In vitro screening of Anti-Fungal Effect of *Averrhoa bilimbi* Leaves Methanolic Extract against *Microsporum canis*

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Abstract

This study was conducted to test the effectiveness of Averrhoa bilimbi leaves extract against Microsporum canis, a fungi that causes skin infections such as tinea capitis. The researchers of the study aimed to help the community especially those people who are experiencing infections caused by the fungi, Microsporum canis, which is often carried by dog and cats. To test the effectiveness of Averrhoa bilimbi methanolic extract against Microsporum canis, agar well diffusion assay method was used. The methanolic extract was placed into the hole of the agar containing the sensitized fungi. The result of the assay revealed enough zone of inhibition to conclude that there is a positive fungal effect of the methanolic extract against Microsporum canis.

Keywords: *Averrhoa bilimbi, Microsporum canis, infection, agar diffusion, methanolic extract*

INTRODUCTION

For the development of health in mankind, the medicinal plants always play an important role. According to World Health Organization, more than 80% of world's population is reliant on medicinal plants to maintain their health and to cure their ailments.

Recent studies showed increasing prevalence of multi-drug resistant organisms as well as strains with reduced susceptibility to the available antibiotics prompted us for the search of new effective therapeutic agents from plants (Ghani, 2003).

One of the most important studies is to explore antimicrobial compound in the plants. Although many scientific studies have been conducted in order to unleash the mystery behind the medicinal plants, yet gaps remain which need to be completed. It seems that the antifungal activity of certain plant species has not been satisfactorily explored. There are about 100,000 species of fungus present in the

environment and more than 100 of them are pathogenic in humans.

Staples (2009) proved in his study that herbal medicines are efficient in treating diseases and infections caused by lots of microorganisms. These herbal medicine products are sold at the market at a reasonable price and yet, as effective as the chemical medicines, which may serve as the herbal medicine advantage over the chemical medicines.

Averrhoa bilimbi has its place to the family Oxalidiaceae. Some mutual name of A. bilimbi include Creole: bimbling plum, blimblin; English: bilimbi, cucumber tree, tree sorrel; Filipino: kamias; French: blimblim, blinblin, carambolierbilimbi. This is attractive, long-lived tropical tree, reaches 16 to 33 ft. (5-10 m) in height; has a short trunk soon isolating into a number of upright branches. A. Bilimbi is native of Moluccas in Indonesia. This plant is also found semi-wild throughout, Brazil, Cuba, Philippines, Sri Lanka, Bangladesh, Myanmar (Burma) and Malaysia. A. bilimbi is used as traditional medicine for treating cough, cold, itches, bmethanolics, rheumatism, syphilis, diabetes, whooping cough, and hypertension in Asia (Mackeen et al, 1997).

In the Philippines, the leaves of Kamias are applied as a paste or poulticed on itches, swellings of mumps and rheumatism, and on skin eruptions. Elsewhere, they are applied on bites of poisonous creatures (Orwa et al, 2009).

Phytochemical research in *Averrhoa bilimbi* verifies that the plant contains; alkaloid, tanin, saponins, flavonoids, cardiac glycosides, triterpenes, phenols, triterpenoid, citric acid, oxalic acid and D-3-hexanal but absence of phytosterols in different extractives. The presence of saponin, triterpenoid, alkaloid, oxalic acid and D-3-hexanal contributes to its anti-fungal agent, anti-microbial agent, anti-lipidemic agent and antioxidant (Polterait, 1997).

Averrhoa bilimbi contains high levels of oxalate. Acute renal failure due to tubular necrosis caused by oxalate has been recorded in several people who drank the concentrated juice on continuous days as treatment for hypercholesterolemia. These people were prompted into consuming this concoction by local media which played up studies done in experimental animals (Pushparah & Natesan, 2004).

Phenols such as phenolic acids, flavonoids and tannins are measured to be the major contributor to the antioxidant ability of plants.

These antioxidants of the plant also possess diverse biological activities, such as anti-carcinogenic, free radical scavenger, anti-atherosclerotic and anti-inflammatory, activities. These activities are related to their antioxidant capacity. Phenolic compounds contribute evidently to antioxidative action and they constitute the major class of natural antioxidants present in plants; therefore, it is necessary to calculate total phenolic content in plant species (Alamgir, et al, 2014)

The anti-fungal activity of the oils against *P. italicum* was attributed to citronellol, octanal, citral, decanal, nonanal, β-pinene, linalool, and γ-terpinene, whereas anti-fungal activity against *P. digitatum* is attributed to octanal, decanal, nonanal, limonene, citral, γ-terpinene, linalool, and α-terpineol (Zhou, 2014).

Microsporum canis is the dermatophyte most frequently recovered from canine and feline ringworm cases. The household environment can be contaminated both by symptomatic animals and through asymptomatic *M. canis* carriage, resulting in a potential human health risk. The load of *M. canis* arthrospores was determined in households harboring infected pets, in order to assess the infectivity of the animals versus the environment (Mancianti, Nardoni, Corazza, D'Achille, & Ponticelli, 2003).

Keratinolytic proteases secreted by dermatophytes are expected to be virulence-related factors. *Microsporum canis* is the leading agent of dermatophytosis in dogs and cats, causes a zoonosis that is regularly reported (Brouta, Descamps, Monod, Vermout, Losson & Mignon, 2002).

Mycotic scalp infection caused by *Microsporum canis* is one of the more unruly disorders, with increasing incidence during the last decade. It has been reported that administration of Itraconazole in 163 children (86 girls, 77 boys) with *M. canis* tinea capitis. In all children, there was both clinical and mycologic cure after a mean treatment period of 39 ± 12 days (range 10–77 days). Eleven children (6.7%) had side effects: diarrhea in five children, cutaneous eruption in four, and abdominal pain in two. Ketoconazole was effective and safe for the treatment of *M. canis* tinea capitis (Ginter et al, 2004). Tinea capitis is a fairly collective superficial fungal infection in children which entails oral antifungal therapy (Ginter et al, 2004)

The warm tropical temperature of the Philippines and its collaboration with cultural rehearses; occupation and immune alertness

contribute to the amplified susceptibility of the Filipinos to fungal infections. Filipinos are prone to *Microsporum spp.* and *Trichophyton spp*. infections (Handog & Dayrit, 2005).

The components of *Averrhoa bilimbi* leaves may have its possible antifungal effect on specific types of organisms. Thus, study was done to validate the claims that specific components of the plant may have an effect on *Microsporum canis.*

Objectives

The study seeks to determine the anti-fungal effect of *Averrhoa bilimbi* leaves methanolic extract against *Microsporum canis* that causes the tinea capitis. It aims to specifically compare the zone of inhibition zone of 95% Averrhoa bilimibi leaves methanol extract with 2mg/g 2% Ketoconazole Cream (positive control) on the test organism.

METHOD

The in vitro study used an experimental approach to control the variables that could have an effect on the results of the study. Observation on the Inhibitory Zone in Diameters (IZD) was gathered.

The *Averrhoa bilimbi* leaves were collected from trees located at Makiling Calamba, Laguna and were verified at Museum of National History located at University of the Philippines Los Banos. It was classified as belonging to the Family Oxalidaceae with a common name, Kamias.

The *Averrhoa bilimbi* leaves were endorsed to University of the Philippines Los Banos Laboratory for extraction. The researchers collected 100 grams of *Averrhoa bilimbi* leaves and were dried under shade, pulverized by a mechanical grinder sieve no. 40. The powder (100g) of the plant was separately extracted with 95% ethanol (300 ml) at room temperature for 7 days with occasional shaking as well as with a Soxhlet apparatus. All the extractives were filtered off through filter paper and the filtrates were concentrated with a rotary evaporator at reduced temperature and pressure to yield 50g extract of 95% *Averrhoa bilimbi*.

The stock culture of *Microsporum canis* were bought at Department of Science and Technology Environment and Biotechnology Division. The organism was sub-cultured in Sabouraud Dextrose Agar at 28°C for 5 days (Nweze, 2010).

Following growth, conidia were harvested in 0.85% NSS and the turbidity are compared to 0.5 McFarland Solution. Mueller-Hinton Agar plates were streaked evenly with a sterile swab dipped in the standardized inoculum suspension (Nweze, 2010). Using a sterile yellow tip, three holes were made for agar well diffusion. Using a sterile syringe, the first well were filled with 95% *Averrhoa bilimbi* leaves methanolic extract , the second were filled with 2mg/g 2% Ketoconazole cream for positive control and the third well were filled with distilled water for negative control. The plates were incubated at 28°C for 3 days.

The experimental results were expressed as mean ± standard deviation (SD).The inhibitory zone in diameter (IZD) of the 95% *Averrhoa bilimbi* leaves methanolic extract was compared to the IZD of 2mg/g 2% Ketoconazole Cream using the T-test.

RESULTS AND DISCUSSION

Table 1 shows the standard values for comparison of collected measurements. The 20mg/g 2% Ketoconazole is the positive control of the study. An average inhibition that shows ≥20mm is considered to be susceptible. 15-19mm of inhibition is considered as intermediate susceptible. ≤14mm of inhibition is considered as resistant.

Table 1: Interpretative standard of Inhibitory Zone in Diameters (IZD) of Ketoconazole (positive control) against *Microsporum canis*

Antifungal agent	Susceptible	Intermediate Susceptible	Resistant
20mg/g 2% Ketoconazole	≥20mm	15-19mm	≤14mm

Source: CLSI

In table 2, 95% *Averrhoa bilimbi* leaves methanolic extract shows an average inhibition of 18mm which means it is intermediate susceptible, 20mg/g 2% Ketoconazole Cream(positive control) shows an average inhibition of 20mm which means it is susceptible and Distilled water (negative control) shows no inhibition which means it is resistant.

Table 2. Zone of inhibition of experimental material and the control

Samples	Trial 1	Trial 2	Trial 3	Mean IZD±SD	Interpretation
95% *Averrhoa bilimbi* leaves methanolic extract	20 mm	16mm	18mm	18mm±1.1547	Intermediate Susceptible
20mg/g 2% Ketoconazole Cream (Positive control)	20mm	22mm	19mm	20mm±0.9122	Susceptible
Distilled water (negative control)	0mm	0mm	0mm	0mm±0	Resistant

Table 3 shows the comparison of the mean zone of inhibition of *Averrhoa bilimbi* leaves methanolic extract and the positive control, 2mg/g 2% Ketoconazole Cream. Results show no significant difference between the two variables. This implies that the experimental material, although it was not able to achieve the desired susceptibility was able to exhibit an equally same potency as the control variable.

Table 3. Comparison of 95% *Averrhoa bilimbi* leaves methanolic extract and 2mg/g 2% Ketoconazole Cream

	Mean	SD	T-value	Sig	Interpretation
95% *Averrhoa bilimbi* leaves methanolic extract	18.00	2.00	-1.257	.336	Not Significant
2mg/g 2% Ketoconazole Cream	20.33	1.53			

The *Averrhoa bilimbi* plants contain chemical constituents that

are proven in recent researches as an effective anti-fungal agent, anti-microbial agent, anti-lipidemic agent and antioxidant (Huda, 2009). Averrhoa bilimbi has triterpenoid group, such as triterpene saponins, together with steroidal saponins, were also isolated as antifungal constituents from medicinal plants (Abad, Ansuategui, & Bermejo, 2007).

Studies has been done in recent years on the antifungal activity of terpenoids of natural origin. These reports concern mainly sesquiterpenes and sesquiterpene lactones. Some of these compounds were isolated by bioassay-guided fractionation, after previously detecting antifungal activity on the part of the plant. Reports of antifungal alkaloids from medicinal species have also been found in the literature. Some of these plants have been reported to be used in folk medicine as anti-infectious agents (Abad et al, 2007).

Many plants have been reported to contain antimicrobial agents. These compounds are the mainly responsible for the plant's therapeutic effect. The result of this study shows that the Kamias (*Averrhoa bilimbi*) may have the potential to inhibit *Microsporum canis*, a fungus that is classified as dermatophytes which causes tinea capitis. Kamias extract produce an average inhibition of 18mm which is concluded as intermediate susceptible against *Microsporum canis*.

CONCLUSION AND RECOMMENDATION

The researchers concluded that the 95% *Averrhoa bilimbi* leaves methanolic extract has enough potency to treat to tinea capitis as the experiment shows an intermediate susceptibility on the inhibition of growth of *Microsporum canis*. Kamias leaves compared to 2mg/g 2% Ketoconazole Cream shows an almost the same average of zone of inhibition. The 95% *Averrhoa bilimbi* leaves methanolic extract compared to 2mg/g 2% Ketoconazole Cream shows no significant difference in the Inhibitory Zone in Diameters (IZD). Thus, the 95% *Averrhoa bilimbi* leaves methanolic extract has a potential to be compared to the efficacy of the 2mg/g 2% Ketoconazole Cream.

The researchers recommend to find other plants with the same chemical constituents to Averrhoa bilimbi Linn and have them tested on *Microsporum canis.* *Averrhoa bilimbi* can also be tested against other organisms such as *Aspergillus* spp, *Tricophyton* spp.

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