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Antiplasmodial Activity of *Psidium guajava* L. Leaf Fractions against *Plasmodium falciparum*

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ABSTRACT: Malaria remains a major global health challenge, particularly in sub-Saharan Africa, where resistance to conventional antimalarial drugs continues to rise. This study aimed to evaluate the in vitro antiplasmodial activity of *Psidium guajava*L. leaf fractions against *Plasmodium falciparum*. Dried leaves were extracted using ethanol and subsequently fractionated into n-hexane, chloroform, ethyl acetate, and ethanol fractions. Phytochemical screening using standard reagents revealed the presence of alkaloids, flavonoids, terpenoids, and saponins in all fractions, while tannins were detected only in the ethyl acetate and ethanol fractions. Antiplasmodial activity was assessed using the Giemsa staining method. All fractions demonstrated concentration-dependent inhibition of parasite growth, with the ethanol fraction exhibiting the highest activity, achieving 84.38% inhibition at a concentration of 10.5 mg/mL. This activity was comparable, although slightly lower, than that of the standard drug artemether/lumefantrine, which showed 95.31% inhibition. The superior activity of the ethanol fraction is likely attributed to its higher content of polar bioactive compounds, particularly flavonoids and tannins, which are known for their antiplasmodial properties. In conclusion, *P. guajava* leaf fractions, especially the ethanol fraction, exhibit significant antiplasmodial activity, supporting their traditional use in malaria treatment. These findings highlight the potential of *P. guajava* as a promising source of natural antimalarial agents and warrant further pharmacological and mechanistic studies.

KEYWORDS: Antiplasmodial; bioassay-guided fractionation; malaria; *Plasmodium falciparum*; traditional medicine.

1. INTRODUCTION

Malaria remains a major global health burden, particularly in sub-Saharan Africa, where it disproportionately affects vulnerable populations such as children under five years of age and pregnant women. Despite ongoing control and prevention efforts, malaria accounted for approximately 249 million cases and 608,000 deaths worldwide in 2022 (World Health Organization, 2023). The increasing emergence and spread of resistance to frontline antimalarial drugs, including artemisinin-based combination therapies (ACTs), present a significant challenge to effective malaria control and underscore the urgent need for the discovery of new and effective therapeutic agents (Beshir et al., 2021).

Natural products have historically played a crucial role in antimalarial drug discovery, as evidenced by the development of quinine and artemisinin, both derived from medicinal plants. This highlights the importance of ethnomedicinal plants as valuable sources of novel bioactive compounds with potential therapeutic applications (Li & Vederas, 2009). In many developing countries, traditional medicine remains widely utilized due to its accessibility, affordability, and cultural acceptance, making it an important foundation for the identification of new drug candidates (Atanasov et al., 2009).

Psidium guajava L. (Myrtaceae), commonly known as guava, is widely used in traditional medicine for the treatment of various ailments, including febrile illnesses associated with malaria (Gutiérrez, 2008). Phytochemical studies have revealed that guava leaves contain a variety of bioactive compounds, such as flavonoids, alkaloids, tannins, and terpenoids, which are known to exhibit diverse pharmacological activities, including antimicrobial and antiplasmodial effects. However, most previous studies have primarily focused on crude extracts, with limited investigation into the specific fractions responsible for antiplasmodial activity and the relationship between phytochemical composition and bioactivity (Shaheen et al., 2020; Ebiloma, 2018).

Therefore, a systematic investigation involving fractionation and activity-guided evaluation is necessary to identify the most active constituents and to better understand their therapeutic potential.

In this study, the in vitro antiplasmodial activity of fractionated leaf extracts of *Psidium guajava* was evaluated against *Plasmodium falciparum*. The leaves were extracted using ethanol and subsequently partitioned into n-hexane, chloroform, ethyl acetate, and ethanol fractions. Each fraction was subjected to phytochemical screening and evaluated for antiplasmodial activity using the Giemsa staining method. This study aimed to identify the most active fraction and to establish a relationship between phytochemical composition and antiplasmodial activity, thereby providing scientific evidence supporting the traditional use of *P. guajava* and contributing to the development of potential novel antimalarial agents.

2. EXPERIMENTAL SECTION

2.1. Plant material collection and authentication

Fresh leaves of *Psidium guajava* L. were collected in June 2023 from Dadin Kowa, Gombe State, Nigeria. Botanical identification and authentication were performed at the Horticultural Department, Federal College of Horticulture, Dadin Kowa, where a voucher specimen (FCHDKH 0031) was deposited.

2.2. Extraction and fractionation

Dried and powdered leaves (350 g) were macerated in 98% ethanol (2 L) at room temperature for 72 h with intermittent agitation. The resulting filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator to obtain a crude ethanol extract (52.8 g). The crude extract was then suspended in an ethanol–water mixture (8:2, v/v) and sequentially partitioned with n-hexane, chloroform, and ethyl acetate to yield four fractions: n-hexane fraction (PHF), chloroform fraction (PCF), ethyl acetate fraction (PEAF), and ethanol fraction (PEF). Each fraction was evaporated to dryness, weighed, and stored at 4°C until further analysis.

2.3. Phytochemical screening

Qualitative phytochemical screening was performed using standard procedures as described by Harborne (1998) to identify the presence of major secondary metabolites. Alkaloids were detected using Dragendorff's reagent (potassium bismuth iodide) and Wagner's reagent (iodine in potassium iodide), indicated by the formation of an orange or reddish-brown precipitate. Flavonoids were identified using the alkaline reagent test (NaOH solution) and lead acetate, producing an intense yellow coloration or precipitate. Terpenoids were determined using the Salkowski test with concentrated H_2SO_4, indicated by the formation of a reddish-brown interface. Steroids were tested using the Liebermann–Burchard reaction (acetic anhydride and concentrated H_2SO_4), resulting in a green or blue coloration. Tannins were detected using ferric chloride ($FeCl_3$) and gelatin solution, producing a blue-black or green precipitate. Saponins were evaluated by the frothing test using distilled water, indicated by the formation of stable foam. Glycosides were assessed using Fehling's solution (A and B) after hydrolysis, indicated by the formation of a brick-red precipitate.

2.4. In vitro antiplasmodial assay

2.4.1. Parasite culture

The chloroquine-sensitive *Plasmodium falciparum* (3D7 strain) was maintained in continuous culture using human O+ erythrocytes at 5% haematocrit in RPMI-1640 medium supplemented with 0.5% Albumax II, 25 mM HEPES, and 25 mM $NaHCO_3$. Cultures were incubated at 37°C under a gas mixture of 5% CO_2, 5% O_2, and 90% N_2.

2.4.2. Drug preparation and assay

Stock solutions of each fraction were prepared in dimethyl sulfoxide (DMSO) and subsequently diluted with complete culture medium to obtain final concentrations of 1.25, 2.5, 5.0, and 10.5 mg/mL, with the final DMSO concentration not exceeding 0.5%. Synchronized ring-stage *Plasmodium falciparum* parasites (2% parasitaemia, 2% haematocrit) were incubated with the test samples in 96-well plates for 48 h. Artemether/lumefantrine (Coartem®) was used as the positive control, while infected erythrocytes containing 0.5% DMSO served as the negative control.

At 24 and 48 h of incubation, aliquots of each culture were smeared onto glass slides, stained using the Giemsa staining method (Method A), and examined under a light microscope. The number of parasitized erythrocytes, identified by red-pink staining, was counted, and the mean percentage of parasite elimination was calculated. The percentage inhibition of parasite growth, representing the antiplasmodial activity of each test sample, was determined using the following equation:

$$\% = \frac{N}{Nx} \text{ x } 100$$

where:
% = percentage activity of the samples,
N = total number of cleared red blood cells,
Nx = total number of parasites in the red blood cells

3. RESULTS

3.1. Extraction yield and phytochemical profile

The ethanol extraction of *P. guajava* dried leaves yielded 52.8 g (15.09% w/w) of crude extract. Sequential solvent–solvent partitioning of the crude extract produced four fractions with varying yields: n-hexane (PHF, 11.93%), chloroform (PCF, 16.10%), ethyl acetate (PEAF, 22.92%), and ethanol (PEF, 34.85%).

The phytochemical composition of the fractions is presented in **Table 1**. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, and saponins in all fractions, while tannins were detected only in the ethyl acetate and ethanol fractions. Steroids and glycosides were absent in all fractions.

Table 1. Phytochemical composition of *P. guajava* leaf fractions.

Phytochemical	PHF	PCF	PEAF	PEF
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Steroids	--	--	--	--
Tannins	--	--	+	+
Glycosides	--	--	--	--
Saponins	+	+	+	+

(+) = present, (--) = absent.

3.2. Antiplasmodial activity

The antiplasmodial activity of the fractions was evaluated using microscopic examination of Giemsa-stained thin blood smears. The results of the activity assay are summarized in **Table 2**. Parasite viability was assessed by comparing the number of parasitized and cleared erythrocytes after incubation. All fractions exhibited a concentration-dependent reduction in parasitaemia after 48 hours, indicating inhibition of *Plasmodium falciparum* growth. A consistent increase in antiplasmodial activity with increasing solvent polarity was observed, with the ethanol fraction (PEF) showing the highest activity.

Table 2. Antiplasmodial activity of *P. guajava* fractions against chloroquine-sensitive *Plasmodium falciparum* (3D7) assessed by Geimsa staining microscopy.

Fractions	Parasite Initial Count	Parasitemia Final Count				Percentage Elimination at the End of Incubation (%)			
Concentration Used (mg/mL)		10.5	5.0	2.5	1.25	10.5	5.0	2.5	1.25
PHF (n-Hexane)	64	18	24	34	41	71.88	62.50	46.88	35.94
PCF (Chloroform)	64	15	22	30	39	76.56	65.63	53.13	39.06
PEAF (Ethyl Acetate)	64	14	21	28	36	78.13	67.19	56.25	43.75
PEF (Ethanol)	64	10	15	21	30	84.38	76.56	67.19	53.13
Artemether/Lumefantrine	64	3	6	11	18	95.31	90.63	82.81	71.88

4. DISCUSSION

The increasing prevalence of drug-resistant malaria necessitates the exploration of alternative therapeutic sources, particularly from medicinal plants with ethnopharmacological relevance (Newman, 2022). In this study, *Psidium guajava*leaf fractions demonstrated significant in vitro antiplasmodial activity against *Plasmodium falciparum*, thereby supporting its traditional use in malaria management. The observed activity was concentration-dependent and varied according to solvent polarity, with the ethanol fraction (PEF) exhibiting the highest inhibitory effect.

The ethanol fraction achieved 84.38% parasite inhibition at 10.5 mg/mL, indicating substantial antiplasmodial activity, although slightly lower than the standard drug artemether/lumefantrine (95.31%). This finding is consistent with the general trend that crude plant extracts exhibit lower potency compared to purified pharmaceutical agents. Nevertheless, the level of inhibition observed falls within the range considered significant for plant-derived antimalarial candidates (Kaushik et al., 2015). Historically, important antimalarial drugs such as quinine and artemisinin have originated from plant sources, reinforcing the relevance of investigating ethnomedicinal plants as potential drug leads.

The superior activity of the ethanol fraction can be attributed to its ability to extract a broad range of polar bioactive compounds, including flavonoids, tannins, and alkaloids. These compounds are known to exhibit antiplasmodial effects through multiple mechanisms, such as inhibition of heme detoxification, induction of oxidative stress, and interference with key enzymatic pathways in the parasite (Oboh, 2023; Tasdemir et al., 2006). In contrast, non-polar solvents such as n-hexane primarily extract lipophilic constituents that generally exhibit lower antiplasmodial activity. The presence of polyphenolic compounds such as quercetin, gallic acid, and ellagic acid may further enhance the observed activity due to their redox-modulating and protein-binding properties (Bero, 2019).

In addition, the presence of multiple phytochemical classes suggests the possibility of synergistic interactions among bioactive compounds, which may enhance efficacy and reduce the likelihood of resistance development. This highlights the potential of *P. guajava* extracts not only as a source of new antimalarial compounds but also as candidates for combination therapy with existing drugs. The absence of steroids and glycosides in the active fraction suggests that these compounds may not significantly contribute to the observed antiplasmodial activity under the tested conditions.

The present findings are consistent with previous studies reporting the antimalarial potential of *P. guajava*. Alozieuwa et al. (2022) demonstrated significant parasitaemia suppression using aqueous leaf extracts in *Plasmodium*

berghei-infected mice, while Wande and Babatunde (2017) reported inhibition of hemozoin formation, suggesting a mechanism similar to chloroquine.

Despite these promising results, further studies are required to fully explore the therapeutic potential of *P. guajava*. Future research should focus on bioassay-guided isolation and characterization of active compounds, followed by structural elucidation using advanced techniques such as nuclear magnetic resonance (NMR) and mass spectrometry. In addition, in vivo studies are necessary to evaluate efficacy, safety, and pharmacokinetic profiles. Mechanistic investigations at the molecular level and evaluation of potential synergistic interactions with existing antimalarial drugs are also recommended. Overall, this study highlights *Psidium guajava* as a promising source of plant-derived antimalarial agents and provides a scientific basis for its continued investigation in drug discovery efforts.

5. CONCLUSION

This study confirms that *Psidium guajava* leaf fractions exhibit significant in vitro antiplasmodial activity against *Plasmodium falciparum*, with the ethanol-soluble fraction demonstrating the highest potency among the tested fractions. These findings provide scientific support for the traditional use of guava leaves in malaria treatment and identify the ethanol fraction as a promising source of bioactive compounds. Further studies are warranted to isolate and characterize the active constituents responsible for the observed activity, as well as to evaluate their efficacy and safety through in vivo investigations. Such efforts are essential to advance the development of *P. guajava*-derived compounds as potential antimalarial agents

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