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Original Research Article

Comparative Study on the Antifungal Property of Methanolic and Ethanolic Extracts of *Psidium guajava*on *Trichophyton mentagrophytes*

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Abstract: This study aims to compare the antifungal property of methanolic and ethanolic extract of *Psidium guajava* leaves and barks to *Trichophyton mentagrophytes*. The leaves and barks were soaked in 70% ethanol and 80% methanol in order for the solvents to penetrate the grinded leaves and barks of *Psidium guajava* to produce the extract. It was tested to determine its antifungal property to a dermatophytic fungus, *Trichophyton mentagrophytes*. It was done by swabbing the Potato Dextrose Agar with the test organism. All assays were in triplicate to compare the extent of zone of inhibition. According to the results obtained 70% Ethanolic leaf extracts showed a greater zone of inhibition compared to 70% ethanolic bark to *Trichophyton mentagrophytes*. In the 80% concentration of Methanolic extract of the different parts of guava (leaves and barks), it was shown that 80% Methanolic leaf extract showed a greater zone of inhibition compared to 80% Methanolic bark extract to *Trichophyton mentagrophytes*.
Keywords: *Psidium guajava*, *Trichophyton mentagrophytes*, methanolic extract, ethanolic extract, potato dextrose agar.

INTRODUCTION

Psidium guajava or commonly known as guava is a small tropical tree that grows up to 35 feet tall which widely grows in tropical countries like Philippines. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [1].Guava is at great importance in rural areas because it has been a traditional to use guava specifically its leaves as a decoction for wounds. This is a great advantage to them because it is readily available and priceless.

Microorganisms have become resistant to many antibiotics due to increase use of drugs and not strictly following the prescribed time to take which decreases its efficiency. So, it has become necessary to find out new antifungal agent. Some antibiotics do not have capability to treat diseases because of drug resistance of pathogens and the immunity of individuals. The uses of herbal treatment are one of the possible ways to treat diseases caused by multi drug resistant fungi.

Guava has demonstrated noteworthy anti-bacterial activities against bacteria such as *Bacillus, Clostridium, E. coli, Shigella, Staphylococcus, Salmonella* and *Pseudomonas* that are responsible for causing general diarrhea. In addition, guava has also shown anti-yeast (*Candida*), anti-fungal, anti-malarial as well as anti-amoebic activities [2].

The long history of guava use has led modern day researchers to study guava extracts which contains over 20 compounds such as phytochemicals such as flavonoids, quercetin, saponins, alkaloids anthraquinones, phlobatannins and cardiac glycosides, tannins, Vitamin C, iron, calcium and phosphorus, potassium and Vitamin A that have been reported present in its leaves, stems, bark and roots [3].These phytochemicals are believed to have different role as anti-diarrheal, anti-bacterial, anti-spasmodic and others[2].

In the study, "Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) on Two Gram-Negative and Gram-Positive Bacteria", the guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water) on *Escherichia coli* and *Salmonella enteritidis* (gram-negative bacteria) and *Staphylococcus aureus* and *Bacillus cereus* (gram-positive bacteria). The study reveals that the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts[1].

Organic extracts from *P. guajava* leaves were investigated for their antifungal effect against clinically important dermatophytic fungi. Compared to control,

the best activity found in our investigation was observed with the hexane extract, which inhibited all the tested dermatophytes[4].

Statement of the Problem

This study aims to compare the antifungal property of methanolic and ethanol extract of *Psidium guajava* leaves and barks to *Trichophyton mentagrophytes*.

Specifically, it aims to answer the following questions:

1. At what concentration of leaf, and bark ethanolic extracts of *Psidium guajava* will give a greater zone of inhibition against *Trichophyton mentagrophytes*
2. At what concentration of leaf, and bark methanolic extracts of *Psidium guajava* will give a greater zone of inhibition against *Trichophyton mentagrophytes*
3. Is there a significant difference in the zone of inhibition of the 70% Ethanol and 80% Methanol extracts of the leaves and barks when compared to the control group?

Hypothesis:

H0: There is no significant difference between the zone of inhibition produced by the ethanolic and methanolic leaves and bark extracts of *Psidium guajava* as an antifungal agent against *Trichophyton mentagrophytes*.

H1: There is a significant difference between the zone of inhibition produced by the ethanolic and methanolic leaves and bark extracts of *Psidium guajava* as an antifungal agent against *Trichophyton mentagrophytes*.

Significance of the study

This study provides an alternative source of treatment for incidence of *Tinea capitis* and other fungal diseases caused by *Trichophyton mentagrophytes*. This study would give the community the idea about the ability of guava as antifungal agent to such infections and not just a basic remedy for some illness. This could serve as a reference in making antifungal products containing guava extracts. This may serve as a basis for the present researchers in conducting parallel and more advanced studies in the future.

MATERIALS AND METHODS

1. Processing and extraction of plant material

a. The leaves and barks of guava was properly washed with distilled water, air dried at room temperature for 1 day and then grinded into fine powder.
b. 20g of the plant material were dissolved in 20mL of 70% ethanol and 80% methanol respectively
c. Mixtures were kept in the dark for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation and exposure to sunlight [3].
d. The mixtures were then transferred into different wide-mouthed ambered bottles and were properly labeled.

2. Media Inoculation

a. Microbial suspension was prepared from 3-5 day old culture of the mold. The suspending medium used was 0.1% peptone water.
b. Pre-poured Potato Dextrose Agar (PDA) agar plates, about 3 mm thick, were inoculated with the microbial suspension by swabbing the agar surface.
c. The cotton swab on an applicator stick was dipped into the microbial suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab.
d. The swab was streaked over the entire agar surface. This procedure was repeated two more times, rotating the plate 60^{o} each time to ensure even distribution of the inoculums.
e. Three equidistant wells were made on the agar plate using a cork borer (10 mm diameter). Two hundred (200) uL portions of the sample were placed in each well.
f. The plates were incubated at room temperature for 3-5 days. The clearing zone was measured in millimeters and the average diameter of the clearing zones was calculated.
g. The Antimicrobial index (AI) was computed using the following formula:

$$AI = \frac{\text{Average diameter of clearing zone} - \text{Diameter of well}}{\text{Diameter of well}}$$

RESULTS AND DISCUSSION

Extent of Inhibition

Table 1: Extent of Inhibition of leaf and bark alcoholic extracts and control groups on *Trichophyton mentagrophytes*

Test Organism	Sample	Replicates	Clearing zone .mm	Antimicrobial Index
Trichophyton mentagrophytes	a. 70% ethanolic bark extract	1 2 3	20 20 20	1.0
	b. 80% Methanolic bark extract	1 2 3	21 21 22	1.1
	c. 70% ethanolic leaf extract	1 2 3	31 31 32	2.1
	d. 80% Methanolic leaf extract	1 2 3	31 31 31	2.1
	Canesten[b](positivecontrol)	1 2 3	45 45 45	3.5

[a] No inhibition of growth of the test organism, [b] Contains 1% clotrimazole

REMARKS:

Sample A and B inhibited the growth of *Trichophyton mentagrophytes* with AIs of 1.1 and 1.0, respectively. Sample C and D inhibited the growth of *Trichophyton mentagrophytes* with AI of 2.1.

SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

The salient findings of the study are as follows:

1. According to the results obtained, 70% Ethanolic leaf extracts showed a greater zone of inhibition compared to 70% ethanolic barkto *Trichophyton mentagrophytes*.
2. In the 80% concentration of Methanolic extract of the different parts of guava (leaves and barks), it was shown that 80% Methanolic leaf extract gave a greater zone of inhibition against *Trichophyton mentagrophytes* compared to 80% Methanolic bark extract to *Trichophyton mentagrophytes*.
3. There is a significant difference on the zone of inhibition against *Trichophyton mentagrophytes* between the 70% Ethanol and 80% Methanol extracts of the leaves and barks of Guava and the positive control.

CONCLUSION

Based on the results and findings, the researcher conclude that alcoholic extracts of the leaves and barks of *Psidium guajava* has antifungal property that was evident at 70% and 80% concentration of Ethanol and Methanol respectively, suggesting inhibition of fungal growth.

RECOMMENDATIONS

In light of the findings and conclusion, the researcher recommends that:

1. Future researchers consider the following:
   - Testing the antifungal property of the used extracts to other dermatophytic fungi.
   - The use of 100% concentration and/or pure extract of the guava leaves, barks and seeds.
2. Pharmaceutical companies come up with & promote *Psidium guajava* antifungal products.
3. In laboratory procedures, utilize *Psidium guajava* extracts as inhibitors of fungal growth on bacterial cultures.

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