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Antioxidant and antilarvicidal potential of guava (Psidium Guajava) leaf extract: A study on free radical scavenging and brine shrimp toxicity

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Abstract
The leaves of guavas (*Psidium guajava*) contain bioactive substances that exhibit strong Antilarvicidal and antioxidant properties. This study examines the toxicity against brine shrimp and its antioxidant potential of an aqueous leaf extract of guava. DPPH, FRAP, ABTS, superoxide free radical neutralization, nitric oxide inhibition, and hydroxyl oxidative stress reduction tests were among the antioxidant assays conducted on the extract obtained from dried guava leaves. Strong ability to scavenge free radicals was demonstrated in the findings, highlighting the *Psidium guajava* aqueous extract's potential to mitigate oxidative stress. Furthermore, the extract's Antilarvicidal effect was evaluated using the brine shrimp cytotoxicity assay. The half-maximal lethal dose (LC_{50}) of Artemia salina nauplii validated its toxic impact on larvae, with mortality increasing proportionally with concentration. The findings suggest that guava leaf extract serves as a valuable reservoir of bioactive molecules and natural antioxidants, with possible applications in agriculture and the pharmaceutical sector. Its therapeutic value should be fully explored, and further research should be conducted to elucidate its mechanism of action.

Keywords: Antilarvicidal activity, Antioxidant potential, Brine shrimp assay, Bioactive compounds

Introduction

Guava, scientifically known as *Psidium guajava* L., belongs to the family Myrtaceae and is a small tropical tree predominantly cultivated in tropical and subtropical regions of the world. Its phytochemical constituents include flavonoids, tannins, alkaloids, phenols, glycosides, terpenes, coumarins, and essential oils, which contribute to its health-promoting properties and make it a valuable ingredient in herbal remedies and cosmetics (Harun *et al*., 2024) [4]. The fruit, leaves, and seeds of Psidium guajava are rich in polyphenols and flavonoids, which offer significant antioxidant properties. These compounds protect cells against oxidative stress and play a key role in enhancing immunity, reducing inflammation, and preventing chronic diseases (Sasan *et al*., 2021 [34]; Kareem and Kadhim, 2024) [18].

Guava's leaves and fruits are also abundant in bioactive constituents such as carotenoids, saponins, vitamin C, tannins, ellagic acid, and catechins. These compounds demonstrate a wide range of pharmacological activities including antimicrobial, antidiabetic, antimutagenic, anticancer, hepatoprotective, antimalarial, anti-inflammatory, wound healing, and cardiovascular benefits (Campos *et al*., 2023 [5]; García-Villegas *et al*., 2023) [10]. Several terpenoids like β-caryophyllene and α-copaene found in guava leaves exhibit potent antioxidant and anti-inflammatory effects, while polyphenols like epigallocatechin-3-gallate (EGCG) are known for their photoprotective and anti-aging activity (Ng *et al*., 2022; Lestari *et al*., 2022) [21].

Flavonoids such as quercetin, kaempferol, and rutin are present in guava in significant amounts, supporting the plant's antioxidant and antimicrobial activity. Compounds such as citric acid, oleanolic acid, and various phenolic acids further enhance its nutraceutical value (Geoffrey *et al*., 2022 [11]; Marina and Noriham, 2014) [23]. Additionally, studies have shown that guava extracts possess larvicidal properties against mosquito larvae. Methanolic extracts in particular were observed to exhibit the highest mortality rate against Aedes aegypti and Culex pipiens larvae, indicating its potential as a natural larvicide (McCook-Russell *et al*., 2019 [24]; Milani *et al*., 2018) [26]. Despite extensive data on the phytochemistry and pharmacology of guava, its mosquito repellent properties remain relatively underexplored, providing an avenue for further research on essential oils or other derived compounds from Psidium guajava (Imam *et al*., 2015 [15]; Kokilananthan *et al*., 2016) [19].

Given its multifunctional bioactive composition, guava holds great potential not only in the food and pharmaceutical industries but also in public health, particularly for vector control applications (Ruksiriwanich *et al*., 2022 [33]; Thaipong *et al*., 2024 [37]; Oliveira *et al*., 2024) [28].

Materials and Methodology

Preparation of aqueous extract of Psidium guajava leaf

A mixture of 50 grams of dried guava leaf powder and 500 milliliters of distilled water was heated to 60 degrees Celsius for half an hour while being continuously stirred. Following cooling, the extract underwent centrifugation at 3000 rpm for 15 minutes. After filtering the supernatant through Whatman No.1. filter paper, it was stored for additional analysis at 4°C (Yousefi *et al*., 2018 [39]; El-Kased *et al*., 2017) [7].

Antioxidant Potential of Psidium guajava Aqueous Leaf Extract

The antioxidant potential of Psidium guajava extract was evaluated through multiple assays. Different concentrations of the extract, which included 10µg/mL, 50µg/mL, 100µg/mL, and 200 µg/mL, were combined with an equal

volume of DPPH solution, left undisturbed for 30 minutes in darkness, and the absorbance at 517 nm was recorded to assess their free radical scavenging ability (Brand-Williams *et al*., 1995) [4]. The ferric reducing potential (FRAP) assay involved measuring absorbance at 593 nm after reacting 100 µL of the extract with the FRAP reagent, which contained acetate buffer, TPTZ, and $FeCl_3 \cdot 6H_2O$, for four minutes at 37°C (Benzie & Strain, 1996) [3].

In the ABTS assay, ABTS was oxidized using potassium persulfate to generate the $ABTS^{\cdot+}$ radical. A volume of 10 µL of the extract was introduced into 1 mL of this solution, and after six minutes, the absorbance at 734 nm was determined to evaluate its scavenging activity (Re *et al*., 1999). To assess superoxide radical scavenging, a reaction mixture containing NBT, NADH, and PMS in phosphate buffer (pH 7.4) was used, to which 1 mL of extract was added. At 560 nm, absorbance was measured after 10 minutes at 25°C (Beauchamp & Fridovich, 1971). For nitric oxide scavenging, the reaction involved the addition of Griess reagent, followed by measuring absorbance at 540 nm after $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ in phosphate buffer (pH 7.4) was incubated for two hours at 25°C (Marcocci *et al*., 1994). Hydroxyl radical scavenging activity was examined using a reaction system composed of ascorbic acid, deoxyribose, $FeCl_3$, EDTA, and H_2O_2. This mixture was incubated for 60 minutes at 37°C, followed by the addition of TBA and heating for 15 minutes at 95°C (Halliwell *et al*., 1987) [13].

Antilarvicidal Activity of Psidium guajava Aqueous Leaf Extract Using the Brine Shrimp Lethality Assay

The antilarvicidal potential of *Psidium guajava* aqueous leaf extract was evaluated using the brine shrimp lethality test. Artificial seawater that had been prepared by diluting 3–8 g of sea salt in 1 liter of double-distilled water, in this seawater, Artemia salina eggs were used to hatch them. The eggs were maintained at 28°C for 48 hours under continuous light and aeration to facilitate the hatching of free-swimming nauplii. Once hatched, the live nauplii were collected for experimental analysis (Kwon *et al*., 2014 [20]; Sorgeloos *et al*., 1978) [35]. The guava leaf extract was prepared using distilled water as a solvent at different concentrations of 10µg/mL, 50µg/mL, 100µg/mL, and 200 µg/mL. Each well of a 24-well microplate was filled with 1 mL of the respective extract concentration and ten nauplii. Distilled water served as the negative control, while potassium dichromate (100 µg/mL) was used as the positive control (Vanhaecke *et al*., 1981 [19]; Carballo *et al*., 2002) [6]. The microplates were incubated at 25 ± 2°C for 24 hours under continuous illumination. Post-incubation, a microscope was employed to count Nauplii fatality number in each well. Nauplii were considered dead if they remained motionless when gently prodded. Mortality percentage was calculated using the formula:

Mortality (%) = (Total number of larvae / Number of dead larvae) × 100

The median lethal concentration (LC_{50}), which represents the concentration of the extract required to induce 50% mortality in nauplii, was determined using Probit analysis (Finney, 1971 [9]; EPA, 2002). All experiments were performed in triplicate to ensure statistical reliability. A lower LC_{50} value indicates greater toxicity and a more potent antilarvicidal effect of the Psidium guajava aqueous leaf extract.

Results

Aqueous extract preparation of *Psidium guajava* leaves

The aqueous extract of guava leaf was successfully obtained by heating 50 g of dried powder with 500 mL distilled water at 60 °C for 30 minutes, followed by centrifugation and filtration. The clear supernatant was stored at 4 °C for further analysis.

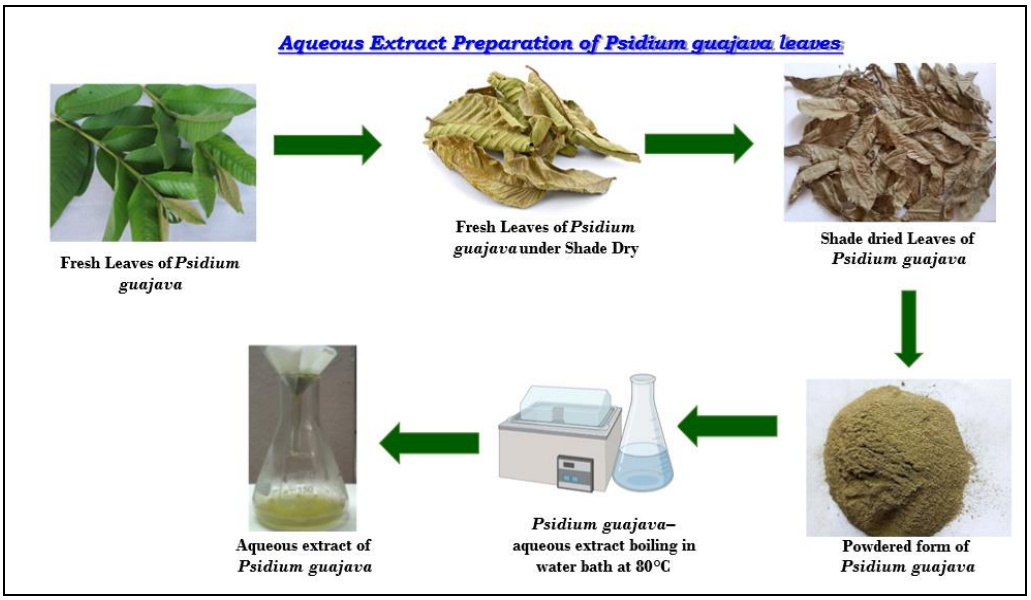


Fig 1: shows the Aqueous extract preparation of *Psidium guajava* leaves

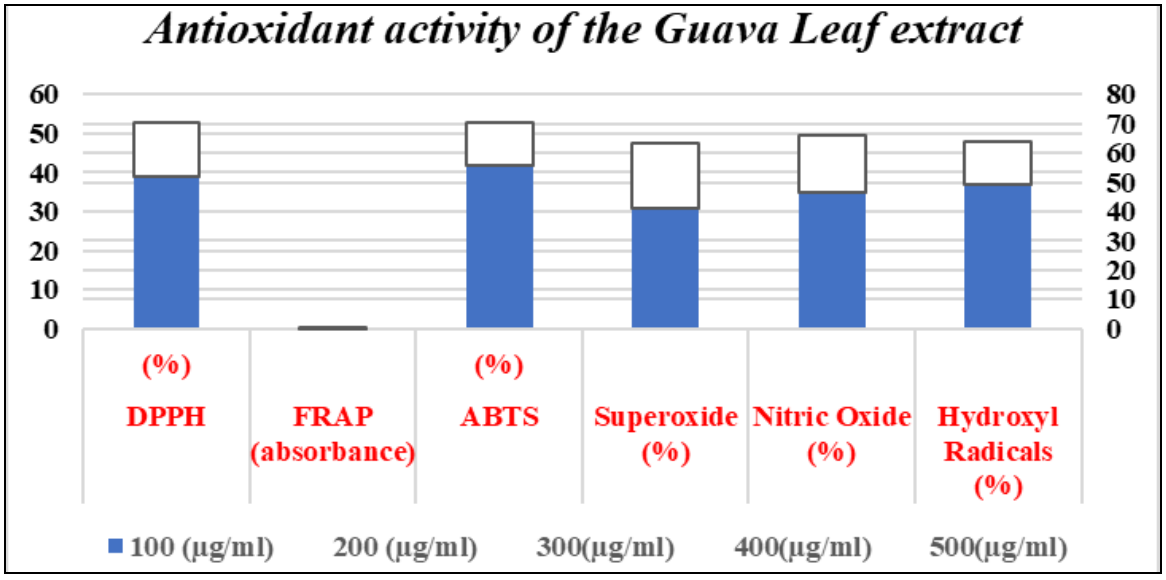
Antioxidant activities of *Psidium guajava* aqueous leaf extract

DPPH, FRAP, ABTS, nitric oxide, superoxide, and hydroxyl radical scavenging assays were among the in vitro tests used to assess the *Psidium guajava* leaf extract's antioxidant capacity. With the maximum percentage inhibition seen at 100 µg/mL, the DPPH radical scavenging activity showed a concentration-dependent inhibition. The

FRAP assay indicated Strong ferric-reducing antioxidant power, which showed a notable increase in Fe (II) equivalents. The ABTS assay also demonstrated successful radical cation decolorization, as evidenced by a discernible drop in absorbance at 734 nm. The guava leaf extract demonstrated a significant diminution in absorbance at 560 nm in the superoxide radical scavenging assay, indicating its effectiveness in scavenging superoxide radicals. The extract's potential was further validated by the nitric oxide scavenging assay, which showed a notable drop in absorbance at 540 nm, suggesting that it could prevent the production of nitric oxide. The hydroxyl radical scavenging assay displayed significant absorbance reduction at 532 nm, demonstrating the extract's strong hydroxyl radical neutralizing capacity. All of these findings point to the guava leaf extract's strong antioxidant capacity, which could support its medicinal uses.

Table 1: Antioxidant activities of *Psidium guajava* aqueous leaf extract

Guava Leaf Extract (µg/ml)	DPPH (%)	FRAP (absorbance)	ABTS (%)	Superoxide (%)	Nitric Oxide (%)	Hydroxyl Radicals (%)
100	45.2	0.25	50.3	35.8	40.1	42.7
200	52.1	0.35	55.8	41.2	46.3	49.2
300	60.3	0.45	62.1	50.7	53.9	55.6
400	65.8	0.55	66.4	58.2	60.5	60.4
500	70.5	0.65	70.5	63.1	65.8	64.1



The graph shows the dose-dependent antioxidant activity of guava leaf extract across various assays (DPPH, FRAP, ABTS, Superoxide, Nitric Oxide, and Hydroxyl Radicals).

Antilarvicidal Activity of *Psidium guajava* leaf extract Using Brine Shrimp Lethality Assay

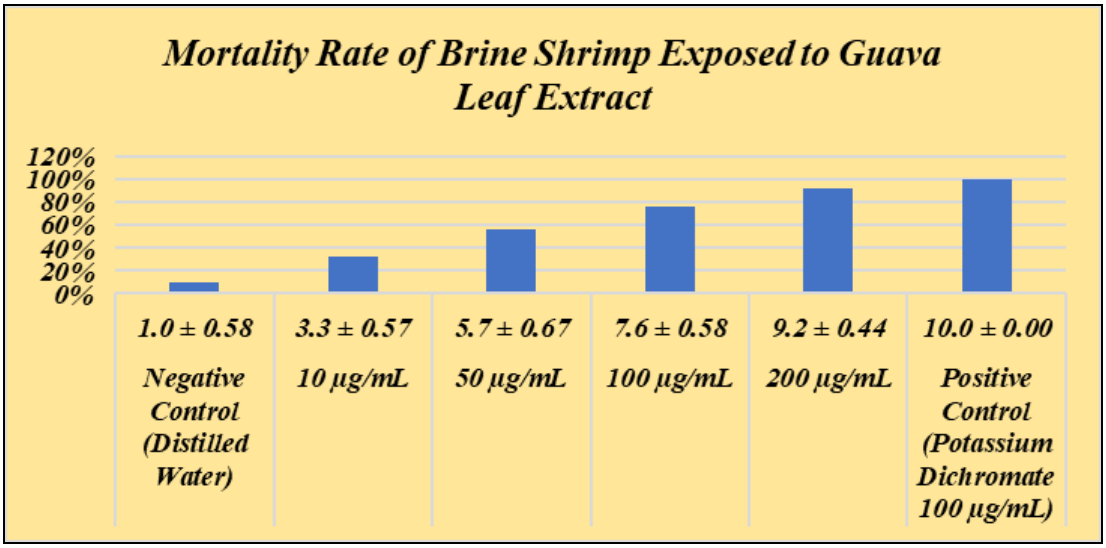
The brine shrimp lethality assay was used to examine the Antilarvicidal properties of *Psidium guajava* leaf extract. After being continuously aerated and exposed to light at 28°C for 48 hours, Artemia salina eggs were incubated in artificial seawater, which is created by adding 3–8 grams of sea salt per liter of distilled water. The eggs then hatched into nauplii. The hatched nauplii were then subjected to varying concentrations of aqueous leaf extract of *Psidium guajava* (10, 50, 100, and 200 µg/mL), while the positive control was potassium dichromate (100 µg/mL) and the negative control was distilled water. A 24-well microplate containing 10 nauplii per well was used for the triplicate experiment. The number of dead nauplii was enumerated using a microscope after the microplates were kept at 25 ± 2°C under constant light for 24 hours. The following formula was used to determine the percentage mortality:

Mortality (percent) = (Total number of larvae\ Number of dead larvae) ×100

The results showed that mortality increased in a dose-dependent manner. Whereas the positive control (potassium dichromate, 100 µg/mL) had the highest mortality, the negative control (distilled water) had the lowest mortality. Probit analysis was used to determine the LC_{50} value of guava leaf extract, which indicated the potential for toxicity. Higher extract Antilarvicidal activity is indicated by a lower LC_{50} value.

Table 1: Mortality Rate of Brine Shrimp Exposed to Guava Leaf Extract

Concentration (µg/mL)	Average Number of Dead Nauplii (Mean ± SD)	Mortality (%)
Negative Control (Distilled Water)	1.0 ± 0.58	10%
10 µg/mL	3.3 ± 0.57	33%
50 µg/mL	5.7 ± 0.67	57%
100 µg/mL	7.6 ± 0.58	76%
200 µg/mL	9.2 ± 0.44	92%
Positive Control (Potassium Dichromate 100 µg/mL)	10.0 ± 0.00	100%



The graph shows a dose-dependent increase in brine shrimp mortality with guava leaf extract, peaking at 100% in the positive control.

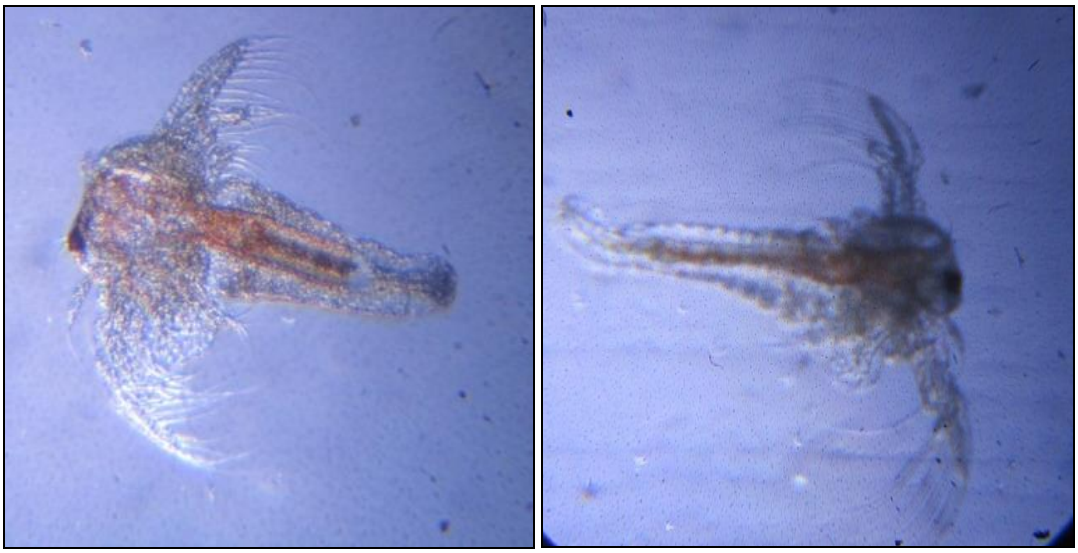


Fig 2: Brine shrimp larvae after exposure to concentrations of Guava leaf extract

Table 2: LC_{50} Value of Guava Leaf Extract

Sample	LC_{50} (µg/mL)
Guava Leaf Extract	54.3 µg/mL
Positive Control (Potassium Dichromate)	15.2 µg/mL

Discussion

The guava *(Psidium guajava)* leaf aqueous extract demonstrated potent antioxidant activity in various in vitro tests. The study found a concentration-dependent increase in the DPPH free radical scavenging activity, with the highest inhibition observed at 500 µg/mL concentration. Similar concentration-dependent antioxidant activities have been reported by Jiménez-Escrig *et al.* (2001) [16] and Riaz *et al.* (2012) [32], who emphasized the role of phenolic compounds in such activities. Significant ferric-reducing potential was shown by the FRAP assay, and successful radical cation decolorization was confirmed by the ABTS assay, aligning with the findings of Thaipong *et al.* (2006) [36], who compared antioxidant methods in fruits including guava.

Furthermore, the extract significantly inhibited the production of nitric oxide, as shown by decreased absorbance at 540 nm, and efficiently scavenged superoxide radicals, as demonstrated by reduced absorbance at 560 nm. These observations are supported by earlier studies such as those by Arima and Danno (2002) [1], which reported nitric oxide inhibition by guava leaf extract, and Miean and Mohamed (2001) [25], who demonstrated strong superoxide radical scavenging from tropical plant leaves. Further confirming the extract's strong antioxidant properties and pointing to possible therapeutic uses was the hydroxyl radical scavenging assay, consistent with results by Gullón *et al.* (2016) [12], who investigated hydroxyl radical inhibition using plant phenolics.

The lethality test evaluated the guava leaf extract's Antilarvicidal properties. The findings showed that mortality increased in a dose-dependent manner, with 92 percent of deaths occurring at the highest concentration (200 µg/mL). The extract's LC_{50} value was 54.3 µg/mL, which indicates moderate toxicity when compared to potassium dichromate, the positive control (LC_{50} = 15.2 µg/mL). These results are in agreement with the studies by Kalyanasundaram and Das (1985) [17] and Rahuman *et al.* (2008) [29], which demonstrated larvicidal activity of plant extracts against Artemia salina and mosquito larvae. These

results highlight the potential of guava leaf extract as a natural Antilarvicidal agent, but they also underscore the necessity of additional research to evaluate its safety and effectiveness in biological applications, as emphasized by Ramos *et al*. (2017) [30] in their review on plant-derived larvicides.

Conclusion

The study's conclusions demonstrate the potent Antilarvicidal and antioxidant properties of *Psidium guajava* leaf extract. Across several antioxidant tests, the extract showed potent free radical scavengers, indicating that it may be used to reduce diseases linked to oxidative stress. A dosage-related toxicity was also confirmed by the shrimp lethality assay, which showed promising Antilarvicidal properties with a median lethal concentration of 54.3 µg/ mL. Though more in vivo research and toxicity assessments are required to confirm its safety and effectiveness for medical and agricultural applications, these findings imply that guava leaf extract may function as a natural antioxidant and biopesticide.

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