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DETERMINATION OF ANTIFUNGAL AND ANTIOXIDANT POTENTIAL OF LEAF AND FRUIT EXTRACTS OF GUAVA (*Psidium guajava* Linn.)

Richa Bhardwaj*, Devyani Naruka and Smita Purohit

Department of Botany, IIS (deemed to be University), S.F.S., Mansarovar, Jaipur – 302 020, Rajasthan (India)
*e-mail: *richa.bhardwaj@iisuniv.ac.in*

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ABSTRACT

The present study was aimed to evaluate the antifungal and antioxidant properties of leaf and fruit extracts of guava (*Psidium guajava* Linn.). Antifungal assays were conducted to assess the efficacy of extracts against clinically relevant fungal strains, *viz.*, *Aspergillus niger* MTCC-4325, *Penicillium chrysogenum* MTCC-2539, *Candida albicans* MTCC-7315, and *Fusarium solani* MTCC-3309. Besides, enzyme activity for catalase (CAT) and peroxidase (POD) were assayed to elucidate the antioxidant potential of extracts. The radical scavenging activities of extracts were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays to quantify the overall antioxidant capacity. Results indicated pronounced inhibitory activity against *F. solani* in methanolic extracts of leaves (21.66 ± 2.05) and fruits (24.66 ± 1.25), with aqueous extracts exhibiting comparatively lesser inhibition across the tested fungal strains. Moreover, the enzyme activity assays revealed higher CAT and POD activities in fresh leaf extract as compared to the fruit extract. Notably, DPPH assay underscored the superior antioxidant potential of *Psidium* leaf extracts relative to fruit extracts, while FRAP assay showed higher radical scavenging capacity of methanolic extract as compared to the aqueous extract.

Keywords: Antifungal, antioxidant, CAT, DPPH, extracts, FRAP, POD

INTRODUCTION

Throughout history, ancient civilizations have relied on botanical remedies for medicinal problems. Despite the advancements in contemporary medicine, botanical resources continue to play a crucial role in addressing various health concerns. This enduring reliance is underscored by the escalating challenge of antimicrobial resistance, particularly concerning the proliferation of multi-drug resistant pathogens (Afanyibo, 2018). Notably, fungal pathogens, like *Aspergillus niger*, *Penicillium chrysogenum*, *Candida albicans*, and *Fusarium solani*, represent formidable adversaries with wide-ranging implications, from food preservation to the manifestation of severe systemic infections (Naglik *et al.,* 2019; Talapko *et al.,* 2021; Fierro *et al.,* 2022; Yu *et al.,* 2022; Nucci and Anaissie, 2023). Furthermore, the pervasive threat of oxidative stress, implicated in a spectrum of life-threatening conditions such as cardiovascular diseases, respiratory disorders, and oncogenesis, necessitates the development of sustainable therapeutic interventions with minimal adverse effects (Kaiser *et al.,* 2021). In response to these exigencies, botanical sources have emerged as promising reservoirs of bioactive compounds, notably anthocyanins, carotenoids, flavonoids, and polyphenols are endowed with potent antioxidant properties. These phytochemicals serve as effective scavengers

of reactive oxygen species (ROS), mitigate oxidative damage at cellular and genomic levels (Batiha *et al.,* 2020; Kaiser *et al.,* 2021). Moreover, their utility extends to food preservation, where they contribute to the maintenance of sensory attributes, thereby offer eco-friendly alternatives to synthetic additives with deleterious effects (Gulcin, 2020; Burcher *et al.,* 2023).

Within the taxonomic framework of plant diversity, Myrtaceae family assumes prominence due to its possession of the diverse array of genera and species. Guava (*Psidium guajava*), a member of this botanical lineage, possesses pharmacological attributes owing to a rich repertoire of secondary metabolites including flavonoids, tannins, phenols, triterpenes, saponins, carotenoids, essential oils, and vitamins A and C (de Araujo *et al.,* 2019; Bogha *et al.,*2020). In present study, we evaluated the antifungal and antioxidant potential of *Psidium guajava* leaf and fruit extracts (aqueous and methanolic extracts) against clinically relevant fungal strains, *viz*., *Aspergillus niger*, *Penicillium chrysogenum*, *Candida albicans*, and *Fusarium solani*. Further, DPPH and FRAP assays were done to assess antioxidant activity.

MATERIALS AND METHODS

The present study was conducted in the Department of Botany, IIS (Deemed to be University) Jaipur (India). The guava (*Psidium guajava* Linn.) fruits and leaves were procured from the local market in Jaipur. The identity of samples was confirmed by the botanists at the Herbarium Department of Botany, IIS, Jaipur. The dried material (10 g) along with 100 mL distilled water for aqueous extracts and an equivalent volume of methanol for methanolic extracts was used for Soxhlet extraction. The extracts were desiccated in a hot air oven for 3 h and subsequently air-dried overnight until whole solvent was evaporated. These extracts were used for antifungal activity, DPPH, and FRAP assays.

Antifungal activity

The antimicrobial activity was assayed by well-diffusion method (Sen and Batra, 2012). The agar well diffusion method determines the minimum inhibitory concentration (MIC) of any extracts. Four fungal species *viz., Aspergillus niger* (MTCC-4325), *Penicillium chrysogenum* (MTCC-2539), *Candida albicans* (MTCC-7315), and *Fusarium solani* (MTCC-3309) were procured from MTCC, Chandigarh (India). The fungal cultures were grown on potato dextrose broth in an incubator shaker for 16-24 h. Then aseptic potato dextrose agar plates were prepared, onto which 150 µL fungal culture was spread and kept as such for 20 min. Then wells of 5 mm diameter were prepared on agar plates, and 30 µL aqueous and methanolic leaf and fruit extracts of *P. guajava* (concentration range: 5-100 mg mL^{-1}) were added. The plates were incubated at 28°C for 16 h, and the lowest concentration inhibiting fungal growth was considered as the MIC (Mohammed, 2015; Berkow *et al*., 2020).

For antifungal activity assessment of guava leaf and fruit extracts, wells of 5 mm dia. were made on agar plates, and 30 µL of each test sample (methanolic and aqueous leaf and fruit extracts) at concentrations of 100 and 500 mg mL^{-1} were added to separate plates, along with positive and negative controls (30 µL each). Ketoconazole (1 mg mL^{-1}) served as the positive control (standard). Petri-plates were sealed with paraffin to prevent contamination and placed in an incubator (28°C) for 16-24 h. The experiment was conducted in a randomized comtrol design with each treatment replicated three times. The inhibition zone was measured, and the results represented as the mean value with standard deviation. Also, the antifungal activity index was calculated using following formula:

$$\text{Antifungal activity index} = \frac{\textit{Inhibition area of the test sample}}{\textit{Inhibition area of the Standard}}$$

Catalase activity

Catalase enzymatic activity was assessed by monitoring hydrogen peroxide degradation as per the established protocols (Haida and Hakiman, 2019; Sita *et al*., 2022). Phosphate buffer (pH, 7) was prepared by mixing 61 mL sodium hydrogen phosphate (0.2 M) with 39 mL sodium dihydrogen

phosphate (0.2 M). Fresh leaves and fruits of *P. guajava* (1 g each) were separately macerated along with phosphate buffer in a mortar and pestle, and centrifuged at 5000 rpm for 10 min. The reaction mixture comprised of 2.7 mL 50 mM chilled phosphate buffer (pH 7), 0.1 mL enzyme extract, and 0.2 mL 200 mM H_2O_2 solution. Absorbance was measured spectrophotometrically at 410 nm for 3 min.

Peroxidase activity

The peroxidase activity was determined by monitoring the decomposition of hydrogen peroxide (Haida and Hakiman, 2019). Fresh leaves and fruits of *P. guajava* (1 g each) were individually macerated along with 50 mM phosphate buffer using a mortar and pestle, followed by centrifugation at 5000 rpm for 10 min. For peroxidase assay, the reaction mixture comprised of 2.8 mL 50 mM phosphate buffer, 0.2 mL enzyme extract, 0.5 mL of 0.5% H_2O_2, and 0.5 mL of 1% guaiacol solution. The absorbance was measured spectrophotometerically at 580 nm for 3 min.

DPPH assay

DPPH assay was performed as per the procedure outlined by Beyhan *et al*. (2014) and Kiran *et al*. (2020). A 0.1 M solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) was prepared using methanol as solvent. The solution was stored in darkness to prevent light-induced reduction. For assessing the free radical scavenging activity, 0.1 mL test sample was mixed with 2.9 mL methanolic DPPH solution and incubated for 30 min in dark at room temperature. Subsequently, absorbance was measured at 517 nm using a spectrophotometer. Various concentrations (20, 40, 60, 80, and 100 µL) of ascorbic acid were used as standard. Crude aqueous, and methanolic leaf and fruit extracts were prepared in methanol. Methanol served as blank, while methanolic DPPH solution acted as control. The % RSA was calculated using the formula:

$$\%\text{RSA} = \frac{1-\text{As}}{\text{Ac}}\ x\ 100$$

where A_c = absorbance of control, and A_s = absorbance of sample

The concentration-response relationship between concentration and percent inhibition was graphed linearly, with IC_{50} values in µg mL^{-1} subsequently calculated. The antioxidant efficacy of each sample was expressed in terms of IC_{50} (the concentration needed to inhibit DPPH radical formation by 50%), derived from the inhibition curve.

FRAP assay

The ferric reducing ability of plasma (FRAP) assay, adapted from Benzie and Strain (1996), was used to assess the antioxidant potential of plant extract. Stock solutions comprised 300 mM 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 HCl), and 20 mM $FeCl_3$.$6H_2O$ solution. A fresh working solution was prepared by mixing 25 mL acetate buffer with 2.5 mL $FeCl_3.6H_2O$ and TPTZ solution, brought to room temperature before use. Plant extracts, dissolved in 100 µL DMSO, were combined with 290 µL FRAP solution and allowed to react in dark for 30 min. The absorbance of resultant coloured complex of ferrous tripyridyl triazine was measured at 593 nm. The %FRAP activity was calculated by using the following equation:

$$\%\ \text{FRAP activity}\ = 100 - \frac{control - Test\ sample}{\text{control}}\ x\ 100$$

A linear graph correlating the concentration of sample with percent inhibition was constructed, and the IC_{50} values expressed in µg mL^{-1}. The antioxidant efficacy of each sample was computed from the inhibition curve in terms of IC_{50}, representing the concentration necessary to inhibit the reduction of ferric ions by 50%.

Statistical analysis

All the experiments were executed in triplicate. Mean values $\pm$ standard deviations were computed for each dataset. Statistical analysis was performed to ascertain the significance of variations among different groups. One-way analysis of variance was performed using MS Excel, and Tukey's honestly significant difference (HSD) post hoc test was conducted using an online tool *https://astatsa.com/OneWay_Anova_with_TukeyHSD/*. The $p > 0.05$ was deemed insignificant, while $p^* < 0.05$ and $p^{**} < 0.01$ indicated significant differences.

RESULTS AND DISCUSSION

The antifungal potential of aqueous and methanolic extracts of guava leaves and fruits was assayed by using the agar well diffusion method. The minimum inhibitory concentration (MIC) values against a spectrum of fungal species revealed a range spanning from 10 to 50 mg mL^{-1}. Notably, aqueous extracts of both guava leaves and fruits exhibited conspicuous antifungal activity, particularly against *Aspergillus niger*. Further, these extracts manifested promising outcome against *Fusarium solani*, especially at lower concentrations, suggesting their candidacy as natural antifungal agents. In comparison, the methanolic extracts showed varying antifungal activity (Table 1). While they displayed heightened effectiveness against *F. solani*, their efficacy against *Penicillium chrysogenum* was comparatively moderate. Comparative analyses with other plant extracts provided additional insights. For instance, Besufekad *et al.* (2017) reported antifungal activity of *Myrtus communis* extracts, elucidating inhibition against *A. niger* and *F. oxysporium*, thereby underscoring the varied spectrum of antifungal properties exhibited among different plant species. Moreover, Berkow *et al.* (2020) observed a concentration-dependent augmentation in antifungal activity, particularly against *A. niger*, with higher concentrations of methanolic leaf extract from guava. This observation suggests the potential for optimizing the efficacy of these extracts through concentration adjustments. Oluwajobi *et al.* (2019) conducted a study on the aqueous guava leaf extract, delineating its antifungal activity against *A. niger*, *Candida tropicalis*, and *Trichophyton tonsurans*, thereby accentuating the broad-spectrum efficacy of guava leaf extracts against clinically relevant fungal pathogens.

Table 1: **Antifungal activity of leaf and fruit extracts of guava against different fungi**

Fungi		Aqueous (mg mL^{-1})				Methanol (mg mL^{-1})			
		Leaf		Fruit		Leaf		Fruit	
		100	500	100	500	100	500	100	500
Aspergillus niger MTCC-4325	IZ	10.7 ± 1.25	11.3 ± 1.25	14.0 ± 0.82	13.0 ± 0.82	12.0 ± 0.82	18.7 ± 2.06	19 ± 1.63	18.3 ± 1.7
	AI	0.32	0.30	0.42	0.35	0.34	0.51	0.54	0.51
Penicillium chrysogenum MTCC-2539	IZ	12.7 ± 1.25	0.33 ± 0.47	0.66 ± 0.94	0	13.7 ± 0.94	8.7 ± 1.25	11.0 ± 0.82	13.7 ± 1.25
	AI	0.40	0.01	0.45	0	0.42	0.25	0.33	0.40
Candida albicans MTCC-7315	IZ	1.33 ± 1.25	0.66 ± 0.94	0.33 ± 0.47	0.66 ± 0.94	14.3 ± 1.25	12.0 ± 1.25	18.0 ± 1.63	14.7 ± 1.25
	AI	0.04	0.20	0.01	0.02	0.43	0.36	0.54	0.44
Fusarium solani MTCC-3309	IZ	14.7 ± 1.25	0.33 ± 0.47	15.7 ± 0.47	0.33 ± 0.47	21.7 ± 2.05	14.0 ± 0.51	24.7 ± 1.25	16.3 ± 0.94
	AI	0.51	0.009	0.54	0.009	0.64	0.42	0.73	0.49

IZ = Inhibition zone in mm, AI = Antifungal activity index)

The antioxidant activity of guava leaf and fruit extracts was evaluated by measuring catalase enzyme (CAT) and peroxidase enzyme (POD) activities, and DPPH and FRAP assays. The study revealed a significant decrease in CAT activity in leaf extracts in comparison to the fruit extract (Fig. 1). Conversely, no substantial change in absorbance was observed for POD activity in either leaf or fruit extracts (Fig. 2), suggesting negligible POD activity in both samples.

The DPPH assay revealed an increase in percent radical scavenging activity (%RSA) with escalating concentrations of crude aqueous and methanolic leaf and fruit extracts (Table 2). The order of antioxidant activity observed was as follows: methanolic leaf extract (IC_{50} = 29.68 ± 1.5 µg mL^{-1}) > aqueous leaf extract (IC_{50} = 30.17 ± 1.62 µg mL^{-1}) > methanolic fruit extract (IC_{50} = 38.07 ± 2.26 µg mL^{-1}) > aqueous fruit extract (IC_{50} = 40.43 ± 1.84 µg mL^{-1}). When compared with the %RSA of ascorbic acid (IC_{90} = 83.16 ± 1.92 µg mL^{-1}), the DPPH radical scavenging activity of leaf extracts was higher than that of fruit extracts, indicating superior antioxidant potential in leaf extracts. In light of the recent advances, these findings contribute to our understanding of the antioxidant properties of guava extracts. The differential CAT activity between leaf and fruit extracts suggests a tissue-specific variation in antioxidant enzyme activity, which warrants further investigation. Additionally, the higher

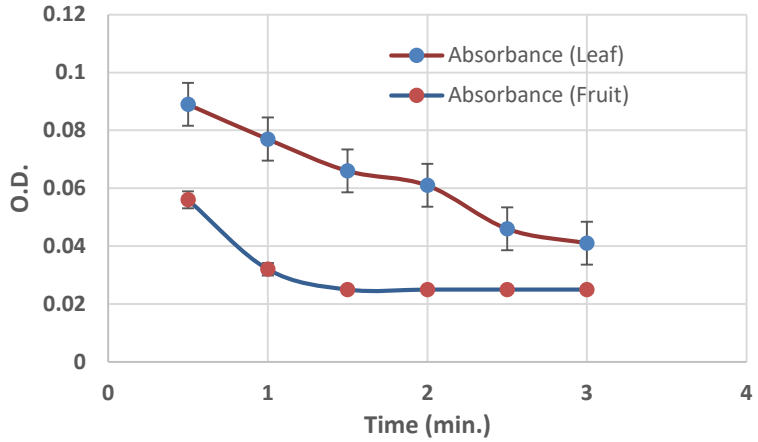

Fig. 1: Absorbance vs. time graph of catalase activity of guava leaf and fruit extracts

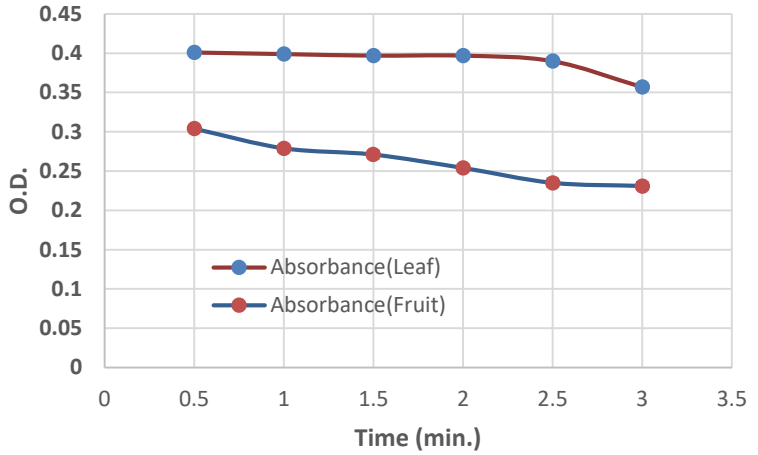

Fig. 2: Absorbance vs. time graph of peroxidase activity of guava leaf and fruit extracts

antioxidant activity observed in leaf extracts underscores the potential therapeutic value of guava leaves as a source of natural antioxidants. Further studies exploring the underlying mechanisms of antioxidant activity and the identification of bioactive compounds responsible for this activity are warranted for the development of anti-oxidant-rich pharmaceuticals and nutraceuticals.

The radical scavenging activity (%RSA) of guava leaf and fruit extracts demonstrated comparable efficacy to ascorbic acid (Table 2). Notably, recent studies on crude methanolic extracts of many *Melaleuca* and *Syzygium* species have reported significant DPPH radical scavenging activity (Khalaf *et al*., 2021). For instance, the methanolic bark extract of *Syzygium caryophyllatum* exhibited considerable DPPH activity with IC_{50} value of 6.20 ± 0.01 µg mL^{-1} (Chandra *et al*., 2022). Similarly, Zhin *et al*. (2017) reported %RSA activity of methanolic extract of guava leaf, which closely aligns with present findings.

Our study used FRAP assay to estimate antioxidant activity. The %FRAP scavenging activity of crude aqueous and methanolic extracts of both leaf and fruit samples increased with increase in concentrations. Notably, the antioxidant activity followed the order: methanolic leaf extract (IC_{50} = 106.39 ± 4.19 µg mL^{-1}) > methanolic fruit extract (IC_{50} = 147.73 ± 3.47 µg mL^{-1}) > aqueous leaf extract (IC_{50} = 150.82 ± 2.67 µg mL^{-1}) > aqueous fruit extract (IC_{50} = 269.12 ± 5.78 µg mL^{-1}) [Table 3]. The comparison with %RSA of ascorbic acid (IC_{90} = 37.6 ± 1.13 µg mL^{-1}) underscores the superior

Table 2: The radical scavenging activity (%RSA and IC_x value (µg mL^{-1}) of ascorbic acid (A.A.), aqueous (Aq.) and methanolic (Me.) guava leaf (LE) and fruit (FE) extracts at different concentrations

Samples	RSA (%) at different concentrations (µL)					IC_x value
	20	40	60	80	100	
A.A.	68.37 ± 0.14	75.98 ± 0.016	81.8 ± 1.67	86.82 ± 1.88	97.36 ± 1.97	IC_{90} = 83.16 ± 1.92
Aq.LE	43.34 ± 0.48	53.88 ± 1.21	70.42 ± 1.02	77.74 ± 1.55	87.11 ± 0.47	IC_{50} = 30.17 ± 1.62
Aq. FE	37.77 ± 1.13	46.85 ± 1.26	65.15 ± 0.61	75.69 ± 2.25	85.65 ± 0.98	IC_{50} = 40.43 ± 1.84
Me. LE	42.60 ±1.11	55.78 ± 0.77	71.01 ± 1.14	81.55 ± 0.99	89.16 ± 1.29	IC_{50} = 29.68 ± 1.50
Me. FE	39.09 ± 1.65	50.51 ± 1.32	63.10 ± 0.96	77.01 ± 1.43	86.09 ± 1.57	IC_{50} = 38.07 ± 2.26

Table 3: FRAP activity (%) and IC_x values (µg mL-1) of ascorbic acid (A.A.) and aqueous (Aq.) and methanolic (Me.) extracts of guava leaf (LE) and fruit (FE) at different concentrations

Samples	%FRAP activity at different concentrations (µL)					IC_x value
	20	40	60	80	100	
A.A.	77.55 ± 0.38	80.46 ± 0.08	84.80 ± 0.22	92.60 ± 0.34	95.37 ± 0.15	IC_{90} = 37.6 ± 1.13
Aq.LE	11.84 ± 0.12	13.72 ± 0.05	16.60 ± 0.08	19.58 ± 0.17	22.47 ± 0.16	IC_{50} = 150.8 ± 2.67
Aq. FE	12.88 ± 0.07	15.37 ± 0.11	18.75 ± 0.21	17.08 ± 0.07	19.37 ± 0.19	IC_{50} = 269.1 ± 5.78
Me. LE	16.14 ± 0.06	17.73 ± 0.04	19.22 ± 0.15	20.52 ± 0.24	33.28 ± 0.17	IC_{50} = 106.4 ± 4.19
Me. FE	17.71 ± 0.13	19.49 ± 0.25	22.22 ± 0.15	25.39 ± 0.04	26.64 ± 0.16	IC_{50} = 147.7 ± 3.47

antioxidant activity of crude methanolic extracts over aqueous extracts. This finding is consistent with Qamar *et al*. (2021) who worked on different solvent extracts of *Syzygium cumini* fruit.

The present study confirms the antifungal attributes inherited in guava extracts. Their demonstrated efficacy against a variety of fungi show their potential as natural alternatives for the management of fungal infections, thereby warranting further exploration into their underlying mechanisms of action and prospective clinical applications. Additionally, our study corroborates the excellent scavenging activity exhibited by aqueous leaf extract of guava as compared to vitamin E, as demonstrated by Alisi *et al*. (2018). These collective findings accentuate the potent antioxidant potential of guava extracts and their promising role in combating oxidative stress-related ailments.

Conclusion: The study revealed remarkable antifungal and radical-scavenging activities of aqueous and methanolic extracts of guava. These extracts serve as valuable natural reservoirs of antioxidants, which effectively counteract the cellular and tissue oxidative stress by neutralizing the free radicals. Notably, the presence of phenolic compounds containing reactive hydroxyl groups demonstrate diverse mechanisms for scavenging free radicals. The therapeutic potential of guava leaf and fruit extracts offers innovative avenues for combating fungal infections and mitigating the oxidative damage caused by the accumulation of reactive oxygen species (ROS). Eco-friendly pharmaceuticals can be developed by harnessing these natural resources through adoption of sustainable synthesis techniques.

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