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Evaluation of the antioxidant potential of leaf and fruit extracts of *Psidium guajava* Linn

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Abstract
The present study was conducted to analyze the antioxidant potential of leaf and fruit extracts of *Psidium guajava* Linn by different assays like CAT, POD, DPPH, and FRAP. Fresh fruit and leaf extracts were used to estimate catalase (CAT) and peroxidase (POD) enzyme activity. For analysis of DPPH and FRAP scavenging activity, aqueous and methanolic leaf and fruit extracts were used. The results were analyzed in comparison to the radical scavenging activity of ascorbic acid as standard. CAT and POD activity of fresh leaf was higher than fruits, DPPH assay also shows that leaf extracts have better antioxidant potential than the fruit extract while FRAP assay indicates that methanolic extracts possess more radical scavenging potential than the aqueous extracts. The bioactive compounds present in the leaf and fruit impart leaf and fruit with excellent antioxidant potential.

Keywords: Antioxidant, CAT, POD, DPPH, FRAP, extracts

Introduction
One of the leading reasons for various life-threatening diseases like cardiovascular diseases, pulmonary diseases, and different types of cancer is oxidative damage caused at the cellular and tissue level. Therefore, it calls for the development of alternative sustainable drugs with lower side effects to scavenge such free radicals that cause oxidative stress [1]. With recent research in phytochemistry, Plant-based products have evolved as better and more sustainable alternatives as they are reported to be naturally rich in compounds like anthocyanins, carotenoids, flavonoids, polyphenols, etc. that have prodigious antioxidant properties. These phytocompounds work by effectively reducing reactive oxygen species (ROS) in cells and tissues and can also prevent damage caused at the DNA level thus preventing mutations [1, 2]. These bioactive compounds are also beneficial in maintaining the flavor, color, and texture of food products during storage apart from their antioxidant efficacy, which makes them a greener alternative over expensive chemically synthesized drugs, which have various adverse effects [3, 4].
Among the edible fruit plant families, Myrtaceae is an important plant family with around 121 genera and 3800-5800 species. It comprises mostly evergreen shrubs and trees found in tropical and subtropical regions [5]. *Psidium guajava* Linn. belongs to the Myrtaceae family and has many pharmaceutical properties because of which it finds its use in traditional medicines, it is a good source of secondary metabolites like flavonoids tannins, phenols, triterpenes, saponins, carotenoid, essential oils, and lectins. It is also a good source of vitamins A and C [6].
In the current study estimation of the antioxidant activity of the leaf and fruit of *Psidium guajava* Linn was done, Catalase activity and Peroxidase activity were measured using fresh plant material, while aqueous and methanolic extract of leaf and fruit were used for DPPH assay and FRAP assay.

Materials and Methods
The study was conducted at the Department of Botany, IIS (Deemed to be University) Jaipur, Fruits and leaves of *P. guajava* Linn were collected from the local market of Jaipur which were then identified by the Herbarium Department of Botany, IIS (Deemed to be University), Jaipur. Extractions were done using a Soxhlet extraction unit for which 10 g of dried and powdered material was used. For aqueous extract, 100 ml of distilled water was used and for methanolic extracts, 100 ml of methanol was used as solvent. The extracts were kept for drying in a hot air oven for 3 hrs. and later at room temperature overnight until the solvent evaporated, leaving dried extract. Later extracts weres used for DPPH and FRAP Assays.

Catalase Activity [7, 9]

It was assayed by measuring the disappearance of H_2O_2. For which, 1gm of fresh leaf and fruit of *P. guajava* Linn. was taken separately and macerated using mortar and pestle along with phosphate buffer and then it was centrifuged. For Enzyme activity, the reaction mixture contained 2.7 ml 50 mM chilled phosphate buffer (pH 7.0), 0.1 ml enzyme extract, and 0.2 ml 200 mm H_2O_2 solution. A decrease in absorbance was recorded at 410 nm for 3 minutes.

Preparation of phosphate buffer: for phosphate buffer preparation, sodium hydrogen phosphate (0.2 M) was prepared by dissolving 3.54 g in 100ml distilled water, and sodium dihydrogen phosphate (0.2 M) was prepared by dissolving 3.12 g in 100 ml distilled water. 61 ml sodium hydrogen phosphate and 39 ml sodium dihydrogen phosphate were mixed and pH was adjusted to 7.

Peroxidase Activity [9, 10]

It was assayed by measuring the disappearance of H_2O_2. For which 1 gm of fresh leaf and fruit of *P. guajava* Linn was taken separately and macerated using mortar and pestle along with 50 mM phosphate buffer and then it was centrifuged. For peroxidase activity, the reaction mixture was containing 2.8 ml of 50 mM phosphate buffer, 0.2 ml enzyme extract, 0.5 ml 0.5% H_2O_2, and 0.5 ml of 1% guaiacol solution. A decrease in absorbance was recorded at 580 nm for 3 minutes. The preparation of phosphate buffer was done by the same method as stated above.

DPPH Assay

DPPH Assay was done by the method mentioned by [11, 12] with slight modification. 0.1M DPPH (1, 1-diphenyl-2,-picryl-hydrazyl) solution was prepared using methanol as solvent. The solution was prepared fresh and stored in the dark to prevent it from reduction by light. For the free radical scavenging activity analysis, 0.1 ml 0f test sample was mixed in 2.9 ml 0f methanolic DPPH solution and was incubated for 30 minutes in dark condition at room temperature, further their absorbance was measured at 517 nm using a spectrophotometer. Different concentrations (20 µl, 40 µl, 60 µl, 80 µl and 100 µl) of ascorbic acid (standard), crude aqueous, and methanolic leaf and fruit extracts were prepared in methanol. Methanol was taken as blank while DPPH methanolic solution was taken as the control.

% RSA was calculated using formula= $(1-A_s/A_c) *100$,

Where A_c = absorbance of control and A_s =absorbance of sample

FRAP Assay

This FRAP test also known as Ferric Reducing Ability of Plasma assay with slight modifications to Benzie & Strain (1996) was used for evaluation of the overall antioxidant potential of the plant extract [13]. The stock solutions contained 300 mM of acetate buffer (0.3 M acetic acid and sodium acetate pH 3.6), 10 mm TPTZ (2, 4, 6-tripyridyl-s-triazine in 40 mm HCI), and 20 mm $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by adding 25 ml acetate buffer to 2.5 ml $FeCl_3.6H_2O$ and TPTZ solution. The solution was brought to room temperature before use. Plant extracts prepared in 100 µl of DMSO and 290 µl of the FRAP solution were allowed to react in the dark condition, for 30 min. The absorbance of the colored complex of ferrous tripyridyl triazine was noted at 593 nm. The % FRAP activity was calculated using the following equation.

% FRAP activity = 100- ((Control-test/control) *100)

Results and Discussions

Fresh materials were used to calculate catalase enzyme (CAT) activity and peroxidase enzyme (POD) activity. CAT Activity is analysed by observing the absorbance of the test sample mixed with hydrogen peroxide. Hydrogen peroxide releases free radicals which are later reduced by the catalase enzyme present in the sample hence leading to a decrease in absorbance with an increase in time period. CAT Activity of leaf extracts shows a significant decrease in absorbance, while for fruit extract, there was no substantial drop in absorbance after 90 seconds (Figure 1), which indicates that CAT Activity is more prominent in the leaf rather than in the fruit. POD Activity is analysed by observing the absorbance of the test sample mixed with hydrogen peroxide. The peroxidase enzyme present in the test sample reduces free radicals produced by hydrogen peroxide. No significant decrease in the absorbance of leaf and fruit extract for POD Activity was observed. (Figure 2), the results indicate that leaf and fruit samples show no significant POD Activity.

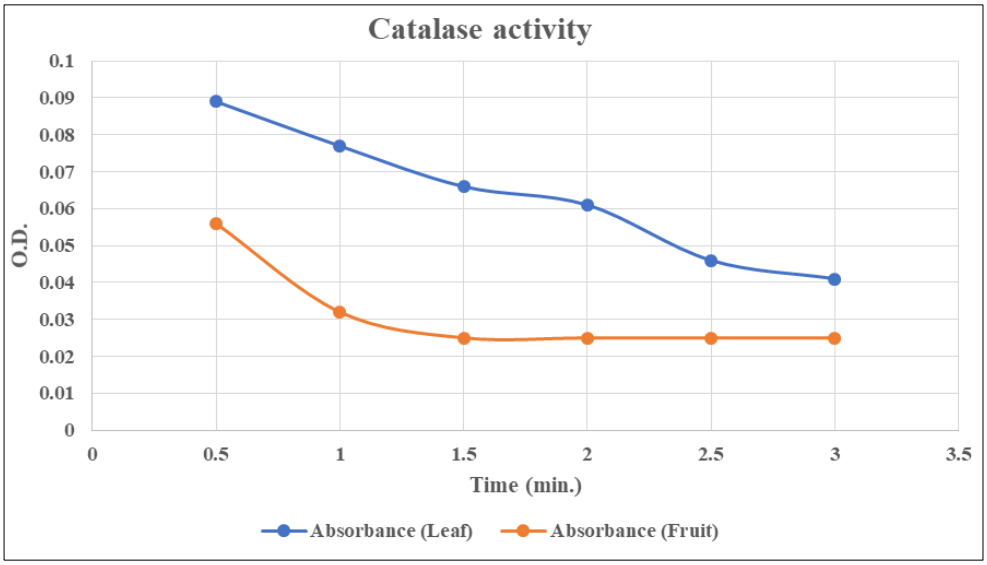

Fig 1: Enzyme activity of catalase present in fresh leaf and fruit of *P. guajava* Linn.

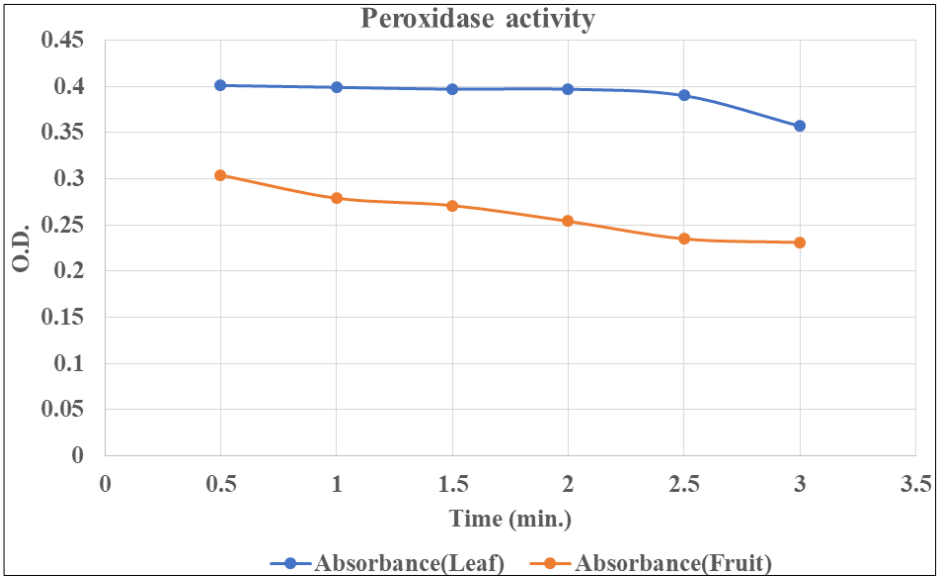

Fig 2: Comparative study of CAT activity of leaf and fruit of *P. guajava* Linn.

In DPPH Assay, antioxidant activity was measured spectrophotometrically. DPPH is a purple-colored stable free radical. When antioxidant compounds present in the sample react with DPPH, its reduction takes place which leads to the change in color of the sample from purple to lighter color. Hence increase in % RSA value with an increase in the concentration of crude aqueous and methanolic leaf and fruit extracts was observed (Table 1). Order of antioxidant activity observed as: Methanolic leaf extract (IC_{50}=1.48 µg/ml)> aqueous leaf extract (IC_{50}=1.52 µg/ml)> methanolic fruit extract (IC_{50}=1.90 µg/ml) > aqueous fruit extract (IC_{50}=2.01 µg/ml).

Table 1: % RSA values of ascorbic acid (A.A.) and aqueous (Aq.) and methanolic (Me.) leaf (LE) and fruits (FE) extracts at different concentrations

Sample	% RSA Value at Different Concentration (µg/ml)					IC Values
	20	40	60	80	100	
A.A.	68.3748	75.9882	81.8448	86.8228	97.3646	IC_{90}=4.15
Aq. LE	43.3382	53.8799	70.4246	77.7452	87.1167	IC_{50}=1.52
Aq. FE	37.7745	46.8521	65.1537	75.6954	85.6515	IC_{50}=2.02
Me. LE	42.6061	55.7833	71.0102	81.5519	89.1654	IC_{50}=1.48
Me. FE	39.0922	50.5124	6.1040	77.0132	86.0908	IC_{50}=1.91

The DPPH radical scavenging activity in comparison with the % RSA of Ascorbic acid was (IC_{90}=4.15). Results indicate that crude leaf extracts have more antioxidant activity than fruit extracts. The leaf and fruit extracts show % RSA activity almost equivalent to the ascorbic acid (Figure 3).

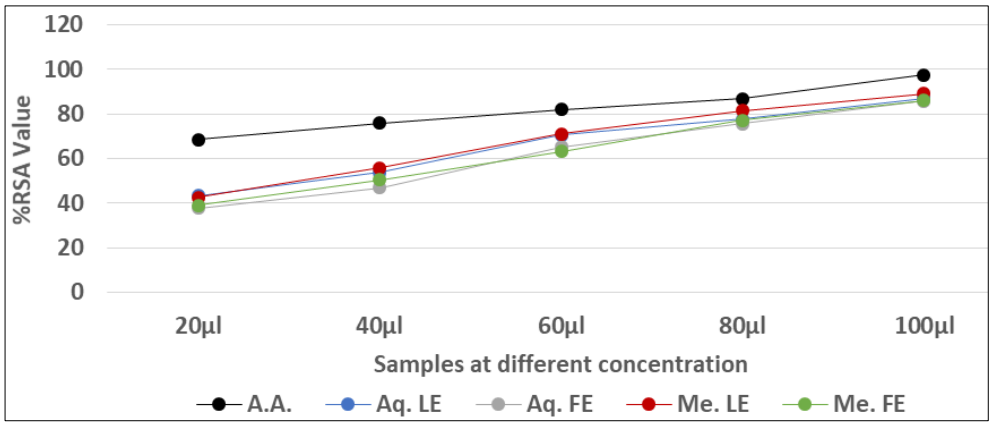

Fig 3: % RSA values of ascorbic acid (A.A.) and aqueous (Aq.) and methanolic (Me.) leaf and fruits extracts at different concentrations.

In recent studies done on crude methanolic extracts of four *Melaleuca* and five *Syzygium species*, good DPPH radical scavenging activity was observed [15]. Methanolic bark extract of *Syzygium caryophyllatum* showed DPPH activity with IC_{50}=6.20±0.01 µg/ml [16]. In a study, the % RSA activity of methanolic extract of *P. guajava* Linn. leaf at 10, 20, 40, 80 µg/ml were 34±1.0, 66.8±4.3, 78.2±1.1 and 85.7±1.8 respectively. The results were almost similar to the current study [17]. FRAP assay for the estimation of antioxidant activity was performed. With an increase in the concentration of the test sample, crude aqueous and methanolic, leaf and fruit extracts, % FRAP scavenging activity also increased (Figure 4).

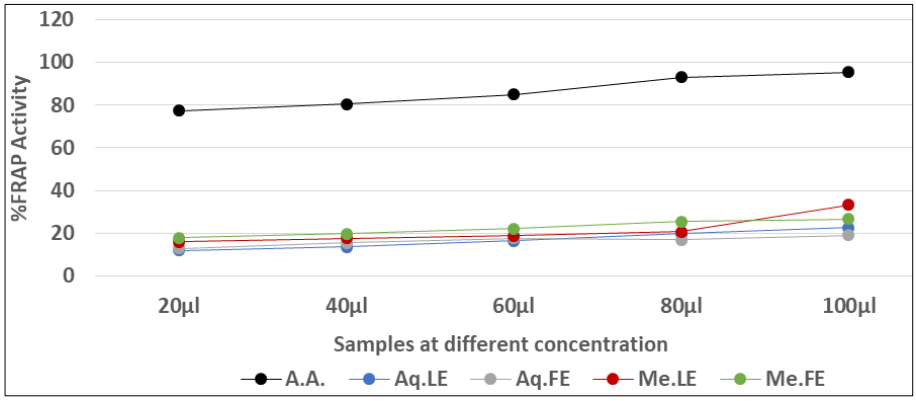

Fig 4: % FRAP Activity values of ascorbic acid (A.A.) and aqueous (Aq.) and methanolic (Me.) extracts of leaf and fruit of *P. guajava* Linn. at different concentration

The antioxidant activity was observed in the following order (Table 2): Methanolic leaf extract (IC_{50}=10.64 µg/ml)> methanolic fruit extract (IC_{50}=14.77 µg/ml)> aqueous leaf extract (IC_{50}=15.08 µg/ml) > aqueous fruit extract (IC_{50}=26.91 µg/ml). The FRAP radical scavenging activity was compared with the % RSA of Ascorbic acid (IC_{90}=3.76 µg/ml).

Table 2: % FRAP Activity values of ascorbic acid (A.A.) and aqueous (Aq.) and methanolic (Me.) extracts of leaf (LE) and fruit (FE) of *P. guajava* Linn. at different concentrations.

Sample	% RSA Value at Different Concentration (µg/ml)					IC Value
	20	40	60	80	100	
A.A.	77.4619	80.4061	84.9746	92.9949	95.533	IC_{90} =3.76
Aq. LE	11.9877	13.7295	16.5984	19.7746	22.6434	IC_{50}=15.08
Aq. FE	12.9098	15.4713	17.6229	17.0082	19.1598	IC_{50}=26.91
Me. LE	16.0861	17.7254	18.8524	20.7992	33.2992	IC_{50}=10.64
Me. FE	17.8279	19.7746	22.1312	25.4098	26.7418	IC_{50} 14.77

Results indicate that crude methanolic extracts have more antioxidant activity than aqueous extracts. The results were almost similar to the study done on the different solvent extracts of *Syzygium cumini* L. fruit extract [18]. Aqueous leaf extract of *Psidium guajava* L. showed excellent scavenging activity compared to vitamin E in a study done by [19].

Conclusion

Present study conducted shows, the significant antioxidant potential of leaf and fruits of *Psidium guajava*. Results concluded that the aqueous and methanolic extracts of the leaf and fruit of guava have promising radical scavenging activity and are a good natural source of antioxidants that can effectively reduce the free radicals from the cells and tissue and prevent oxidative damage. Phenolic compounds with reactive hydroxyl groups convene the potential to scavenge free radicals via a broad range of mechanisms, A significant correlation between phyto-compounds and free radical scavenging potential has been stated in recent research. Hence, analysis of the potential of these extracts opens doors to novel options to explore as replacements for chemical-based drugs to treat diseases and to minimize the effects of damages that are caused by due to the presence of excessive ROS.

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Conflict of Interest: There are No potential conflicts of Interest.

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