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Safety and Efficacy of *Psidium guajava* Stem Bark Extracts in the Management of Oral Thrush

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Abstract

Psidium guajava L. has long been used in traditional medicine for managing infectious diseases. However, there is limited scientific data on its antifungal potential and safety. This study investigated phytochemical composition, acute oral toxicity, and antifungal efficacy of stem bark extracts of *P. guajava* against *Candida albicans* isolated from patients with oral thrush. The stem bark was extracted using cold maceration with water and ethanol. Standard qualitative phytochemical screening was conducted. Acute toxicity testing followed OECD guideline 423 using mice at doses up to 4000 mg/kg. Antifungal activity was assessed using the disc diffusion method, while MIC and MFC were determined using broth microdilution. Data were analyzed using one-way ANOVA at a 95% confidence level. Both extracts contained phenols, tannins, terpenoids, flavonoids, alkaloids, and glycosides, while saponins were present only in the aqueous extract. No mortality or observable signs of toxicity were recorded up to 4000 mg/kg, indicating a wide safety margin (LD_{50} > 4000 mg/kg). Ethanolic extract showed significantly higher potency than the aqueous extract (p = 0.004). At 250 mg/mL, the ethanolic extract (17.67 mm) demonstrated comparable efficacy to fluconazole (18.67 mm, p = 0.355). *P. guajava* stem bark extracts exhibit promising antifungal properties, with the ethanolic extract showing comparable efficacy to fluconazole at higher concentrations. The extracts are safe at the tested doses and contain diverse bioactive compounds, supporting their ethnomedicinal use in managing oral candidiasis.

INTRODUCTION

Oral candidiasis, also known as thrush, is a common mucosal infection primarily caused by *Candida albicans.* It is also increasingly caused by non-albicans species. The infection mostly affects neonates, the elderly, people wearing dentures, and people with compromised immune systems [1,2]. A major global public health concern is the rising incidence of fungal infections and antifungal resistance, especially in low- and middle-income nations where access to potent antifungal medications is restricted [3, 4]. Topical and systemic antifungal agents, like azoles and polyenes, are frequently linked to unfavorable side effects, exorbitant expenses, and treatment failures because of resistant strains. This leads to a desperate need for new, secure, and efficient treatment options [5-7]. Because of their accessibility, affordability, and chemical diversity, plant-based antifungal agents are becoming more and more popular as alternative or complementary therapies [4, 8-10]. *Psidium guajava* L., a perennial tropical plant which is a member of the *Myrtaceae* family, is used extensively in traditional medicine for the treatment of gastrointestinal and oral conditions. Its antimicrobial, anti-inflammatory, and antioxidant qualities have drawn scientific attention [11,12]. Its edible fruit and therapeutic qualities make it widely grown throughout tropical and subtropical regions [13]. Several studies revealed the presence of bioactive substances like flavonoids, tannins, phenolic compounds, alkaloids, triterpenes, saponins, carotenoids, lectins, and essential oils, which are abundant in the plant's leaves, fruits,

bark, and roots. These substances have been linked to a wide range of pharmacological activities [14]. Also, the plant is an excellent source for the creation of natural therapeutic agents because of these phytoconstituents. The plant's antimicrobial, antioxidant, anti-inflammatory, analgesic, and wound-healing qualities are also due to the presence of these compounds [15].

Other previous studies revealed that the plant's leaves contain a rich mixture of bioactive constituents such as phenolic acids, flavonoids, tannins, terpenes, and alkaloids, which plausibly underlie its antimicrobial effects [16,17]. These substances have been reported to suppress fungal growth by interfering with energy metabolism, changing membrane permeability, and disrupting cell wall synthesis [18]. *P. guajava* leaf extracts demonstrated antifungal activity against *Candida* spp. in a number of *in vitro* investigations. The strongest fungal inhibitory activity is frequently observed in the flavonoid and tannin fractions [19]. Formulations based on nanoparticles made from guava extracts have also shown improved antifungal efficacy [20].

A wide range of illnesses, such as ulcers, bacterial infections, fungal diseases, diabetes, diarrhea, and malaria, have been treated using *P. guajava* extracts [21]. Its use against fungi like *Candida albicans*, the cause of candidiasis, and dermatophytes that cause superficial mycoses is also interesting [21]. Methanolic extracts of the plant, and certain phytoconstituents, like myricetin and guaijaverin, have been demonstrated in numerous studies to inhibit the growth of *Candida albicans in vitro*, sometimes with activity that is on par with or better than that of conventional antifungal agents [22, 23]. The safety profile of *P. guajava* extracts is crucial for its consideration as a therapeutic candidate, even though it shows encouraging antimicrobial potential. Aqueous and methanolic extracts showed a high margin of safety in several animal studies. In albino rats, for instance, oral administration of aqueous leaf extract up to 5000 mg/kg body weight resulted in no mortality and no discernible alterations in the levels of hepatic enzymes [24].

Similarly, rats given doses of up to 5000 mg/kg of methanolic extracts did not exhibit any acute toxic effects; these results classify the *P. guajava* extracts as "practically nontoxic" according to the Hodge and Sterner toxicity scale. [25]. Other experimental reports have demonstrated high oral LD_{50} values (>2000–5000 mg/kg) and absence of significant mortality or major biochemical alterations, suggesting a favorable safety margin for guava extracts [26,27]. Considering the clinical burden of oral candidiasis, the reported antifungal potential of *P. guajava*, and encouraging signals from preclinical studies on its safety, an integrated study that combines phytochemical profiling with acute oral toxicity testing and antifungal efficacy against *Candida* isolates from oral thrush is necessary. The present study, therefore, aimed at characterizing the phytochemical composition of *P. guajava* stem bark extract, determining its acute oral toxicity profile, and assessing its antifungal efficacy against *Candida* species isolated from patients with oral thrush.

MATERIALS AND METHODS

Study location

This study was conducted at the Microbiology Laboratory of Sa'adu Zungur University, Gadau, Bauchi State, Nigeria, where fungal identification and antifungal assays were carried out. Acute toxicity experiments were performed under controlled environmental conditions in the institution's animal house.

Ethical Approval

This study was approved by the Research and Ethics Committee of Extreme Hospital, Azare Bauchi State, (approval number: EXT/ETH/2025/019I) on 3rd February, 2025. All participants provided written informed consent prior the sample collection. Animal experiments were approved under the same committee's protocol number (EXT/ETH/2025/019II), and carried out following ARRIVE guidelines.

Sample Collection and Laboratory Investigations

Fungal Isolates

In collecting oral swab samples from patients with of oral, sterile swab sticks were used. The samples were transported to the microbiology laboratory of Sa'adu Zungur University, Gadau for microbiological investigations. To isolate the fungal pathogens, the samples collected were inoculated on Sabouraud dextrose agar (SDA), prepared following the manufacturer's guide. The agar was complemented with chloramphenicol and incubated at 30 °C for up to 7 days. The fungal isolates were identified using colony morphology, lactophenol cotton blue staining, germ tube testing, and sub-culturing on Chromagar Candida [25]. Stock cultures were maintained at 4 °C on SDA slants and sub-cultured before use in antifungal testing.

Plant material

The stem bark of *P. guajava* was collected in fresh form from Chilankori village of Azare, Katagum local government area, Bauchi State, Nigeria, in the month of June 2025. The plant material was identified and authenticated at the Department of Biological Sciences, Sa'adu Zungur University, Gadau, and a voucher specimen (SAZU233C) was deposited in the departmental herbarium for reference.

Preparation and Extraction of the Plant Material

The stem bark collected was washed, air-dried under shade at 28 °C for 28 days. The dried stem bark was crushed into fine powder using a mechanical grinder. Extraction was done using ethanol and water, following the method described by Metwally *et al.* (2010) with slight alterations. Cold maceration as described by Edeoga *et al*. [26] was used for the extraction process.
Exactly 100 g of the powdered stem bark of the plant was soaked in 0.5 L of distilled water for 3 days at 28 °C with episodic shaking. This was repeated using 99% ethanol. The mixtures were filtered using Whatman No. 1 filter paper. The filtrates were concentrated with a water bath at 40°C. The concentrated extracts were stored at 4°C for further use [26].

Phytochemical Analysis

The phytochemical analysis was carried out following the standard phytochemical screening procedures established by Trease & Evans [27] and Sofowora [28]. This is to detect active compounds like tannins, flavonoids, alkaloids, phenols, glycosides, steroids, and saponins.

Assessment of Acute Toxicity of *Psidium guajava* Extracts

The aqueous and ethanolic extracts of *P. guajava* were assessed for acute oral toxicity according to the guidelines of the Organization for Economic Cooperation and Development (OECD guideline 423) [29]. Mice weighing 20–30 g were used for the test [30]. The dose limit of 4,000 mg/kg was used in this study. The test mice were kept fasting overnight before administration of the extracts. The mice were divided into five groups (three mice per group). The first group served as a control, in which the mice received distilled water. The second, third, fourth, and fifth groups served as test groups and received doses of 500 mg/kg, 1000mg/kg, 2000 mg/kg, and 4000 mg/kg, respectively. After the first 4 hours of the administration of the

extracts, the mice were examined for toxic effects. The mice were then periodically observed for a period of 14 days for possible toxic effects. Also, changes in behavior, body weight, urination, food intake, water intake, respiration, eye color, skin color, and body temperature. Other effects like constipation, tremor, and convulsion were also observed [30].

Assessment of the Antifungal Potency of *Psidium guajava* Stem Bark Extracts

In assessing the antifungal activity of the plant extracts, the disc diffusion method was used. Different concentrations of the extracts were prepared, including 250 mg/mL, 125 mg/mL, and 62.5 mg/mL. Exactly 5 mm diameter filter paper discs were prepared and soaked in each of the concentrations for 24 hours. These were then air-dried and stored in a cool and dry place before use [27]. Sabouraud Dextrose Agar (SDA) was prepared following standard microbiological approaches. The agar was sterilized and poured into sterile Petri dishes.

The plates were dried at 45 °C in a hot air oven before use. Each plate was flooded with the fungal spore suspension adjusted to 1×10^6 spores/mL. The density of the inoculum was standardized using a 0.5 McFarland. The plates were air-dried before the extract-impregnated discs were placed onto them. Fluconazole (50 mg/disc) was used as the positive control, and Dimethyl sulfoxide (DMSO, 5%) as the negative control. The plates were incubated at 28 °C for 48 hours. The zones of inhibition produced were measured and recorded as mean inhibition diameters [31]. All tests were done in triplicate.

Determination of the minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations (MICs) of *P. guajava* aqueous and ethanolic extracts were tested using the broth microdilution method following the CLSI guidelines [32]. Sterilized Sabouraud Dextrose Broth (SDB) was distributed at 1 mL per test tube. Exactly 1 mL of each of the extract concentrations (250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.8 mg/mL, 3.9 mg/mL and 1.9 mg/mL) was added to the tubes. About 0.1 mL of the fungal suspension (1×10^6 spores/mL) was dispensed into each of the test tubes.

Control tubes were prepared from only the broth and fungal suspension. The tubes were then incubated at 30°C for 48 hours. The MIC was recorded as the lowest concentration of the extract with no visible fungal growth [31]. On the other hand, the minimum fungicidal concentration (MFC), was obtained by sub-culturing samples from tubes with no visible growth, on a fresh Sabouraud Dextrose Agar (SDA). The plates were then incubated at 30°C for 48 hours and assessed for fungal growth. The MFC was recorded as the lowest concentration with no fungal growth on the agar plates [31].

Statistical Analysis

All experiments were performed in triplicate, and the results were recorded as mean ± standard deviation. Comparison between the extracts was done using one-way ANOVA, with Turkey HSD post hoc. These were performed using SPSS version 23.0, with significance determined at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

The current study showed that qualitative phytochemical analysis revealed the presence of phenols, tannins, terpenoids, flavonoids, alkaloids, and glycosides in both the aqueous and ethanolic extracts of *Psidium guajava* stem bark. Saponins, however, were detected only in the aqueous extract, making its phytochemical profile slightly broader than that of the ethanolic extract. These are presented in **Table 1** below.

Table 1. Phytochemical compositions of the ethanolic and aqueous extracts of the stem bark of *Psidium guajava.*

Phytochemical Compositions	Aqueous extract	Ethanolic extract
Flavonoids	+	+
Glycosides	+	+
Phenols	+	+
Terpenoids	+	+
Saponins	+	-
Tannins	+	+
Alkaloids	+	+

+ : Present
- : Absent

Our results showed that the presence of saponins in the aqueous extract only suggested that water-based extraction may be more effective in isolating this particular compound from the stem bark of *P. guajava*. This observation aligns with the findings of Metwally *et al*. [33], who also reported slight differences in the phytochemical profiles of different extraction solvents. These emphasized the influence of solvent polarity on phytochemical yield. Similarly, the observation also agrees with findings from other studies, which have reported that different solvents can lead to varied phytochemical concentrations [34]. For instance, a study by Möwes *et al.* [35] reported that a 70% methanol extract had the most extensive phytochemical profile, comprising alkaloids, flavonoids, phenols, tannins, steroids, saponins, and terpenoids [35]. In contrast, the same study by Möwes *et al.* [35], reported that alkaloids and flavonoids were absent from pure methanol extracts [35].

These findings highlighted that the addition of water to an alcoholic solvent can improve the polarity of the resulting solvent and enhance its extraction efficiency for polar phytochemicals like flavonoids from the herbal matrix. For example, aqueous-methanol can be better at isolating flavonoids than pure methanol or water in general [35]. In comparison with earlier studies, such as those by Metwally *et al*. [33] and Gupta *et al*. (2020), our results confirmed the presence of core phytochemicals like tannins, flavonoids, and alkaloids in the stem bark of *Psidium guajava*. However, the detection of saponins exclusively in the aqueous extract contrasts with Gupta *et al*. [36], who observed saponins in all extracts of the leaf, raw fruit, and ripe fruit. This inconsistency may reflect differences in plant part composition, environmental factors influencing phytochemical content, or variations in extraction procedures.

Furthermore, the absence of phlobatannins in the stem bark extracts analyzed in our study is consistent with Gupta *et al*. [36], who reported that phlobatannins were restricted to the leaf and raw fruit. This suggests that phlobatannins may be more localized to specific tissues within the plant. The broader phytochemical profile of the aqueous extract compared to the ethanolic extract also supports previous studies [33], which emphasized the solvent-dependent nature of phytochemical extraction.

Acute Toxicity Assessment

Table 2 of the current study showed that, across all administered doses (500 to 4000 mg/kg body weight), no mortality was recorded in any treatment group. Similarly, there were no observable signs of toxicity or changes in the key physiological parameters such as respiration rate, urination, body temperature, eye colour, behaviour, body weight, and food or water intake.

Table 2. Acute toxicity of *P. guajava* stem bark extracts.

	Ethanolic Extracts								Aqueous Extracts							
Dose ($mgkg^{-1}$)	Mo	RR	Ur	BT	EC	BW	Bh	FI	Mo	RR	Ur	BT	EC	BW	Bh	FI
Distilled water	0/3	-	-	-	-	-	-	-	0/3	-	-	-	-	-	-	-
500	0/3	-	-	-	-	-	-	-	0/3	-	-	-	-	-	-	-
1000	0/3	-	-	-	-	-	-	-	0/3	-	-	-	-	-	-	-
2000	0/3	-	-	-	-	-	-	-	0/3	-	-	-	-	-	-	-
4000	0/3	-	-	-	-	-	-	-	0/3	-	-	-	-	-	-	-

Note:
Mo: Mortality
RR: Respiration Rate
Ur: Urination
BT: Body Temperature
EC: Eye Color
BW: Body Weight
Bh: Behavior
FI: Food Intake
+ : Changed
- : Not Changed

Our acute toxicity assessment findings confirm that the extracts were well-tolerated and safe at the tested concentrations. This indicates an LD_{50} greater than 4000 mg/kg, which makes it classified as lowest toxic (category 5) under OECD 2001/2002 Harmonized System. This finding is supported by a number of other studies. For instance, Hermione *et al*. [37] observed no abnormality or mortality in rats at a dose of 5000 mg/kg, suggesting that the LD_{50} of the methanolic bark extract is greater than 5000 mg/kg. Similarly, Sekhar *et al*. [38] reported that the *P. guajava* aqueous bark extract has an LD_{50} greater than 5000 mg/kg. Atik *et al*. [39] also emphasized that the *P. guajava* ethanolic fruit extract administered in mice has an LD_{50} greater than 5000 mg/kg. These results suggest that the extracts are relatively nontoxic since substances with an oral LD_{50} between 2000 mg/kg and 5000 mg/kg are considered to have the lowest toxicity [37].

It is important to note that some studies have reported different results. For example, a study by Igwe *et al*. [40] revealed that two mice died at 5000 mg/kg, indicating that the LD_{50} was less than 5000 mg/kg. Also, an LD_{50} of 1.352 mg/kg was reported by Onyekwe *et al*. [41]. These inconsistencies may be attributed to variations in the plant parts used in the studies. Extraction methods employed and the specific animal models used in the studies may also be the cause of the variations. These warrant further investigation into the safety of the plant extracts.

Antifungal activity of the plant extracts

The results of the antifungal activity of the plant against *Candida albicans* revealed a clear dose-dependent increase in the inhibition zones for both aqueous and ethanolic extracts. The ethanolic extract was more effective than the aqueous extract, particularly at higher concentrations. At 62.5 mg/mL, the aqueous and ethanolic extracts produced mean inhibition zones of 8.67 mm and 10.33 mm, respectively. Statistical analysis indicated no significant difference between the two extracts (p = 0.102). Fluconazole, which was used as a positive control in our study, exhibited significantly greater activity than both the ethanolic and aqueous extracts (18.67 mm, p = 0.000). Post hoc comparisons confirmed that the observed variation was attributable to the higher potency of fluconazole rather than differences between the plant extracts (**Tables 3** and **4**).

At 125 mg/mL, the zone of inhibition increased to 10.67 mm for the aqueous extract and 14.33 mm for the ethanolic extract. The difference between the two extracts was statistically significant (p = 0.004). Fluconazole also maintained significantly greater activity than the aqueous extract (p = 0.000) and the ethanolic extract (p = 0.002). Interestingly, the gap between the ethanolic extract and fluconazole narrowed compared to the lower dose (**Tables 3** and **4**).

At 250 mg/mL, the aqueous extract achieved an inhibition zone of 13.67 mm, while the ethanolic extract reached 17.67 mm. at this concentration, the difference between the ethanolic extract and fluconazole (18.67 mm) was statistically insignificant (p = 0.355). This indicates a comparable efficacy between them. However, the aqueous extract remained significantly less effective than both the ethanolic extract (p = 0.002) and fluconazole (p = 0.001) (**Tables 3** and **4**).

Table 3. Antifungal Activity of the Extracts of *P. guajava* Stem Bark against *Candida albicans.*

Treatment	62.5 mg/mL Mean (mm) ± SD	125 mg/mL Mean (mm) ± SD	250 mg/mL Mean (mm) ± SD
Aqueous extract	8.67 ± 0.57	10.67 ± 0.57	13.67 ± 0.57
Ethanolic extract	10.33 ± 0.57	14.33 ± 0.57	17.67 ± 0.57
Fluconazole (Cont)	18.67 ± 1.15	18.67 ± 1.15	18.67 ± 1.15
DMSO (5%)	0.00	0.00	0.00

± : standard deviation (SD)

Table 4 of the current study showed that the ethanolic extract demonstrated a lower MIC (7.8 mg/mL) compared to the aqueous extract (15.625 mg/mL), reflecting greater inhibitory potency. Similarly, its MFC (125 mg/mL) was half that of the aqueous extract (250 mg/mL), confirming stronger fungicidal capacity against *Candida albicans*.

Table 4. Minimum inhibitory and minimum fungicidal concentrations of *P. guajava* stem bark extract against *Candida albicans*.

Isolates	CRT (mg/mL)	MIC (mg/mL)	MFC (mg/mL)
Aqueous Extract	1.9-500	15.625	250
Ethanolic Extract	1.9-500	7.8	125

Note:
MIC: Minimum Inhibitory Concentration
MFC: Minimum Fungicidal Concentration
CRT: Concentration Range Tested

Table 4. Tukey HSD Comparing the antifungal efficacies of the plant extracts and fluconazole.

(I) Antifungals	(J) Antifungals	62.5 mg/mL			125 mg/mL			250 mg/mL		
		Mean Difference (I-J)	SE	Sig	Mean Difference (I-J)	SE	Sig	Mean Difference (I-J)	SE	Sig
Aqueous extract	Ethanolic extract	-1.67	0.67	0.102	-3.67*	0.67	0.004	-4.00*	0.67	0.002
	Fluconazole	-10.00*	0.67	0.000	-8.00*	0.67	0.000	-5.00*	0.67	0.001
Ethanolic extract	Aqueous extract	1.67	0.67	0.102	3.67*	0.67	0.004	4.00*	0.67	0.002
	Fluconazole	-8.33*	0.67	0.000	-4.33*	0.67	0.002	-1.00	0.67	0.355
Fluconazole	Aqueous extract	10.00*	0.67	0.000	8.00*	0.67	0.000	5.00*	0.67	0.001
	Ethanolic extract	8.33*	0.67	0.000	4.33*	0.67	0.002	1.00	0.67	0.355

*. The mean difference is significant at the 0.05 level.

Our antifungal evaluation against *Candida albicans* revealed a clear, dose-dependent increase in inhibition zones for both the aqueous and ethanolic extracts, with the ethanolic extract consistently outperforming the aqueous extract, particularly at higher concentrations. This is a crucial finding that highlights the potential of *P. guajava* as a source of antifungal agents. This validates its traditional use for treating candidiasis as earlier reported by Ugbogu *et al*., [9]. The antifungal properties of *P. guajava* have also been confirmed in other studies. For example, Möwes *et al*. [35] reported that pure acetone leaves extracts exhibited the highest inhibitory effect (22.33 mm ± 3.21) against *C. albicans* compared to other extracts. The same study also noted that the aqueous extract, prepared as per traditional methods, showed a significant inhibitory effect (9.00 mm ± 7.81) against *C. albicans* [35].

The antifungal activity of the plant is likely due to the joint effects of its diverse phytochemical constituents. Tannins, which are plentiful in its stem bark, exercise antifungal action by binding to the fungal cell-wall proteins and extracellular enzymes. These result in the loss of enzymatic function and structural disruption in the fungal pathogen [42]. Additionally, Flavonoids have been reported to interact with fungal membrane lipids, causing increased membrane permeability, leakage of intracellular contents, and induction of oxidative stress. This damages vital fungal cellular structures [43]. Furthermore, Terpenoids disrupt mitochondrial respiration and compromise membrane integrity. This leads to energy exhaustion and fungal cell death [44]. Also, Saponins contribute additional antifungal effects by interacting with ergosterol in the fungal cell membrane, forming pores that result in cytoplasmic leakage [45]. These specific biochemical mechanisms provide a strong mechanistic basis for the antifungal potential of *P. guajava* and likely account for the inhibitory patterns demonstrated in the present study.

CONCLUSION

This study demonstrated that both aqueous and ethanolic stem bark extracts of *Psidium guajava* are rich in bioactive compounds with notable antifungal activity against *Candida albicans*. The ethanolic extract was significantly more potent than the aqueous extract. Its activity at 250 mg/mL was comparable to fluconazole. Acute oral toxicity testing revealed no mortality or observable signs of toxicity at doses up to 4000 mg/kg. This indicates that the extracts are safe and well-tolerated at the tested doses. These findings scientifically validate the traditional use of *P. guajava* for treating fungal infections such as oral thrush. The findings also highlight the potential of *P. guajava* as a source of safe and effective plant-based antifungal agents. Future research should aim to isolate and characterize the active compounds, clarify their mechanisms of action, and conduct *in vivo* studies to establish therapeutic efficacy and dosage profiles for clinical application.

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