

Full Length Research Paper

Antifungal activity of *Psidium guajava* organic extracts against dermatophytic fungi

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Hexane, acetone and methanol extracts obtained from *Psidium guajava* leaves were studied for their antifungal properties against *Trichophyton rubrum*, *Trichophyton tonsurans*, *Sporotrix schenckii*, *Microsporum canis*, *Cryptococcus neoformans*, *Candida parapsilosis*, and *Candida albicans* by using the agar disk diffusion technique. Compared to control, hexane extract showed the best antifungal activity, being active against all the tested dermatophytes. Methanol and acetone extracts also showed relevant activity. The phytochemical analysis of the hexane extracts revealed the presence of flavonoids, terpenoids and coumarins, whereas alkaloids, carbohydrates and saponins were not detected. Since the bioactive compounds in the hexane extract inhibit the growth of microorganisms, it could be considered for future development of new anti-skin disease agents.

Key words: Antimycotic activity, dermatophytes, inhibition zone, crude extracts, *Psidium guajava*.

INTRODUCTION

Many skin diseases such as tinea and ringworm caused by dermatophytes are prevalent in tropical and subtropical regions. In general, these fungi live in the dead, top layer of the dermis and in moist areas of the body. They can penetrate into the cells and cause itching, swelling, blistering and scaling. Dermatophytes are important causes of acute or chronic deep-seated human infections, especially recurrent mucosal, cutaneous, or nail infections that can be severe in debilitated or immunocompromised individuals (Debruyne and Coquerel, 2001; Welsh et al., 2010). However, the toxicity of currently available antifungal therapies, as well as the increasing of drug-resistance among the etiologic agents has driven the research towards the study of new antimicrobial agents from natural products (Khan et al., 2012; Ajose, 2007). Based on reports that plants have

developed mechanisms of defense to protect themselves against biotic and abiotic threats, including infections by pathogens like fungi, bacteria, and viruses, recent interest has been focused to the search of plant-derived fungicides and antimicrobials (Gurgel et al., 2005; Wojtaszek, 1997).

Medicinal plants are considered a rich source for antimicrobial agents, with the advantage that most of the natural products used in traditional medicine are readily available in rural areas at relatively lower cost than modern medicines (Mahesh and Satish, 2008; Mann et al., 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and pharmaceutical drugs. In relation to this, the common guava tree (*Psidium guajava* Linn.), a member of the Myrtaceae family, has been reviewed extensively by Gutiérrez et al. (2008) in order to highlight the pharmacologic effects of the extracts obtained from its fruits, leaves, bark or roots. The investigators found that extracts from *P. guajava* have antispasmodic and antimicrobial properties for the

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Table 1. Antifungal activity of *P. guajava* leave extracts

Microorganism	Zone of inhibition (mm)			
	Organic extract			Control*
	MeOH	Acetone	Hexane	
<i>Candida albicans</i>	11 ± 2	17 ± 2	14 ± 1	45 ± 5
<i>Candida parapsilosis</i>	17 ± 3	10 ± 1	10 ± 2	25 ± 5
<i>Cryptococcus neoformans</i>	11 ± 1	18 ± 3	15 ± 1	35 ± 3
<i>Microsporium canis</i>	-	-	16 ± 3	30 ± 4
<i>Microsporium gypseum</i> ,	-	-	10 ± 1	43 ± 5
<i>Trichophyton tonsurans</i>	19 ± 3	-	16 ± 3	44 ± 4
<i>Trichophyton rubrum</i>	10 ± 2	13 ± 1	16 ± 2	50 ± 6
<i>Sporotrix schenckii</i>	-	11 ± 2	10 ± 1	47 ± 4

*Ketoconazole was used as positive control.

treatment of diarrhea and dysentery. Additional pharmacological properties attributed to extracts from *P. guajava* include antioxidant, hepatoprotective, antiallergic, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, anticough, anti-inflammatory, antinociceptive, hypoglycemic and antidiabetic activities, thus, supporting its uses in traditional medicine. Previous works have also showed important antifungal activity of the tinctures (Dutta et al., 2000). Because little is known about the antifungal properties of organic extracts prepared from *P. guajava* leaves, this study was performed to assess the efficacy of the organic extracts against dermatophyte fungi.

MATERIALS AND METHODS

Sample collection and processing

P. guajava (Linn) (Myrtaceae) was collected in San Nicolás de los Garza, Nuevo León, México, during May and June, 2000; it was identified in the Department of Botany by Dr. Marcela González Alvarez. A plant specimen was deposited in the ethnobotanical collection of the FCB-UANL herbarium (voucher specimen number: 024884).

Leaves from the plant were dried at room temperature, and 30 g of the dry-powdered material were sequentially extracted by maceration with hexane, acetone and methanol (3 times, 24 h each). The plant:solvent ratio was 1:5 (w/v). After filtration and concentration under reduced pressure, the percentage (w/w yield) of extracts from *P. guajava* was hexane (5.5), acetone (11.6) and methanol (19.8).

Microbial suspension

Clinical isolates of *Candida albicans*, *Candida parapsilosis*, *Cryptococcus neoformans*, *Microsporium canis*, *Microsporium gypseum*, *Trichophyton tonsurans*, *Trichophyton rubrum*, and *Sporotrix schenckii* were maintained at 4°C in Sabouraud Dextrose Agar (SDA) culture medium. The dermatophytes were subcultured on SDA slants and incubated at 35°C for 7 to 14 days, depending on the microorganism. The mycelial growth was scraped aseptically and suspended thoroughly in sterile distilled water. The suspension was standardized spectrophotometrically to an absorbance [also called optical density (OD)] of 0.600 at 450 nm. These adjusted

suspensions approximately corresponded to 0.5 to 2.5 × 10³ cells/ml and were used as inoculum for antifungal susceptibility testing (Chandrasekaran and Venkatesalu, 2004).

Antifungal assay

Antifungal activity tests were performed by using the disk diffusion agar method (Bauer et al., 1966). Test plates were prepared with 20 ml of sterile SDA. The standardized fungal suspension was applied on the solidified culture medium by using sterile cotton swabs and allowed to dry for 5 min. A sterile paper disk (Whatman AA disk, 6 mm) was impregnated with 10 µl of a stock solution (50 mg/ml) from each crude extract. The disks were aseptically transferred on the inoculated agar plates and incubated for 48 h to 7 days, depending of the tested fungi. Antifungal activity was determined by measuring clear zones of inhibition around the test crude extract discs. The clear zones indicated the fungicidal effect while fungi static effect referred to the unclear zone of inhibition. Ketoconazole (250 µg) disks were used as a standard reference or positive controls, and the solvent or empty disks were used as negative controls. All assays were performed in triplicate.

Phytochemical screening

The phytochemical constituents of the plant were determined in accordance with the methods described by Harborne (1984). The colour intensity of extracts and/or the appearance of solids in them during the identification reactions allow a semi-quantitative evaluation of the presence of secondary metabolites.

RESULTS

Organic extracts from *P. guajava* leaves were investigated for their antifungal effect against clinically important dermatophytes fungi. Compared to control, the best activity found in our investigation was observed with the hexane extract, which inhibited all the tested dermatophytes (Table 1). However, methanol and acetone extracts showed relevant activity against 70% of the strains. *C. neoformans* was the most sensitive fungi, and the acetone extract was the most active (18 ± 3). *M. gypseum* and *M. canis* were inhibited by the non polar

Table 2. Phytochemical screening of *P. guajava* leaf extracts.

Organic extract	Alk	Flav	Coum	Sap	Phenolics	Sesq	CHO	Terp
Hexane	-	+	+	-	+	+	-	++
Acetone	-	+	-	+	+	-	+	+
Methanol	-	++	+	+	++	+	+	++

Alk, Alkaloid; Flav, flavonoids; Coum, coumarins; Sap, saponins; Sesq, sesquiterpene lactones; CHO, carbohydrates; Terp, terpenoids; (++) , abundant; (+), present; (-), absent.

extract, whereas *C. parapsilosis* was the most resistant, with inhibition zones of 25 ± 5 mm showed by the positive control (Ketoconazole) and 17 ± 3 mm by the methanol extract.

The secondary metabolites that were identified in the non-polar extract were flavonoids, terpenoids and coumarins; only the methanol and acetone extracts showed carbohydrates and saponins; no alkaloids were detected in any extracts (Table 2).

DISCUSSION

Previous studies have shown the fungicidal effect of organic extracts derived from plants, and also has been shown that the activity of secondary metabolites may vary depending on the type of solvent used. In accordance with the last, antifungal activity has been reported in polar compounds such as glycosylated flavonoids, and saponins isolated from polar extracts (Kim et al., 2010; Lanzotti et al., 2012), and, in non-polar compounds, like terpenoids (Wang et al, 2011; Singh et al., 2011).

In the present study, methanol and acetone extracts showed comparable activity against the fungal strains; similar results were obtained by Nair and Chanda (2007), with inhibition zone diameters of 7.5 to 18 mm against *Candida* spp and *C. neoformans* (9 ± 1.15), the best antifungal activity was showed by the hexane extract. With regard to our results, diverse authors have found that antifungal activity relies on the organic solvents used. For example, Machado et al. (2009) demonstrated antimicrobial activity in the methanol extracts, while Tay et al. (2004) and Cardoso et al. (2010) reported activity with acetone and hexane extracts, respectively. The observed activity for the hexane extract is acceptable, considering that a crude extract was used, and the active compound could be diluted. It is possible that isolating the active compound or compounds will provide better fungicide activity. The above results suggest that *P. guajava* could be an important source of non-polar compounds with antimicrobial activity. Regarding to the last, reports about antibacterial and antifungal compounds isolated from leaves of *P. guajava* showed that in the polar extract (alcoholic), flavonoids such as quercetin and its glycosides derivatives are responsible of the strong antibacterial activity, including against *C. albicans* (Arima

and Danno, 2002; Metwally et al., 2010). In the non-polar extract (toluene), the terpenoids betulinic acid and lupeol were isolated; these compounds showed antifungal activity against *Colletotrichum camelliae*, *Fusarium equisetiae*, *Alternaria alternata*, *Curvularia eragrostidis* and *Colletotrichum gleosporoides* (Ghosh et al., 2010). Both triterpenoids have been previously reported possessing antifungal activity against *S. schenckii*, *M. canis*, *C. albicans* and *C. neoformans* (Shai et al., 2008). Taking the last reports into account, and the similitude of our results, it can be possible that the same compounds could be responsible for antidermatophyte activity of the *P. guajava* leaves.

Conclusion

The results of this study indicate that the leaves of *P. guajava* contain bioactive compounds, like flavonoids and terpenoids which inhibit the growth of dermatophytic fungi thereby providing an additional alternative source of antifungal compounds.

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