ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT FROM BANANA (*MUSA ACUMINATE*) PEELS AGAINST *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Philippines possesses a rich source of banana plants enough to provide us with its many uses. The banana peels have been used for their property of producing a substantial amount of ethanol; however, their efficacies against Staphylococcus aureus have not been studied. The present investigation involved the study of antimicrobial activity of ethanol from banana (Musa acuminate) peels against S. aureus. In the present study, banana peel waste were subjected into two phases – ethanol production and anti-microbial susceptibility testing. In producing ethanol, Musa acuminate peels were gathered and underwent pre-treatment before going through fermentation. Two samples were conditioned at a temperature of 30 °C and the pH value was set to 5.0–5.5. The samples underwent fermentation process for seven days and three days, respectively, by the culture of Saccharomyces cerevisiae. The ethanol product from the banana peels was distillated using a distillation apparatus and two samples of 10mL and 8mL ethanol were extracted. The ethanol extract of Musa acuminate was investigated for antimicrobial activity at 100 µg/ml concentrations by using disc diffusion method against gram positive Staphylococcus aureus. After the 24- hour incubation, the zone of inhibition was compared with standard antibiotics ciproflaxin (100 µg/ mL) for comparing the results obtained with. Against the first sample of ethanol extract, Staphylococcus aureus showed a higher inhibition zone of 9 mm, whereas for the second sample, Staphylococcus aureus showed a lesser inhibition zone of about 5 mm. It was then observed that ethanol extract of Musa acuminate was potent against the S. aureus bacteria.

Keyword: *Ethanol, Banana peel, Musa acuminate, Staphylococcus aureus, Antimicrobial effect*

INTRODUCTION

Musa acuminate, known as Lacatan in the Philippines, is one of the most common banana cultivars belonging to *Musaceae* family. Being one of the most popular tropical fruits distributed all over the world, banana is grown in 122 countries worldwide. It also has a great medicinal relevance that dates way back to the ancient time and is indicated as medicinal containing an antibacterial principle that has big benefits to the society and mankind. It has a lot of healthy uses in human life. It can be used to keep cholesterol level low because it is rich in fiber that can prevent heart diseases such as heart attack and stroke. It is also rich in lutein that helps protect vision. It also plays a role in preventing cataracts and macular degeneration. (Hautea et. al, 2002)

On the other hand, the banana peel which is the skin of the banana fruit is usually stripped off and discarded. Many people find the skin of the banana useless but there are various researches supporting the usefulness of banana peel in many aspects. According to Morton (1987), banana fruit peels have been a valuable source for maintaining human health. The peel of a banana can be rubbed on mosquito bites which will help the stinging sensation to stop and the swelling to reduce. Moreover, the peel and pulp of bananas have antifungal and antibiotic properties which protect the plant against fungal and bacterial infections. The use of fruit peels extracts for antimicrobial properties can be of great significance in therapeutic treatments. Banana peel waste's extracts could be potential antimicrobial alternatives and may be effective to utilize as a natural source of antimicrobial agent in pharmaceutical industries.

Ethanol or ethyl alcohol has existed since the beginning of recorded history. The ancient Egyptians produced alcohol by naturally fermenting vegetative materials. In ancient times, the Chinese discovered the art of distillation, which increases the concentration of alcohol in fermented solutions (Otulugbu, 2012). Alcohols are useful disinfectants as they evaporate quickly without leaving excess. They are effective on dissolving lipids which makes them functional against lipid-wrapped viral cells such as HIV and hepatitis A. As a disinfectant, alcohol functions by denaturing proteins and diffusing membranes which can effectively kill many types of bacterial and viral cells. It is typically used in concentrations of 70 percent for the reason that higher

concentrations evaporate rapidly and lower concentrations are not fully effective (Health E, n.d.).

According to UCSB Science Line, ethanol kills bacteria in mainly two schemes: protein denaturation and dissociating the cellular membrane. In protein denaturation, protein must defuse in water for it to function properly. If protein is placed in an ethanol, it cannot function properly and may denature. In the disassociation of cellular membrane, ethanol kills the bacteria by dissolving its cellular membrane. When an ethyl had been applied to the bacteria cell membrane, it will react with the ethanol causing this membrane to fall apart.

Staphylococcus is a group of bacteria that can cause a number of infectious diseases in various body tissues. Diseases from staph are due to direct infection or due to the production of toxins by the bacteria (Stoppler, 2017). One type of staph bacteria called *Staphylococcus aureus* has long been recognized as one of the most important bacteria that cause disease in humans. It stains Gram positive and is non-moving small round shaped or non-motile cocci.

Staphylococcus aureus is the most dangerous of all many staphylococcal bacteria mainly because this kind of bacteria can be easily spread by having direct contact with an infected person, by using a contaminated object, or by inhaling infected droplets dispersed by sneezing or coughing (Bush & Schmidt, ND). Staph bacteria often spread through skin-to-skin contact — the bacteria can be spread from one area of the body to another if someone touches the infected area.

To further study alternative medicine in replacement of the antibiotics that *S. aureus* has grown resistant to, various researchers studied different plants and substances that can potentially kill *S. aureus*. Various compounds found in plants have been investigated on its antibacterial potential against these bacteria. Curcumin, the major constituent of turmeric, is found to be effective against *Staphylococcus aureus*. Hence, this natural product derived from plant is believed to have profound medicinal benefits and could be potentially developed into a naturally derived antibiotic in the future. (Sin-Yeang et. al, 2016)

Moreover, antimicrobial activity of the ethanolic extract of propolis, a compound produced by bees, were found to be effective *Staphylococcus aureu*s as well. It was found that higher temperature and acidic pH enhanced the antibacterial activity of the ethanolic extract of propolis. (Li et. al, 2002)

In modern days, bacteria strains are slowly becoming resistant to drugs and they will pass this mutation to their offspring that will have full resistance to drugs. This has been a problem for the doctors as it becomes harder to control. In a study in the United States, the estimated number of *S. aureus*–related hospitalizations from 1999 through 2005 increased 62%, from 294,570 to 477,927and the estimated number of methicillin-resistant *S. aureus*or MRSA-related hospitalizations more than doubled, from 127,036 to 278,203(Klein et. al, 2007). The current problem associated with emerging bacteria strains presents a serious global medical crisis, requiring constant surveillance, which continuously challenges the scientific community. The diminishing efficacy and increasing toxicity of synthetic drugs further aggravate this problem; thus, scientists are directed to seek more natural or organic materials for solutions.

Traditional medicine has been practiced worldwide for centuries, particularly the application of plants for therapeutic purposes. Philippines possesses a rich source of banana plants enough to provide us with its many uses, even in alternative remedies. The banana peels have also been used for their property of producing a substantial amount of ethanol which is deemed as potential source of bio-fuel (Bhatia et. al, 2010 & Gebregergs et. al, 2016). However, the efficacies of banana peel ethanol against *Staphylococcus aureus* are not yet studied. In developing countries, it is imperative that effective but less expensive antibacterials should be developed to accommodate all patients, regardless of financial status, in order to eliminate some of the human factors that can cause drug resistant bacteria.

In recent years, there are increasing published reports showing successful antimicrobial activities of various traditional medicinal plants against multiple drug resistant bacteria. In a study by Andrade et.al (2015), favorable antagonistic activities against various MDR bacteria were exhibited by the ethanol extracts of four Philippine medicinal plants, namely, P. betle, P. guajava, P. niruri and E. microphylla. Banana plants are also reported to have successfully shown antimicrobial activities. Ighodaro (2012) studied the antibacterial activity of banana peel extract (*M. paradisiaca*) against human pathogenic bacteria and he found that its extract showed inhibition against *S. aureus*, *Escherichia coli*, and *Proteus mirabilis*. Chabuck *et al.* (2013) studied antimicrobial activity of aqueous banana peel extract on clinical isolates of two Gram-positive (*S. aureus* and *Streptococcus pyogenes*), four Gram-negative (*Enterobacter aerogenes*, *Klebsiella pneumoniae*, *E. coli*, and *Moraxella catarrhalis*), and one yeast (*Candida albicans*). Banana extract showed highest antibacterial activity against both *M. catarrhalis* and *S. aureus* followed by *S. pyogenes*, *E. aerogenes*, and *K. pneumoniae* and no effect against *E. coli* and *C. albicans*.

As previously mentioned, banana peels has known antimicrobial properties, but the efficacies of its ethanol extract against *Staphylococcus aerus* have not been well-documented in the local medical literature or in other countries. In this regard, this research paper aims to (1) investigate the antimicrobial activities of ethanol from banana peelings against *Staphylococcus aureus* isolated from a tertiary medical center in the Philippines, (2) determine if the banana peel can produce ethanol after fermentation and distillation, and (3) examine if *S. aureus* is susceptible to the ethanol extracted from the banana peels. Specifically, the paper aims to answer the following questions: (1) What is the effect of ethanol extract from banana peelings to the bacteria Staphylococcus aureus? (2) What is the antibacterial potency of the ethanol

against the bacteria? (3) Is there an inhibition of bacterial growth after the treatment?

METHOD

The study was conducted to determine the anti-microbial activity of *Musa acuminate* ethanol extracts on *Staphylococcus aureus*. Specifically, the study wants to identify the highest antimicrobial activity and the concentration that has the highest mortality rate.

The design that researchers used in conducting this research paper is experimental wherein researchers can manipulate and control their tests to understand casual processes more. Researchers used this to know the antimicrobial activity of ethanol extract from banana peels against *Staphylococcus aureus* and to find out the answers to the research questions. The procedures of the study enabled the researchers to maintain control over all factors that may affect the result of the experiment.

The overall experimentation process of the research was conducted in two phases. First was the ethanol extraction wherein *Musa acuminate* peels were gathered, fermented, and distilled. The extracted ethanol samples then went through the anti-microbial susceptibility testing wherein the ethanol was tested against the *Staphylococcus aureus* bacteria.

In the evaluation of the anti-microbial activity of the different concentrations, the presence of inhibition zones on the petri dishes containing *Staphylococcus aureus* treated with different samples of ethanol were considered.

Production of Ethanol from Banana Peels

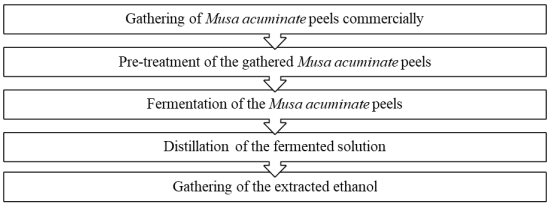

Figure 1: Ethanol Extraction

In the ethanol extraction process, different materials and methods were used in producing ethanol from *Musa acuminate*. The used procedures came from the studies researched by the proponents of this study and were later modified.

Gathering of banana peels

The first batch of *Musa acuminate* peels were obtained commercially. Experts from the National Museum of the Philippines were consulted for the proper identification of the plant sample. The first sample of collected banana peels was dried for approximately one (1) week. It was heated in a convection oven at 385°F for 15 minutes. The banana peels were ground into powder using a grinder. A total of 30g of powder from the first sample were obtained. The sample powder was boiled with distilled water for 20 minutes at the ratio of 30 grams: 240mL. Meanwhile, the 24 grams of baker's yeast (*Saccharomyces cerevisiae*) was added to boiling water then cooled down to room temperature. The proportion used was 24 grams: 240mL of water. Both solutions were mixed then poured in a sealed plastic container.

The next batch of *Musa acuminate* peels were sun dried for approximately three (3) days. It was also heated in a convection oven at 385°F for 15 minutes. The banana peels were ground into powder using a grinder. A total of 50g of powder from the second sample were obtained. The sample powder was boiled with distilled water for 20 minutes at the ratio of 50 grams: 400mL. Meanwhile, the 40 grams of baker's yeast (Saccharomyces cerevisiae) was added to boiling water then cooled down to room temperature. The proportion used was 40 grams: 400mL of water. Both solutions were mixed then poured in a sealed plastic container.

Fermentation

The two samples were conditioned at a temperature of 30 °C and the pH value was set to 5.0–5.5. The first sample was left to ferment for seven days. The second sample was left to ferment for three days. It was stirred every few hours to preserve the homogenous mixture.

Distillation

The distillation apparatus was set up in the biology laboratory of Colegio de San Juan de Letran for the fractional distillation. This process is the separation of a liquid mixture into fractions differing in boiling point (and hence chemical composition) by means of distillation. The liquid mixture is our fermented sample and the fraction we wanted to be separated is the ethanol.

For the first trial, the researchers used an alcohol lamp as source of heat. The thermometer inside the distillation flask used was submerged in the solution. The researchers guarded the thermometer at 78.37 °C since the said temperature is the boiling point of ethanol. The researchers distilled several hours for the first three days. However, no ethanol was produced.

After three days of failed ethanol extraction from the first sample, fractional distillation errors were corrected. The thermometer was placed higher up and the researchers used hot plate as the source of heat. The first sample was distilled once more after six days of fermentation. The second sample fermented for three days was distilled following the correction of previous laboratory errors.

Antimicrobial Susceptibility Testing

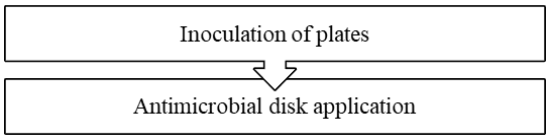

Figure 2: Agar Disk Diffusion Method

Agar disk diffusion method is a convenient, efficient and low-cost method in antimicrobial testing. It is the most widely used method for determining antimicrobial resistance.

Inoculation of plates

The antimicrobial testing was held at Metropolitan Medical Center. It was conducted by the proponents of this research paper with assistance from a pathologist, Dr. Grig Misiona, MD. Two agar medium plates and the *Staphylococcus aureus* bacteria were provided by the hospital.

The antimicrobial susceptibility testing that was done started with the inoculation of the agar plates. A sterile cotton swab was dipped into the *Staphylococcus aureus* bacteria suspension, then the excess inoculum was removed by lightly pressing the swab against the wall of the test tube. The *Staphylococcus aureus* bacteria was spread by streaking the cotton swab onto the agar medium, plates were rotated to ensure that there is an even distribution of the inoculum in both dishes. The surface medium was dried for three minutes to allow absorption of excess moisture.

Antimicrobial disk application (Kirby-Bauer Test)

The ethanol extract obtained was tested for the antimicrobial activity against one bacterial strain, the *Staphylococcus aureus*. These strains were clinical isolates from human beings and collected from the Metropolitan Medical Center in Manila. The ethanol then underwent disk diffusion method. Ciprofloxacin was taken as a standard compound for comparing the results obtained with. Two sets (100 µg/ml) each of ethanol extract and ciprofloxacin were prepared. Sterile nutrient agar plates were prepared and incubated at 37° for 24 h to check for any sort of contamination. Two sterile filter paper discs were soaked in two sample of ethanol extract and placed in appropriate position of the surface of the flooded plate, marked as quadrants at the back of the Petri dishes. The Petri dishes were incubated at 37° for 24 h and the diameter of zones of inhibition use measured in mm. Similar procedure was adopted for the pure ciprofloxacin and the corresponding zone diameters were compared accordingly.

RESULTS

Ethanol Production

The result of the first phase of the experiment which is the ethanol production showed that the fermented banana peels produced a significant amount of ethanol. The volumetric production of ethanol was varied into two samples according to the variations in water concentration and yeast concentration. It was also varied according to fermentation time.

Approximately 10mL of ethanol were successfully extracted from the first sample that was fermented for approximately 7 days. For the second sample that was fermented for only 3 days, it was only able to produce 8mL of ethanol.

This paper notes that Erlenmeyer flasks were used in measuring the volumes of the ethanol produced, resulting to mere approximate values presented as data.

Table.1 Percentage of the ethanol produced from banana peels at varying concentrations and fermentation time.

Sample	Banana Peels (g): Water (mL)	Yeast (g): Water (mL)	Fermentation duration (days)	Ethanol Produced (mL)
1	50 g: 400 mL	40 g: 400 mL	7 days	**10 mL**
2	30 g: 240 mL	24 g: 240 mL	3 days	**8 mL**

Disk Diffusion Method

The disk diffusion method for antimicrobial susceptibility testing was initially performed to determine the antimicrobial activities of the ethanol from banana peels against *S. aureus*. Using the zone of inhibition, one can read, measure and analyze data from the antimicrobial susceptibility testing that was done. The zone of inhibition is the point at which there is no growth of bacteria that can be seen by the unaided eye. Zone of inhibitions are circular shaped zones that surrounds the disk that was placed on the plates. Results can also be as Resistant (R), Intermediate (I), or Susceptible (S). Rulers are also used to measure data in agar disk diffusion. They measure the size of the inhibition by its diameter and round it up to the nearest millimeter.

The proponents of this research conducted a series of two trials in the disk diffusion method before successfully coming up with the third and final test. For the first trial, there were no zones of inhibition that appeared on the agar plate after incubation. The researchers suspected that the filter paper discs might not have been sterilized enough, resulting to the test showing that the ethanol has no inhibitory effects to the bacteria. For the second trial, there were still no zones of inhibition that appeared on the agar plate because of an error wherein the sterile filter paper discs that was soaked in the produced ethanol was placed on the agar plate while it was still wet. For the final trial, the researchers

corrected this error by allowing the disc to dry first. The researchers now observed proper impregnation of ethanol to the sterile filter-paper paper discs which resulted to the data below.

The result of zone of inhibition of the ethanol extract and its comparison with standard antibiotic ciprofloxacin (100 μg/ml) was recorded in Table 2. After the 24-hour incubation, results revealed that against the first sample of ethanol extract, *Staphylococcus aureus* showed a higher inhibition zone of about 9 mm, whereas for the second sample, *Staphylococcus aureus* showed a lesser inhibition zone of about 5 mm. From the results of zone of inhibition values and their competition to that of the standard ciprofloxacin, it is evident that the ethanol extract is active against the bacteria *Staphylococcus aureus.*

Table.2 Zones of inhibition produced by the ethanol extract and ciprofloxacin

Sample	Bacteria	Ethanol Extract (100μg/ml)	Ciproflaxacin (100μg/ml)
1	*S. aureus*	9 mm	25 mm
2	*S. aureus*	5 mm	22 mm

DISCUSSION

There are existing studies that have successfully evaluated the antimicrobial activities of different parts of diverse plants for the treatment of microbial infection. These are deemed as possible alternatives to synthetic drugs to which many infectious microorganisms have developed resistance (Igohadro, 2012). Banana peels contain secondary metabolites such as glycosides, alkaloids, saponins, volatile oil, flavonoids, and tannins. (Ehiowemwenguan et. al, 2014) Ehiowemwenguan suggested that the presence of glycosides and alkaloids in *Musa sapientum* peels may be attributed to it being used by traditional medicine practitioners in the treatment of some bacterial infections such as cough, fever, cold and venereal diseases. Moreover, it is also reported that flavonoids, which is present in banana peels, are responsible for the antimicrobial activity associated with some ethnomedicinal plants. (Singh and Bhat, 2003)

Although banana peel and its phytochemicals, minerals and nutrient components are well-documented, the antimicrobial activity of ethanol extracted from banana peels is not. With the aim to include the study of banana peel ethanol and its antimicrobial properties to the medical literature, this research paper's results suggest that ethanol from *Musa acuminate* peels showed antimicrobial activity against *Staphylococcus aureus*. It is shown that the bacteria is susceptible to the ethanol, evidenced by the inhibition of bacterial growth after testing.

Against the first sample of ethanol extract that fermented for seven days, *S. aureus* showed an inhibition zone of 9mm as compared with ciprofloxacin's 25 mm. Against the second sample that was fermented for three days, *S. aureus* showed an inhibition zone of 5 mm as compared with ciprofloxacin's 22 mm. However, this result should be interpreted with caution because the correlation of the ethanol's fermentation time with its potency against *S. aureus* is not within the scope of the study. Generally, this result suggested that ethanol extract of banana peel showed antibacterial activity against *S. aureus* bacteria.

The antimicrobial activity of banana peel against Gram- positive bacteria in our study was agreed with different studies. Ighodaro (2012) studied the antibacterial activity of *M. paradisiaca* peel extract against pathogenic bacteria and he found that the extract showed inhibition against *S. aureus*, *Escherichia coli*, and *Proteus mirabilis*. Chabuck *et al.* (2013) studied antimicrobial activity of aqueous banana peel extract on clinical isolates of *S. aureus* and *Streptococcus pyogenes (Gram-positive), Enterobacter aerogenes*, *Klebsiella pneumoniae*, *E. coli,* and *Moraxella catarrhalis* (Gram-negative), and *Candida albicans* (yeast). Banana extract showed highest antibacterial activity against both *M. catarrhalis* and *S. aureus* followed by *S. pyogenes*, *E. aerogenes*, and *K. pneumoniae* with no effect against *E. coli* and *C. albicans*.

In this study, some unavoidable limitations were present. First, because of the insufficient resources and the long duration of the fermentation and distillation processes, only two samples were produced. Second, the percentage of ethanol was not tested due to financial constraints and the failure to meet the standards of the testing center. Department of Science and Technology Chemistry Laboratory requires at least 100 mL of ethanol to be tested, whereas the ethanol we produced were 8mL and 10mL only. The only way the researchers determined they successfully extracted ethanol is through flame test.

The results obtained in this study suggest the use of banana peel as a potential component for an antibiotic alternative. This study also enlightens a new line of approach for further study of different types of banana peels against different pathogens. These results may encourage researches to be done on various clinical isolates of bacteria with different varieties of banana and its ethanol extracts.

CONCLUSION AND RECOMMENDATION

Musa acuminate peels is a good substrate for ethanol production due to the successful extraction of ethanol evident by the volumetric samples acquired from the experiment. Moreover, the produced ethanol from *Musa acuminate* peels is potent in inhibiting bacterial growth of *Staphylococcus aureus*, as evidenced by the results of the antimicrobial susceptibility testing. More data can be determined by further testing of the researchers who wish to extensively investigate on this matter.

For future studies, the researchers recommend performing the distillation correctly and concisely. Moreover, they suggest incorporating more variables for

the production of ethanol such as varying fermentation time, pH level, and temperature. They also recommend testing the ethanol content of the extract produced in order to provide substantial data that will further allow the comparison of the efficiency of the samples. Future researchers may also delve on other effects of banana peels such as production of alternative efficient biofuel that can contribute knowledge on the issue of oil crisis.

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