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Effect of aqueous extract of leaf and bark of guava (*Psidium guajava*) on fungi *Microsporum gypseum* and *Trichophyton mentagrophytes*, and bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*

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ABSTRACT

In this study, we investigated the effects of *Psidium guajava* on organisms responsible for skin disorders, specifically the fungi: *Microsporum gypseum* and *Trichophyton mentagrophytes*, and bacteria: *Staphylococcus aureus,* and *Staphylococcus epidermidis.* The leaves and bark of the *P. guajava* plant was harvested from Obasa farm Ijero, Ekiti-State, Nigeria, during the beginning of rainy season in March, 2009. Aqueous solutions were obtained by grinding the leaves and the bark. Mueller-Hinton agar was used to grow the bacteria *S. aureus* and *S. epidermidis*. Sabouraud Dextrose broth was used to grow the fungi *Trichophyton mentagrophytes* and *Microsporum gypseum*. Analysis of the antibacterial action of the extracts of guava leaves and bark was carried out at different concentrations, by comparing the mean diameter of the inhibition haloes as a variable. Values were represented as mean ± S.E. An ANOVA Tukey's test was performed to determine the mean difference between the control and the two treatments (S1 and S2). In comparing the tetracycline positive control to both solutions, tetracycline had a significantly ($p < 0.05$) stronger inhibition effect than both solutions. This could be due to the fact that tetracycline is a pure chemical while the *P. guajava* solutions were crude extracts. Both *P. guajava* solutions were effective against inhibiting the growth of bacteria *S. aureus* and *S. epidermidis*, and fungi *M. gypseum* and *T. mentagrophytes*. This supports the reported use of *P. guajava* in many countries as a traditional herbal medicine.

Keywords: *Psidium guajava*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, tetracycline.

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INTRODUCTION

Guava is a small tropical tree that grows up to 35 feet tall; it is widely grown for its fruit in tropics. It is a member of the *Myrtaceae* family, with about 133 genera and more than 3800 species. The leaves and bark of *Psidium guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi et al., 2008). The leaves and bark of the guava plant have been used to treat diarrhea, other gastrointestinal disorders, toothaches, colds, and swelling, in areas such as (Weiner, 1971), India (Dutta et al., 2000), Africa (Rabe and van Staden, 1997; Tona et al., 1998; Tona et al., 2000; Lin et al., 2002), Hawaii (Nagata, 1971), Malaysia and Panama

(Lutterodt, 1989). In Tahiti (Weiner, 1971), Guatemala (Caceres et al., 1990, 1993), the Philippines (Weiner, 1971), Northeast India (Weiner, 1971), and West Bengal (Gupta and Banerjee, 1972), guava is used for skin disorders such as an astringent for acne, rashes, and ringworm. The Southeast Nicaraguan indigenous communities of Cuna and Waunana, make tea from the leaves and bark of the guava for treatment of diarrhea and dysentery, while the Sumu, Panamahka, Twahka, Ulwa, and Bawihka tribes use it for upset stomachs, vertigo, and to regulate menstrual cycles (Coe and Anderson, 1999).

The long history of guava use has led modern day researchers to study guava extracts. Many experiments have examined the antimicrobial properties of *Psidium guajava* (Matsuo et al., 1994). This medicinal plants has anti-proliferative effects on human mouth epidermal carcinoma and murine leukemia cells using MIT assay, guava leaf showed anti-proliferative activity, which was 4.37 times more than Vincristine (Manosroi et al., 2006). Bark and leaf extracts were shown to have *in vitro* toxic action against numerous bacteria. Gallocatechin isolated from the methanol extract of guava leaf showed antimutagenic activity against *Escherichia coli (*Manosroi et al., 2006). Water and chloroform extracts of guava were effective in activating the mutagenicity of *Salmonella typhimurium (*Grover and Bala, 1993). The antimicrobial activities of *P. guajava* and leaf extracts, determined by disk diffusion method (zone of inhibition), were compared to tea tree oil (TTO), doxycycline and clindamycin antibiotics. It was shown that *P. guajava* leaf extracts might be beneficial in treating acne especially those that have anti-inflammatory activities (Qadan, 2005). The active flavonoid compound - quercetin-3-Oalpha- l-arabinopyranoside (guaijaverin) - extracted from leaves has high potential anti-plaque activity by inhibiting the growth of *Streptococcus mutans* (Limsong et al., 2004).

Jaiarj (1999) tested the anti-cough and antimicrobial activities of *P. guajava* leaf extracts. The guava juice showed some positive effects on reducing coughs and the leaves demonstrated some antimicrobial activity on *S. aureus* and beta-streptococcus groups. Gnan and Demello (1999) also performed experiments on the inhibition of *S. aureus* by aqueous Gioba (*P. guajava)* extracts. The Gioba leaf and fruit extracts showed antimicrobial activity against the nine different strains of *S. aureus*.

Guava leaf extract inhibited the growth of *Streptococcus aureus* in a study carried out by disc diffusion method (Abdelrahim, 2002). Lin et al. (2002) tested *P. guajava* extracts for anti-microbial activities against different species of diarrheagenic *E. coli*, *Salmonella*, and *Shigella*. *P. guajava* showed inhibitory activities against two species of *Salmonella, Shigella flexneri, Shigella virchow,* and *Shigella dysenteriae*, and two varieties of enteropathgenic *E. coli.* Guava sprouts (young leaves of *P. guajava*) have been used in Brazilian medicine for gastrointestinal disorders, the microbicidal activity of *P. guajava* showed inhibition on *E. coli* and *S. aureus, Salmonella typhi, Shigella flexneri* and *Shigella dysenteriae* (Caceres et al., 1990, 1993). Dutta et al. (2000) tested *P. guajava* against dermatophytes *Trichophtyon tonsurans, Trichophyton rubum* and *Microsporum fulvum*. Almost all dermatophytes showed no growth with the exception of two: *Trichosporon beigelli* and *Candida albicans*. Extracts from both the bark and leaves were used, although the extracts from the bark were more efficient in inhibiting the dermatophytes than the leaves. The leaves and bark also act as an antidiarrhoeic (Lutterodt, 1989, 1992; Tona et al., 1998; Tona et al., 2000).

Existing literature has reported that *P. guajava* has demonstrated considerable anti-fungal and anti-bacterial effects. In this research, an experiment was designed to determine the effects of *P. guajava* on organisms responsible for skin disorders, specifically the fungi *M. gypseum, T. mentagrophytes*, and bacteria *S. aureus* and *S. epidermidis.*

MATERIALS AND METHODS

Plant material

The leaves and bark of the *P. guajava* plant was harvested from Obasa farm Ijero Ekiti State, during the beginning of rainy season in March, 2009. The plant was taken to the Department of Botany, Olabisi Onabanjo University, Ogun state and identified by Dr Sannu Achidi, Ogun State, Nigeria for authentication. After authentication, a voucher sample of the plant was deposited in the herbarium of the University.

Preparation of *Psidium guajava* aqueous leaf and bark extracts (PGALBE) and its fractions

The leaves of *Psidium guajava* were rinsed in distilled water to remove dirt, dried in an air-oven at 40°C for 3 days, and then pulverized into fine powder that passed through a 30-mesh sieve.

Aqueous solutions were obtained by grinding the leaves and the bark. After grinding, 100 ml of ethyl alcohol was added. Two solutions were prepared using traditional methods. One solution was left out for two days at room temperature and then placed in the refrigerator. The other solution was immediately placed in the refrigerator. Both solutions were agitated twice daily for 5 min, after 7 days both solutions were strained through sterile cheesecloth, and placed back inside of the refrigerator.

Test for antimicrobial and anti-fungal effect

Mueller-Hinton agar was used to grow the bacteria *S. aureus* and *S. epidermidis*. Sabouraud Dextrose broth was used to grow the fungi *T. mentagrophytes* and *M. gypseum*. A modified Kirby-Bauer method was performed to test the effectiveness of the guava extract on the bacteria *S. aureus* and *S. epidermis*. 25 µl of each solution was added to sterile discs and placed on the Mueller-Hinton agar of each bacterium. A negative control blank disc, a positive Tetracycline control disc, and an ethanol control disc were

Table 1. *Staphylococcus aureus* growth inhibition diameter in millimeters (mm).

Parameter	Mean ± S.E	P-value
B- Blank disc (negative control)	0	0
T- Tetracycline (positive control)	36.5 ± 1.93**	P < 0.05
S1- Solution 1 (25 µl)	21.6 ± 1.87	P < 0.05
S2- Solution 2 (25 µl)	20.2 ± 2.45	P < 0.05
E- Ethyl alcohol control	4 ± 2.71	P > 0.05

Values are expressed as mean ± S.E, **p < 0.01

Table 2. *Staphylococcus epidermidis* growth inhibition diameter in millimeters (mm).

Parameter	Mean ± S.E	P-value
B- Blank disc (negative control)	0	0
T- Tetracycline (positive control)	38.4 ± 2.50**	P < 0.05
S1- Solution 1 (25 µl)	24.9 ± 3.07	P < 0.05
S2- Solution 2 (25 µl)	24.6 ± 2.56	P < 0.05
E- Ethyl alcohol control	2.2 ± 2.56	P > 0.05

Values are expressed as mean± S.E, **p<0.01

Table 3. *Microsporum gypseum* growth results for each test group.

M. gypseum		*M. gypseum* w/ antifungal		*M. gypseum* w/ solution 1		*M. gypseum* w/ solution 2	
Tube 1	++++	Tube 1	-	Tube 1	-	Tube 1	-
Tube 2	++	Tube 2	-	Tube 2	-	Tube 2	-
Tube 3	+++	Tube 3	-	Tube 3	-	Tube 3	-

Positive growth ranging from 4+ to 1+.

also placed on the agar of each dish and were incubated for 24 h. After incubation, the diameters of the inhibition zones were measured in millimeters. Each test was conducted with 15 replicates. An ANOVA Tukey's test was performed.

Twenty-four tubes of Sabouraud Dextrose broth containing extract (1:400 v/v) were inoculated, twelve tubes of fungi *M. gypseum* and twelve tubes of *T. mentagrophytes*. As a negative control, a tube was inoculated with fungi only and as a positive control tube two drops of a Tolnaftate topical 1% anti-fungal solution was placed in the inoculated broth. The tubes were incubated in a shaking water bath for two days. After incubation, the tubes were observed for fungi growth, and a qualitative reading with the degree of growth represented from four plus (+) to one plus (+). Three replicates were performed for each test group.

Statistical analysis

Analysis of the antibacterial action of the extracts of guava leaves was carried out at different concentrations, by comparing the mean diameter of the inhibition haloes as a variable. When the halo produced surpassed the measurement of the diameter of the disc (6.00 mm), we considered that there had been growth inhibition of the tested strains. Values were represented as mean ± S.E. An ANOVA Tukey's test was performed to determine the mean difference between the control and the two treatment solution of *P. guajava*.

RESULTS

The bacteria *S. aureus* and *S. epidermidis,* and fungi *M. gypseum* and *T. mentagrophyte* were inhibited by *P. guajava* aqueous extracts. *S. aureus* (Table 1) and *S. epidermidis* (Table 2) *P. guajava* solutions were effective in inhibiting bacterial growth. Neither extract was as effective as the positive control (Tetracycline), but both were more effective compared to the ethyl alcohol control and the negative control (blank disc). This confirms that the *P. guajava* extract was the active compound inhibiting the growth and not the solvent.

ANOVA Tukey's test indicated a significant ($p < 0.05$) difference between the average of both controls and the two treatments for *S. aureus* and *S. epidermidis* (Tables 1 and 2). There was no significant difference between the zones of inhibition for the two different preparations of *P. guajava* solutions for both *S. aureus* and *S. epidermidis*.

Due to the rhizoid-type growth which remained clumped in broth culture, the growth of fungi *M. gypseum* (Table 3) and *T. mentagrophyte* (Table 4) could not be measured quantitatively. However, in the presence of the commercial anti-fungal solution and the two *P. guajava*

Table 4. *Trichophyton mentagrophytes* growth results for each test group.

T. mentagrophyte		*T. mentagrophyte* w/ antifungal		*T. mentagrophyte* w/ solution 1		*T. mentagrophyte* w/ solution 2	
Tube 1	++++	Tube 1	-	Tube 1	-	Tube 1	-
Tube 2	+++	Tube 2	-	Tube 2	-	Tube 2	-
Tube 3	++	Tube 3	-	Tube 3	-	Tube 3	-

Positive growth ranging from 4+ to 1+.

solutions, fungi growth was prevented.

DISCUSSION AND CONCLUSION

In comparing the Tetracycline positive control to both solutions, Tetracycline had a significantly ($p < 0.05$) stronger inhibition effect than both solutions. This could be due to the fact that the Tetracycline is a pure chemical while the *P. guajava* solutions were crude extracts. Our results support the findings of Vieira et al. (2001), which also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of *S. aureus*. Gnan and Demello (1999) testing guava leaf extract, found good antimicrobial activity against nine different strains of *S. aureus.* Berdy et al. (1982) and Caceres et al. (1993) described the antibiotic activity of the aqueous extract of dried leaves and bark of *P. guajava* to guajaverin and psidiolic acid.

Both *P. guajava* solutions were effective against inhibiting the growth of bacteria *S. aureus* and *S. epidermidis*, and fungi *M. gypseum* and *T. mentagrophytes*. This supports the reported use of *P. guajava* in many countries as a traditional herbal medicine.

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