Potential antiradical and reducing activity assessment of selected species of *Psidium* (Myrtaceae)

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***ABSTRACT**: During the current study the antioxidant potentialities of the methanolic extract of leaves of Psidium guajava and Psidium guineense were tested. The results of the reducing power assay showed a dose dependant relationship between concentration of the extract used and the AEAC value. Similar results were obtained during DPPH assay also. In the case of nitric oxide scavenging assay, though the percentage of inhibition was dose dependant, the numerical value was slightly lesser than that of the other two assays. Based on the observations of the present work and previous reports, it may be concluded that the antioxidant potentialities of the methanolic extracts of Psidium guineense observed during the present study may be due to the ability of the various chemical components present in the extract to scavenge the free radicals.*

***Key Words:** Methanolic extract, Psidium, Antioxidant, Free radicals.*

INTRODUCTION

People have become more health conscious in recent years due to the outbreak of many new and fatal diseases. Many of these diseases are caused by the adverse environment or different chemicals that comes into the body by various means. It may be through the air we breathe, the food we consume or the water we drink. In most of these cases the ultimate etiologic agent implicated is oxidative stress due to the generation of excess pro-oxidents. Pro-oxidants in biological system include excited states, free radicals and other related species mainly derived from oxygen and nitrogen [1].

A free radical is an atom or molecule with at least one unpaired electron in the outermost shell [2]. These uncoupled electrons are highly reactive with bio-molecules such as carbohydrates, lipids, proteins and nucleic acids causing cellular damage (Kuhn, 2003). They are mainly derived from oxygen (Reactive Oxygen Species / ROS) and nitrogen (Reactive Nitrogen Species / RNS). Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases [3]. Pro-oxidants are generated in the body during the normal metabolic processes. They are produced as a protective mechanism [4]. However, the presence of excess amount of free radicals within the body have a significant role in the development and progression of many diseases like heart disease, heart failure, hypertension, cerebro-vascular accidents, and diabetic complication. In a healthy organism, the pro-oxidants are deliberately balanced by the antioxidant defense [5].

Antioxidants are compounds which when present at low concentration significantly delays or prevent oxidation of cell contents [6]. These can stabilize or inactivate free radicals before they attack the cellular structures. There are various factors that can shift the balance between pro-oxidants and antioxidants in either of the directions. Human body has the inherent mechanism to produce various enzymatic or non-enzymatic antioxidants. At certain times these natural protective mechanisms may not be sufficient for scavenging the excess free radicals. Supplementation with exogenous antioxidants may have a protective role in the body during such situations [7].

In last few decades natural herbal products have received maximum attention owing to their wide utility. A number of herbal preparations both in crude form and their fractioned components have been proved to render protective effects [8]. *Psidium* is known widely for its food and nutritional value. The medicinal properties of *Psidium* fruits, leaves and other parts are also well known in traditional systems of medicine. It has been used traditionally as medicinal plant throughout the world for a number of ailments. Guava leaf extract has analgesic, anti-inflammatory, antimicrobial and hepato-protective activities. Many researchers have demonstrated the presence of a wide variety of bioactive compounds in the leaf, seed and bark of *Psidium* that are beneficial to human health [9, 10].

In this context, the present work is an attempt to evaluate the antioxidant potentiality of methanolic leaf extracts of two different species of *Psidium* such as *Psidium guajava* and *Psidium guineense* using various assay systems.

MATERIALS AND METHODS

The intension of the present study was to evaluate the antioxidant activity of the leaf extract of two species of *Psidium* such as *Psidium guajava* and *Psidium guineense* by evaluating the free radical scavenging activity and reducing power. The description of the plant material, assay system, reagents and procedure adopted are described here.

ABOUT THE SELECTED PLANTS

A. *Psidium guajava*

Fig.1 Image of *Psidium guajava* L.

Scientific name : *Psidium guajava* L.
Family : Myrtaceae
Fl. & Fr. : Mar- May
Common name : Guava
Malayalam Name : Pera maram

Small tree. Stem smooth with pealing bark. Young stem 4-angled. Leaves l6-11 x 2.5-5 cm, elliptic-oblong, base rounded to obtuse-cuneate, apex acute-apiculate, hirsute on both sides when young, glabrous on ageing except the nerves, thin-coriaceous, lateral nerves prominent; petioles 0.6-1 cm long. Cymes axillary, 1-3-flowered; peduncles 0.5-1.2 cm long; pedicel short or 0. Calyx tube 4-9 mm long, ovoid, densely hirsute; lobes 4, unitedland closed in bud. Petals 4, white, 1-1.5 cm long, broadly ovate, caducous. Stamens many. Ovary globose, many-celled; ovules numerous; style subulate. Berry 2.5-3.5 cm diam., globose crowned by persistent calyx lobes. Seeds many, embedded in fleshy pulp. (Fig 1) [11].

B. *Psidium guineense*

Fig.2 Image of *Psidium guineense* Sw.

Scientific name : *Psidium guineense* Sw.
Family : Myrtaceae
Fl. & Fr. : Jul- Oct
Common name : Guava
Malayalam Name : Mundiri pera

Shrubs to small trees, branchlets terete, pubescent. Leaves subopposite or opposite, subcoriaceous, 8-10 cm, broadly elliptic-oblong, pellucid dotted, entire, pubescent beneath; lateral nerves 8-10 pairs, looping; petiole 1-1.5 cm long. Flowers slightly fragrant. Calyx tube adnate to the ovary, imperfectly 5-lobed, green, pubescent without. Petals 5, caducous, white, spathulate, 1.5 x 1 cm. Stamens c. 200, white;

filaments 1-1.2 cm long; anthers oblong, 0.1- 0.5 cm long, introrse, dehiscing longitudinally. Ovary many-celled, ovules many in each locule; style 1.3 cm long, white, stigma capitate. Berry globose, 2-3 cm diameter, pubescent, yellow when ripe; seeds many, embedded in the creamy-yellow flesh. (Fig 2) [11].

Preparation of Alcoholic Extract

Mature leaves were used for the preparation of methanolic extract. The leaves of *Psidium guajava* were collected from Calicut district, Kerala and were authenticated by using available Floras and Literature. The fresh leaves were collected and were cleaned properly using running tap water. Water was removed from the leaves by blotting using tissue paper. Then the leaves were chopped into small pieces and spread in steel trays. It was dried using hot air oven at 35^{o}C. The dried pieces were made into powder using motor and pestle. The powder was stored in glass bottles. 25g of the leaf powder was added to 250ml methanol and kept on magnetic stirrer for 3 hours for proper mixing. Later the suspension was centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred to glass beakers and was kept in hot air oven at 35^{o}C to evaporate the methanol completely. The methanolic extract thus obtained was scrapped from the beaker and was transferred to amber coloured bottle. The bottles were labeled properly and were stored under refrigeration. In the present study five different concentrations of methanolic extract such as 0.05%. 0.1%, 0.2%, 0.4% and 0.8% were used. At first 0.8% solution of the extract was prepared using the respective solvent. Other concentrations tested during the present study were prepared by proper dilution of this 8% solution. The assays were conducted in triplicate for each of the concentrations. Distilled water was used as the solvent for dissolving the extract.

Antioxidant Assays

Three different assays were conducted to find out the antioxidant activity of the leaf extracts of *Psidium guajava* and *Psidium guineense*.

A. Reducing power assay

Reducing power of all extracts was determined according to the method of Yen and Chen [12]. 1.25 ml each of this solution was transferred to three test tubes. 1.25 ml of 1% of potassium ferricyanide was then added to each test tube. The mixture was incubated at 50 degree for 20 minutes. 1.25 ml of this mixture was added to 1.25 ml of 10% trichloro acetic acid solution. After mixing, it was centrifuged at 1500 rpm for 10 minutes. Mixed 1.25ml of supernatant with 1.25 ml distilled water and 0.25ml of 1% ferric chloride solution. It was kept for 10 minutes at room temperature and measured the absorbance at 700 nm. The reaction mixture without plant extract was used as the blank

The reducing power was expressed in relation to the reducing power of ascorbic acid, which was used as the positive control. Ascorbate Equivalent Antioxidant Capacity (AEAC) was calculated by following equation.

$$AEAC = C_A \times A_S / A_A$$

Where, C_A – final concentration of ascorbic acid in µg/mL

A_S - absorbance of the sample

A_A – absorbance of ascorbic acid.

Increase in absorbance of reaction mixture indicated the increase in reduction power. The results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

B. DPPH Assay

Determination of the scavenging of DPPH (2,2-Diphenyl-1-picryl hydrazyl), a commercially available and stable free radical, was carried out with different concentrations of extracts. This method is based on the reduction of DPPH solution in presence of a hydrogen donating antioxidant, resulting in the formation of the non radical form of DPPH called diphenyl picryl hydrazine. In its radical form, DPPH has an absorption maximum at 515nm and disappears on reduction by antioxidant.

Reaction mixture was prepared by mixing 1.8 ml of 0.1 mM freshly prepared DPPH solution (in methanol) and 0.2 ml of the different concentrations of the plant extracts. The mixture was incubated at room temperature under darkness for five minutes and the absorbance was measured at 515 nm [13]. Reaction mixture, where methanol was added instead of plant extract was used as the control. Percentage of DPPH radical scavenging activity was expressed as percentage of inhibition calculated using the formula

$$\text{Percentage of inhibition} = \frac{ABS_c - ABS_t}{ABS_c} \times 100$$

Where ABS_c = Absorbance of the control

ABS_t = Absorbance of the test

The results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

C. Assay of nitric oxide radical scavenging activity

Sodium nitroprusside solution at physiological pH (7.2 -7.4) was used as the source of nitrate radical. The amount of the nitrate radicals produced by the interaction of nitric oxide with oxygen was measured by Grives reaction. The efficacy of the plant extract to scavenge these free radicals was measured according to the procedure developed by Green *et al.,* [14].

3 ml of reaction mixture containing sodium nitroprusside (10 mM) in phosphate buffered saline and various concentrations of plant extract were incubated at 25^0C for 150 minutes. At the end of incubation, 0.5 ml of Grives reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was measured at 546 nm. A control group was maintained without any plant extract, but with an equal amount of buffer.

The percentage of inhibition of nitric oxide generation was measured by comparing the absorbance value of control group and that of the reaction mixtures containing different concentrations of plant extracts using the formula,

$$\text{Percentage of inhibition} = \frac{ABS_c - ABS_t}{ABS_c} \times 100$$

Where ABS_c = Absorbance of the control

ABS_t = Absorbance of the test

RESULTS AND DISCUSSION

Reducing power assay

The reducing ability was measured by the Fe^{3+}- Fe^{2+} transformation in the assay system in presence of methanolic extracts of plants. The increase absorbance of the reaction mixture indicates an increase reducing power. The reducing power of the different concentrations of extracts from individual extract was compared with the reducing power of the ascorbic acid – a standard reducing agent using the same assay system. The data obtained were tabulated in Table 1 and are represented in Fig 3. The AEAC values were dose dependent in experiments with methanolic extracts of leaves of both *Psidium guajava* and *Psidium guineense*. The results gave a clear picture about the reducing power of these methanolic extracts. Among the extracts investigated during the current study, the extract of *Psidium guineense* (Table 1, Fig 3) showed the higher degree of reducing power.

Table 1: AEAC values of extracts of leaves of *Psidium guajava* and *Psidium guineense*

Sl. No.	Concentration of the extract (%)	AEAC value (Mean ± Standard error)	
		Psidium guajava	*Psidium guineense*
1.	0.05	9.17 ± 0.28	12.67 ± 0.12
2.	0.1	14.59 ± 0.32	21.05 ± 0.55
3.	0.2	17.46 ± 0.17	29.63 ± 0.18
4.	0.4	22.20 ± 0.10	36.52 ± 0.14
5.	0.8	24.51 ± 0.54	41.09 ± 0.99

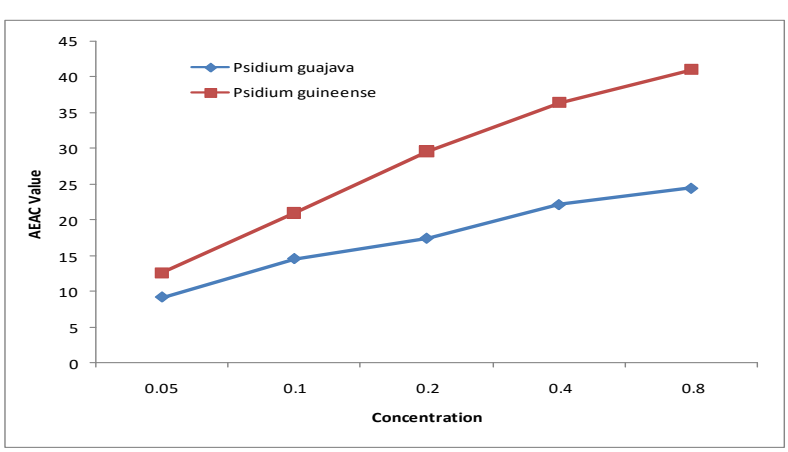

Figure 3: Graph showing the AEAC values of different concentrations of extracts of leaves of *Psidium guajava* and *Psidium guineense*.

A. DPPH radical scavenging assay

The results of the DPPH radical scavenging assay using the different concentrations of leaf extracts of *Psidium guajava* and *Psidium guineense* showed a dose dependent antiradical activity (Table 2, Fig 4).

Table 2: DPPH radical scavenging activity of extracts of leaves of *Psidium guajava* and *Psidium guineense*

Sl. No.	Concentration of the extract (%)	Percentage of inhibition of DPPH radical (Mean ± Standard error)	
		Psidium guajava	*Psidium guineense*
1.	0.05	24.53 ± 0.33	56.14± 0.21
2.	0.1	37.43 ± 0.56	64.73 ± 0.87
3.	0.2	41.25 ± 0.38	70.97 ± 0.40
4.	0.4	53.57 ± 0.43	73.97 ± 0.32
5.	0.8	61.89 ± 0.30	77.45 ± 0.91

At different concentration of the extract, the activity was higher for *Psidium guineense* than *Psidium guajava.* The results thus clearly indicate the potentiality of the methanolic leaf extract of *Psidium guajava* and *Psidium guineense* to remove the DPPH free radicals from the reaction mixture. This in turn indicates the antioxidant potentiality of these extracts.

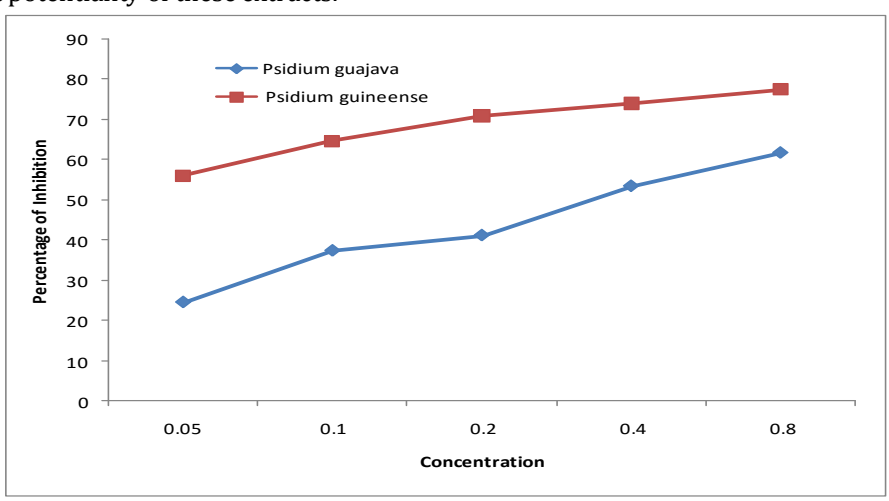

Fig 4: Graph showing the DPPH radical scavenging activity of extracts of leaves of *Psidium guajava* and *Psidium guineense*

B. Nitric oxide radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Grives reaction. The results of the present study (Table 3 and Fig. 5) indicated the dose dependent inhibition of the nitric oxide radical generation from sodium nitroprusside by the methanolic extracts of leaves of *Psidium guajava* and *Psidium guineense.*

Table 3: Nitric oxide radical scavenging activity of extract of leaves of *Psidium guajava* and *Psidium guineense.*

Sl. No.	Concentration of the extract (%)	Percentage of inhibition of nitric oxide generation (Mean ± Standard error)	
		Psidium guajava	*Psidium guineense*
1.	0.05	05.55 ± 0.52	16.54 ± 0.40
2.	0.1	10.05 ± 0.20	22.65 ± 0.42
3.	0.2	12.31 ± 0.82	31.76 ± 0.10
4.	0.4	13.50 ± 0.43	34.69 ± 0.42
5.	0.8	14.35 ± 0.88	41.75 ± 0.01

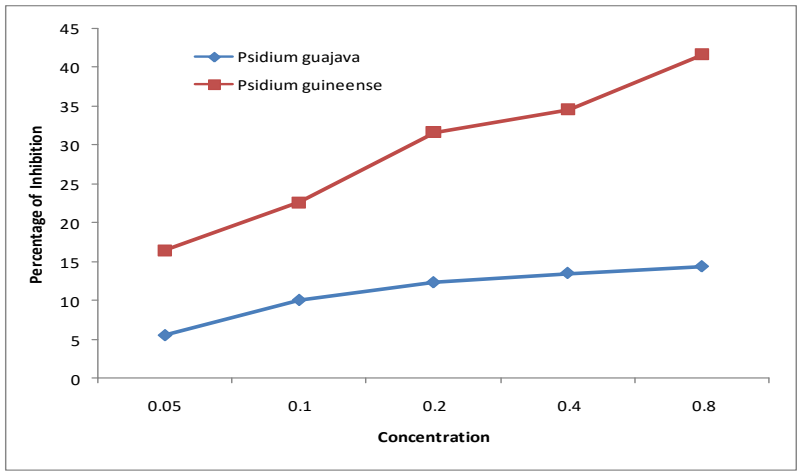

Fig 5: Graph showing Nitric oxide radical scavenging activity of extract of leaves of *Psidium guajava* and *Psidium guineense*.

The results obtained from three different antioxidant assays showed somewhat similar pattern of observations. In all the three different assays the activity was dose dependent. Similarly in all the three sets of the experiments, the activity was higher for extracts of *Psidium guineense* than extract of *Psidium guajava*. Free radicals are unstable atoms or molecules with an unpaired electron. These are naturally formed in the body as a result of chemical reactions during normal cellular processes. Majority of them belongs to Reactive Oxygen Species (ROS) - various forms of activated oxygen, which includes superoxide ions, hydroxyl radicals and non free radical species such as hydrogen peroxide [15].

During their attempt to get stabilized, they may attack other bio-molecules leading to their activation which in turn results in cell damage triggered by the formation of new free radicals from the chain of reactions [16]. Excess amount of reactive oxygen species generated in the body may have an important role in the initiation and promotion of many diseases. Thus the oxidative stress has been implicated in numerous pathological conditions [17].

It was demonstrated that oxidative stress could cause chromosome damage, induce many type of DNA protein crosslinks, depurination and depyramidiation [18]. Oxidative stress also damages certain cellular defense enzymes such as catalase and superoxide dismutase. Thus free radicals often attack DNA, proteins, enzymes etc. leading to alterations in genetic material [19]. All aerobic organisms, including human being have an antioxidant defense that protects the organism from toxic effect of free radicals [20].

Antioxidants are compounds that inhibit or retard the many oxidation reactions caused by free radicals, thereby preventing or delaying damage to the cells and tissues. Their mechanism of action include scavenging reactive oxygen and nitrogen free radicals species there by reducing molecular oxidation potential thus preventing the further generation of free radicals. In this way antioxidants limit the free radicals from oxidizing the vital bio-molecules [21]. However this natural antioxidant mechanism may be insufficient during some instances and hence dietary intake of antioxidant compound is important. Reports indicated that there is an inverse relationship between dietary intake of antioxidant rich food and the incidents of human diseases [22]. This has resulted in an increase in the information about various natural plant and animal products that may be used as food supplements and nutraceuticals.

The present study aimed at elucidating the antioxidant potentiality of the methanolic extracts of leaves of *Psidium guajava* and *Psidium guineense* is also an attempt in this direction. The results of the reducing power assay showed high AEAC values indicating their high antioxidant property. The AEAC value of extracts of both *Psidium guajava* and *Psidium guineense* showed a dose dependent increase during the present study. It was also noticed that the reducing power was more for the extract of *Psidium guineense* than *Psidium guajava*.

Results of the DPPH assay also showed a similar pattern of observations, where the percentage of inhibition was dose dependant. The inhibition was more for extract of *Psidium guineense* than that of *Psidium guajava*. But the percentage of inhibition of nitric oxide generation showed only lower numerical values. But in this case also the activity was dose dependant and was more for *Psidium guineense* than *Psidium guajava*. Hence it may be concluded that the methanolic extract of leaves of *Psidium guajava* and *Psidium guineense,* show different levels of antioxidant activity against different free radicals.

Phytochemical screening of the leaves of *Psidium* by Manikandan *et al*., [23]. revealed the presence of several bioactive compounds like alkaloids, flavones, tannins and phenols which could be responsible for the antioxidant property observed during the present study. Consumption of the raw fruits of *Psidium* may have a positive effect on human health due to the presence of various biochemical ingredients in it. Extensive investigations on the pharmacodynamics, kinetics, standardization and clinical trials are needed to exploit their therapeutic utility to combat various diseases.

CONCLUSION

The present study aimed at elucidating the antioxidant potentiality of the methanolic extracts of leaves of *Psidium guajava* and *Psidium guineens.* The AEAC value of extracts of both *Psidium guajava* and *Psidium guineense* showed a dose dependent increase during the present study. It was also noticed that the reducing power was more for the extract of *Psidium guineense* than *Psidium guajava*. Similarly the results of the DPPH assay also showed a similar pattern of observations, where the percentage of inhibition was dose dependant. The inhibition was more for extract of *Psidium guineense* than that of *Psidium guajava*. Hence it may be concluded that the methanolic extract of leaves of *Psidium guajava* and *Psidium guineense,* show different levels of antioxidant activity against different free radicals.

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