Journal of Complementary and Alternative Medical Research

Volume 25, Issue 11, Page 147-157, 2024; Article no.JOCAMR.125885
ISSN: 2456-6276

Evaluation of Antifungal Activity of *Psidium guajava* (Guava) Leaf Extracts

Bibi R. Ally-Charles [a*], Ede Tyrell [a], Rebecca Khatun [a], Richard Lall [a], Bibi Yassin [a], Martin King [a], Devi Rajnarine [a], Basil Dey [b], Narita Singh [a], Andrew Hutson [a], Obena Vanlewin [a] and Karishma Jeeboo [a]

[a] College of Medical Sciences, University of Guyana, Georgetown, Guyana.
[b] Faculty of Natural Sciences, University of Guyana, Georgetown, Guyana.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jocamr/2024/v25i11589

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc. are available here: https://www.sdiarticle5.com/review-history/125885

Original Research Article

Received: 01/09/2024
Accepted: 03/11/2024
Published: 09/11/2024

ABSTRACT

Aims: This study aimed to evaluate the effectiveness of four *Psidium guajava* leaf extracts against three strains of *Candida albicans* and a strain of *Cryptococcus neoformans*.
Study Design: Experiment-observational study.
Place and Duration of Study: *P. guajava* leaves were collected from specific areas in Regions Three and Four in Guyana; and the preparation of the leaf extracts and the treatment against the fungi were performed in two laboratories at the University of Guyana from August 2022-September 2023.
Methodology: *P. guajava* leaves were washed with water, air-dried at room temperature, and ground in a disinfected food mixer. Extracts were prepared by soaking the ground leaves in

**Corresponding author: E-mail: bibi.ally-charles@uog.edu.gy;*

Cite as: *Ally-Charles, Bibi R., Ede Tyrell, Rebecca Khatun, Richard Lall, Bibi Yassin, Martin King, Devi Rajnarine, Basil Dey, Narita Singh, Andrew Hutson, Obena Vanlewin, and Karishma Jeeboo. 2024. "Evaluation of Antifungal Activity of Psidium Guajava (Guava) Leaf Extracts". Journal of Complementary and Alternative Medical Research 25 (11):147-57. https://doi.org/10.9734/jocamr/2024/v25i11589.*

different solvents, and then using the rotary evaporator to reduce the solutions and obtain crude extracts. Filter paper discs were prepared and allowed to soak in various concentrations of the crude extracts. The discs were then subjected to antifungal susceptibility testing on Potato Dextrose agar seeded with an appropriate fungus according to Clinical Laboratory Standards Institutes guidelines. The plates were incubated and the zone of inhibition (ZOIs) was measured.
Results: *P. guajava* leaves were effective against all four strains of fungi evaluated. The largest ZOI was observed for *C. neoformans* (25 mm) with the ethyl acetate extract at 100 mg/mL concentration. Large ZOI was also observed for the three strains of *C. albicans* (10-15 mm). For one of the *C. albicans* strains, a ZOI significantly higher than the antifungal agents (ketoconazole and fluconazole) was observed with the *P. guajava* ethyl acetate extract at 100 mg/mL.
Conclusion: It can be concluded that *P. guajava* leaves have antifungal activity against *C. albicans* and *C. neoformans*, potentially greater than current antifungal agents like ketoconazole and fluconazole.

Keywords: P. guajava; C. albicans; C. neoformans; solvents; concentration; antifungal agents.

1. INTRODUCTION

Mankind has depended on medicinal plants ever since its existence [1]. Although science and the pharmaceutical industry have grown drastically, many people use medicinal plants to improve their health, especially in rural areas [2]. According to the World Health Organization (WHO), 60% of the population globally, and a whopping 80% in developing countries depend on medicinal plants [3]. Medicinal plants produce a variety of metabolites that show important biological activity and, are, therefore useful for a wide range of conditions [4]. Metabolites such as phenols, flavonoids, alkaloids, tannins, and saponins are found in the plants' leaves, roots, and stems [5]. Plant metabolites have several beneficial properties, such as anti-inflammatory, antihypertensive, anti-ageing, antibacterial, antifungal, antiviral, anti-cancer, antioxidants, neuroprotective, and anticoagulant effects [6]. For instance, the metabolite flavonoid is associated with antioxidant activity [7] and saponins are associated with antimicrobial activity [8]. There has been an increasing interest in plants and their medicinal value over the last few decades [9].

Psidium guajava is a fruit-bearing plant commonly known as Guava, belonging to the *Myrtaceae* family. It mostly grows in tropical and subtropical regions of the world, including Asia, the Caribbean, and South America, and is widely cultivated for its fruits and medicinal purposes [10]. The fruit is rich in vitamin C and is extensively used in the food industries to prepare of jam, jellies, and fruit spreads. It is also used in the beverage industries to produce fresh juices, and also in medicine to regulate high blood pressure [11].

The leaves are traditionally used to treat diarrheal infections and other diseases [11]. *Psidium guajava* leaves contain several phytochemicals, including quercetin, saponins, tannins, alkaloids, anthraquinones, phlobatannins, and cardiac glycosides [12]. Quercetin derivatives are the primary flavonoids in guava leaves, and they have spasmolytic properties. The pharmacological effects of quercetin include the inhibition of intestinal motility and the reduction of capillary permeability in the abdominal cavity [12]. The presence of these phytochemicals in *Psidium guajava* leaves and the bark suggests their potential as natural remedies for various ailments.

Fungi cause a wide variety of diseases. They cause superficial, subcutaneous, and systematic infections [13]. Fungi cause serious infections in immunosuppressed patients [14] and patients with co-morbidities [15]. Over the last few decades, there has been a rapid increase in fungal infections mainly because of climate change, the virulence of fungi, and the increase in immunocompromised patients [16], nosocomial fungal infections (especially in the ICU), community-acquired fungal infections and COVID-19 infections [15]. Fungi diseases linked to mortality are predominantly caused by fungi belonging to four genera, namely *Aspergillus*, *Candida*, *Cryptococcus*, and *Pneumocystis* [17].

Certain systematic fungal infections have always been problematic to treat [18]. Besides this, fungal treatment has become limited because of the emergence and reemergence of resistance to conventional antifungal agents (AFAs) [19]. Resistance to AFAs can occur naturally for certain species without prior exposure to the agent. For example, resistance by *Candida sp.* to

fluconazole and *Cryptococcus sp*. to echinocandins has been recorded. Resistance to AFAs develops among previously susceptible strains post-exposure to the AFAs and is based on altered gene expression. For example, *Candida albicans* and *C. neoformans* strains are resistant to fluconazole [20, 21]. Some current AFAs also have serious side effects, especially for prolonged users [22]. The burden of fungal treatment and antifungal resistance has become a crucial concern, especially for patients with immunosuppression and co-morbidities.

Therapy for *Candida albicans* and *Cryptococcus neoformans* is becoming more complex [23, 24]. Therefore, new methods of treating fungal diseases will remain a priority for the foreseeable future. Medicinal plants offer an alternative for dealing with the prevalence of resistant strains of fungal species. Plant products provide an efficient, cost-effective, and safer option to conventional medicines [25]. A review of the literature shows that *Psidium guajava* leaves have demonstrated antifungal activity against *Saccharomyces cerevisiae* and *Aspergillus niger* [26], dermatophytes [23, 27], *Candida sp*. and *Cryptococcus neoformans* to an extent [23]. However, it is unclear whether the subspecies of *P. guajava* found on Guyana's coast, has antifungal activity against *Candida sp* and *Cryptococcus neoformans*.

There seems to be a lack of scientific information about the antifungal activity of *Psidium guajava* leaves in the literature. Furthermore, there is a paucity of information and even awareness about *Psidium guajava* leaves in Guyana, and it is not well known except in a few rural communities. The objective of this study was to evaluate the effectiveness of extracts from the leaves of *P. guajava*, against *Candida albicans* and *Cryptococcus neoformans,* using different solvents. We sought to determine which solvent extract had the most antifungal activity.

2. METHODS

2.1 Collection and Preparation of Plant Materials

Psidium guajava leaves were collected from specific areas in Regions Three and Four in Guyana, namely Lusignan, Hope, Parfaite Harmony, and an area in the Mahaicony River. The plants were identified and verified by the Centre for Study of Biological Diversity, University of Guyana (Fig. 1). The leaves were washed with running tap water and left to air dry at room temperature for 3-4 weeks, carefully avoiding sunlight. A disinfected food mixer was used to grind the leaves into a coarse powder. The powdered plant material was packed into sealed bags and stored for the next process (Fig. 2).

Fig. 1. *Psidium guajava* leaves

Fig. 2. Ground leaves

Fig. 3. Maceration of ground leaves with different solvents.

2.2 Extraction of Compounds

One hundred grams (100 g) of dried pulverised *P. guajava* leaves were macerated in 500mL of four different solvents namely: hexane, ethyl acetate, methanol, and 95% ethanol (Fig. 3). Maceration of the ground leaves was carried out in tightly sealed and dark bottles which were placed in a dark cupboard for 24 hours under occasional shaking. The different extracts were filtered using sterile Whatman No. 1 filter paper and a sterile filtration apparatus. The extraction was repeated three times with the same amount of solvents, and filtration was performed each time. The filtered extracts were consolidated and reduced to dryness by evaporating the solvents under reduced pressure using a rotary evaporator at 45 °C. All the crude extracts were stored at 4°C in the dark until needed.

2.3 Antifungal Susceptibility Testing

2.3.1 Preparation of media

Potato Dextrose agar (PDA) was prepared for antifungal susceptibility testing using dehydrated powder and distilled water according to the manufacturer's water-to-powder ratio. After reconstitution, this mixture was heated and stirred, to fully dissolve all components for at least 5 minutes. Sterilization of PDA was obtained by autoclaving at 15 lb/psi pressure for 15 minutes. The media was allowed to cool and then poured into sterile petri dishes, and left to solidify, and store until needed.

2.3.2 Preparation of various concentrations and controls (treatments)

Preparation of various concentrations of each solvent extract was done through serial dilution. The crude extracts were used as the 100 mg/mL concentration and these samples were serially diluted to obtain 50 mg/mL, 25 mg/mL, and 12.5 mg/mL concentrations. Pure solvents were used as controls. The antifungal agents' ketoconazole and fluconazole were used as positive controls. One percent (1%) of solutions was prepared for each antifungal agent. Five milliliters (5 mL) of each concentration were placed in sterile vials. This was also done for the controls. All vials were then set aside until needed. The *P. guajava* leaf extracts for the different concentrations, the positive and the negative controls were the treatments used against the fungi.

2.3.3 Preparation of antifungal discs

Whatman No. 3 filter papers were used to prepare discs for antifungal susceptibility testing. Six millimeters (6 mm) discs were punched using a paper puncher, and placed in a secured petri dish, and then sterilised using the autoclaved. Approximately 15 discs were placed into each vial and soaked for 24 hours. These discs were then ready to be used for antifungal susceptibility testing with known microorganisms.

2.3.4 Preparation of fungal cultures

Three strains of *C. albicans* were used in this study. They were *C. albicans* ATCC 24058 and two in-house strains (In-house A and In-house B). One in-house strain of *C. neoformans* was used. The in-house strains were obtained from two clinical laboratories in Georgetown, Guyana, while the ATCC strain was sourced from the United States. All four strains of fungi were revived or subcultured onto fresh PDA and incubated overnight. The cultures were then set aside until needed.

2.3.5 Susceptibility testing

Antifungal susceptibility testing was performed according to the Kirby-Bauer disc diffusion

method. Potato Dextrose Broth (PDB) was aseptically seeded with an appropriate fungus and adjusted to 0.5 McFarland standards. A dry, sterile cotton swab was used to inoculate PDA plates with the appropriate PDB suspension. The PDA plates were inoculated to obtain a lawn of growth. Disinfected forceps were subsequently used to pick up discs from respective vials and place them on the PDA plates seeded with fungi. Three discs were placed on each PDA plate. The plates were then incubated at 37°C for 48-72 hours. Inhibition zones were measured in millimeters, and expressed as mean ± standard deviation. A mean ZOI greater than 10.0 mm was considered an effective treatment. Six millimeters (6 mm) meant no ZOI was observed and the fungus grew up to the disc.

2.3.6 Statistical analysis

In this study, the different treatments were said to be independent variables; and the zone of inhibitions was the dependent variable. All statistical analyses, including means and standard deviation, were calculated using SPSS Version 20. One-way analysis of variance (ANOVA) test was performed to detect the statistical differences among the ZOIs for the various treatments ($P < 0.05$). The LSD test was used to perform multiple comparisons.

3 RESULTS

3.1 Antifungal Activity against *C. albicans* ATCC

Table 1 shows the ZOIs obtained with the different treatments against *C. albicans* ATCC. The ethyl acetate extract at 100 mg/mL recorded the largest ZOI and was significantly higher than all other treatments, including the standard antifungal agents (Ketoconazole and Fluconazole) (Fig. 1). The ethanolic extract at 100 mg/mL recorded a ZOI of 10.3 ± 0.6 mm. This was significantly lower than the ethyl acetate extract at 100 mg/mL but significantly higher than all other treatments (P=0.001).

3.2 Antifungal Activity against *C. albicans* In-House A

Table 2 shows the ZOIs obtained with the various treatments against *C. albicans* In-house A. The standard treatments: ketoconazole and fluconazole performed best against *C. albicans* In-house A, with ketoconazole recording a significantly higher mean ZOI than all other treatments. The average ZOI for fluconazole was lower than ketoconazole but was significantly higher than all the treatments with *P. guajava* leaf extracts (P=0.001). The methanolic and ethanolic extracts at 100 mg/mL concentrations recorded the largest mean ZOI when compared to the other *P. guajava* leaf extracts (12.0±0.0 mm and 12.0±1.7 mm respectively); the average ZOI for these two concentrations were statistically similar to mean ZOI of the methanolic extract at 50 mg/mL, the ethanolic extract at 50 and 25 mg/mL, and the ethyl acetate at 100 mg/mL (Fig. 5).

3.3 Antifungal Activity against *C. albicans* In-house B

Table 3 shows the ZOIs obtained with the various treatments against *C. albicans* In-house B. The standard conventional treatment, ketoconazole, had the highest ZOI (23.3±5.8 mm) and was significantly higher when compared to all other treatments (P=0.001). The methanolic extract at 100 mg/mL recorded the second-highest mean ZOI against *C. albicans* In-house B (15.0 ± 2.0 mg/mL); this was significantly lower than the mean ZOI for ketoconazole but statistically similar to the average ZOI for ethanol extract at 50, 25 and 12.5 mg/mL (Fig. 6), and ethyl acetate extract at 100 mg/mL.

3.4 Antifungal Activity against *C. neoformans*

Table 4 shows the ZOIs obtained with the various treatments against *C. neoformans*. The standard treatment, ketoconazole, was the most effective against *C. neoformans*, with an average ZOI of 41.0±1.0 mm. Fluconazole recorded the second highest ZOI (32.7±0.6 mm). This was significantly lower than the ZOI for ketoconazole and significantly higher than the ZOI for all the *P. guajava* leaf extract (P=0.001). For the *P. guajava* leaf extracts, the ethyl acetate extract at 100 mg/mL was the most effective against *C. neoformans*. This ZOI was significantly higher than the ZOI for all other extracts.

It is worth noting that no ZOI was observed for the hexane extracts against the different fungi or for the negative controls.

Table 1. Zone of inhibition for treatments used against *C. albicans* ATCC

Treatments	ZOI (mm)
Methanol extract at 100 mg/ml (N=3)	6.0 ± 0.0^{d}
Methanol extract at 50 mg/ml (N=3)	6.0 ± 0.0^{d}
Methanol extract at 25 mg/ml (N=3)	6.0 ± 0.0^{d}
Methanol extract at 12.5 mg/ml (N=3)	6.0 ± 0.0^{d}
Ethanol extract at 100 mg/ml (N=3)	**10.3 ± 0.6^{b}**
Ethanol extract at 50 mg/ml (N=3)	8.0 ± 2.0^{c}
Ethanol extract at 25 mg/ml (N=3)	7.0 ± 1.0^{cd}
Ethanol extract at 12.5 mg/ml (N=3)	6.0 ± 0.0^{d}
Ethyl Acetate extract at 100 mg/ml (N=3)	**15.0 ± 1.0^{a}**
Ethyl Acetate extract at 50 mg/ml (N=3)	6.3 ± 0.6^{d}
Ethyl Acetate extract at 25 mg/ml (N=3)	6.0 ± 0.0^{d}
Ethyl Acetate extract at 12.5 mg/ml (N=3)	6.0 ± 0.0^{d}
Ketoconazole (N=3)	6.0 ± 0.0^{d}
Fluconazole (N=3)	7.3 ± 2.3^{cd}
P – Value	**0.001**

**means with the same letter are statistically similar*

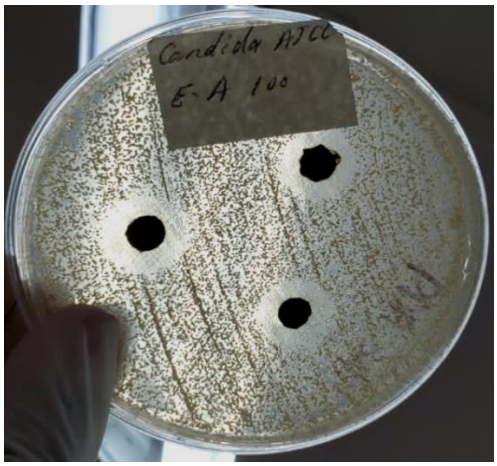

Fig. 4. ZOI of *P. guajava* leaf ethyl acetate extract against *C. albicans* ATCC at 100 mg/mL concentration

Table 2. Zone of inhibition for treatments used against *C. albicans* In-House A

Treatments	ZOI (mg/mL)
Methanol extract at 100 mg/ml (N=3)	**12.0 ± 0.0^{c}**
Methanol extract at 50 mg/ml (N=3)	**11.0 ± 1.0^{cd}**
Methanol extract at 25 mg/ml (N=3)	6.0 ± 0.0^{e}
Methanol extract at 12.5 mg/ml (N=3)	6.0 ± 0.0^{e}
Ethanol extract at 100 mg/ml (N=3)	**12.0 ± 1.7^{c}**
Ethanol extract at 50 mg/ml (N=3)	**11.0 ± 1.0^{cd}**
Ethanol extract at 25 mg/ml (N=3)	**11.0 ± 1.0^{cd}**
Ethanol extract at 12.5 mg/ml (N=3)	9.7 ± 0.6^{d}
Ethyl Acetate extract at 100 mg/ml (N=3)	**11.7 ± 3.5^{cd}**
Ethyl Acetate extract at 50 mg/ml (N=3)	6.0 ± 0.0^{e}
Ethyl Acetate extract at 25 mg/ml (N=3)	6.0 ± 0.0^{e}
Ethyl Acetate extract at 12.5 mg/ml (N=3)	6.0 ± 0.0^{e}
Ketoconazole (N=3)	**41.7 ± 2.9^{a}**
Fluconazole (N=3)	**24.0 ± 1.7^{b}**
P – Value	**0.001**

**means with the same letter are statistically similar*

Fig. 5. ZOI of *P. guajava* ethyl acetate extract against *C.albicans* In-house A at 100 mg/mL concentration

Table 3. Zone of inhibition for treatments used against *C. albicans* in-house B

Treatments	Zone of inhibition (mg/ml)
Methanol extract at 100 mg/ml (N=3)	9.7 ± 0.6 ^{d}
Methanol extract at 50 mg/ml (N=3)	9.0 ± 1.0 ^{d}
Methanol extract at 25 mg/ml (N=3)	6.0 ± 0.0 ^{e}
Methanol extract at 12.5 mg/ml (N=3)	6.0 ± 0.0 ^{e}
Ethanol extract at 100 mg/ml (N=3)	**15.0 ± 2.0** ^{b}
Ethanol extract at 50 mg/ml (N=3)	**14.3 ± 1.5** ^{b}
Ethanol extract at 25 mg/ml (N=3)	**14.0 ± 1.0** ^{b}
Ethanol extract at 12.5 mg/ml (N=3)	**12.3 ± 0.6** ^{bc}
Ethyl Acetate extract at 100 mg/ml (N=3)	**12.0 ± 0.0** ^{bc}
Ethyl Acetate extract at 50 mg/ml (N=3)	**10.0 ± 1.0** ^{cd}
Ethyl Acetate extract at 25 mg/ml (N=3)	6.0 ± 0.0 ^{e}
Ethyl Acetate extract at 12.5 mg/ml (N=3)	6.0 ± 0.0 ^{e}
Ketoconazole (N=3)	**23.3 ± 5.8** ^{a}
Fluconazole (N=3)	6.0 ± 0.0 ^{e}
P – Value	**0.001**

**means with the same letter are statistically similar*

Fig. 6. ZOI of *P. guajava* ethanolic extracts against *C. albicans* In-house B at 12.5 mg/mL concentration

Table 4. Zone of inhibition for treatments used against *C. neoformans*

Treatments	ZOI (mm)
Methanol extract at 100 mg/ml (N=3)	**11.7 ± 0.6^{d}**
Methanol extract at 50 mg/ml (N=3)	**10.3 ± 1.5^{de}**
Methanol extract at 25 mg/ml (N=3)	9.0 ± 1.0^{ef}
Methanol extract at 12.5 mg/ml (N=3)	7.3 ± 0.6^{f}
Ethanol extract at 100 mg/ml (N=3)	8.7 ± 0.6^{ef}
Ethanol extract at 50 mg/ml (N=3)	8.3 ± 0.6^{ef}
Ethanol extract at 25 mg/ml (N=3)	7.0 ± 0.0^{f}
Ethanol extract at 12.5 mg/ml (N=3)	6.3 ± 0.6^{f}
Ethyl Acetate extract at 100 mg/ml (N=3)	**25.0 ± 3.6^{c}**
Ethyl Acetate extract at 50 mg/ml (N=3)	**10.0 ± 1.0^{cd}**
Ethyl Acetate extract at 25 mg/ml (N=3)	6.0 ± 0.0^{f}
Ethyl Acetate extract at 12.5 mg/ml (N=3)	6.0 ± 0.0^{f}
Ketoconazole (N=3)	**41.0 ± 1.0^{a}**
Fluconazole (N=3)	**32.7 ± 0.6^{b}**
P – Value	**0.001**

**means with the same letter are statistically similar*

4. DISCUSSION

Antifungal resistance is a major concern both globally and regionally. Therefore, the leaves of the *P. guajava* plant were investigated to determine its antifungal activity against four strains of fungi. This plant leaf was effective against all four strains of fungi evaluated. We found that the leaf extracts were more effective against *C. neoformans* than the *C. albicans* strains. In contrast, in a similar study, Beatriz et al. (2012), found a slight difference between the activity against *C. neoformans* and *C. albicans* with the *P. guajava* leaf extracts [23].

Three of the *P. guajava* leaf solvent extracts (methanol, ethanol, and ethyl acetate) showed antifungal activity, whilst one (hexane) did not. The present study found that the semi-polar, ethyl acetate extract showed the greatest antifungal activity, as greater than 10 mm diameter zones were obtained in all four fungi tested at the 100 mg/mL concentration and up to 50 mg/mL for *C.albicans* B and *C. neoformans*. The ethyl acetate extract was more effective against *C. neoformans* than the *C. albicans* strains. Beatriz et al. (2012) in a similar study showed that the acetone extract was slightly more effective against *C. neoformans* (18) than with *C. albicans* (17 mm) [23]. The ZOI observed by Beatriz et al. (2012) was slightly lower than what we found in our study of *C. neoformans,* but higher than what we found for *C. albicans*. It is crucial to point out that acetone is also semi-polar with a polarity of 0.3 and is said to be similar to ethyl acetate, which has a polarity of 0.2 [28].

The next best effective solvent extract was ethanol, where antifungal activity was seen against three of the fungi tested (the three *C. albicans* strains) at 100 mg/mL concentration and up to 25 mg/mL concentration for *C.albicans* In-house A, and up to 12.5 mg/mL for *C.albicans* In-house B. The extract worked best for *C. albicans* In-house B, where the ZOI was greater than 15 mm in diameter. A recent study done in 2023 showed that the ethanolic extract of *P. guajava* leaves showed a larger ZOI than what we found for *C. albicans* (18 mm) [29]. In this current study, the ethanolic extract was ineffective against *C. neoformans.* However, we did not find any study that investigated the effectiveness of *P. guava* leaf ethanolic extract against *C. neoformans.*

The methanolic extract showed antifungal activity against two fungi, *C.albicans* In-house A at 100 mg/mL concentration and *C. neoformans* up to 50 mg/mL concentration. Results from the study by Beatriz et al. (2012) showed the same ZOI for *C. neoformans* with the methanolic extract but a slightly larger ZOI for *C.albicans* than what was found in this study [23]. This current study showed that the methanolic extract had the largest ZOI against *C.albicans* In-House A at 100 mg/mL concentration. However, a higher ZOI (20 mm) was noted in a similar study done for *C.albicans* with the methanolic extract [29], and a lower ZOI (8 mm) was noted in another study [30].

Surprisingly, the hexane extracts were ineffective against all the fungi at all concentrations since Beatriz et al. (2012) showed that *P. guajava* leaf

hexane extract was effective against both fungi, with ZOI as high as 14 mm for *C. albicans* and 15 mm for *C. neoformans* [23]. The ethyl acetate, ethanolic, and methanolic extracts worked within the same range (ZOI=10-15 mm) for *C. albicans*, while the ethyl acetate extract worked best for *C. neoformans* compared to the other extracts.

The 100 mg/mL concentration was the most effective against all the fungi. The ZOI obtained against the fungi seems to decrease as the concentration of the extracts decreases, a finding confirmed by many other studies [31, 32].

The *P. guajava* leaf extracts showed significant activity against *C. neoformans*; however, the antifungal agents, ketoconazole and fluconazole showed significantly greater activity. Good antifungal activity was noted against *C.albicans* ATCC, but surprisingly, the antifungal agents, ketoconazole and fluconazole did not. The extracts also showed good activity against *C.albicans* In-House A; however, ketoconazole and fluconazole showed greater activity against this strain of fungi. The *P. guajava* leaf extracts also showed good activity against *C. albicans* In-House B; however, ketoconazole was much more effective, while fluconazole was ineffective. Clearly, *P. guajava* leaf extracts worked better than the positive controls in some cases. Beatriz et al. (2012) showed that the antifungal agent ketoconazole was more effective against *C. neoformans* and *C. albicans* than the *P. guajava* leaf extracts [23].

A perusal of the literature shows that only a few studies on the antifungal activity of *P. guajava* leaf extracts against *C. albicans* and *C. neoformans* were carried out. Therefore, we are excited about the inhibition effects of all three extracts against all four strains of fungi we tested.

5. CONCLUSION AND RECOMMENDATIONS

P. guajava leaf extracts have considerable antifungal activity, and could therefore be an excellent complementary and alternative medicine for treating infections caused by *Candia albicans* and *Cryptococcus neoformans*. It is recommended that this study, be carried out using another antifungal sensitivity test, such as the micro-dilution technique, to confirm our preliminary results. In addition, other pharmacological elements such as dosage and toxicology should be investigated along with the identification and quantification of metabolites in *P. guajava* leaves through phytochemical analysis. Furthermore, more solvent extracts should be considered against a wider range of fungi including antifungal-resistant strains.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

This was not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yuan H, Ma Q Ye, L, Piao G. The Traditional medicine and modern medicine from natural products.Molecules. 2016;21(5):559.
2. Allkin B. Useful plants-medicines. State of the World's Plants 2017- NCBI Bookshelf; 2017.
3. Khan MSA, Ahmad I. Herbal Medicine. InElsevier eBooks. 2018;3–13.
4. Ali MC, Chen J, Zhang H, Li Z, Zhao L, Qiu H. Effective extraction of flavonoids from *Lycium barbarum* L. fruits by deep eutectic solvents-based ultrasound-assisted extraction.Talanta. 2019;203:16–22.
5. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4):564–582.
6. Lesellier E, Lefebvre T, Destandau E. Recent developments for the analysis and the extraction of bioactive compounds from *Rosmarinus officinalis* and medicinal plants of the Lamiaceae family.Trends in Analytical Chemistry. 2021;135:1166158.
7. Dias MC, Pinto DCGA, Silva AMS. Plant flavonoids: Chemical characteristics and biological activity. Molecules. 2021; 26(17).
8. Cankaya IIT, Somuncuoglu EI. Potential and prophylactic use of plants containing saponin-type compounds as antibiofilm

agents against respiratory tract infections.Evidence-based Complementary and Alternative Medicine. 2021;1-4.

9. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: Current approaches and prospects. The Nucleus. 2020;65(3):399–411.
10. Kumar M, Tomar M, Amarowicz R, Saurabh V, Nair MS, Maheshwari C et al. Guava (*Psidium guajava* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Foods. 2021;10(4):752.
11. Serunjogi D. Active phytochemicals present in the Guava Tree (*Psidium guajava*) leaf Extracts that grow in Uganda. Student's Journal of Health Research Africa. 2020;1(12):6-6.
12. Metwally AM, Omar AA, Harraz FM, El Sohafy SM. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. Pharmacognosy Magazine. 2010;6(23).
13. Thambugala KM, Daranagama DA, Tennakoon DS, Jayatunga DPW, Hongsanan S, Xie N. Humans vs. Fungi: An Overview of Fungal Pathogens against Humans. Pathogens. 2024;13 (5):426.
14. Garnacho-Montero J, Barrero-García I, León-Moya C. Fungal infections in immunocompromised critically ill patients.Journal of Intensive Medicine. 2024;4(3):299–306.
15. Søgaard KK, Baettig V, Osthoff M, Marsch S, Leuzinger K, Schweitzer M et al. Community-acquired and hospital-acquired respiratory tract infection and bloodstream infection in patients hospitalized with COVID-19 pneumonia. Journal of Intensive Care. 2021;9(1).
16. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: Human fungal infections.Sci Transl Med.2012;19(4):165.
17. Buscaino A. Chromatin-mediated regulation of genome plasticity in human fungal pathogens.Genes.2019;10- 855.
18. Benedict K, Richardson M, Vallabhaneni S, Jackson BR, Chiller T. Emerging issues, challenges, and changing epidemiology of fungal disease outbreaks. The Lancet Infectious Diseases. 2017;17(12):403-411
19. Wiederhold NP. Emerging fungal infections: New species, new names, and antifungal resistance. Clinical Chemistry. 2021;68(1):83–90.
20. Alves SH,LopesJO,CostaJM,KlockC. Development of secondary resistance to fluconazole in*Cryptococcus neoformans*isolated from a patient with AIDS. Rev Inst Med Tropical Sao Paulo. 1997;39:359-61.
21. Marichal P,Koymans L,Willemsens Set al.Contribution of mutations in the cytochrome P450 14alpha-demethylase (Erg11p, Cyp51p) to azole resistance in*Candida albicans*. Microbiology. 1999;145(10):2701-13.
22. Antifungals. Cleveland Clinic; 2024, May 1. Available:https://my.clevelandclinic.org/health/drugs/21715-antifungals
23. Beatriz PM, Ezequiel VV, AzucenaOC, Pilar CR. Antifungal activity of *Psidium guajava* organic extracts against dermatophytic fungi. Journal of Medicinal Plants Research. 2012;6(41):5435-5438.
24. Díaz-de-Cerio E, Verardo V, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A. Health Effects of *Psidium guajava* L. Leaves: An overview of the last decade. International Journal of Molecular Sciences. 2017;18(4):897-927.
25. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: Current approaches and prospects. The Nucleus. 2022;63(3):399-411.
26. Das, M., Goswami, S. Antifungal and antibacterial property of guava (*Psidium guajava*) leaf extract: Role of phytochemicals.International Journal of Health Sciences and Research. 2019;9(2):39-45.
27. Fugaban-Hizon C. Comparative study on antifungal property of methanolic and ethanolic extracts of *Psidium guajava* on *Trichophyton mentagrophytes.*Scholars Academic Journal of Biosciences. 2016;4(2):158-160.
28. Abubakar A, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences. 2020;12(1):110.
29. Thombre NA, Laddha UD, Ahire ED, Tajanpure AB, Surse SN, Kakad SP et al. Formulation development and evaluation of herbal antifungal cream by using *Psidium guajava*leaf extract. International

Journal of Drug Delivery Technology. 2023;13(4):1229-1234.

30. Nair R, ChandaS. *In-vitro* antimicrobial activity of *Psidium guajava* l. leaf extracts against clinically important pathogenic microbial strains.Brazilian Journal of Microbiology. 2007;38(3):452-458.
31. Kebede B, Shibeshi W. *In vitro* antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. Veterinary Medicine and Science. 2022;8(4):1802–1815.
32. Akinduti PA, Emoh-Robinson V, Obamoh-Triumphant HF, Obafemi YD, Banjo TT. Antibacterial activities of plant leaf extracts against multi-antibiotic resistant Staphylococcus aureus associated with skin and soft tissue infections. BMC Complementary Medicine and Therapies. 2022;22:47.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/125885