BERMUDA GRASS (CYNODON DACTYLON) AS AN ALTERNATIVE ANTIBACTERIAL AGENT AGAINST STAPHYLOCCOCUS AUREUS

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ABSTRACT

Antibacterial products have been found to have harmful chemicals like Triclosan. There is currently a need for alternative antibacterial agents to replace Triclosan in many antibacterial products like shampoo, toothpaste, and soaps. This experimental study, which utilized post-test only control group design, aimed to determine the effect of Bermuda grass (Cynodon dactylon) oil as an antibacterial agent that inhibits the growth of Staphylococcus aureus. Sensitivity disks testing was done to find out the efficacy of Bermuda grass oil extract against S. aureus. The application of water served as negative control and that of Amoxicillin solution as the positive control. With the use of t-test and mean, it was found that there is a significant difference in the measure of the area of the zone of inhibition between the negative control group (treated with water) and the experimental group. There is a significant difference in the measure of the area of the zone of inhibition between the positive control group (treated with amoxicillin) and the experimental group. Further, Bermuda grass oil has a positive result in inhibiting the growth of Staphylococcus aureus bacteria, though amoxicillin shows a greater positive effect on the said bacteria. This study would be of great benefit to pharmaceuticals, making Bermuda grass as a potential source of organic antibacterial substances.

Keywords: Cynodon dactylon, Staphylococcus aureus, antibacterial agent, zone of inhibition

INTRODUCTION

One of the most conventional medicines that are profusely used by people all over the world is antibiotics. The individual user or prescriber has little concern about the loss of effectiveness of these antibiotics as the resistance only affects the following patient. Antibiotic resistance is accelerated due to this combined characteristic, (Gelband et al., 2015). According to Van Boeckel et al. (2014), a 10-year period between 2000 and 2010 shows a 30 percent increase from approximately 50 billion to 70 billion standard units. Data were accumulated from 71 countries including the most highly populated countries.

Triclosan is an antibacterial component included in a variety of common home hygiene products. Triclosan includes two phenol functional groups, and its structure is similar to that of numerous anthropogenic estrogen as well as other possible estrogenic or androgenic endocrine disrupting compounds (Wang and Tian, 2015). In 2016, the United States of America ranked #1 in the world. The Food and Drug Administration (FDA) issued the final rule, Safety, and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use, stating that 19 specific ingredients, including triclosan, were no longer generally

recognized as safe and effective and were misbranded. The FDA's new guideline prohibits companies from advertising antibacterial soaps or washes that include one or more of the 19 chemicals mentioned above. The FDA's new guideline prohibits companies from marketing antibacterial soaps or washes that include one or more of these 19 compounds, which include triclosan (U.S. Environmental Protection Agency, 2009).

It was discovered that triclosan causes problems such as breast cancer progression (Lee et al., 2014), breast, ovarian, prostate, and testicular cancer, preterm and low birth weight babies, precocious puberty in girls and undescended testicles in boys, weakened heart muscle function, endocrine issues, and bone deformation. Triclosan has been linked to embryonic bone deformities in mice and rats, which may be due to hormonal influences. Triclosan may potentially interfere with cell signaling in the brain, heart, and other tissues (Davis UC Health, 2018).

Antibacterial items such as soaps, creams, and other medications have become an important part of people's lives in the Philippines, as in other nations. According to Kantar Stannard (2018) statistics, bath basics like shampoo, hair conditioner, and soap, in particular, dominate Pinoy consumers' shelves, with the country's humid environment cited as one driving reason. Expenditure on shampoo and hair conditioner increased by 6% and 7%, respectively, in 2016; bar soaps, which accounted for almost 94 percent of total bath soap sales, also saw a 7% increase in spending. Oral health treatment also increased by 7%.

In an attempt to find an alternative, the researchers were driven to tap the potential of naturally occurring substances found in plants like shrubs, herbs, and grasses. As stated by Yadav et al., (2012), medicinal plants play a very important role in developing alternative drugs to overcome the pitfalls and hazards possessed by synthetic drugs. Natural Home Remedies (n.d.) revealed some of the clinical remedies of Bermuda grass which include detoxifying the body, curing of eczema and scabies, treating anal itch and urinary tract infection, and reducing blood sugar levels.

Staphylococcus aureus is one of the five most common causes of hospital-acquired infections and is often the cause of postsurgical wound infections. It can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis (MedlinePlus, 2020).

With the desire to find a naturally organic-based alternative to fight bacteria, this investigated if Bermuda grass (*Cynodon dactylon)* can be used as an alternative antibacterial agent against *Staphylococcus aureus.*

OBJECTIVES OF THE STUDY

This study investigated the effects of Bermuda grass (*Cynodon dactylon)* oil to *Staphylococcus aureus*, especially its antibacterial property.

More specifically, it aimed to answer the following questions:

1. To measure the area of the zone of inhibition in every group after twenty-four (24) hours of intervention with:
   1.1. water
   1.2. amoxicillin solution
   1.3. Bermuda grass oil.
2. To determine if there is a significant difference in the measure of the area of the zone of inhibition between the negative control group (treated with water) and the experimental group (treated with Bermuda grass oil)?
3. To determine if there is a significant difference in the measure of the area of zone of inhibition between the positive control group (treated with amoxicillin) and the experimental group?

METHODOLOGY

This experimental study used Post-test Only Control Group Design. This design follows all the same steps as the classic pre-test/post-test design except that it omits the pre-test. There are many situations where a pre-test is impossible because the participants have already been

exposed to the treatment, or it would be too expensive or too time-consuming, (California State University, 2015).

Figure 2 shows the model of Post-test Only Control Group Design which was adopted in this research, where the positive control group and experimental control group were exposed to treatments, while the negative control group was only treated with water. Post-test was conducted through data gathering of the zone of inhibition after the intervention of the experimental group and control groups.

	(Post-test)
Experimental	O_E^1 O_E^2 O_E^3
Control Groups	O_N^1 O_N^2 O_N^3
	O_P^1 O_P^2 O_P^3

Figure 2. Model Design of the Study

The treatments indicated below were done in three (3) replications to identify the average zone of inhibition of subjects through measuring the diameter of the zone of inhibition in each treated disk.

NC – applied with water (negative control)
PC – applied with Amoxicillin solution (250mg/5 mL)
EC – applied with Bermuda grass oil

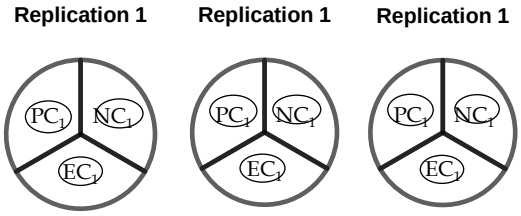

Figure 3. Experimental Layout of the Study

Figure 3 exhibits the experimental layout of the study, with the three (3) petri dishes of Mueller-Hinton Agar cultured with *Staphylococcus aureus* used in this study. All three (3) petri dishes were divided into three (3) divisions. Each petri dish was introduced with one (1) filter paper disk bathed in water as a negative control, one (1) filter paper disk bathed in amoxicillin solution as the positive control, and one (1) filter paper disk bathed in Bermuda grass oil as experimental control, a total of three (3) positive controls, three (3) negative controls, and three (3) experimental controls. The zones of inhibition were measured on the 24th hour after the intervention, thus there will only be one recording.

The subject of this study is the *Staphylococcus aureus*, a gram-positive coccal bacterium found mostly on skin diseases specifically in boils and it is still one of the five most common causes of postsurgical wound infections. This research has three (3) petri dishes of cultured *Staphylococcus aureus.* Data were interpreted through Mean and T-test. These statistical treatments functioned to quantify the results and establish relationships between variables.

RESULT AND DISCUSSION

1. Experimental Procedure

These scientific processes were followed accordingly during the conduct of the research:

1.1. Pre-intervention

Preparation of bacteria

The researchers asked for permission to utilize the medical laboratory of the Tagum Doctors Hospital. This was done for the cultivation of the *Staphylococcus aureus* bacteria, Sensitivity Testing, and the data gathering through measuring the diameter of the zones of inhibition of all control groups.

The preparation and culture of bacteria were done by the researchers with the assistance of the Registered Medical Technologists of the Tagum Doctors Hospital, Inc. The procedures were as follows:

a. Protective gears were properly used for safety purposes.
b. Materials needed such as an alcohol lamp, inoculating loop, and Blood Agar plate was prepared.
c. The inoculating loop was heated for sterilization and was allowed to stand for several seconds.

d. The pure culture of *Staphylococcus aureus* was prepared. The opening of the test tube was heated after opening the lid.
e. The sterilized inoculating loop was carefully inserted inside the test tube but not unto the medium of the organism. The surface of the medium of the bacteria was slightly touched.
f. The opening of the test tube was heated and was covered afterward.
g. With the same inoculating loop, a vertical line on the plate was gently streaked.
h. The inoculating loop was heated and was allowed to stand for several seconds.
I. A horizontal line on top of the vertical line was streaked and the streaking was repeated downwards but with decreasing length.
j. Again, the loop was heated and allowed to stand for several seconds. The same process of streaking was done, but this time the horizontal streaking started at the end of the first streak on the previous streaking. The process for the third horizontal streaking was repeated. The loop was heated before streaking.
k. The lid of the Agar plate was covered and labeled with SAu in B.A.P.
l. The Agar plate with the label was placed inside the oven with a temperature of 34.4 degrees Celsius in order to let the organisms proliferate.

Preparation of Bermuda Grass Oil

The researchers asked permission to have the Chemical Analyst of the University of Immaculate Conception conduct the oil extraction method through the process of Maceration. The said method of oil extraction will be as follows:

a. The needed materials; Petroleum ether, grass sample, Erlenmeyer Flasks, Heat source, Beaker were prepared.
b. Approximately 200 grams of Bermuda grass leaves were measured.
c. The grass leaves were soaked together with 1000 ml ether in an Erlenmeyer flask.
d. The soaked grass leaves were water bathed at a temperature of 40 degrees Celsius to let the ether evaporate.
e. The extracted oil from the sample was collected.

A. Intervention

The researchers asked for permission to use the medical laboratory with the supervision of the Medical Technologist from Tagum Doctors Hospital. This was granted for the intervention phase of the research.

a. The protective gears were properly worn for safety purposes.
b. After 24 hours, the *S. aureus* in Blood Agar Plate and other materials needed such as inoculating loops, alcohol lamp, 3 Mueller-Hinton agar plates, 6 mm filter paper disks with treatments, Normal Saline Solution, cotton swabs, marker, and ruler were prepared.
c. The 3 Mueller-Hinton Agar plates were divided into three equal parts using the ruler and marker.
d. The equally divided three parts were labeled as the following; N.C. for Negative Control, P.C. for Positive Control, and E.C. for Experimental Control
e. The inoculating loop was prepared and heated and was allowed to stand for some time.
f. The inoculating loop was used to get the colony from the Blood Agar plate.
g. The cover of the NS Solution was opened and the mouth of the test tube was pre-heated.
h. The inoculating loop was inserted inside the NS Solution and was swished to let it scattered inside into the solution.
i. The mouth of the test tube was heated and covered back.
j. The inoculating loop was heated again and was left aside.
k. After the sterilized cotton swab was prepared, the NS Solution was opened and the mouth was preheated.
l. The cotton swab was stirred inside the solution and was gently rubbed in the sides of the test tube to get rid of excess liquid. The mouth of the test tube was heated and covered.
m. The cotton swab was gently streaked unto the whole MH agar plate from left to right, top to bottom.

n. The MH Agar plate was turned 90 degrees and the streaking was done with the same cotton swab. The same procedure was repeated twice.
o. The side of the agar plate was encircled with the cotton swab to ensure that no area was left untouched.
p. The cotton swab was properly disposed of after.
q. The procedures from procedure k to procedure p were repeated.
r. 6millimeter filter paper disks with treatments were prepared. The Negative treatment (water) was placed first to prevent contamination on later procedures.
s. Forceps was pre-heated and allowed to stand for some time.
t. With the use of forceps, one 6millimeter filter paper disk bathed in the water was placed in the first Mueller-Hinton Agar with the label NC.
u. Procedure t was repeated but this time on the second and third Mueller-Hinton Agar with the label NC.
v. Procedures t to u were repeated but this time on the Experimental treatment.
w. The same procedure for Positive treatment was done.
x. All three (3) Agar plates were placed inside the oven for 24 hours before observation at 34.1 degrees Celsius.

B. Post-intervention

After 24 hours, the diameter of the zone of inhibition by millimetres was measured using a ruler.

After the gathering of data, the researchers asked the help of the Registered Medical Technologist for the disposal of the materials, treatments, and bacteria.

2. Summary of the Area of the Zone of Inhibition in every Group after Twenty-Four (24) hours of Intervention

Table 1 illustrates the area of the zone of inhibition of the groups treated with water amoxicillin solution and Bermuda grass oil after twenty-four (24) hours of intervention. With an average diameter measure of 39.67 millimetres (mm), it is evident that the area of the zone of inhibition is greatest in the groups treated with amoxicillin, which served as the positive control.

Table 1
Summary of the Area of the Zone of Inhibition in every Group after Twenty Four (24) hours of Intervention

Group	Rep 1	Rep 2	Rep 3	Mean Area of the Zone of Inhibition (mm)
Water	00.00	00.00	00.00	0.00
Amoxicillin Solution	41.00	44.00	34.00	39.67
Bermuda Grass Oil	27.00	26.00	27.00	27.67

It is followed by the experimental group – the one treated with Bermuda grass oil - that is having an average diameter measure of 27.67 mm. While the two groups show a positive result, the negative control group shows no result as indicated by the mean measure of 0.00 mm.

This affirms the study of Nayaka et al. (2014) which proved that *Portulaca oleracea* has a zone of inhibition by isolating its Flavonoid, Apigenin, and thus has inhibitory properties. Amoxicillin acts by inhibiting bacterial cell wall synthesis.

3. Difference in the Area of Zone of Inhibition between the Negative Control Group and the Experimental Group

Table 2
Difference in the Area of Zone of Inhibition between the Negative Control Group and the Experimental Group

Groups	Mean (mm)	T-value @ *a*=0.05; df=4		Decision on Ho	Decision on Difference
		Computed	Tabulated		
Negative Control (Water) vs. Experimental (Bermuda grass oil)					
Negative Control	.00	-41.500	+/- 2.776	Rejected	Significant
Experimental Group	27.67				

Table 2 flashes the difference in the measure of the area of the zone of inhibition between the negative control and experimental group.

Comparing the negative group and the experimental group, the result reveals that

computed t-value of 41.500 is greater than the tabulated value of 2.776. Thus, the null hypothesis is rejected, which means that there is a significant difference between the groups treated with water alone and groups treated with Bermuda grass oil.

The result further indicates that Bermuda has the capacity to inhibit or repel *Staphylococcus aureus* bacteria, considering that its mean has a far value from the 0.00 mm measure of the control, which shows no effect on the bacteria.

This supports the study that presents the fact that Bermuda grass (*Cynodon dactylon*) contains Flavonoids called Apeginin which is proven by the study of Johnson et al., 2002 and Annapurna et al., 2013.The study of Nayaka et al (2014) entitled *"Antibacterial Attributes of Apigenin, Isolated from Portula caoleracea L."* proved that the Apigenin in *Portula caoleracea L* has inhibitory properties.

4. Difference in the Area of Zone of Inhibition between The Positive Control Group and the Experimental Group

Table 3
Difference in the Area of Zone of Inhibition between the Positive Control Group and the Experimental Group

Groups	Mean (mm)	T-value @ *a*=0.05; df=4		Decision on Ho	Decision on Difference
		Computed	Tabulated		
Positive Control (Amoxicillin) vs. Experimental (Bermuda grass oil)					
Positive Control	39.67	3.952	+/- 2.776	Rejected	Significant
Experimental Group	27.67				

Table 3 shows the difference in the measure of the area of the zone of inhibition in the experiment groups.

Comparing the experimental groups with regards to the area of the zone of inhibition, the result reveals that the computed t-value of 3.952 is greater than the tabulated t-value of 2.776. Thus, the null hypothesis is rejected, which means that there is significant difference between the areas of the zone of inhibition among the experimental groups.

This further means that amoxicillin has a more inhibitory effect on *Staphylococcus aureus* than Bermuda grass oil. However, despite the difference, the mean value of the experimental group indicates that Bermuda grass extract proves its capacity to impede or repel growth of the said bacteria.

Silver nanoparticles were synthesized using *C. dactylon* leaf extract. The biologically synthesized AgNPs showed dose-dependent cytotoxicity against HepG2 cells and concentration dependent antimicrobial activity against E*. coli, S. aureus, M. luteus, and S. typhimurium* (Supraja, 2017). Lack of bacterial cell wall results in death due to lysis of bacteria. So amoxicillin is useful only for actively growing and cell wall synthesizing bacteria (National Information Program on Antibiotics, 2016).

CONCLUSIONS

Based on the results, the researchers have deduced to the following conclusions:

1. Bermuda grass oil has far greater zone of inhibition, thus an effective inhibitory agent against *Staphylococcus aureus* than the negative control which is water showing no zone of inhibition.
2. The Amoxicillin solution has greater zone inhibition than Bermuda grass oil and thus, more effective against *Staphylococcus aureus.*
3. Comparing the negative group and experimental group, the null hypothesis is rejected. There is a significant difference in the measure of the area of zone of inhibition between the negative control group (treated with water) and the experimental group (treated with Bermuda grass oil).
4. Comparing the positive group and experimental group, the null hypothesis is rejected. There is a significant difference in the measure of the area of zone of inhibition between the positive control group (treated with amoxicillin) and the experimental group

RECOMMENDATIONS

The researchers were able to come up with the following recommendations that are believed to be beneficial:

1. The area of the zone of inhibition of the Bermuda grass oil is greater than that of the negative control. Thus, it is encouraged to the manufacturers of antibacterial products to use Bermuda grass oil as an antibacterial agent.
2. Since that the Bermuda grass oil has the ability to inhibit the growth of *Staphylococcus aureus* and an organic agent, it is recommended that manufacturers should prioritize its application than Triclosan, which in studies was found to impair muscle activity.

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Neil Ryan B. Ado, LPT, Ph.D., finished his Bachelor of Secondary Education Major in General Science as Magna Cum Laude in St. Mary's College of Tagum, Inc. (SMCTI) in 2008. He completed his master's degree in the same school in 2014. He also acquired his Doctor of Education Major in Educational Leadership at the University of the Immaculate Conception in 2021. Currently, he is the Research Director and a professor in the Graduate Education Program of SMCTI. As a passionate researcher, he devoted most of his time to assisting student-researchers as an adviser and a panel member and collaborating with other institutions in research projects.

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Honey Grace L. Jorgeo, LPT, finished her Bachelor of Secondary Education Major in Biology in St. Mary's College of Tagum, Inc. Currently, she is a Teacher I in Libaylibay National High School Division of Davao de Oro. At present she is actively engaging herself with the different webinars, workshops, and trainings that the Department of Education is offering for continuous professional enhancement.

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