and flavonoids in the species(Khandelwal, 2000). Phenolics, flavonoids and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier(Kumar *et a.,l* 2000). Hence, these may be responsible for the observed activity in these species. The present study proved the antioxidant potential of *Psidium guajava* plant.

Table 1: IC_{50} Values of *In Vitro* antioxidant study of aqueous and ethanolic extracts of *Psidium guajava* and standard (ascorbic acid) [Values are mean ± SEM of 3 replicates]

S.No	Models studied	IC_{50} (µg/ml)		
		Aqueous extract	Ethanolic extract	Standard
1	ABTS activity	94.25 ± 0.84	85.48 ± 0.52	31.01 ± 0.62
2	DMSO activity	102.45 ± 1.28	103.40 ± 0.39	88.72 ± 0.47
3	Nitric oxide scavenging	85.71 ± 2.10	86.43 ± 1.31	82.69 ± 0.49
4	Superoxide dismutase activity	91.84 ± 0.71	90.20 ± 2.20	84.73 ± 1.08
5	Reduction of ferric ions	96.65 ± 1.97	96.32 ± 1.59	91.78 ± 1.75
6	Total antioxidant activity	100.71 ± 0.67	86.49 ± 0.82	84.28 ± 0.20
7	Lipid peroxidation activity	93.15 ± 1.03	95.05 ± 0.12	85.12 ± 2.61

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