Antibacterial Activity Against Staphylococcus aureus from Leaf Extracts of Talus (Homalomena philippinensis) and Boracan (Merremia peltata L. Merr.): Ethnobotanical Plants

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Abstract – Ethnobotany is the study of the relationship between man and plants. An Ati tribe located at Brgy. Nagpana, Barotac Viejo used plants from their vicinity against 23 ailments, such as boils. Boils is commonly caused by *Staphylococcus aureus.Homalomena philippinensis* (talus) and *Merremia peltata L. Merr.* (boracan) are the plants used by the tribe against boils. This study, specifically, aims to compare the antibacterial effects of talus, boracan and the antibiotic, Ampicillin, and determine the minimum concentration of both plant extracts that can kill Staphylococcus aureus. The plants were collected from the Brgy. Nagpana, Barotac Viejo and identified and confirmed by an expert from University of the Philippines - Visayas. The bacteria was obtained from the Clinical Laboratory of University of San Agustin, where the experiment was also conducted. In conclusion, Talus and Boracan crude extracts showed no antibacterial inhibitory effect against *Staphylococcus aureus.* Only Ampicillin showed antibacterial activity against Staphylococcus aureus. Talus and Boracan also showed no minimum inhibitory concentration that can kill *Staphylococcus aureus.*

Introduction. – Ethnobotany is defined as the study of the relationship between man and plants. The more inclusive way of defining ethnobotany is the study of the uses, technological manipulation, classification, agricultural systems, magico-religious concepts, conservation techniques and general economic and sociological importance of plants in primitive or preliterate societies. The first ethnobotanist is one of our ancestors. It started when they started classifying plants; those used for alleviating pain or improving illness; and those used for poisoning. It was not long after man used plants to cure illness, and these men were called shamans[1].

Using medicinal plants to cure illness is also part of the culture in the Philippines. The Philippines is one of the megadiverse countries in the world, according to the 2014 Philippines Fifth National Report in the convention on Biological Diversity[2].Being abundant with natural resources, early folk utilized plants for medical purposes. In fact one of the early ethnobotanical projects in the Philippines documented that at least 1,297 plants have ethnomedical values[3] originally cited from Tan (1980).

In Panay region, a study conducted by Madulid et al. [3] documented 46 medicinal plants that were used by the Ati tribe at Nagpana, Barotac Viejo, Iloilo. The documented medical plants were used by the tribe to cure or relieve the pain of 23 ailments. One of the 23 ailments that are listed is boils.

A boil is a skin infection that starts in a hair follicle or oil gland. It can form anywhere in the body, but they are most commonly found in the face, neck, armpits, shoulders, back and buttocks. Areas that are hairy, sweaty and experiences friction are common sites where boils appear. Staphylococcus aureus is the bacteria that usually causes boils. Even a perfectly healthy man carries this bacteria on their skin without noticing it. This germ enter the skin through tiny cracks, cut, or scrapes. When not treated, this may turn into a more serious infection called a carbuncle [4].

Utilizing the medical plants that can be found within their vicinity, Ati tribe came up with their own solution

to tackle the boil infection. Two medicinal plants namely talus (*Homalomena philippinensis*), and boracan (*Merremia peltata L. Merr.*) are used as solutions for the stated ailment. The part used in talus and boracan are the leaves and applied to the afflicted area[3].

Thus, this study aims to confirm the practices of the Ati tribe at Nagpana, Barotac Viejo, Iloilo through an antibacterial test. At the same time, it aims to identify the minimal inhibitory concentration of the plants that they used.

Methods. – This chapter outlines the experimental design in the procedures that were done, starting from plant collection until the disposal of materials.

Research Design. In this study, the complete block design was used. For the disk diffusion assay, there were 10 replicates. In each plate, there will be two treatments, one positive control (antibiotic ampicillin) and one negative control (distilled water). For the minimal inhibitory concentration assay, the first 10 test tubes will contain the different concentrations of the plant extracts and the last 3 test tubes will contain the positive control, the negative control and the media control.

Materials. This study made use of the following materials: *Staphylococcus aureus*, Talus (*Homalomena philippinensis*), Boracan (*Merremia peltata L. Merr.*), petri dishes, media bottles, Mueller-Hinton broth and agar, beakers, metal spatula, culture tubes, volumetric flasks, filter paper, inoculating loop, forceps, caliper, vortex, autoclave, hot plate, incubator, refrigerator, Biosafety Cabinet, alcohol lamp, McFarland 0.5 Standard Solution, anhydrous ampicillin, sodium chloride, distilled water and proper lab wear.

Procedure. The methodology used for the antibacterial testing, from the preparations to the assays, was adapted from Quinto and Santos [5].

Talus (*Homalomena philippinensis*), and Boracan (*M. peltata L. Merr.*) were gathered from Brgy. Nagpana, Barotac Viejo, Iloilo. Plant taxonomy and verification was confirmed by an expert from the University of the Philippines - Visayas. The *S. aureus* pure culture was obtained from the Clinical Laboratory of University of San Agustin.

Preparation of Materials. All materials needed for the antibacterial assays were washed with distilled water and placed inside an autoclave at 121C and 15 psi for 15 minutes to exterminate any microbial contamination such as bacteria, fungi and viruses that might affect the experiment.

The extracts of the Talus plant and the Boracan plant were prepared by washing and crushing the leaves. The extracts were then stored in the cold, at temperatures between 0-5C.

The Mueller-Hinton broth was prepared by suspending 10.5 grams of the medium in 500 mL of distilled water. The mixture was then mixed and boiled over a hot plate then autoclaved for 15 minutes with a setting of 121C and 15 psi.

The Mueller-Hinton agar was prepared in a media bottle by suspending 20 grams of the medium in 500 mL of distilled water. The mixture was then boiled over a hot plate, autoclaved for 15 minutes with a setting of 121C and 15 psi. The cooled agar was poured into sterile petri dishes on a level, horizontal surface to give uniform depth.

Three loopfuls of the bacterial test organism was inoculated using an inoculating loop into 15 mL of Mueller-Hinton broth. The concentration was adjusted corresponding to the 0.5 McFarland standard. 0.1 mL of the 0.5 McFarland standardized inoculum was diluted further to a final volume of 15.0 mL with Mueller Hinton broth.

Anhydrous ampicillin (0.10 g) was weighed and placed into a sterilized 150 mL beaker using of a sterilized metal spatula. 70.0 mL of sterile distilled water was added, then the contents were gently swirled to completely dissolve the solute. The dissolved contents were transferred to a 100 mL volumetric flask and about 30 mL of the sterile water was added to rinse the beaker. The contents were again swirled. The total rinsing was transferred to fill the volumetric flask to its 100 mL mark. The stock solution was stored in a media bottle. When used as an antibiotic standard, 10 mL of the stock solution was taken and diluted to a final volume of 100 mL with sterile isotonic saline.

Disk Diffusion Assay. Approximately 15 mL of Mueller-Hinton agar was poured into dry and sterile petri dishes. The medium was left to solidify for an hour. One loopful of bacteria was taken from the pure culture then streaked over the entire surface of the agar plate evenly. A pair of forceps was flame-sterilized. Using the forceps, a Whatman # 1 filter paper disc was picked up and immersed into the extracts for one hour. The bacteria was inoculated using a inoculating loop. After about 2 minutes, the moistened filter disc was laid gently on the seeded agar plate. The plates were inverted then incubated for approximately 18 hours.

Minimal Inhibitory Concentration. Thirteen screw capped 13 mm x 100 mm test tubes were sterilized and numbered accordingly. Using a 1.0 mL serological pipette, 1.0 mL of Mueller-Hinton broth was introduced into the 2nd to the 11th tube. For the 12th tube, 2.0

mL of Mueller-Hinton broth was introduced. Two mL of the prepared plant extract was pipetted into the first and second tubes. The second tube was vortexed for five seconds. Using a sterile 1.0 mL serological pipette, 1.0 mL of the contents of the second tube was aseptically withdrawn and transferred to the third tube, which was vortexed afterwards. The same process was continued until 1.0 mL has been withdrawn from the ninth tube and subsequently added to the tenth tube. The contents of the tenth tube was vortexed. From the tenth tube, 1.0 mL was pipetted off the contents and it will be discarded.

One mL of the diluted bacterial inoculum was introduced into the tubes 1 to 11 and tube 13. In the 13th tube, 1.0 mL of the antibiotic standard was introduced. All tubes was tightly capped then the contents was vortexed. The tubes were incubated at 35C for 16-18 hours. After the incubation period, the tubes were examined for bacterial growth by checking the turbidity in the tube. The tube with the lowest concentration of plant extract at which no growth or turbidity is observed was reported as the minimal inhibitory concentration (MIC) of the plant extract against S. aureus.

The MIC Assay was considered valid since the negative control tube has visible growth, the media control tube has no visible growth and the positive control tube has no visible growth.

Disposal of materials. Proper waste disposal methods were followed for all materials. All used agar and broth cultures were decontaminated immediately after the conduct of the experiment by mixing in hypochlorite for approximately an hour inside the biosafety cabinet. All of the spent agar media and broth were disposed of in the hazardous wastes bin. All glassware, including the culture tubes and dishes cleaned using antibacterial soap.

Statistical Tools. The data from the disk diffusion assay was analyzed using One-Way ANOVA of the zones of inhibition with a 0.05 margin for error. Post HOC Least Significant Difference (LSD) was used to analyze the significance between each treatments.

Results. – The main objective of this study is to conduct antibacterial tests to verify the effectiveness of medicinal plants used by the Ati tribe at Nagpana, Barotac Viejo, Iloilo in treating boils. This chapter contains the findings of the test for antimicrobial property, findings for the Minimum Inhibitory Concentration Assay and the implications of these findings.

Talus extracts were green then turned yellow after filtration. The extract has a low viscosity. Boracan extracts were brown before and after filtration. The extracts were highly viscous.

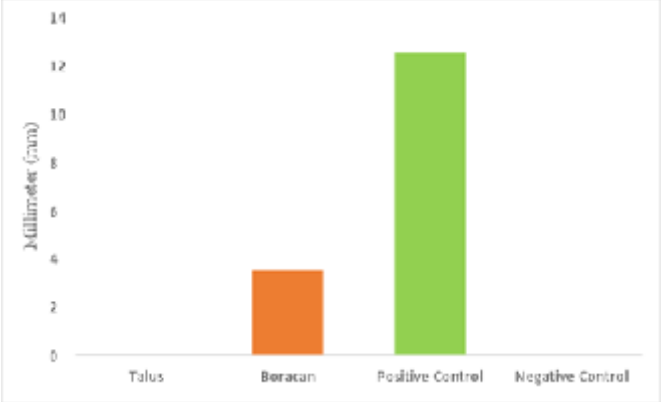

Fig. 1: Mean Zone of Inhibition of All the Treatments.

Antimicrobial Property. One antibacterial test that was conducted in this study was the disk diffusion method. This method measures the antibiotic resistance of the extracts through the zone of inhibition formed around the filter paper disks[6].

See Fig. 1. The graph shows that the positive control (ampicillin) has the largest zone of inhibition with a mean of 12.5 mm compared to the talus, boracan, and negative control (water). Talus extracts together with the negative control didnt show any zone of inhibition against S. aureus.

After using One-Way ANOVA to compare the means of the four treatments the P-value is 0.027. The P-value is less than the alpha value which is 0.05 therefore there is a significant difference between the means of the four treatments.

To further compare the means of each treatment LSD Post Hoc Tests was conducted. This test revealed which treatments have a significant differences from each other. Comparing the positive control with the treatment of talus extracts gave a significant value of 0.010 which is less than the alpha value of 0.050, therefore there is a significant difference between the two. Comparing the positive control with the treatment of boracan extracts gave a significant value of 0.029 which is also less than the alpha value, therefore there is also a significant difference between the positive control and the boracan extracts. Lastly, there are no significant differences between the talus extracts, boracan extracts and negative control. Therefore, it can be inferred that the two plant extracts are not as effective as the positive control.

The disk diffusion method used in this study. In a study by Lehtopolku et. al [7], they tested the inaccuracy of the disk diffusion method when compared to the agar diffusion method when testing for antimicrobial suscepti-

Table 1: Turbidity of Tubes.

Concentration %	Talus Extracts	Boracan Extracts
100	+	+
50	+	+
25	+	+
12.5	+	+
6.25	+	+
3.125	+	+
1.5625	+	+
0.718	+	+
0.391	+	+
0.195	+	+
Negative Control	+	+
Media Control	-	-
Positive Contol	-	-

Legend: + indicates turbidity; - indicates no turbidity.

bility. Their results show that the disk diffusion method is not a reliable tool for the testing of antimicrobial susceptibility against Campylobacter spp. It has been also recommended in their study that further studies are needed to assess whether the disk diffusion test method could be improved [7].

In this study, the crude extracts of the plants were used, however it seemed to be less effective in comparison to ampicillin in the disk diffusion assay. It would be better if a solvent, such as ethanol and methanol, was used to extract the plants. More nonpolar solvents are most likely to extract the antimicrobial compounds of a plant [8].

The results in this study also show the mild resistance of *S. aureus* against ampicillin. In a study by Gentillini et al [9], a total of 206 *S. aureus* strains isolated from bovine mastitis in Argentina were investigated for susceptibility against several antimicrobial agents. No resistance was detected against ampicillin-sublactam. However, in a study by Braga et al [10], the post antibiotic effect of ampicillin against *S. aureus* lasted for around two to three hours. Some *S. aureus* strains may have developed a resistance against the antibiotic ampicillin.

Minimum Inhibitory Concentration Assay. See Table 1. The percent concentration decreased by half due to the serial diffusion method. All of the tubes containing plant extracts in the Minimum Inhibitory Concentration Assay contained precipitates which gradually decreases as the amount of plant extracts decreases. For the tubes containing talus extracts, yellow precipitates were present while the tubes containing boracan extracts had presented green precipitates. The same amount of bacterial inoculum was placed in the said tubes. Before incubation, all tubes were vortexed, therefore any present precipitates were not clearly visible. During the incubation time of 18 hours, the precipitates of the plant crude extracts might have settled at the bottom of the tube. It can be assumed that the precipitates in tubes containing plant extracts are plant precipitates since crude extracts were used.

Conclusion. – Based on the current findings, the following conclusions were drawn: (1) Talus and Boracan crude extracts showed no antibacterial inhibitory effect against *Staphylococcus aureus*. Only Ampicillin showed antibacterial activity against Staphylococcus aureus. (2) Talus and Boracan showed no minimum inhibitory concentration that can kill *Staphylococcus aureus*.

Recommendations. – The researchers in the study recommend that the plants used in the study be tested for their anti-inflammatory activity, since inflammations are also present in boils. It is also recommended that extraction methods using solvents, especially the nonpolar, such as ethanol and methanol be used in similar studies testing the antibacterial properties of Talus and Boracan as it may ensure the extraction of antibacterial components.

The agar well method could also be used to determine the zone of inhibition since, in this method, the antimicrobial agents diffuse in the agar medium and inhibits the growth of the microbial strain tested. This method also lacks the intervention of a paper disc, which may be a source of other microorganisms if not properly sterilized. It is also recommended that the minimum bactericidal concentration assay be performed to confirm the presence of bacterial colonies in tubes since turbidity can be caused by other factors such as the presence of plant precipitates. For a direct quantification of antimicrobial susceptibility of microorganisms, the Epsilometer test can be used in order to detect low levels of resistance. Other common antibiotics that are used for treating S. aureus infections can be used as the positive control for more comparison. Lastly, other microorganisms that cause boils can be used in similar studies that will test the antibacterial properties of Talus and Boracan.

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