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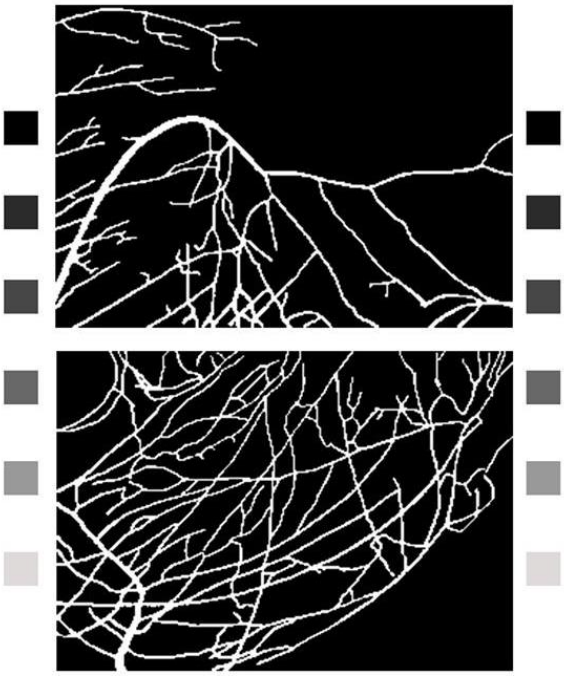
About the Cover



The cover features a binary image of a chick egg's chorioallantoic membrane with the white lines representing the blood vessels on the surface. Blood vessels follow a fractal geometry which essentially means that new blood vessels will take forms geometrically similar to the parent branch. The complexity of such natural phenomenon is mathematically analyzed, exemplifying the universality of mathematics as a language of science that can be incorporated in the exploration of any field. The presence of fractals in biological elements embody the unification of STEM fields, appropriate in the journal of highly diverse scientific contributions.

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Foreword

The Philippine Science High School's new 6-year curriculum culminates with the successful accomplishment of the 3-year Research Program of our scholars who have dedicated their time and hard work on going through the entire research process that is celebrated in this very first publication of PSHSWVC's ***Publiscience***, a compilation of all research journals of Grade 12 scholars.

Research collections are full of inspiring stories behind the victory achieved in finally completing research studies and eventually having these published. These stories may tell of times of frustrations and failures experienced in the process, but all worth the journey of learning and unlearning as well.

These works shall not speak only of the scientific minds and skills of our very first graduates under the new PSHS curriculum, but also of their resilience and perseverance- life skills that will aid them to become better researchers and scientists in the future.

This output shall live as a testament of the dedication of Batch 2018 to pursue Truth, Excellence and Service to God and Country- core values of a true-blooded Pisay graduates. They sought truth as they passionately pursued their scientific investigations; they exemplified excellence as they delivered quality research outputs, and they practiced service as they addressed needs and knowledge gaps in the community and this book serves as a way of communicating Research to all.

I am very pleased of this initiative from the Research Unit to publish all the research studies of Batch 2018 scholars. It is hoped to become a continuing effort until the next batches as they leave this valuable legacy to their alma mater, and to be shared to the community and other research enthusiasts. This is something which the future generation can consult and re-consult, making what has become old, new again.

Very special thanks go to our Research students and teachers who have worked to pull this journal together. Congratulations!

SHENA FAITH M. GANELA, Ph.D.
Campus Director, PSHSWVC

Editorial Page

We are pleased to present the first ever journal of high school researches of first time researches. We are excited to introduce how we pushed the frontier from the usual binding of manuscript to writing of journal-ready article. We hope that through this scheme, we encourage young researchers to read published works in a concise format. Likewise, the journal serves as a compilation of the annual research outputs for easier retrieval of previous works. Lastly, we would like our young researchers to fully complete the research process by publishing their work in a journal. Therefore, it is with great delight and pride that we introduce ***Publiscience***.

Publiscience is a convergence of 'public' and 'science'. The journal embodies the aspiration of Philippine Science High School Western Visayas to bring science closer to public. In doing so, we also provide an on-online access of the articles in this journal at wvc.pshs.edu.ph/products-and-services/research/publiscience. We also would like to distribute this journal to as many recipients as possible. By doing so, we can inspire other schools to do the same or welcome contributions from other secondary schools.

The journal article underwent peer-review; however, it is not as stringent unlike other journals. We ensure the quality of journal articles by asking their fellow researchers, respective research adviser, and an outside expert to review the submitted articles. We would like to caution readers to be critical in citing the information in this journal. We guarantee though that each article is a product of the ultimate effort of work unit in coming up with the journal article.

Finally, we would like to thank the contributors for their steadfast commitment to uphold the research philosophy. Above all else, these journal articles forge their desire to become the future scientists.

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Foreword

When PSHSWVC embraced the K to 12 Curriculum, we committed ourselves to the vision of developing future globally competitive scientists. The PSHSWVC Research Program has to move a little bit further.

Research has become a memorable adventure to most of our scholars for it tested every student's commitment, dedication, perseverance, and grace. This experience has to be captured and documented.

Knowledge generated from research work should be shared to the community. Before, we were just contented to have one or two projects presented in conferences with a chance of having the paper published. For a high school first time researchers, having papers published in peer-reviewed and referred journals is a challenge. Thus, publications were mostly as abstracts or part of the proceedings. Most of the other projects were left as traditionally hard-bound paper kept in multiple copies inside the school's library or research hub.

The idea of having school's student journal was opened and was given a nod. This is the maiden issue of *Publiscience,* the research journal published by PSHS Western Visayas Campus. This contain the research papers of PSHSWVC Batch 2018. These researches are made of PSHSWVC scholars as output on their Research Class. The projects were done under the supervision of their respective research advisers and to some with outside consultants. This is not peer-reviewed thus we would like to encourage our readers to have wisdom over the contents of this journal.

We are now dedicated to make this a yearly publication, not just as a class output, but as PSHSWVC's commitment to spread culture of science.

HAROLD P. MEDIODIA

Research Unit Head
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The Use of Convex Lens as Primary Concentrator for Multi-Junction Solar Cells

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Abstract – A concentrator lens system was designed for a multi-junction solar cell, CDO-100-C3MJ, with an added feature-a convex lens was added above the Fresnel lens, in order to improve the efficiency of the setup. The convex lens setup was tested with the Fresnel lens setup over a three-day photoperiod by measuring the voltage, current, irradiance, and temperature at every hour. The results showed that the convex lens setup produced 1.04 percent more power, but only at around midday. The power difference caused by the convex lens was determined by the Wilcoxon Signed Test to be significant for the photoperiod, as it focuses a greater amount of sunlight on the solar cell over the course of the day.

Introduction. – Solar cells can be composed of a single pair of semiconductor materials, called single junction solar cells (SJSCs), or multiple layers of different semiconductor materials, called multi-junction solar cells (MJSCs). These MJSCs can absorb a wider spectrum of wavelengths due to the different semiconductor materials present in the cell, making it more efficient. The highest recorded efficiency for SJSCs is only 28 percent, while MJSCs with concentrator lenses have reached an efficiency of 46 percent with the latest design from Fraunhofer Institute for Solar Energy Systems[1].

Efficiency in any device is measured by dividing the output over the input. It is measured with the use of a sourcemeter as it has varying resistances. The output from a solar cell is called performance. It is measured in Watts which can be measured by multiplying the voltage with the current. Several factors affect solar cell performance. These factors are dependent on the manufacturing and installation processes of the solar cell. Among those dependent on the manufacturing process are material composition, which determines the spectrum of wavelengths absorbed by the solar cell, and surface area, which determines the size of the cell. On the other hand, factors dependent on the installation of the cell include environmental factors such as cell temperature, wind exposure, and the amount sunlight received by the cell. In photovoltaic systems, light from the sun translates to a power input of around 1 kW/m^2. When light hits the solar cell, electrons are excited from a lower energy to an excited state where they can move freely. Electric current is then produced when the free electrons are extracted; thus, resulting to an electrical output[2].

To efficiently harness the sun's energy, there has to be a great amount of sunlight directed to a solar cell in order for it to generate a greater electrical output. Concentrators are able to reduce materials cost while at the same time increase the solar cells efficiency by concentrating a large surface area of sunlight onto a smaller and therefore cheaper solar cell. The most commonly used lens concentrator is the Fresnel lens[3], which has been used since the 1950s, when plastics were starting to be used for lenses. Plastics were determined to be effective due to their thermal stability and transmissivity which matches the solar spectrum and their index of refraction which is similar to that of glass.

Of the several factors affecting solar cell performance, the- for the full article visit http://bit.ly/2HZqGMb.

Development of Cellulose-based Bioplastic from Corn Stalks

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Abstract – Due to the abundance of corn available, there is also a large amount of corn residue. The study aims to develop a cellulose-based sheet from corn stalks. The cellulose-based sheet was tested of its density, tensile strength, and percent elongation, and was analyzed with an fourier-transform infrared spectroscopy (FT-IR) to determine whether it is chemically similar with commercial cellophane. Overall, the cellophane sheet was similar to commercial cellophane in terms of the functional groups present but was weaker in terms of it mechanical properties.

Introduction. – Plastics are artificial materials with long, strong chains of molecules known as monomers bonded together and is usually composed of carbon, hydrogen, oxygen, nitrogen, chlorine and sulfur. These are formed usually by the processing of natural products such as oil, coal, petroleum, and natural gases[1]. It can be found everywhere and is used to make up a lot of things such as Polyethylene bottles and grocery bags. For decades, plastics have been widely used due to its functionality and versatility. It is easy to manufacture and cheap to produce. It is also designed to be durable, lightweight, and water resistant in order to meet the needs of people.

Due to the rapid growth of population and industrialization, the demand for plastic has increased which also lead to an increase in human waste. As of 2015, the Philippines places third around the world as to the amount of plastic being deposited into the ocean[2]. This is an alarming news to the country because the Philippines is a group of islands surrounded by bodies of water where unique, diverse aquatic species can be found[3].

Plastics made from renewable raw materials or from non-food source are called bioplastics[4]. Bioplastics can also be defined as plastics having either biodegradable properties or if it is made from biological sources, or having the property of both[5]. Intensive research has been done mostly on two resource materials: cellulose and starch. Starch and cellulose-based plastics are inexpensive to reproduce and have widely available materials. Since starch is used more often as an organic raw material in chemical industries, this study will opt to use cellulose as a potential raw material for bioplastic by utilization of major crop residues after harvesting since they are rich in cellulose[6].

One of the most important crops in the Philippines is corn. Corn also plays an important role to livestock and poultry, serving as the major ingredient to their feeds[7]. When corn is harvested, the remaining residues are mainly corn stalks. Cellulose is most abundant in corn stalks wherein the stalks consist of nearly 50 percent cellulose.

Álvarez-Chvez et al.[8] recommended the usage of agricultural byproducts, one of them being corn stalks, to produce bioplastics to be able to reduce production cost and land used for polymer production while giving economic value to waste products. By subjecting the cellulose-based bioplastic sheet to FT-IR, the molecular compounds present can determined and assessed. In this study, the important analysis needed using FT-IR is the identification of the organic and inorganic compounds with which we could compare with the conventional compounds present in cellophane.

This study aims to develop cellulose-based bioplastic from corn stalks. It specifically aims to:

(i) measure its mechanical properties in terms of its density, tensile strength and percentage elongation;

(ii) compare these properties with that of commercial cellophane;

(iii) identify the functional groups present in the cellulose-based bioplastic using Fourier-Transform Infrared Spectroscopy (FTIR) spectrometer; and

(iv) compare the spectra of the cellulose-based bioplastic with that of commercial cellophane.

By subjecting the developed cellophane sheet to FT-IR, the functional groups present can be determined and assessed. In this study, the analysis using FT-IR serves to identify the functional groups present in the bioplastic formed which can be compared with the functional groups present in commercial cellophane.

Methods. – The methods was done in five general steps and in five repetitions. The five general steps include preparation of stalks, extraction of cellulose, viscose process, casting and drying of sheets, and testing of sheets. Out of the five repetitions, only the first and the fourth repetitions were successful in producing sheets. All of the repetitions followed the general steps unless stated otherwise or specified.

Preparation of stalks. The corns stalks were removed of its dirt and leaves, and the outer sheath were peeled to reduce impurities and to produce a smoother surface. The stalks were chopped, blended, and used immediately after. Using a top loading balance set to 0.1 milligrams accuracy, 200 g of the blended corn stalks were weighed in preparation for the next procedure. The outer sheath was remained intact in the first repetition, but since it resulted to a rough bioplastic sheet in the end, the outer sheath was peeled off in the succeeding repetitions.

Extraction of cellulose. In order to delignify the corn stalks, two separate bath solutions were prepared: 15% sodium hydroxide and 15% sodium sulfite. The chemicals weighed were 150 g of sodium hydroxide and 150 g of sodium sulfite, and were each dissolved separately to produce 1L of each solution. The first bath is the 15% sodium hydroxide and was placed on top of a hotplate set to 150°C. The weighed corn stalk samples were slowly added and were heated for two hours. The cooked corn stalks were then filtered using a strainer. Further delignification was performed using the bath of 15% sodium sulfite solution placed on top of a hot-plate set to 150°C for two hours. The cooked corn stalks were then filtered using a strainer to remove absorbed liquids. All of the repetitions followed this method except for the second repetition which used formic acid (90% v/v). However, extraction of cellulose was unsuccessful, which resulted to its end on this repetition.

Viscose process. In order to convert the cellulose pulp to alkali cellulose, the sodium hydroxide solution was prepared by making a 500-mL solution of 18% sodium hydroxide. All of the cellulose pulp are then poured into the beaker containing the solution which was then sealed for 60 minutes, and after which was hand-pressed in a strainer as dry as possible. After breaking up any large lumps into crumbs, the alkali cellulose was transferred to a one-liter media bottle for xanthation. Xanthation was accomplished by adding 7.70 g of carbon disulfide to the alkali cellulose in the bottle and maintaining the sealed bottle at 30C for two hours. The bottle was rotated periodically to insure uniform xanthation. After two hours, the yellow pulp turned to a deep orange color after reacting with the carbon disulfide. The media bottle was opened in the fume hood and 240.28 mL of cold water (5°C) and 48.56 g of 18% sodium hydroxide were added. The mixture started to turn viscous as a thick, orange-colored liquid was formed. The mixture was transferred to a one-liter beaker and was stirred using a handheld electric mixer for two hours. The thick orange-colored solution that resulted was "viscose." The viscose was transferred into a clean media bottle and stored at 5°C in a refrigerator for 24 hours.

Casting and drying of sheets. The cellophane casting stage is when the viscose is poured onto the glass slides and bathed into the chemical baths of 40% ammonium sulfate and 12% sulfuric acid-18% sodium sulfate. In a 250-mL beaker, 200 g of ammonium sulfate was weighed to create 500 mL of 40% solution of ammonium sulfate was created. Subsequently, the sulfuric acid-sodium sulfate bath was made by adding 60 mL of sulfuric acid into a one-liter graduated cylinder. After sulfuric acid, 90 g of sodium sulfate was added. The solution produced was a 12% sulfuric acid-18% sodium sulfate solution. The two baths, ammonium sulfate and sulfuric acid-sodium sulfate baths, were heated over a hot plate up to 45°C.

The sheet of bioplastic was prepared by spreading a thin, layer of viscose on the 1x3x8 inch glass plate using a rubber spatula. The viscose was allowed to coagulate by immersing the sheets in an ammonium sulfate bath (45°C) for 60 seconds. After the immersion, the coagulated sheet that was still on the plate was immersed in a sulfuric acid-sodium sulfate bath (45°C) for two minutes. Upon contact, the yellow sheets gradually turned white and a bubbling effect was observed. The cellulose-based bioplastic films were then soaked in hot distilled water (80°C) for 10 minutes. In order to obtain a more flexible bioplastic, the bioplastic sheets were treated with 5% glycerin solution for 15 minutes. The bioplastic sheets were flipped during the glycerin treatment to allow even plasticizing. The sheets were air-dried for three days at room temperature.

In the first repetition, the viscose was spread onto the inside of the glass slides, which surrounds the viscose with walls. Sheets were successfully made. In the fourth repetition, the chemical used for the first bath was ferrous ammonium sulfate instead of ammonium sulfate. Moreover, the underside of the glass plates which had no walls

surrounding was used instead of casting the sheets inside the glass plates with walls. The sheets were then immersed into pans filled with the coagulating and regenerating reagents. Sheets were successfully made. In repetitions three and five, the sheets would tear at every attempt to remove them from the glass since they adhered to the surface of the glass plate.The films were not appropriate for testing since they would tear easily and the sizes of the film were too small.

Testing of sheets. The first and the fourth repetitions were successful in producing solid sheets that could be tested. The first batch was tested of its mechanical properties, particularly the density, percent elongation, and tensile strength as these were the only available tests in Central Philippine University - Packaging Engineering. The fourth batch was tested of its chemical composition using the FT-IR Spectroscopy in Philippine Science High School Western Visayas Campus. All the tests were compared with commercial cellophane.

Results and Discussion. – The results of the study are divided into three sections, specifically appearance of the sheet, mechanical properties, and the results of the FT-IR test. Two sheets are used, namely the sheet produced from the first repetition, and the sheet produced from the fourth repetition.

Appearance. The first sheet produced was rough, thick, and opaque. Additionally, prominent fibers were visible on its surface. The sheet was brownish-yellow in appearance. The bioplastic formed was thick and coarse in appearance since the viscose used for casting was a thick liquid. Due to the viscous nature of the liquid, the sheet was difficult to spread in the glass plate using a spatula. Additionally, the viscose used for the first sheet contained corn stalk fibers since it was considered the first batch of viscose among the five repetitions, resulting to its coarse texture. Figure 1 shows the appearance of the sheet.

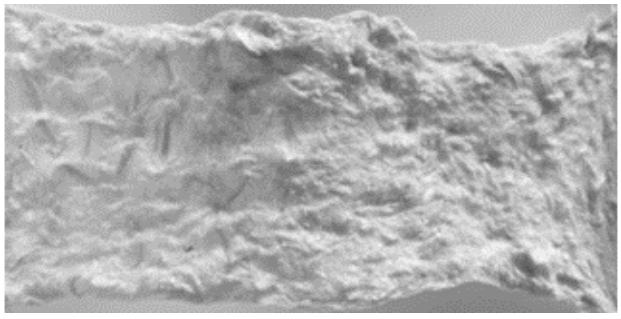

Fig. 1: Appearance of the sheet on the first repetition.

The fourth sheet produced was significantly different compared to the first sheet since it was smooth, thin, and slightly opaque. The fourth sheet was brownish-gray in color due to the presence of Fe3+ ions. Compared to the previous sheet which was thicker and coarser in appearance, the viscose used for casting in the fourth sheet was thin and runny. Hence, the sheet formed was smooth. Figure 2 shows the appearance of the sheet.

Fig. 2: Appearance of the sheet on the fourth repetition.

Mechanical properties. The mechanical properties test was only conducted to the sheets in the first repetition. The results were compared with the commercial cellophane.

Table 1 shows the comparison of the properties between the developed bioplastic sheet and that of the control which is commercial cellophane.

Table 1. Properties of the bioplastic versus commercial cellophane.

	Bioplastic	Commercial Cellophane
Density (g/mL)	0.49	1.24
Tensile Strength (MPa)	1.81	90.12
Percentage Elongation (%)	7.70	8.67

The values of the bioplastic in terms of density, tensile strength, and percentage elongation were lower than that of the commercial cellophane, which served as the control. Compared with the commercial cellophane sold in the market, the bioplastic made from corn stalks had a density which was 60.48% lower, tensile strength which

was 98% lower, and percent elongation which was 10.27% lower.

The lower density of the cellulose-based bioplastic could be attributed to its higher thickness and mass. The leftover hemicellulose in the crude alkali cellulose could have deteriorated the strength in the final viscose product which lowered the quality of the film[9]. Consequently, it affected the tensile strength of the cellulose-based bioplastic. Due to the leftover hemicellulose, it may have consumed the chemical substituents during the process before the chemicals had time to react with the cellulose polymers, thus it reduced the availability of chemicals for the cellulose derivatization. This lead to irregularities in the product quality and thus, may have greatly affected its tensile strength and percentage elongation [10].

FT-IR Results. The FT-IR test was only conducted to the sheets in the fourth repetition. Subsequently, the results were compared with the commercial cellophane. Figure 3 represents the comparison in appearance of the spectra of the fourth sheet versus the spectra of commercial cellophane.

The analysis on the spectra will be limited to the region to the left of 1400 cm-1 which is called the functional group region. The range of 600 to 1400 cm-1 which is found in the right region of the spectrum is called the fingerprint region. This region is a complex area, making it complex and difficult to interpret reliably due to the overlapping bands. Lastly, since the researchers are beginners in analyzing the spectrum, discussion will focus on the left region of the spectrum or the functional group region since this is where most of the stretching frequencies occur.

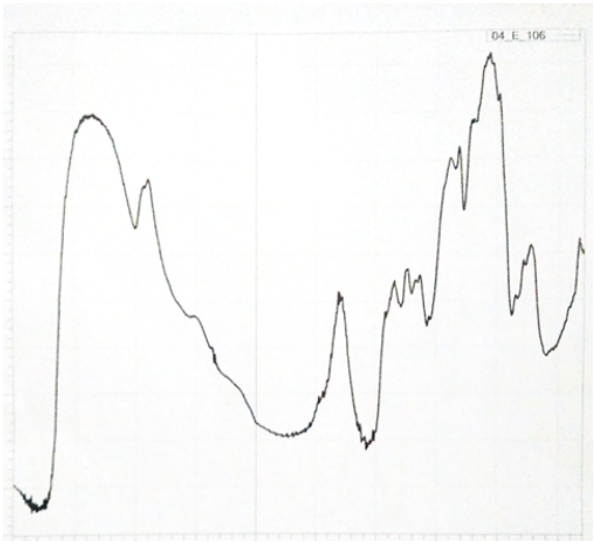

Fig. 3: Spectra of the bioplastic sheet from the fourth repetition.

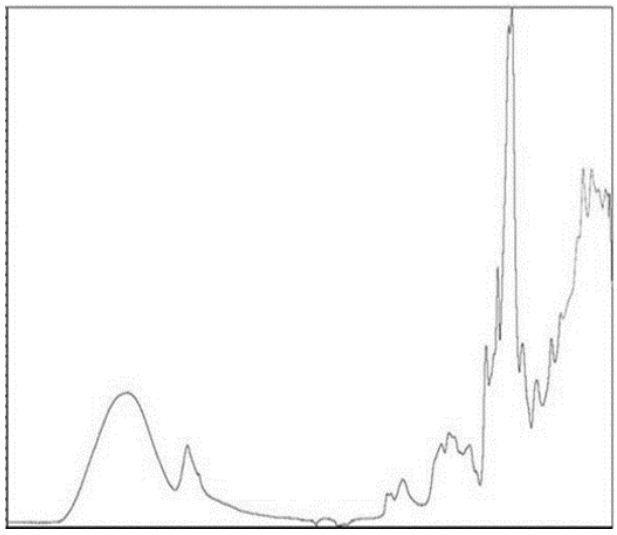

Fig. 4: Spectra of commercial cellophane from Specac (2017).

When the spectra of the fourth sheet (Fig. 3) is compared to the spectra of commercial cellophane (Fig. 4), a general similarity in the peaks can be observed. The spectra used for commercial cellophane (Fig. 4) was based on the analysis done on polymer samples by Specac, a company that manufactures FT-IR accessories and other products related to spectroscopy (Specac 2017). When the two spectra (Fig. 3 and 4) are compared, there is a difference in the intensity of absorption between the bioplastic and the commercial cellophane which is presented in the difference in peaks based on the y-axis. Generally, the spectra for the bioplastic has a higher intensity of absorption. According to the Beer-Lambert law, the thickness of a material is directly proportional to its thickness[11]. Since the bioplastic the researchers produced did not utilize rollers during the process, the sheets were thicker than commercial cellophane which are generally thin and transparent.

Additionally, a larger peak intensity increase indicates that there are more identical functional groups[12]. Figures 5 and 6 present the functional groups present in the spectra. Specifically, the common functional groups present among the two spectra are alcohol and carboxylic acid. Basing on the graph, it indicates that the cellulose-based bioplastic (Fig. 6) has more functional groups, namely alcohol and carboxylic acid, since it has a higher peak compared to commercial cellophane.

Conclusion. – A cellulose-based bioplastic made from corn stalks was possible. However, its mechanical properties are weaker compared to commercial cellophane. Its weaker properties may be attributed to the impurities present in the sheet, and the methods used were limited to a laboratory scale, which is Millers method[13], which does

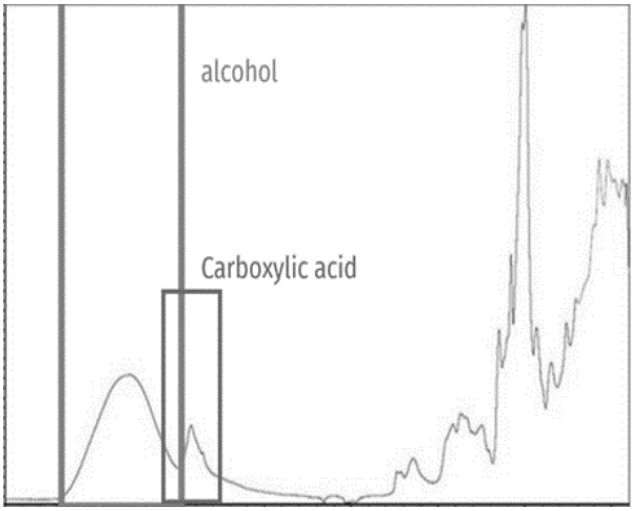

Fig. 5: Functional groups present in the cellophane spectra. The peaks in the cellophane sheet indicate the presence of functional groups, such as alcohol and carboxylic acid.

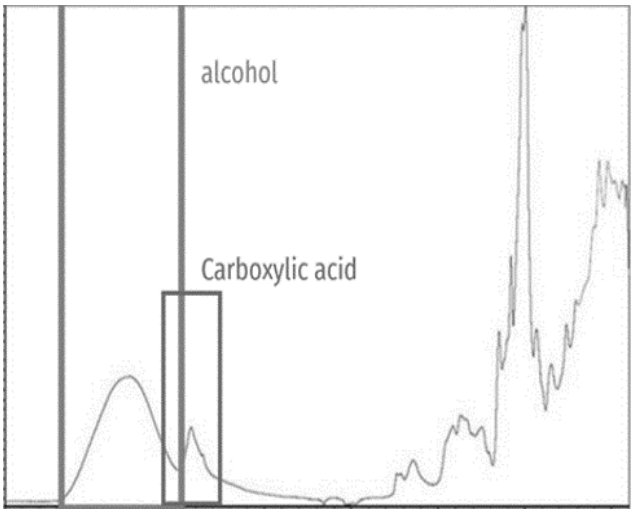

Fig. 6: Functional groups present in the bioplastic spectra. The peaks in the bioplastic sheet indicate the presence of functional groups, such as alcohol and carboxylic acid.

not utilize high-power equipment. The cellulose-based bioplastic is similar to commercial cellophane in terms of the functional groups present. The difference between the cellulose-based bioplastic and commercial cellophane is their appearance wherein the cellulose-based bioplastic is thicker, coarser, and more opaque. Among the five attempts done to develop a cellulose-based bioplastic, the fourth repetition which used ferrous ammonium sulfate yielded to a sheet which is similar to cellophane chemically. However, the current study is limited to the basic knowledge of the researchers in terms of analyzing the FT-IR results.

Recommendations. – In order to obtain bioplastic that possesses better characteristics, it is highly recommended to:

a) Use the corn stalks freshly harvested to ensure the cellulose inside does not degrade over time.

b) Opt for mature stalks than young stalks because plants generally have more cellulose as they age.

c) Change the variety of the corn used may also be done to determine which variety yields to more cellulose.

d) Use a cellulose extraction procedure specifically for corn stalks which results to high yield and purity.

e) Search for a recent method on how to cast cellophane which enables the user to cast thin sheets.

f) Use a more delicate procedure in casting and in bathing the sheets so that the regeneration of the sheets will be even and will not cause holes when pouring the reagents on the sheets.

g) Search and utilize a more efficient method in casting in order for the sheet to be more consistent chemically and physically.

h) Consult an expert who specializes in using the FT-IR to enable more in-depth analysis and interpretation of the sheets.

i) Calibrate the FT-IR machine properly before use.

j) Use a Scanning Electron Microscope to determine whether the commercial cellophane tested is indeed microfibrillated cellulose.

k) To determine what causes the eccentric waves in the spectras.

* * *

This research would not be possible without the guidance of the Research Faculty of Philippine Science High School - Western Visayas Campus, the help given by the peers and reviewers of the researchers, and the assistance of Sir Ron Adriane Dionaldo, supervisor in the Packaging Department at the Central Philippine University. Additionally, the researchers would also like to thank their families for supporting them financially and emotionally despite the constant trials facing their way. Lastly, the researchers extend their wholehearted gratitude to Ma'am Lani Estilo and Sir Aris Larroder for giving the researchers encouragement throughout the gruel research process.

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Bio-Enzymatic Degradation with Pectinase Enzyme and Activators of UV-Irradiated Low-Density Polyethylene (LDPE)

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Abstract – Low-density polyethylene (LDPE), classified as plastic number four, is a kind of plastic with long degradation period, low recyclability potential, and is harmful to marine habitats. Due to this, LDPE plastic strips were subjected to treatments that can hasten its degradation. They were irradiated to ultraviolet for 14 days. Bio-enzymatic degradation was then conducted using pectinase enzyme with barium, magnesium, and calcium ions as activators. The assessments for the degradation were in terms of weight loss and carbonyl index of the LDPE strips. The pectinase with magnesium activator is the most effective treatment as it was able to show a significant difference with the two parameters.

Introduction. – For the past few years, plastic materials are widely used in food packaging, clothing, transportation, shelter, construction, medical, and recreational industries ranging from basic needs to leisure activities and innovations of industry [31]. Plastics, also called polymers, are products of the conversion or synthesis of chemicals extracted from oil, coal, and natural gas [23]. The Society of Plastics Industry (SPI) formulated a Resin Identification Code system on plastics as a reference for recyclers to identify the resin content in plastics. At the latest, there are only seven (7) types of plastics identified by the system [47].

Despite their uses, plastic materials are disadvantageous because of their long degradation period. In the normal environment, plastic bag degrades at 10 to 20 years, plastic film container at 20 to 30 years, and plastic beverage at 450 years [29]. Plastics can also cause aesthetic, health, and environmental problems. It affects maritime activities and tourism [45]. Plastic debris have been ingested by animals that results in impaired movement and feeding, reduced reproductive output, lacerations, ulcers and death. Floating debris can also be colonized by marine organism, and due to its long life and the current, it can facilitate to the transport of foreign species to other waters [45].

Low-density polyethylene (LDPE), coded by SPI as number 4, is a plastic made from repeating units of the monomer CH_2 or ethylene [14]. LDPE is a common packaging material due to its mechanical properties, barrier properties against water, light weight, low cost, and versatility [12]. LDPE, being soft due to its added softeners, poses a problem in recycling where it gets caught in the wheels and gears of recycling machines [15]. These softeners, also called plasticizers, are substances added to the plastic that contribute to its flexibility, resiliency, and versatility [37]. LDPE is hydrophobic because it is made up of a nonpolar ethylene monomer. It also has long carbon chains which result in a high tensile strength. However, the tensile strength of LDPE is relatively weaker compared to a High-Density Polyethylene (HDPE) plastic due to the branching structures of polymer chains reducing the intermolecular forces of attraction. The hydrophobic and long carbon chain properties of LDPE make it resistant to environmental degradation [38].

Philippines is one of the five countries that contribute most to more than 80 percent of land-based sources of ocean plastics. Out of that 80 percent, 75 percent comes from uncollected wastes, and 21 percent of the uncollected wastes are plastics with medium residual value such as LDPE [33]. Due to this, plastic degradation should be practiced in-land to prevent these leakages into the oceans. Photodegradative effects are also significantly

decreased in seawater due to lowered temperature and oxygen availability, and the rate of hydrolysis of most polymers is insignificant in the ocean [48]. Due to its long degradation process, low potential as recycling material, and consequences in oceanic waters, research must be made to hasten the degradation process of LDPE while it is in-land.

Degradation is the change in physical or chemical properties of polymer [41]. Generally, polymers starts with chemical and physical degradations as a precursor to biodegradation. Biodegradation is a form of degradation through assimilation by microorganisms or degradation by enzymes. Fungi species such as Aspergillus sp. are suitable candidates for LDPE degradation[20]. These fungi secrete the enzyme pectinase, which has a very high specific enzymatic activity. Pectinase is a commercial enzyme that breaks down pectin, a polysaccharide substrate that is found in cell walls of plants.

Enzymatic activity is the rate at which enzymatic reactions proceed. It is affected by different factors such as temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators [16]. These factors must be exposed to optimum conditions for the enzyme to function most efficiently. In the case of pectinase, optimum conditions vary on depending on the organism producing the enzyme. It has been found out that barium, calcium, and magnesium ions can be activators to increase the activity of pectinase [1].

This study aims to investigate the difference in degradation capabilities of the pectinase enzyme with different enzymatic activators in hastening LDPE degradation with primal photodegradation by ultraviolet radiation. The degradation would be measured by percentage of weight loss and change in carbonyl index obtained from Fourier Transformed Infrared Spectroscopy (FTIR) spectra analysis.

Materials and Methods. – *Photodegradation.* Fifteen samples of 5.0 x 1.0 cm LDPE strips were irradiated under a UV lamp for 12 hours a day in a span of two weeks.

Bio-Enzymatic Degradation. Five treatments were prepared for biodegradation: no enzyme, pure pectinase solution, and the enzyme added with salts of barium, calcium, and magnesium salts at 1.0 mg/mL concentration. The strips were put in a petri dish with the treatment and stored in an incubator at 40 degrees Celsius. Sterile buffer was added every day to maintain the pH. After 14 days, the plastic strips were washed with tap water and immersed in distilled water to separate the films from the enzyme, and oven-dried at 40 degrees Celsius for 72 hours.

Pre-assessment and Post-assessment. The LDPE strips were assessed by weight and infrared spectra before and after degradation.

Results and Discussion. – *Physical Appearance.* The physical appearance of the plastic strips were different before and after degradation in terms of visual appearance and texture. The strips became smoother and turned into a light yellow color.

Weight Loss. LDPE strips subjected to degradation using pectinase enzyme added with magnesium as activator was able to show a significant difference in weight loss as compared to other treatments. While the treatment without enzyme had a weight loss due to photodegradation.

Some studies have shown that the final weight of the plastic strips may increase after biodegradation. In this study, the pectinase enzyme adheres to the LDPE which acts as subtrate, resulting in a weight gain.This may explain why plastic strips treated with pure pectinase enzyme has increased in weight after degradation, as compared to the decrease in weight in other treatments. Similarly, the results of the study of Gajenderin et al. (2016) shows an increase in the weight of the plastic due to the microbes used in degrading the plastic. They got the weight loss through the proportionality to the surface area of plastic strips. Their study reports a 35 percent weight loss after 90 days of incubation. At their 15th day of incubation, they were able to measure a 3 percent weight loss. This study was able to obtain almost 5 percent weight loss after just a 14-day enzymatic biodegradation period, considering it was also pre-treated with UV-irradiation.

Some limitations regarding the weight loss results may be possible due to the weighing equipment which can only weigh up to the fourth decimal place in grams, and there are only two signigicant figures. The analytical balance used can only weigh up to the fourth decimal place in grams, and there are only two significant figures. The actual difference between the weight of the plastic strips before and after degradation may not have been completely shown because the data value is rounded off. The cleaning of the strips after the enzymatic biodegradation and possible contamination, despite the precautionary measures executed, may have also affected the weight of the plastic strips.

Carbonyl Index. LDPE strips subjected to degradation added with pectinase enzyme and magnesium ion as activator was able to show a significant difference in the carbonyl index. Although the negative control has the highest change in the carbonyl index, the difference was not significant as compared to the difference in the carbonyl index of plastics treated with magnesium ion

as activator. Data for the carbonyl index was obtained through the initial and final infrared spectra of the LDPE strips.

Normally, after degradation, the carbonyl indices would increase due to the supposed increase of the carbonyl groups. The increase in carbonyl groups is mainly caused by abiotic degradation [21]. In this study, the abiotic degradation was through the irradiation of ultraviolet light. The hydrophilicity of a polymer increases in proportion to the increase of carbonyl groups, making the polymer more available for biodegradation [21].

Factors that may have affected the results of the infrared spectra include the use of different FTIR equipments for the pre-assessment and post-assessment of the plastics. The plastics were also subjected to FTIR analysis 23 days after the biodegradation was completed.

Overall. The metal ions were added in the same concentration at 1.0 mg/mL despite the difference in molar masses. Of these metal ions, magnesium has the lowest molar mass resulting to the highest molarity in the three treatments with activators. It is possible that the magnesium ion activator was able to yield the highest percentage of weight loss, and change of carbonyl index because it had the greatest amount of molar concentration in all the treatments.

Conclusion. – The treatment in the enzymatic biodegradation with pectinase enzyme and magnesium ion as activator has the highest percentage weight loss of 4.94 percent after 14 days of UV-irradiation and 14 days of bio-enzymatic degradation. It also has the highest carbonyl index difference that is significant. If the photodegradation has, considerably, contributed 0.94 percent weight loss, prior to the enzymatic biodegradation, then, 4 percent is the highest percentage weight loss. Among the treatments with activators, the magnesium ion is the most effective as it was able to show a significant difference in both weight loss and carbonyl index.

Recommendations. – It is recommended to add the same molarity of the activator to the pectinase enzyme.

It is also recommended to use the same FTIR equipment for the pre-assessment and post-assessment of degradation as different equipments may yield different infrared spectra.

It is also recommended to use a microbalance to weigh the samples. This is to ensure a more precise measurement of the weight loss of the plastic due to degradation.

It is also recommended to have a longer bio-enzymatic degradation period to provide more significant difference and higher percentage weight loss.

It is also recommended that the LDPE strips are assessed after UV-irradiation and before bio-enzymatic degradation.

It is also recommended to use scanning electron microscope to assess the surface of the LDPE strips before and after the treatment.

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Immune Boosting Activity of Aqueous Lemongrass (Cymbopogon citratus) Leaf Extract on Native Chickens (Gallus gallus domesticus) Challenged with Newcastle Disease

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Abstract – Newcastle Disease is a highly infectious disease which can cause up to a 100% mortality in native chickens and no treatment has been yet found for it. Lemongrass (Cymbopogon citratus) is a plant that has alkaloid, saponin, tannin, and flavonoid to combat Newcastle disease. This study aims to measure and compare the antibody titre gain of Philippine native chickens treated with different dilutions of C. citratus aqueous extract. Fofty-four two-month-old chickens were infected with live virus-concentrated solution. Blood was collected from all the samples prior to infection and after the treatment Samples were grouped into four treatment groups A, B, C, and D, according to the dilution factor. Samples from the treatment group A were given with 1 mL undiluted C. citratus extract, treatment group B with 5-fold dilution, treatment C with 10-fold dilution, and treatment D with 20-fold dilution. Antibody titre gain was determined by hemagglutination inhibition. Result show that there is a significant increase with the antibody titre gain for unvaccinated and vaccinated groups. Unvaccinated samples do not require a treatment diluted in a specific volume of distilled water while vaccinated samples require a treatment diluted in 9 mL distilled water for an optimal antibody increase when infected with ND. Treated samples have an increase in the average live weight with treatments B and C exhibiting a significant increase. Survival rate of the chickens is also high with 85.21%.

Introduction. – Poultry is the most progressive animal enterprise today and has been a significant contributor to the country's agriculture sector (Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development 2011). According to the Philippine Statistics Authority (PSA), it accounted for 15.72 percent of the total agricultural production from April to June 2016.

The chicken industry constitutes a large part in the poultry sector. As of July 2016, the countrys chicken inventory has approximately 17.5 million, of which 44.2% are native chickens (Philippines Statistics Authority or PSA 2016a). Native chickens belong to a population of chickens with no extensive information about the breeds. These are the local backyard chickens raised by farmers often showing large phenotypic diversity (Cabarles et al. 2012).

The raising of native chickens is an integral part of the farming systems of Filipinos as they are the main source of eggs and meat for backyard farmers. According to PCAARRD (2016), native chicken meat has always been preferred over that of commercial broilers because of its unique taste, distinct flavor and texture, presence of functional compounds, and lower fat content. Also, the study of Sokoowicz et al. (2016) has found that native chicken meat contains less fat and has a healthier ratio than that of broilers. However, two main problems entail the native chicken industry: the unstable supply of slaughter, and a high chicken mortality rate of 40% caused by poultry diseases (PCAARRD 2016).

The chicken production from April to June 2016 slightly declined by 0.02 percent. The drop was attributed to the low disposition of native chickens due to the incidence of

Newcastle Disease that prevailed in Luzon (PSA 2016b). Linden (2016) reported that 41,000 birds died in Luzon because of Newcastle disease (ND). Barnes (2016) also reported that 109,255 chickens died of ND infection in the northern region as of February 29. In La Union, the same virus also infected 25,000 chickens (Alhambra 2016).

According to the Department of Agriculture (2016), one contributing factor of the spread of Newcastle disease virus was the change of weather that weakened the immune system of chickens. The spreading worsened due to contact of chickens in cockfights allowing the virus to spread from one poultry farm to another (Reano 2016).

Newcastle disease (ND) is a highly infectious disease in domestic poultry and wild birds. ND is caused by a single-strand, non-segmented, negative-sense RNA virus known as Avian paramyxovirus 1 (APMV-1) and is regarded as one of the most important avian diseases (Catolli et al. 2011; Chollom et al. 2012). Birds infected with ND experience respiratory and nervous attacks. Effects further include paralysis of wings and legs, twisting of the head and neck, rapid drop of egg production and sudden death (Mississippi State University 2014). Humans can also be affected by ND. Effects include mild conjunctivitis, influenza-like symptoms, and laryngitis (Badr date unknown).

Chickens are the most susceptible to clinical disease among most avian species susceptible to infection with the virus. It may cause outbreaks with up to 100% mortality and is considered a major threat to both commercial and village poultry flocks (Chollom et al. 2012).

There is still no specific treatment for the disease (Butcher et al. 2015). Vaccines are recommended to prevent the occurrence of ND among healthy chickens. However, Newcastle disease remains unabated despite various vaccination programs (Chollom et al. 2012). Also, another way of transmission aside from direct contact is through contamination of poultry vaccines (Badr date unknown).
Lemongrass (Cymbopogon citratus) or locally known as tanglad, contains phytochemicals such as alkaloids, flavonoids, saponins, and tannins found in plants are considered as antiviral agents (Nambiar and Matela 2010). Alkaloids bind with RNA and can cause chain terminations, thus inhibiting the synthesis of virions (Mcdaniel date unknown); saponins induce the production of SOCS2 (Lee et al. 2012); tannins inhibit the virus from attaching to the host cell (Cheng et al. 2004); and flavonoids inhibit the neuraminidase activity of the virus disabling the removal of siallic acid on the surface of the host cell, thus not allowing infection of other cells (Hanh et al. 2014).

Native chickens play an essential role to the economy of the country. Since it is free range, it is perceived as chemical and antibiotic free. There is a global trend from recent years showing the shift of consumers interest into organic and naturally produced products, which makes native chicken more preferable compared to commercial chickens (PCAARRD 2016). It is also a cheap source of animal protein and provides extra income to families in rural areas (Cabarles et al. 2012). The sustained use of native chickens in the traditional poultry production system implies the need to consider the value of native chickens (Padhi 2016). Given the recent ND outbreaks, there is a need for a treatment when the virus breaches the immunity of chickens given by vaccines.

This study aims to determine the immune boosting activity of C. citratus aqueous leaf extract on native chickens challenged with Newcastle disease. Its objective is To determine whether C. citratus aqueous leaf extract increases antibody titre, live weight, and survival rate of native chickens challenged with Newcastle disease, specifically, to measure and compare the antibody titre, live weight gain, and survival rate of chickens treated with different concentrations C. citratus aqueous leaf extract.

There has been no treatment found against ND. If the bird begins to show symptoms, vaccines are ineffective. Since the diagnosis for this disease is poor, there is a 100 percent mortality rate once infected, which can therefore affect the poultry economy (Foster and Smith 2016). Thus, it provides the need for a treatment to boost the immunity of the chickens against the disease.

Methods. –

Collection and Identification of Plant Material. Six kilograms of C. citratus were obtained from the Central Philippine University - College of Agriculture, Resources and Environmental Science (CPU - CARES) farm in Leon, Iloilo. The plant samples were cut at the stem using a sickle. The collected plant materials were certified by Dr. Jaime Cabarles, dean of CPU - CARES. The samples were then washed with distilled water.

Preparation of Stock Solution. Preparation of the aqueous extract was done according to the methodology of Hindumathy (2011). C. citratus aqueous extract was obtained by juicing one kilogram of C. citratus leaves with 1 L of distilled water using a juicer provided by CPU - CARES.

Preparation of Serial Dilutions. From the stock solutions, serial dilutions were prepared to obtain the test solutions of different concentrations of 1 mL extract/1 mL, 1 mL extract/5 mL, 1 mL extract/10 mL, and 1 mL extract/20 mL respectively.

Experimental Birds and Cultural Management. This study utilized 54 two-month-old native Philippine chickens of unspecified breeds. Chickens were kept in premade

cages with the dimensions 2 ft 2 ft 45 cm and enclosed in a 3 m 7 m sized knotted nylon mesh netting. Samples were divided into nine groups including the control group. They were given one week upon transfer for acclimatization to their new environment to prevent possible stress. Samples from four of the groups were already vaccinated against ND. The control group was separated from the treated group to avoid contaminations. Birds were fed with 58 g of CPU Feeds Chick Grower daily. Each of the samples was also given water in their corresponding waterer.

Weighing of Samples. Live weight of samples were measured days before infection and after treatment. Samples were each taken from their cages and set on top of the digital weighing machine.

Virus Infection. Preparation of Virus-concentrated Solution. The LaSota vaccine, bought from a local livestock supply store, was diluted with a vaccine solvent specifically for freeze-dried avian vaccines provided by CPU - CARES. The ratio of the vaccine to the solvent was modified by the Department of Agriculture (DA) so that the final solution would be more concentrated with live virus.

Infection of Samples. The final solution was injected to either the thigh or the breast part of the chicken samples. Cottons were also soaked with the solution and was taped at the top of the cages to contaminate the air with the virus. This was done under the supervision of Dr. Jaime Cabarles Jr. of CPU - CARES and Dr. Jonic Natividad of DA.

Treatment. Infected samples were drenched using 1 mL syringes with aqueous extracts from the C. citratus plant every afternoon. Groups from vaccinated samples vaccinated against ND were labeled as Group VA (vaccinated, group A) , Group VB (vaccinated, group B), Group VC (vaccinated, group C), and Group VD(vaccinated, group D). Groups from the unvaccinated samples were labeled as Group UA (unvaccinated, group A), Group UB (unvaccinated, group B), Group UC (unvaccinated, group C), and Group UD (unvaccinated, group D). Samples from groups VA and UA were administered with aqueous extract dosed at 1 mL extract, groups VB and UB with 1 mL extract/5 mL, groups VC and UC with 1 mL extract/10 mL, and groups VD and UD with 1 mL extract/20 mL. Survival rate and live weight gain were measured 14 days after the start of treatment. Survival rate was computed by treatment group using the equation (total number of chickens - number of deaths)total number of chickens) x 100 . With regards to the side effects of the phytochemicals on chickens, the study of Raza et al. (2015) showed that plants containing alkaloids, flavonoids, saponins, and tannins that were tested against the ND virus in vitro and in vivo exhibited positive results and did not show any adverse reactions on chickens.

Blood Collection and Serology. Blood for serum samples was collected through either the brachial or jugular vein of the samples. Collection was done five days before infection and 14 days after treatment, following the method of Orajaka and Ezema (2004) with modification on the number of days before infection. The chicken was held horizontally on its back, holding the legs and under the back to support the chicken and pull its wing towards the holder. The wing vein is the bifurcating form (V-shaped) that ran between the biceps and the triceps muscles and with tendon of the pronator muscle running across it. Small feathers that obscured the vein were plucked. Seventy percent by volume alcohol was swabbed around the bleeding site for disinfection. A 23 mm gauge needle was inserted not too deeply under the tendon and directed into the wing vein towards the blood flow, keeping clear of the ulnar nerve. The plunger of the syringe was gently pulled once the tip of the needle was in the vein. If blood did not flow, the end of the needle was repositioned slightly, and if haematoma formed, the other wing was used. After bleeding, pressure was applied to the vein for a few seconds to prevent further bleeding. The needle was removed and the cap was placed on its end to prevent leakage (FAO 2005). The collection of blood was performed quickly and gently to avoid vein damage. Minimizing the blood loss lessens trauma to the chickens. The collection of blood was conducted under the supervision of Dr. Jaime Cabarles Jr. of CPU - CARES and Dr. Jonic Natividad, certified veterinarian of DA - R6. ND virus antibody titres in the sera of inoculated hens were examined through hemagglutination inhibition (HI) assays carried out by the Department of Agriculture - Region 6 Regional Animal Disease Diagnostic Laboratory.

Safety and Disposal. To guarantee the safety of this experimental study, biosafety level (BSL) was be considered. According to USDA, Newcastle disease virus has a biosafety level 2 (BSL-2). This means that Newcastle disease virus is an agent that can be associated with some human diseases such as mild conjunctivitis. The guidelines of U.S. Department of Health and Human Services (2009) was followed in the containment of infected samples, and the protocol of The Ministry of Health and Welfare Notification No. 1997-22 was used to transfer blood samples for HI testing.

Dead samples were removed from their cages and then collectively burned in an excavation. After the conduct of the study, all remaining samples were also incinerated. The excavation was then covered back with soil after the burning process.

Statistical Analysis. Data for initial and final antibody titre and live weight were analyzed using paired-sample t-test. Mean antibody and live weight difference of vaccinated samples and vaccinated samples were compared using independent-sample t-test. Vaccinated and unvaccinated treatment groups were compared separately using one-way ANOVA in IBM SPSS Statistics version 22

Table 1: The mean antibody titre gained sample groups.

Sample		Mean Antibody Titre Gain
Treated	Vaccinated	167.67
	Unvaccinated	74.67
	Overall	121.17
Control	85.33	

software.

Results. – This study aimed to determine the antiviral activity of C. citratus aqueous leaf extract by measuring and comparing the antibody titre of chickens treated with a) undiluted 1 mL, b) 5-fold dilution, c) 10-fold dilution, and d) 20-fold dilution of C. citratus aqueous extract. Thirty two-month-old Philippine native chickens were infected with Newcastle disease. Six chickens served as control and the remaining 24 chickens were infected and treated. From the treated group, 24 unvaccinated samples were each divided into four subgroups, A, B, C, and D. Subgroups were then given with undiluted 1 mL, 5-fold dilution, 10-fold dilution, and 20-fold dilution of C. citratus aqueous extract respectively. Final and initial live weight and blood of the samples were measured and collected before infection and after treatment of C. citratus extract.

Antibody titre. The treated group, which comprises the vaccinated and the unvaccinated samples, has a greater mean antibody titre gain of 117.74, compared to the control group that has 85.33 antibody titre mean. The vaccinated group has a 171.11 mean antibody titre gain, which is higher than the unvaccinated groups with 68.00 mean antibody titre gain. Both are greater than the control group.See Table 1

The average antibody titre of the treated sample increased. Vaccinated samples with 20-fold dilution of C. citratus aqueous extract had the highest antibody titre gain against Newcastle disease. Samples given with 1 mL undiluted C. citratus aqueous extract had the lowest antibody titre. The average antibody titres of vaccinated samples increase as the dilution factor increases. Unlike in vaccinated samples, unvaccinated samples given with undiluted 1 mL C. citratus aqueous leaf extract had the highest average antibody titre among all the other treatments in the unvaccinated group. Moreover, samples treated with 20-fold dilution of C. citratus aqueous had the lowest antibody titre in the unvaccinated group. The average antibody titres of unvaccinated samples decrease as the dilution factor increases. See Fig 1.

The treatment group C had the most significant p-value among all the vaccinated treatment groups. This implies that it had the most significant mean antibody titre gain. It is closely followed by treatment group B, and then C. On the other hand, the mean antibody titre gain of treatment group A was not significant.See Table 2

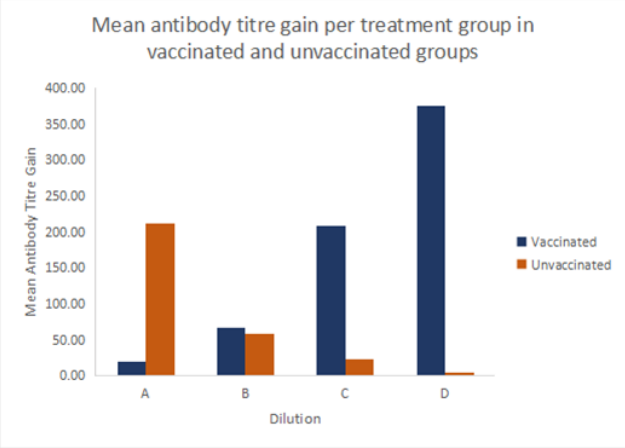

Fig. 1: Mean antibody titre gain per treatment group in vaccinated and unvaccinated groups

Live Weight. The treated group has a mean live weight gain of 78.93 g. This result is higher than the control which has a mean live weight gain of 47.33 g. The mean live weight gain of the unvaccinated samples has the highest mean live weight gain of 95.77 g, followed by the vaccinated with 62.08 g, while the control has gained the least weight.See Table 3

Among the treatment groups in vaccinated samples, samples treated with 1 mL undiluted C. citratus aqueous leaf extract had the highest mean live weight gain of 85.75 g, while samples treated with 10-fold dilution of C. citratus aqueous extract had the lowest with 31.50 g. Samples treated with 10-fold dilution C. citratus aqueous extract had the highest mean live weight gain among all the treatment groups in unvaccinated samples with 112.00 g. Meanwhile, samples treated with 5-fold dilution of C. citratus aqueous extract had the lowest among all the treatment groups in unvaccinated samples with 75.83 g of mean live weight gain.See Fig 2.

Survival Rate. The treated group had an overall survival rate of 81.25%. The unvaccinated group had a higher survival rate compared to the unvaccinated group. The control group has a 100% survival rate, higher compared to the survival rates of vaccinated and unvaccinated treatment groups. The vaccinated samples had the lowest average survival rate of 79.17%.See Table 4

Vaccinated chickens treated with 10-fold dilution of C. citratus aqueous extract is observed to have the highest survival rate among vaccinated treatment groups with a 100%, which means that none of the samples died. On the other hand, vaccinated samples from treated with undiluted 1 mL C. citratus aqueous extract and samples treated with 20-fold dilution of C. citratus aqueous extract have the lowest survival rate among vaccinated samples, with a rate of of 66.6%, indicating two deaths. The lowest survival rate in unvaccinated samples also

Table 2: Mean antibody titre gain per treatment group in vaccinated and unvaccinated groups

Vaccinated Treatment Groups	P-value
A	0.278
B	0.001
C	0.000
D	0.020

Table 3: Mean antibody titre gain per treatment group in vaccinated and unvaccinated groups

Group	Mean Live Weight Gain (g)	
Treated	Vaccinated	62.08
	Unvaccinated	95.77
	Overall	78.93
Control		47.33

come from the samples from treated with 20-fold dilution of C. citratus aqueous extract. Unvaccinated chickens from treated with 5-fold dilution of C. citratus aqueous extract and treated with 10-fold dilution of C. citratus aqueous extract had zero deaths indicating a 100% survival rate.See Fig 3. Vaccinated chickens treated with 10-fold dilution of C. citratus aqueous extract is observed to have the highest survival rate among vaccinated treatment groups with a 100%, which means that none of the samples died. On the other hand, vaccinated samples from treated with undiluted 1 mL C. citratus aqueous extract and samples treated with 20-fold dilution of C. citratus aqueous extract have the lowest survival rate among vaccinated samples, with a rate of of 66.6%, indicating two deaths. The lowest survival rate in unvaccinated samples also come from the samples from treated with 20-fold dilution of C. citratus aqueous extract. Unvaccinated chickens from treated with 5-fold dilution of C. citratus aqueous extract and treated with 10-fold dilution of C. citratus aqueous extract had zero deaths indicating a 100% survival rate.

Possible causes of deaths were considered with the guidance of Dr. Jaime Cabarles, Jr. Five out of eight deaths were speculated to be due to bacterial infection and environmental condition. Two were due to stress, possibly from mishandling.See Table 5

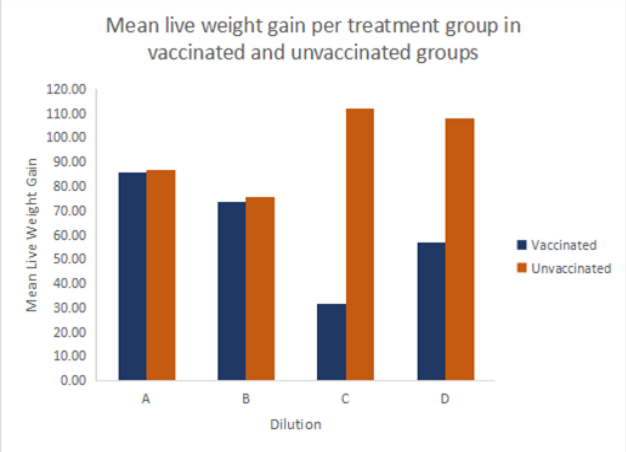

Fig. 2: Mean live weight gain per treatment group in vaccinated and unvaccinated groups

Table 4: Mean survival rate of sample groups

Group	Survival Rate	
Treated	Vaccinated	79.17
	Unvaccinated	87.50
	Overall	81.25
Control		100.00

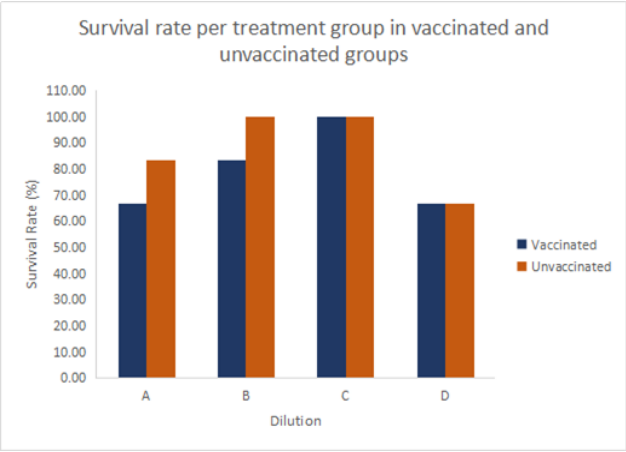

Fig. 3: The survival rate per treatment group in vaccinated and unvaccinated groups

Discussion. – The initial mean antibody titres in overall treated group comprised by the vaccinated and vaccinated samples significantly increased (p¡0.001) after given the treatment of C. citratus aqueous leaf extract, indicating the efficacy of C. citratus aqueous extract in boosting immunology of native chickens. Additionally, mean antibody titre gain of vaccinated samples is not significantly different (p¿0.05) from the mean antibody titre of unvaccinated samples. This implies that vaccination does not contribute to the effectiveness of C. citratus in increasing the antibody titre of native chickens infected with ND. Vaccination enhances the bodys immunity against diseases by using antigens to trigger the immune systems production of antibodies. The induced immune response enables the immune cells to quickly recognize and react to a specific disease-causing organism (Pharmaceutical Research and Manufacturers of America

Table 5: Possible causes of death of samples that died throughout the duration of the study

Sampl	Possible cause of death
VA5	stress after blood collection
VA6	unknown
VB4	coryza
VD2	extreme heat
VD3	stress
UA2	coryza
UD1	coryza
UD2	extreme heat

2013). However, failures in vaccination attenuation, vaccine regimes or administration can cause vaccine-related failures. Host-related failures are caused by genetics, immune status, age, health or nutritional status of the host (Wiedermann 2016). The vaccine loses its effectiveness due to these failures, thus the lack of the vaccine-induced antibody titre (Siegrist n.d.).

Mean antibody titre gain of vaccinated treatment groups differ significantly from each other (p¡0.05). The treatment group given with the 20-fold dilution of C. citratus aqueous leaf extract had the most significantly different mean antibody titre gain. The vaccinated group given with 5-fold, 10- fold, and 20-fold exhibited a significant increase in mean antibody titre gained. This means that the lower the concentration the higher is the antiviral activity against ND which contradicts the results of Hindumathy (2011) which states that the higher the concentration, the higher is its inhibition of microbial growth. The difference can be because Hindumathys study measured the bacterial activity of lemongrass. Bacteria are different from viruses because they can survive on their own, while virus reproduce inside a host cell, and is bigger compared to virus (National Institute of Allergy and Infectious Diseases 2009). Among the 5-fold, 10-fold, and 20-fold, the group receiving the 10-fold treatment exhibits the most significant increase followed by 5-fold, then by the 20-fold. After reaching 10-fold, the antibody gain decreases. According to Arnason et al. (1995), the antiviral activity is expressed as the virus titer reduction at the maximal nontoxic dose (MNTD) of the test substance. Toxic doses of the plant extract are dilutions that can deteriorate and degenerate the monolayer which causes the inability to determine the viral titre. In this case, 1-fold solution is a toxic dose of the extract that causes no significant increase in the antibody and dilutions more than 10-fold approaches the toxic dose, thus weakening the antiviral activity of C. citratus against ND.

The mean antibody titre of unvaccinated treatment groups on the other hand, do not significantly differ (p¿0.05) from each other. This implies that unvaccinated samples do not require a treatment diluted in a specific volume of distilled water for their antibody to increase when infected with ND. The initial mean live weight of the treated samples has increased significantly (p¡0.05) after given treatment of C. citratus aqueous leaf extract. There is no significant difference between the mean initial and the final live weight of samples from the control group. This indicates that C. citratus contributed to the growth of the treated samples. This can be attributed to the significant increase in the antibody of the samples because one of the symptoms of ND infection is loss of appetite, which leads to weight loss, but then administration of C. citratus treatment could have induced antibody production and eventually resulted to a significant increase in the live weight of the sample (Grimes 2002). C. citratus increased the level of protection of the treated samples. The survival rate of treated samples is higher than the normal survival rate of native chickens in the Philippines (Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development 2016) which suggests that C. citratus aqueous extract is effective for the protection of chickens against Newcastle disease.

It was found that there were eight recorded deaths which all came from the treated group. Only one death occurred after infection and during the treatment period. The other four deaths occurred during the acclimatization period, and then the other three after the infection but before the treatment (See Table 3 in appendix A). Factors such as stress, vitamin deficiency, and bacterial diseases can be attributed to the deaths before the virus infection. Stress can weaken the immune system of the chickens that make them prone to bacterial, fungal, and other types of diseases. Malfunction in the metabolism can also be a result to stress (Antony 2013). Heat stress, specifically, is one of the most important stressor that affects poultry production (Lara and Rostagno 2013). The housing utilized for the study was enclosed with plastic to avoid the spread of the airborne virus (Figure 1 in appendix C). This caused the heat to be trapped inside and eventually weakened the chickens. Vitamins and minerals are very important components of a chickens diet (The Poultry site 2008). According to Natividad (2017), the chicken samples used were showing signs of vitamin deficiency before infection. This deficiency, according to Davis (2015), can change the antibody count. For the duration of the study, the infection of coryza was observed (Figure 2.2 in appendix C). Coryza is an infectious disease caused by the bacterium Haemophilus paragallinarum that is characterized by sneezing, swollen face, and mucus from the eyes (Luciana et al. 2016). Before infection, some samples were already infected with coryza which could have affected their vulnerability to the Newcastle disease virus.

Conclusion. – In conclusion, C. citratus aqueous leaf extract is effective in boosting the immunity of native chickens when challenged with Newcastle disease. In addition, it is more practical to not vaccinate samples because unvaccinated samples infected with ND, when treated with C. citratus, has an increase in antibody titre and

live weight just as much as when it is vaccinated, and saves expenses on vaccination, and do not require a treatment diluted in a specific volume of distilled water for their antibody to increase when infected with ND. Vaccinated samples on the other hand require a treatment diluted in 9 mL distilled water for an optimal antibody increase when infected with ND.

Recommendations. – The 10-fold dilution of aqueous C. citratus leaf extract is recommended as an immune booster to chickens that have been infected with ND. It is also recommended to conduct further related studies to utilize an equal number of samples among the overall treated group and the control group. External factors such as heat stress and bacterial infections were observed. It is recommended to conduct the study in a more controlled laboratory. Also, the use of isolated Newcastle disease virus is recommended.

* * *

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Determination of the Golden Ratio in Selected 16^{th} to Mid - 19^{th} Century Churches of Panay Island, Central Philippines

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Abstract – Using numerical analysis and geometric construction, this study aimed to find out whether the Golden ratio is present in churches constructed during the 16th to mid-19th century in Panay, an island in central Philippines. The study focused on churches that met the criteria set by the National Historical Commission of the Philippines as historically significant namely: Pan-ay Church, Sta. Barbara Church, and Jaro Cathedral. A measuring device, that utilizes a laser, was used to take measurements. The values obtained were used to construct a floor and facade plan then proportionalized to determine their ratios. Ratios within five percent error margin of the Golden ratio were observed which showed that all three churches sampled exhibited the Golden ratio. However, access to historical documents is needed to conclude a purposeful intent of using the Golden ratio in the churches design.

Introduction. – The Spanish occupation of the Philippines lasted for over 300 years, during which the Spaniard colonists introduced many European influences into every facet of Filipino culture. One of these facets is architecture in the Philippines. Throughout the colonial period, the Spaniards oversaw the construction of many notable historic buildings across Luzon and Visayas, such as parks, mansions, and churches. Many of these buildings have been preserved to some degree by the efforts of the National Historical Commission of the Philippines.

In many historic religious buildings of the European world, there occurs an architectural phenomenon where the buildings designer intentionally utilizes the Golden Ratio in the dimensions of certain aspects of the building. Philippine architecture was influenced by the Spanish conquest. Along with the colonization of the Philippines, the Spaniards incorporated some of their culture into the countrys. One aspect of their culture, which was adapted into the archipelagos, was the building of churches as public places of Christian worship The Golden Ratio is a mathematical concept where the ratio between a larger quantity and a smaller quantity is the same as the ratio between the sum of both quantities and the larger quantity. The Golden ratio can be expressed into a line segment divided into two parts such that the ratio between the longer part to the shorter part is the same as the whole segment to the longer part. Studies have found that buildings utilizing the golden ratio subconsciously register as more pleasing to look at for the majority of people compared to buildings without the ratio.In knowing the ratio that occur in the buildings, we may have a basis of preserving our historical structures. This may also lead to finding a new ratio being used in the Philippines.It would also add an extra layer of cultural and historical value of the chosen heritage sites which in turn would help in the preservation efforts that are being allocated to the buildings.

The Spanish occupation of the Philippines lasted for over 300 years, during which the Spaniard colonists introduced many European influences into every facet of Filipino culture. One of these facets is architecture in the Philippines. Throughout the colonial period, the Spaniards oversaw the construction of many notable historic buildings across Luzon and Visayas, such as parks, mansions, and churches. Many of these buildings have been preserved to some degree by the efforts of the National Historical Commission of the Philippines.

Materials and Methods. – The methods are composed of five main parts; Illustration, Procurement, numerical analysis, geometric construction and percent deviation analysis. Buildings that were made during the Spanish colonial rule which was from 16th century until

the end of 19th century. The buildings should also be considered historically significant according to the criteria of the National Historical Commission of the Philippines (NHCP)

Illustration of Diagrams.. Diagrams of the facades and floor plan were drawn. The measurements taken in the next step were put onto these diagrams so the numerical analysis could be conducted accurately.

Procurement of Measurements. Availability of blueprints for the chosen buildings was checked. The chosen buildings had no available blueprints. Manual measurement was done using a laser measuring device and tape measure. The measurements in meters were taken at least three times and then averaged. Since the laser measurement device has an area for error of 1.5 mm, then each measurement obtained should be placed in a range of + 1.5 mm.. A laser measuring device was used to acquire the dimensions of specific parts of the churches facades and floor plans. Dimensions measured were marked on a diagram of the church by the researchers.

Numerical Analysis. The analysis was carried out by dividing a, the greater length, by b, the lesser length, then comparing the value obtained to the golden ratio given by the formula (1)

$$Ratio = a/b \approx 1.61812297 \tag{1}$$

Subsequent calculations follow using the same basic idea of dividing the greater by the lesser.

Geometric Construction. The measurements were inputted into Geogebra graphing software in order to construct a proportionally accurate diagram of the churchs features. Analysis was conducted on the diagrams through Geometrical Construction as described by Salleh where a rectangle with the Golden Ratio was generated and overlaid on the diagram.

Percent Deviation Analysis. The percent deviation was calculated by getting the absolute value of the value obtained by the equation (2)

$$(\frac{DerivedRatio}{GoldenRatio} * 100) - 100 \tag{2}$$

Only ratios that deviated from the Golden Ratio by 5% or less were considered relevant to the studys goal. Materials. A Bosch laser measuring device with a 70m effective range was used to take measurements of the churches features. During the data gathering, a pencil diagram was used to take note of the measurements of specific areas of the structures. Data was analysed through use of scientific calculators, Microsoft Excel spreadsheets, and Geogebra graphing software.

Results. – Numerical data was visually verified through geometric construction on the diagrams.

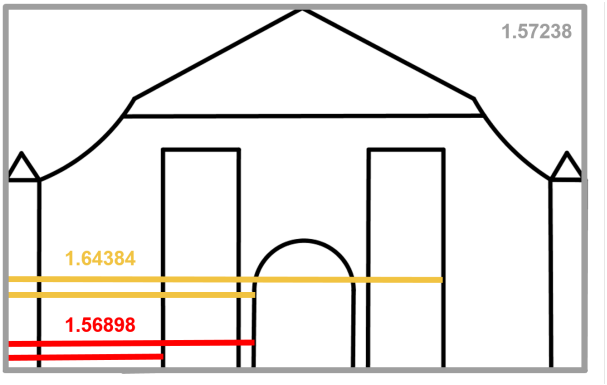

Fig. 1: Golden ratios found on the front facade of Jaro Cathedral

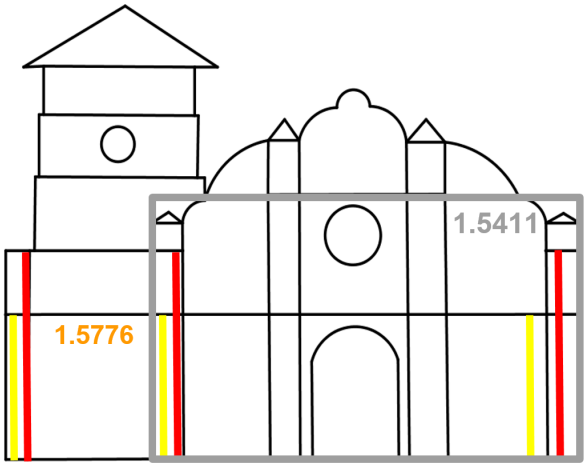

Fig. 2: Golden ratio found on the front facade of Pan-ay Church

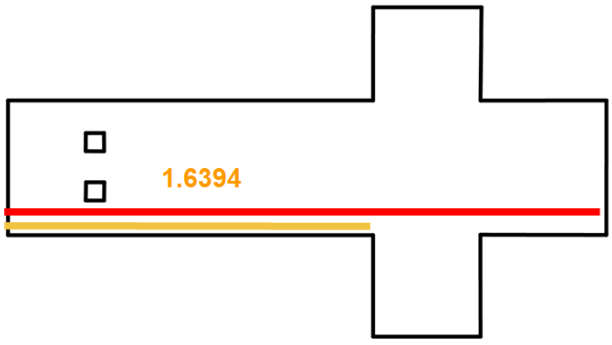

Fig. 3: Golden ratio found on the floor plan of Pan-ay Church

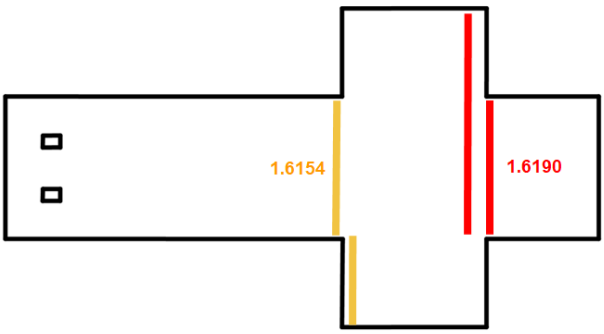

Fig. 4: Golden ratio found on the floor plan of Sta. Barbara Church

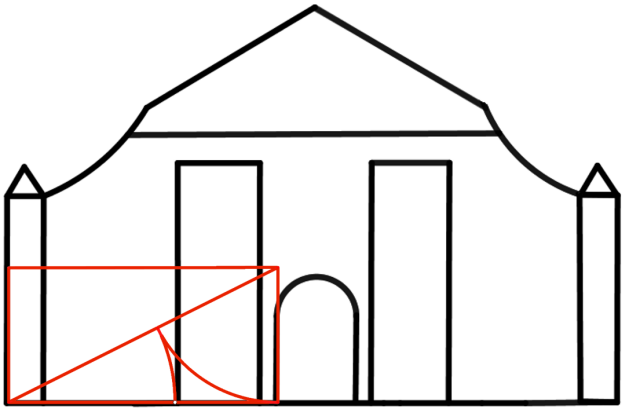

Fig. 5: Geometric construction to verify golden ratio in Jaro Cathedral facade.

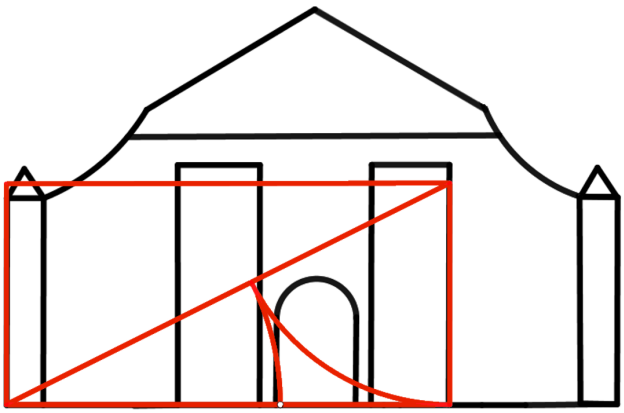

Fig. 6: Geometric construction to verify golden ratio in Jaro Cathedral facade.

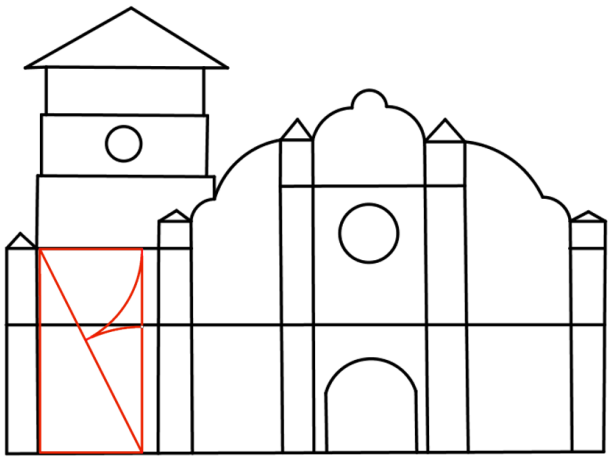

Fig. 7: Geometric construction to verify golden ratio in Pan-ay Church facade.

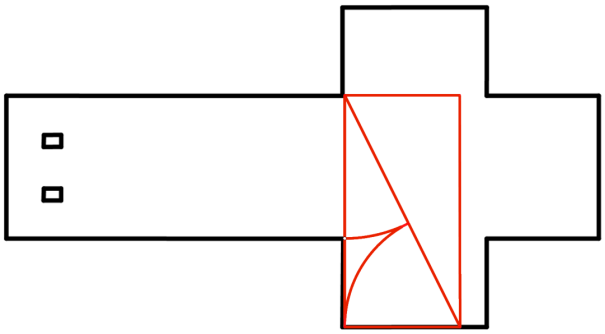

Fig. 8: Geometric construction to verify golden ratio in Sta. Barbara Church floorplan.

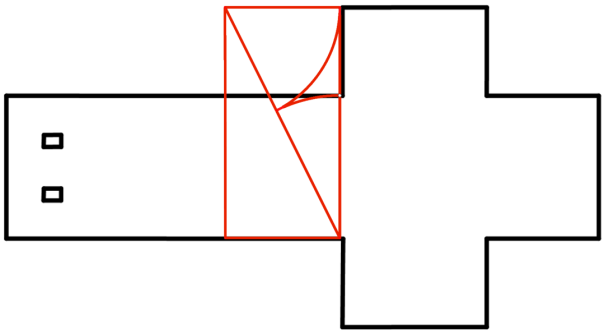

Fig. 9: Geometric construction to verify golden ratio in Sta. Barbara Church floorplan.

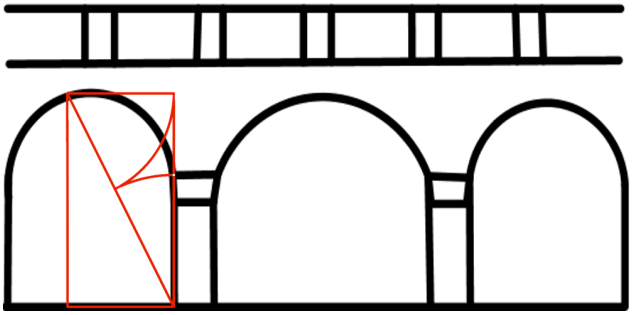

Fig. 10: Geometric construction to verify golden ratio in Sta. Barbara Church arches.

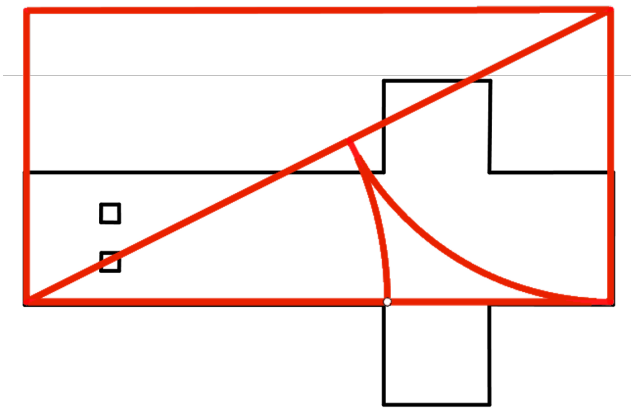

Fig. 11: Geometric construction to verify golden ratio in Pan-ay Church floorplan.

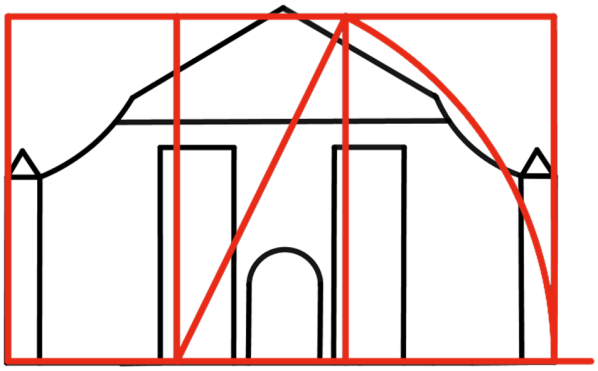

Fig. 12: Geometric construction to verify golden rectangle in Jaro Cathedral facade.

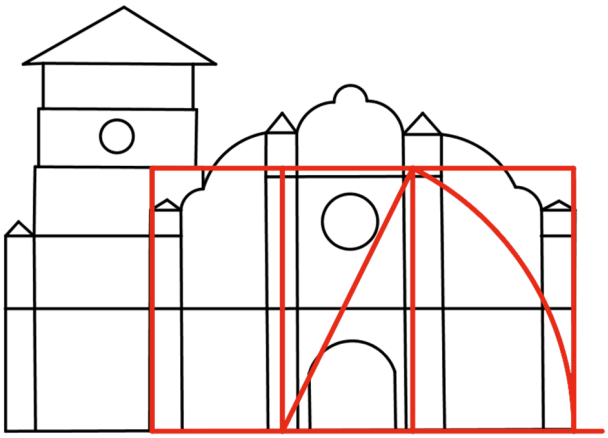

Fig. 13: Geometric construction to verify golden rectangle in Pan-ay Church facade.

Discussion. – The data that was collected showed varying values for the percent deviation with eight being found to be relevant to the Golden ratio. For the front facade of the Jaro Cathedral, the first ratio is denoted by letters A and a in Figure 1. The shorter length is represented by A from the leftmost part of the facade to the left-most of the column to the left of the door. The longer length, represented by B, runs from the leftmost part of the facade to the left of the door. The second ratio has the lengths C and D. The third ratio, unlike the previous two, does not have lengths aligned on one axis. Rather, it is a Golden rectangle with the longer length being the width of the facade F and the shorter length being its height E.

From the front facade of Pan-ay Church, one can observe two significant ratios. The first has lengths represented by letters G and H in Figure 2. Starting from the floor, they measure up to the horizontal section above the door and the horizontal section above that respectively. The second is another Golden rectangle with the width being the facade minus the section including the bell tower, J, and the height being the top of the column second from the floor, I. From Pan-ay Churchs floor plan, we have found one observable ratio. Its lengths were from the door to the other end of the building, K and from the door to where the floor begins to split into the side entrances, L.

The ratios found in Sta. Barbara Church were all within the floorplan as shown in Figure 4. Although three ratios are shown to be significant, two of those can be counted as one. The ratios represented by lengths M and N, and O and P are deemed to be the same ratio. This is because M and P are of the same length and N is merely the sum of lengths O and P. This can be said because both of the side entrances are approximately the same width. The last

ratio was found not exactly within the floorplan, but on an arch inside the church. The longer length of this ratio is the height of the arch R, and the shorter length is the height of a column that is part of the arch itself Q.

In addition, geometrical construction was done to show a more concrete visualization of the golden ratio using the data gathered from the numerical analysis. The geometrical construction was made using the significant ratios found with their P and Q values being used as basis for the initial dimensions of the squares and rectangles needed for the geometrical construction. Figure 5 , Figure 6 , and Figure 12 used the significant ratios from Jaro church. Figure 7, Figure 11 ,and Figure 13 used the significant ratios from Pan-ay church. Figure 8 , Figure 9 , and Figure 10 showed the significant ratios from Sta. Barbara church. The geometric constructions aligned with the location of significant ratios on the diagram of the churches. These constructions confirmed the areas and precision of the ratios that was derived from the numerical analysis.

Conclusion. – Evidence and traces of the Golden ratio can be found in each of the churches which are represented by the highlighted marks on the tables. These ratios are within 5% error deviation from the Golden ratio and have been derived from the floor plans and facades of the churches that were studied. Although the Golden ratio exists in each of the churches, one cannot conclude that it was intentionally incorporated into the entire design of the buildings. This can be said considering that the significant ratios found were either concentrated in one facade or floor plan, or scattered across the building.

* * *

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We would also like to thank our parents for their ever present support and financial assistance; and We would finally like to thank God, for creating the beauty of nature, architecture, culture and math for humankind to appreciate and learn more on.

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Antimicrobial Activity of Callyspongia sp From Culasi, Antique Against Ice-Ice Promoting Bacteria, Bacillus cereus, Brevundimonas diminuta, and Vibrio alginolyticus

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Abstract – Seaweeds are one of the Philippines' major exports. However, seaweed farms are often damaged by ice-ice, a disease condition that turns the branches of the seaweeds into white, fragile branches that easily come off. Previous studies have shown that *Bacillus cereus*, *Brevundimonas diminuta*, and *Vibrio alginolyticus* are known to be associated with and causes ice-ice disease. Methods known to use other organisms to control or inhibit the bacteria that cause the disease are polyculture cultivation and bioprotection. *Callyspongia sp.* was concluded to have potential as bioprotector in seaweed cultivation; however, other studies suggest that sponges at different locations may result to different antimicrobial activity due to the different symbionts present in different environments, which are responsible to the production of many chemical compounds. This study tested the antibacterial activity of *Callyspongia sp.* from Mararison Island, Culasi, Antique against *Bacillus cereus*, *Brevundimonas diminuta*, and *Vibrio alginolyticus*. *Callyspongia sp.* crude extract was extracted using 200 mL of methanol for every 25 g of *Callyspongia sp.* Broth dilution test was used and serial dilution was performed to produce an eight-fold concentration. Each bacterium was inoculated into the treated test tubes containing the crude extract and three replicates were used. Data was collected using a spectrophotometer and was analyzed using ANOVA. Results showed that the crude extract had significant effects in the growth of *Bacillus cereus* and *Vibrio alginolyticus* especially in 4.8x10-3 g/mL and 4.8x10-4 g/mL concentrations and for *Brevundimonas diminuta*, extract concentration 4.8x10-3 g/mL significantly inhibited its growth. Results show that *Callyspongia sp.* has the ability to inhibit the growth of bacteria associated with and causing ice-ice disease and has the potential to be used as a bioprotector in seaweed cultivation.

Introduction. – Seaweeds are one of the Philippines major exported products, which constitutes 60 percent of the Philippines aquaculture produce. In fact, the Philippines was once considered as the worlds leading supplier of seaweeds, exporting up to 400,000 metric tons of fresh seaweeds which comprises about 80 percent of the total world supply [1].

Seaweed farms are often damaged by ice-ice, a disease condition that turns the branches of the seaweeds into white, fragile branches that easily come off with little disturbance [2]. It has caused a negative growth of 42.8 percent in the aquaculture production of Zamboanga and a drop of 22 percent in the aquaculture industry of the Philippines in 2003 [3]. It has been reported by Largo (2002) that seaweed farms in Bohol, Batangas, and Iloilo have ceased to exist due to ice-ice disease. According to statistics, the Philippines had a total seaweed production of 84,500 tons and semi-refined carrageenan was only 2,592 metric tons in 2010 [4].

Ice-ice outbreaks are often associated with environmental stress such as change in salinity, temperature, and amount of light received by the organisms. When the seaweeds are stressed, they release organic substances that attract bacteria belonging to the *Vibrio- Aeromonas* and *Cytophaga-Flavobacterium* complexes, a group of bacteria that has the ability to break down cells and cause diseases. The most common bacteria discovered to promote ice-ice disease are *Vibrio sp.* and *Cytophaga sp.* [5]. Results from the study of Aris (2011) showed that bacterial species isolated from *Kappaphycus alvarezii* suffering from

ice-ice disease were *Vibrio alginolyticus*, *Brevundimonas cepacia*,*Flavobacterium meningosepticum*, *Brevundimonas diminuta* and *Plesiomonas shigelloides*, while in the study by Tokan et al. (2014), they were able to identify the specific species of bacteria that causes ice-ice disease namely, *Brevundimonas nigricaciens*, *Brevundimonas fluorescens*, *Vibrio granii*, *Bacillus cereus* and *Vibrio agar liquefaciens*.

One of the methods done in order to control ice-ice is planting other species of seaweeds such as *Achantophora spicifera*, *Caulerpa racemosa*, etc, in the same field to control the bacteria promoting ice-ice disease [6]. This technique is called polyculture cultivation, a process where different species of plants are cultivated in the same field resulting in the reduction of pests [7]. However, species such as Achantophora spicifera and Caulerpa racemosa are considered pests or invasive plants due to their fast growth. Although they may protect the seaweed farm from ice-ice disease, their invasive growth could potentially hinder the seaweed farms growth instead [8] [9].

Similar to polyculture cultivation, another method is to use live organisms in order to suppress a pest or pathogen to an acceptable level. These organisms serve as bioprotectors, biological control, or biosecurity. The goal of using biological control is to suppress pests or pathogens and to restore the native plant community [10]

In order to determine the potential of an organism as a bioprotector against ice-ice disease promoting bacteria, it must be a natural source of antibacterial compounds, chemical compounds that can kill bacteria, which it naturally releases into its environment [6].

Tokan and Lodo (2008) showed that three species of sponges, *Callyspongia biru*, *Callyspongia subarmigera* and *Callyspongia sp.* have antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*. Consequently, another study was conducted in order to determine whether *Callyspongia* species have antibacterial effects or potency against ice-ice causing bacteria and can be a potential bioprotector against ice-ice disease on seaweeds. It was observed that the three types of *Callyspongi*a showed different effects on the inhibition of bacteria. *Callyspongia biru* showed the most potent antibacterial activity especially having the most vulnerability against *Acinetobacter*. Based on the observations, *Callyspongia* has the potential to be used as a bioprotector to biologically control ice-ice disease [6]. Currently, there are no other known biological controls other than *Callyspongia* that are used against ice-ice disease on seaweeds.

A study of Qian et al. (2006) demonstrated that two *Callyspongia* species from the same genus that were collected from two bio-geographically different zones accommodated distinct bacterial communities but produced relatively similar secondary metabolites. Based on this, it can be inferred that at different locations, sponges may have a different composition of microbial symbionts, which are known to be responsible to the production of many chemical compounds extracted from sponges [11] [12]. There is also the possibility that different environments may cause the differences between metabolic activities of sponges [13]

This study aims to determine the antimicrobial activity of Callyspongia sp. found in Mararison Island, Culasi, Antique against ice-ice disease promoting bacteria and its potential as a bioprotector in cultivation of seaweeds.

Methods. – *Acquisition of bacteria.* Cultures of *Bacillus cereus*, *Brevundimonas diminuta*, and *Vibrio alginolyticus* were purchased from the National Institute of Molecular Biology and Biotechnology (BIOTECH) in Los Baos, Laguna, and were stored in the refrigerator at 4C. Under the laminar flow hood, *Brevundimonas diminuta* was directly inoculated into a test tube containing Tryptic Soy Broth while *Bacillus cereus* and *Vibrio alginolyticus* were directly inoculated into test tubes containing 6 percent NaCl Nutrient Broth in order to obtain subcultures. The test tubes were covered with Parafilm and were incubated at an optimal temperature of 30C for two days.

Media Preparation. In a 500 mL media bottle, two batches of broth media were prepared to obtain a total of 620 mL of 6 percent NaCl Nutrient broth and one batch was prepared to obtain 330 mL of Tryptic Soy Broth. The prepared broth was transferred into their respective test tubes, 33 for TSA and 62 for 6 percent NaCl NB. Each test tube was capped using cotton balls, covered with aluminum foil and gathered for sterilization. The prepared media was autoclaved at optimal temperature of 121C and pressure of 15 psi for 15 mins, cooled and stored in the refrigerator prior to usage.

Collection of samples. One kg of Callyspongia sp. was collected eight to ten meters underwater in the seas of Mararison Island, Culasi, Antique by local divers. They were then cleaned and transported to Philippine Science High School - Western Visayas Campus.

Drying. The collected samples were sundried for 6 hours under sunlight and air dried in the laboratory at night for two days until no more moisture was present in the sponge. They were then minced into the finest possible particle that can be obtained.

Extraction. The crude extract was extracted by using 200 mL of methanol for every 25 g of dried Callyspongia sp. The mixture was brought to the Department of Science and Technology (DOST) to collect the extract by evaporating the methanol using a rotary evaporator. The remaining evaporation was done in Philippine Science High School Western Visayas Campus using a water bath at 40C. The final amount collected was 0.53 grams and diluted to obtain an initial concentration of 4.8×10^{-4} g/mL.

Antibacterial Screening. Serial dilution was performed in order to dilute the extract in the prepared broths in 8-fold concentrations. The bacteria was then inoculated into the treated test tubes. The test tubes were then incubated for 18-24 hours under optimal temperature of 30C. The turbidity of each test tube was measured using UV-2100 Spectrophotometer with a wavelength of 600 m.

Statistical Analysis. One-way ANOVA was used to find the significant difference between the treated and untreated groups for every concentration. Using the LSD Post-hoc Test, each concentration were compared with each other to determine whether they are significantly different from each other.

Results and Discussion. – The mean absorbances of *Bacillus cereus* treated with the different concentrations of crude extract are shown to be lower than that of the untreated group. The first two concentrations exhibited the lowest absorbance while concentrations $4.8x10^{-6}$ g/mL and $4.8x10^{-8}$ g/mL exhibited the highest as shown in Figure 1. After statistical analysis using One-Way ANOVA, the extract showed an effect in the growth of Bacillus cereus. All of the concentrations showed a significant difference when compared to that of the untreated group. The LSD post-hoc test showed that absorbance reading for concentrations $4.8x10^{-5}$ g/mL to $4.8x10^{-10}$ g/mL are not significantly different from each other and among all the concentrations, absorbance is significantly lower when Bacillus cereus is exposed to extract concentrations of $4.8x10^{-3}$ g/mL and $4.8x10^{-4}$ g/mL.

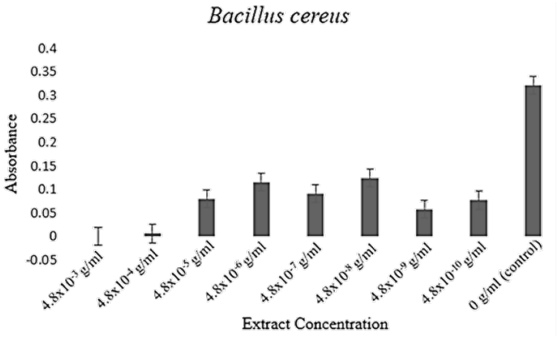

Fig. 1: Absorbance level means of both treated and untreated groups in different concentrations against *Bacillus cereus*.

The results of the treatment against *Vibrio alginolyticus* show that the means were relatively lower than that of the untreated group. The one with the highest concentration ($4.8x10^{-3}$ g/mL) showed the lowest absorbance while the lowest concentration ($4.8x10^{-8}$ g/mL) exhibited the highest as shown in Figure 2. Statistical analysis using One-Way ANOVA showed that all of the concentrations had significant effect on the growth of *Vibrio alginolyticus*. Similar to the results of *Bacillus cereus*, the LSD post-hoc test showed that absorbance reading for concentrations $4.8x10^{-5}$ g/mL to $4.8x10^{-10}$ g/mL are not significantly different from each other and among all the concentrations, absorbance is significantly lower when *Vibrio alginolyticus* is exposed to extract concentrations of $4.8x10^{-3}$ g/mL and $4.8x10^{-4}$ g/mL.

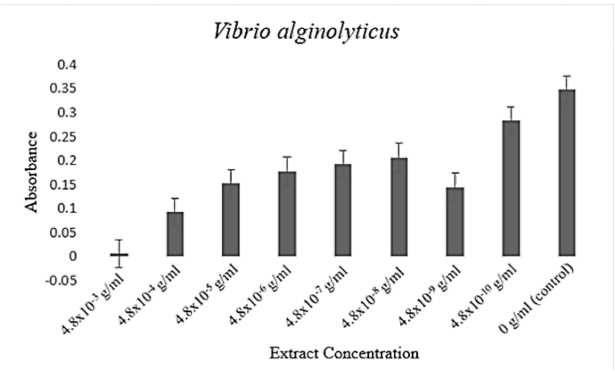

Fig. 2: Absorbance level means of both treated and untreated groups in different concentrations against *Vibrio alginolyticus*.

The means of the treated group for *Brevundimonas diminuta* were all lower than the untreated group. The lowest absorbance values were from concentrations one ($4.8x10^{-3}$ g/mL), five ($4.8x10^{-7}$ g/mL), and six ($4.8x10^{-8}$ g/mL) while the highest ones were from concentrations two ($4.8x10^{-4}$ g/mL) and three ($4.8x10^{-5}$ g/mL) as shown in Figure 3. One-way ANOVA showed that the mean absorbance reading of *Brevundimonas diminuta* treated with different extract concentrations are all significantly lower than the untreated group. The LSD post-hoc test showed that absorbance reading for concentrations $4.8x10^{-4}$ g/mL to $4.8x10^{-6}$ g/mL and $4.8x10^{-9}$ g/mL to $4.8x10^{-10}$ g/mL are not significantly different from each other and among all the concentrations, absorbance is significantly lower when the bacteria is exposed to extract concentrations of $4.8x10^{-4}$ g/mL, $4.8x10^{-7}$ g/mL, and $4.8x10^{-8}$ g/mL.

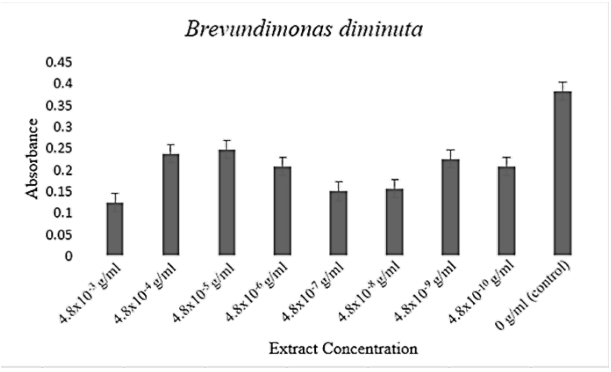

Fig. 3: Absorbance level means of both treated and untreated groups in different concentrations against *Brevundimonas diminuta*.

Previous studies have shown that Callyspongia sp. has

anti-larval, antifungal and antimicrobial activity. Different species of Callyspongia sp has inhibited the growth of microorganisms such as tubeworms, fungus and bacteria [14] [15] [16] [17]

Diverse and unique bioactive metabolites have been isolated from sponges. There are various antibiotics that are active against human pathogens and other bacteria foreign to sponges. Examples of these are plakortin from *Plakortis halichondroides*, manoalide from *Luffariella variabilis*, furospongin-1 from *Spongia officinalis*, and aerothionin from *Aplysina gerardogreeni* [18] The group of secondary metabolites, 3-alkylpiperidine alkaloids, serve as a chemical marker for the order where *Callyspongia sp.* belongs. It has been recorded that these compounds exhibit antifungal, antimicrobial, and anticancer activity [19] However, sponges from different locations have varying metabolic activities [13]. This is due to the different bacterial communities found in varying environments [20] and this may affect the composition of the microbial symbionts, which are known to be responsible to the production of many chemical compounds extracted from the sponges [11] [12]. This poses a possibility that different sponges from different places will not have the same effect on the same bacteria. The results have shown that the sponge collected from Mararison Island, Culasi, Antique have antimicrobial activity against certain ice-ice disease-causing bacteria.

The sponge extract notably had the highest bacterial inhibition against *Bacillus cereus* and the lowest against *Vibrio alginolyticus*. The resistance of *Vibrio alginolyticus* against the sponge extract may be due to it being a gram-negative bacteria. Gram-negative bacteria are known to have more layers of cell membranes which make it harder for antibiotics to penetrate the bacteria and inhibit the bacterial colonies growth. Meanwhile, gram-positive bacteria are more susceptible to antibiotics due to having only a single layer of cell membrane - even with its thick peptidoglycan layer [21] [22]. In a study by Mustapha et al (2013), it has been shown that *Vibrio alginolyticus* is generally resistant to antibiotics like penicillin and vancomycin. Its resistance is also more prominent when compared to other strains from the same family like *Vibrio cholerae*. *Bacillus cereus* is a gram-positive bacteria making it less resistant to the extract as compared to the other two bacteria which are gram-negative. This has also been shown in the study of McCaffrey and Endean (1985), where the gram-positive bacteria were generally more susceptible to the sponge extracts than the gram-negative bacteria as exhibited by the greater inhibition of *Thorecia vasiforis* against *Staphylococcus aureus*, a gram-positive bacteria, than *Pseudomonas aeruginosa*, a gram-negative bacteria . Contrastingly, the studies of Bergquist and Bedford (1978) and Amade et al. (1982) have exhibited that the gram-negative strains were more sensitive to the antimicrobial activity of sponges than the gram-positive bacteria. This has led them to propose that sponges contain active constituents that make them improve the capturing and digestion of particulate organic matter by prompting the slight clustering of vulnerable bacteria, therefore enlarging the size of particles that are captured at the choanocyte chambers. This theory of Bergquist and Bedford, however, seems unlikely due to the contrasting results from the study of McCaffrey and Endean (1985) as well as in an earlier study conducted by Burkholder and Ruetzler (1969). Out of the three bacteria that were tested, only *Vibrio alginolyticus* exhibited a trend in relation with the concentration of the extract. Meanwhile, the other two - *Brevundimonas diminuta* and *Bacillus cereus* - had fluctuating sets of data despite having all of them having significant differences when compared to the untreated counterparts. With the help of One-Way ANOVA, it has been shown that there are sets of data with greater standard deviations compared to others. This may be due to the presence of erroneous data. According to Dr. Ananya Mandal [10], biosecurity or bioprotection refers to measures that are taken to stop the spread or introduction of harmful organisms to human, animal and plant life. The measures taken are a combination of processes and systems that have been put in place by bioscience laboratories, customs agents and agricultural managers to prevent the use of dangerous pathogens and toxins. Its goal is to protect human health and to increase and protect agricultural produce through the prevention, control and management of biological risk factors. In order to determine the potential of an organism as a bioprotector against ice-ice disease promoting bacteria, it must be a natural source of antibacterial, a chemical compound that can kill bacteria, which it naturally releases into its environment [6]. Studies have shown that sponges naturally have antimicrobial compounds which they release in their surrounding environment. It has been observed that the sponge surfaces, including surrounding waters and nearby substrates where these sponges are located, rarely get affected by fouling organisms. However, some of these antimicrobial substances are shown to be selective and specific to certain strains of bacteria and other microorganisms [18]. With the results of the study, it can be said that Callyspongia sp. from Mararison Island, Culasi, Antique has the potential to be a bioprotector in the cultivation of seaweeds as it exhibited potent antibacterial activity against the strains of bacteria that cause ice-ice disease. However, it should be considered that the antibacterial compounds from the sponge were extracted from it and a high concentration is needed in order to effectively inhibit the growth of the pathogens. Although at lower concentrations, the extract can still significantly reduce the pathogens, the antimicrobial activity is still lower when compared to high concentrations. There may be a difference as to how the sponge will act when it is put in the natural environment. The antimicrobial compounds it will release in its surroundings may not be the same concentration as the extract needed to inhibit the growth of the bacteria that causes ice-ice disease.

Conclusion. – Callyspongia sp. from Mararison Island, Culasi, Antique has the potential to be a bioprotector against ice-ice disease causing bacteria. It has shown potent antimicrobial activity against the bacterial strains, *Bacillus cereus*, *Vibrio alginolyticus*, and *Brevundimonas diminuta*. The most effective extract concentrations against *Bacillus cereus* are $4.8x10^{-3}$ g/mL and $4.8x10^{-4}$ g/mL; for *Vibrio alginolyticus* extract concentrations $4.8x10^{-3}$ g/mL and $4.8x10^{-4}$ g/mL significantly inhibited its growth; and for *Brevundimonas diminuta*, the most effective concentration is $4.8x10^{-3}$ g/mL.

Recommendations. – In order to further isolate the specific compounds that causes the antimicrobial activity, it is recommended to perform thin-layer chromatography on the sponge extract.

Additionally, a more accurate method for determining the antimicrobial activity of the sponge extract is to use the disk diffusion assay since it measures the zone of inhibition which effectively quantifies the bacterial growth.

Furthermore, the spicules of the collected sponge should be investigated by an expert to validly confirm its taxonomic identity. Moreover, field experiments can be done in order to test whether this antimicrobial activity is also exhibited in the natural environment. Instead of extracting the sponges, the secreted enzymes may be tested to determine its antimicrobial activity in a natural setting.

* * *

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Compound Identification of the Methanolic Gut and Gonad Extracts of T. gratilla

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Abstract – Taking to account that microbial populations in seawater may reach numbers as high as 106/mL, it can be concluded that marine organisms, including sea urchins, are exposed in much harsher conditions in comparison to their terrestrial counterparts. Previous researches found out that various parts of *Tripneustes gratilla* manifest antimicrobial properties against an array of pathogenic bacteria, wherein the highest antimicrobial effect resulted from the guts and gonad extracts; however, the bioactive compounds present in this extract are still unknown. Identification of such bioactive compounds is necessary for the confirmation and correlation of the bactericidal effects and also the possibility of discovering novel compounds from this species. The extract was subjected to gas chromatography-mass spectrometry. The oven temperature was programmed from 110°C, with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. The whole sample ran for 36 minutes with the results identified by the systems spectral database. The chromatogram results showed a total of six prominent peaks. The compound n-hexadecanoic acid, which manifests antibacterial properties, was present in the sample alongside benzo[a]pyrene, 2,4-diisocyanato-1-methyl-benzene, both of which are carcinogenic and methadone N-oxide, a urinary metabolite; the presence of the two compounds suggest combustion of the sample due to the high temperatures utilized for the GC-MS procedure. From this, it can be concluded that certain compounds are present in the *Tripneustes gratilla* gut and gonads extract that can be attributed for its antimicrobial activity.

Introduction. – Sea urchins are globular, spiny, hard-shelled marine organisms that inhabit the seabed or burrow into the sand. There are about 1,000 sea urchin species known worldwide, 64 of which are from the Philippines (Appeltans et al. 2012). They are popular for their roe (gonad) and have been described as a delicacy in many countries (Andrew et al. 2002). The class comprising sea urchins are home to myriads of bioactive compounds. Species such as *Paracentrotus lividus*, and *Strongylocentrotus droebachiensis* have been used as sources for novel antimicrobial peptides (Schilacchi et al. 2013; Li et al. 2008). On the other hand, *Diadema setosum*, and *Salmacis virgulata* exhibit antibacterial activity on bacterial species such as *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Marimuthu et al. 2015; Shankarlal et al. 2011). Their ovaries and shells, respectively, served as sources for extracts with antimicrobial activity. Similar to other sea urchin species, the polar and nonpolar extracts from the gonad and body wall of the sea urchin *Tripneustes gratilla* have shown various antioxidant activities (Chen et. al 2015). In a separate study, it was shown that the gut and gonad extracts of *T. gratilla* have shown capacity for antimicrobial activity against various test microorganisms such as *E. coli*, *S. typhi*, *P. aeruginosa*, *S. sonnei*, *S. aureus* and *Penicillium* spp (Abubakar et al. 2012); however, the biochemical composition of the methanolic extracts of *T. gratilla* has still not been studied. This study aims for the identification of the compounds present in the methanolic gut and gonad extracts of *T. gratilla*.

Methods. – ***Collection and Transport of Tripneustes gratilla samples*** Four *T. gratilla* sea urchins, 5 cm to 10 cm in diameter, were collected at Brgy. Can-

hawan, Nueva Valencia, Guimaras.

Extraction and Separation of Guts and Gonads - The gut and gonads were collected by cutting through the circumference using a dissecting scalpel and surgical scissors. The collected samples were then homogenized. Filtration process involved the use of Whatman filter paper. The extract was then concentrated using a rotary evaporator with a heating temperature of 40°C. Sep-PakTM C18 cartridge, used to remove fats and nonpolar substances, was prepared by injecting one millimeter of 100 methanol into the column. The cleaned-up sample was then subjected to GC-MS analysis at UP Visayas Freshwater and Aquaculture laboratory.

Gas Chromatography -Mass Spectrometry analysis - GC- MS analysis is generally used for the identification of bioactive compounds. GC analysis was performed in partial accordance with the methods of Vanathi Devi and Selvaraj (2015). The sample that was directly injected in a Claurus 600 Perkin ElmerTM gas chromatography system with 99.9995 He as carrier gas. The GC inlet temperature was set at 280°C, and source temperature set at 230°C. The oven temperature was programmed from 110C, with an increase of 10C/min until reaching 200°C, and 5°C/min until 280°C, ending with a 9 minute isothermal at 280°C. The whole sample was run for 36 minutes. The identification was carried out using a Claurus 600T Perkin ElmerTM Mass Spectrometer with a spectral database installed with the system. The MS method involved identification of compounds from 50.00 to 550.00 amu; electron ionization with 255 L/sec turbomolecular pump with EI voltage of 70 eV. The libraries for identification are the following: NIST 08 MS Library and MS Search Program v.20f.; NIST/EPA/NIH Mass Spectral Library 2008 (NIST 08) and NIST Mass Spectral Search Program (Version 2.0f). The significant peaks recorded were identified with their corresponding compounds.

Solid Phase Microextraction (SPME) - Solid phase microextraction is an extraction method for sample matrix usually consisting of purge-and-trap or headspace methods for the concentration of volatile and semi-volatile compounds. Prior to the GC-MS injection process, SPME was first performed using polyacrylate fibre specific for the procurement of polar semi-volatile compounds. The procedure was only set at 40°C to prevent the degradation of any compound in the sample. At this specific temperature, the extract was allowed to evaporate and accumulate in the fibre injected around the headspace area of the container. After this process, the polyacrylate fibre was then injected to the GC-MS system.

Data Analysis - A GC-MS analytical report was made regarding the compounds identified in the gut and gonad fractions of *Tripneustes gratilla*.

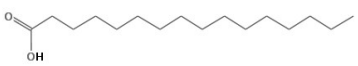
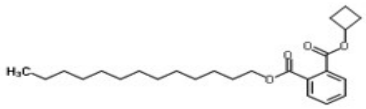
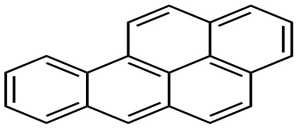
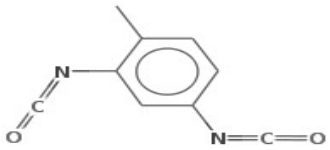
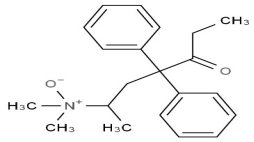
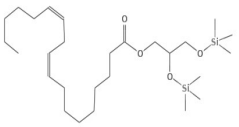
Results and Discussion. – The GC-MS analysis identified six compounds pooled from both direct injection and SPME methods. The compounds are the following: n-hexadecanoic acid, phthalic acid, cyclobutyl tridecyl ester, benzo[a]pyrene, 2,4-diisocyanato-1-methylbenzene, methadone N-oxide, and 1-Monolinoleoylglycerol trimethylsilyl ether.

The methanolic guts and gonad extracts of *T. gratilla*, as stated by the study of AbuBakar and others (2012), have been recorded to exhibit antimicrobial activity on an array of bacterial species. From the GC-MS analysis it was noted that there were six recorded compounds: n-hexadecanoic acid; phthalic acid, cyclobutyl tridecyl ester; benzo[a]pyrene; 2,4-diisocyanato-1-methylbenzene; methadone N-oxide; and 1-monolinoleoylglycerol trimethylsilyl ether.

The major component of the extract is n-hexadecanoic acid, which is known for its antibacterial, antioxidant, hypocholesterolemic and anti-inflammatory properties (Sermakkani and Thangapandian 2012). This saturated fatty acid can normally be found in the different tissues of plants and animals; specifically, it is a component of the embryos of sea urchin *Strongylocentrotus purpuratus*, as well as a constituent of the roe of sea urchin species *A. crassipina* and *P. depressus* (Bolanowski et al. 1984). Aside from appearing in the tissues of urchins, n-hexadecanoic acid was also present in numerous organisms that *T. gratilla* feeds on. Its variant, hexadecanoic acid, was found to comprise the antibacterial fractions of microalgal species *Cystoseira myrica* and *Sargassum boveanum*. The two aforementioned species are of the same genus as *Cystoseira trinodis* and *Sargassum flavicans* with the latter two being the most preferred microalgal species in the diet of sea urchin *T. gratilla* (Seymour et al. 2013). Additionally, hexadecanoic acid methyl ester, another variant of palmitic acid, has also been observed to be a constituent of the sea grass species *Syringodium isoetifolium*, which is also a preferred food option for *T. gratilla* (Vaitlingon et al. 2003). The active fractions of the sea grass exhibit antifouling and toxic properties which coincide with the documented antibacterial activity of the sea urchin (Iyapparaj et al. 2014). With the accumulated literature, it can then be inferred that the n-hexadecanoic acid recorded in the GC-MS analysis is either contained in the sea urchin tissues or is ingested by the sea urchin as it feeds upon seagrasses or microalgae.

In various researches, cyclobutyl tridecyl ester phthalate ($C_{25}H_{38}$) was found to constitute plant extracts of species such as *Hertia cheirifolia*, *Lagerstroemia speciosa*, and *Dracocephalum heterophyllum* (Numunov et al. 2013; Durai and Balamurugan). The three plant species exhibited antibacterial activity on bacterial species such as *Staphylococcus aureus*; however, the three species are not marine inhabitants and thus, the presence of this com-

Table 1. Compound Information

Name of Compound	Synonyms	Chemical Structure
n-hexadecanoic acid	palmitic acid hexadecanoic acid	
phthalic acid, cyclbutyl tridecyl ester	Cyclobutyl tridecyl phthalate 1,2-Benzenedicarboxylic acid, cyclobutyl tridecyl ester	
benzo-a-pyrene	3,4-Benzopyrene; Benzo[pqr]tetraphene	
benzene, 2,4-diisocyanato-1-methyl-	2,4-Diisocyanato-1-methylbenzene, 2,4-Tolylene diisocyanate;	
methadone N-oxide	N/A	
1-Monolinoleoylglycerol trimethylsilyl ether	9,12-Octadecadienoic acid (Z,Z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester; 2,3-Bis[(trimethylsilyl)oxy]propyl (9Z,12Z)-9,12-octadecadienoate	

pound in their systems cannot be compared to this study. No literature was found that highlighted the presence of this compound on other marine organisms.

In contrast with the compounds derived from plant or animal sources, benzo[a]pyrene ($C_{20}H_{12}$), a polycyclic aromatic hydrocarbon (PAH), was identified. This compound, along with other PAHs, are formed naturally through incomplete combustion of organic materials such as coal, wood, and gasoline. Classified as a mutagen and carcinogen (NCBI), the said compound has no known bactericidal activity. Numerous studies have shown the presence of such PAHs in the island of Guimaras, which is consequential to the sinking of Motor Tanker Solar I 16 nautical miles southeast of the island in 2006. BaP was found to accumulate in the tissues of mangrove clam *Geliona erosa* collected from several areas of Nueva Valencia, Guimaras, the same site where the sea urchins in this study were collected (Peralta and Serrano Jr 2014). The assessment of the accumulation of PAHs in shellfish and bivalves inhabiting the island has indicated the presence of BaP (Uno et al. 2017). It was shown that there was a decrease in total PAH concentrations, with the presence of BaPs already under the prescribed EU regulations; however, the total PAH concentration accumulated by the test organisms is still eight times higher compared to the concentrations prior to the oil spill. In this study, the presence of benzo(a)pyrene can be indirectly attributed to the recent oil spill and that PAH accumulation occurs on different marine organisms situated in the island.

2,4-diisocyanato-1-methyl-benzene ($CH_3C_6H_3(NCO)_2$),

also known as toluene diisocyanate, was also recorded from the analysis. It is a known skin irritant or allergen utilized for the synthesis of polyurethane foams. There are no studies specifically implying the effects of this compound on sea urchins; however, this compound is still classified as a pollutant and poses risks to the organisms inhabiting the coastlines of Canhawan. In a study conducted by Sullivan and Due (1973), it was reported that methadone N-oxide is not found among the urinary metabolites but rather was only formed from the storage of urine, which consists of methadone.

The two latter compounds mentioned are both elicited during combustion processes. The said identification of the two compounds may not be rooted to being constituents of the extracts but due to the sample being subjected to extremely high temperatures during the GC-MS procedure. It should be noted that the GC-MS temperature utilized ranged from 110°C to 280°C which could have possibly damaged the sample through excessive heat prior to the mass spectrometry identification; thus, allowing the identification of the two aforementioned compounds. Other studies which have used methanolic solvents set their initial oven temperature to only 40°C/70°C prior to increasing the temperature to 250°C/300°C respectively. (Kanthal et al. 2014; Ghosh et al. 2015).This variation in initial oven temperature may have affected the compound identification process.

Methods such as nano-electrospray ionization mass spectrometry (nESI-MSMS) and electron ion mass spectrometry (EI-MS) are used for the identification of pigments, peptides, and amino acids in sea urchin species *Paracentrotus lividus*, *Strongylocentrotus franciscanus*, and *Strongylocentrotus droebachiensis*. The difference in compounds identified on profiling studies are affected by the methods used as well as the installed library of compounds on the equipment. Studies which identified the presence of pigments normally use liquid chromatography-mass spectrometry (LC-MS) or electron ion-mass spectrometry (EI-MS).

In this study, the method was limited to gas chromatography-mass spectrometry (GC-MS). The use of GC-MS is ideal for identifying and quantifying organic compounds that are volatile in nature; thus, it is optimal for outgassing studies, residual solvent tests, contaminant evaluation, and impurity identification. This, however, cannot identify non-volatile compounds this issue is normally solved through the use of LC-MS instead. The library installed in the GC-MS device also affects its capacity for identification; it is only limited to the compounds that have embedded molecular weight data in that library. In this study, the library used was not specific for natural products.

Conclusion. – Out of the six compounds identified from the spectral database of the GC-MS system, only two compounds have been identified to exhibit properties related to antimicrobial activity. The two compounds are: n-hexadecanoic acid and cyclobutyl tridecyl ester phthalate.

Aside from the two compounds mentioned above, there are also compounds that are classified as pollutants and carcinogens. These compounds are: benzo[a]pyrene; 2,4-diisocyanato-1-methyl-benzene; and methadone N-oxide. The presence of such compounds do not have any relation to antimicrobial activity or bioactivity. Their presence may be attributed to the GC-MS procedure which utilized high temperatures ranging from 383 K to 553 K; thus, damaging the sample and eliciting compounds resulting from combustion.

To sum, it can be concluded that the methanolic gut and gonad extracts contain compounds that are antimicrobial in nature; nonetheless, the analysis also shows the presence of compounds that are not from the sea urchin itself and might have been accumulated from its environment. To add, the compounds are also carcinogenic and may provide ample harm to the community of marine organisms in the area.

* * *

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Assessment of the Combination of Anthocyanin and Quercetin as an Angiogenic Inhibitor through a Chick Embryo Chorioallantoic Membrane Assay

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Abstract – Angiogenesis is a normal physiological process where new capillary branches sprout from preexisting blood vessels. Excessive angiogenesis can lead to the development of cancer, atherosclerosis, rheumatoid arthritis, etc. Anthocyanin is known for inhibiting angiogenesis in human endothelial cells. Quercetin has also shown an inhibitory effect when it was administered to the chorioallantoic membrane of chicken eggs. However, it has been proven that single flavonoids are less effective compared to when they are combined. This study aimed to assess the combined effect of anthocyanin and quercetin in inhibiting blood vessel growth in a chick embryo chorioallantoic membrane. Anthocyanin, quercetin, and their combination were each prepared with a concentration of 15 mg/L. The treatments were administered to seven-day-old chicken eggs and dimethyl sulfoxide served as the positive control. The appearance of each chrorioallantoic membrane was evaluated using the software ImageJ through calculation of fractal dimension values three days after the treatments were introduced. The values were then statistically analyzed through Kruskal-Wallis where it was found out that there was no significant difference between all treatments when compared to the controls. Of the three flavonoid treatments, the combination of anthocyanin and quercetin appeared to be the most effective in reducing blood vessel growth. This shows that the combination of anthocyanin and quercetin is more efficient in inhibiting angiogenesis. This treatment could be a novel source of treating pathological angiogenesis.

Introduction. – Angiogenesis is a normal physiological process primarily occurring in embryo development where new capillary branches made of endothelial cells sprout from preexisting blood vessels (Ucuzian et al. 2010). It occurs in the healthy body after ovulation and also for the restoration of blood flow to injured tissues to provide oxygen and nutrients for repairing cells [9]. Normally, angiogenesis is modulated by the angiogenic switch where there is a balance of pro- and antiangiogenic factors secreted by both host and tumor cells which when balanced in favor of pro-angiogenic factors lead to new vessel formation [2]. However, when this balance is disturbed, excessive or insufficient angiogenesis can occur which could lead to the pathology of various diseases such as solid cancers, coronary artery disease, stroke, diabetic blindness, and psoriasis affecting more than one billion people worldwide.

Natural remedies such as curcumin, quercetin, ginger, proanthocyanidin have been discovered to have a high degree of antiangiogenic activity. For example, the synergistic effect of turmeric tea powder made of curcumin, quercetin, vitamin E, and vitamin C suppressed angiogenesis in the chorioallantoic membrane of 10-day old duck eggs. This is shown by the decrease in blood vessel sprouting as the concentration of the powder increased [14]. Vitamin E and vitamin C are vitamins while curcumin and quercetin are flavonoids. Flavonoids are phytochemicals that give color to most flowers, fruits, and seeds. They have been abundantly studied for their inhibitory capacity in angiogenesis by: (1) prevention of capillary sprout formation and endothelial cell proliferation and migration, (2) modulation of angiogenic receptor signaling pathways like VEGF and FGF, and (3) inhibition of degradation of the basement membrane [27]. The problem is that although single flavonoids have al-

ready been proven to be effective as angiogenic inhibitors, evidence has shown that single antiangiogenic agents have limited efficacy [20]. It is suggested that synergistic activity of natural products be studied due to the range of complex organic chemicals they possess which can inhibit angiogenesis by interacting with multiple pathways and other ways that can affect cell signaling [20].

The pairing of anthocyanin and quercetin has already been proven by Hidalgo et al. (2009) to exhibit a synergistic effect in terms of antioxidant activity. However, there is no study conducted on the combination of these two bioflavonoids as an angiogenic inhibitor. Theoretically, these two flavonoids are expected to react in such a way that the chemical compositions of anthocyanin and quercetin will be retained. There will be no new compound formed, only a mixture wherein each of the two flavonoids will target the blood vessels separately using the same mechanism which is the prevention of proliferation of endothelial cells responsible for the formation of new capillaries.

Methods. –

Acquisition of materials. One hundred fifty 0-day old chicken eggs were bought from the Hatchery and Incubation Unit of West Visayas State University - Calinog. Twenty-five grams each of anthocyanin and quercetin was purchased from Sinuote Biotech and shipped from China. Other materials were bought from Mercury Drug, E. Lopez Street, Iloilo City, Berovan Marketing Inc., 100 Com Civil, JM Basa Street, Iloilo City, and Citi Hardware, Diversion Road, Iloilo City and SM Savemore Jaro, Libertad St, Jaro, Iloilo City.

Incubation of chicken eggs. All chicken eggs purchased were put inside a manual industrial egg incubator with a temperature range of 38C to 38.5C and relative humidity of 57%. The eggs were candled on the third day of incubation to check their fertility, as measured by the visible growth of blood vessels in the egg when viewed under a candler. All infertile eggs were properly disposed of after candling. During the entire incubation period, the eggs were turned to a 45- degree angle from their vertical positions six times a day for seven days to ensure balanced temperature flow throughout the eggs and prevent the blood vessels from sticking to the shell.

Preparation of flavonoid treatments. For the treatment with anthocyanin only and quercetin only, 0.0151 g [5] each of anthocyanin and quercetin was weighed on an analytical balance. The two flavonoid powders were then separately transferred to 50-mL beakers and mixed with 50 mL of dimethyl sulfoxide (DMSO) [16]. The solutions were stirred until the powders dissolved. Each solution was transferred to a one-liter beaker where it was diluted with distilled water until the 1 liter line was reached. The mixture was stirred. For anthocyanin and quercetin, 0.0076 each of anthocyanin and quercetin were combined and the same process was followed. All treatments were then transferred to reagent bottles and stored in a refrigerator for 24 hours at normal refrigeration temperature, 1.6 C.

Administration of treatments. All chicken eggs were taken out of the incubator on the seventh day and their shell surfaces were individually cleaned with 70% ethyl alcohol using a common paper towel. A slit was cut using a 12-inch hacksaw on the center of the blunt side of the egg where the air sac was located. A micropipette was then used to fill the barrel of an insulin syringe with 20 L of 15 mg/L (DMSO, 0.5%) of each treatment. The insulin syringe was inserted into the slit on each egg and positioned on the lower half of the air sac. Each treatment was then administered. The eggs were put back in the industrial egg incubator and further incubated for three days before viewing of blood vessels [21].

Determination of Fractal Dimension. All 300 x 210 px photographs of the chorioallantoic membrane were analyzed using the software ImageJ. The blood vessels on the image were first reinforced by being manually overlaid with black using any image - editing software to have a strong contrast against the background. These reinforced images were then converted to 8 - bit grayscale in ImageJ and the range of dark colors that represent the blood vessels were isolated from the background. The blood vessels were skeletonized and the fractal dimensions were retrieved using the box - counting method set to a maximum of 64 boxes on ImageJ.

Statistical Analysis. Kruskal-Wallis statistical analysis with a level of confidence of 0.05 was performed in order to compare the means of the average fractal dimensions of the treated eggs within and between treatments. If the asymptotic significance value is less than the desired level of significance of 0.05, it is declared that it is significant.

Results. – The means were calculated from fractal dimensions of each treatment of each replicate. A lower mean (See table 1) corresponds to a lower fractal dimension which suggests a stronger antiangiogenic effect, thus making the fractal dimension values inversely proportional to the antiangiogenic effect exhibited. The fractal dimension in anthocyanin-treated samples was lower compared to the quercetin-treated samples. This indicates that anthocyanin was more effective in inhibiting angiogenesis, only in terms of the values gathered, between the two substances being individual flavonoid treatments. The eggs that were not administered with any treatment displayed the highest fractal dimension mean of 1.306 among the five samples. Despite the consistency of values for the four samples, however, the calculated mean for dimethyl sulfoxide turned out to be higher than that of the samples treated with the combination of anthocyanin and quercetin. In theory, as the DMSO-treated is the positive control group, it must

Table 1: Fractal dimensions (Mean SD) of treatments after administration on chorioallantoic membranes of Darag native chicken embryos

Treatments	Mean SD	Description
No treatment	1.306 ±0.115	Control (-)
Quercetin	1.264 ±0.081	Weakest antiangiogenic effect
Anthocyanin	1.239 ±0.050	Moderate antiangiogenic effect
Anthocyanin + Quercetin	1.189 ±0.106	Strongest antiangiogenic effect
Dimethyl sulfoxide (DMSO)	1.240 ±0.053	Control (+)

exhibit the strongest antiangiogenic effect, but such was not the case. It also had a higher fractal dimension by 0.001 compared to the anthocyanin-treated experimental group which indicates that anthocyanin had a stronger antiangiogenic effect than DMSO, although the difference is negligible.

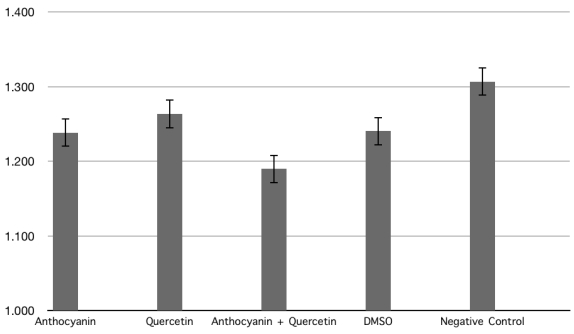

Fig. 1: Mean fractal dimension of each group

The Kruskal-Wallis statistical test was the primary test used for data analysis. Prior to the said test, the data set of replicate means for each treatment was initialized using the box-and-whisker plot by observing its distribution pattern and was found to be not normally distributed, which led to the choice of statistical test. For the data to be considered significant, the calculated asymptotic significance must be lesser than 0.05 and such was not the case as it turned out to be equal to 0.645 (See Table 2and 3).

In summary, between anthocyanin only, quercetin only, and their combination, the samples treated with combined anthocyanin and quercetin had the lowest blood vessel density as displayed by the low fractal dimension. Consequently, anthocyanin had a lower fractal dimension than quercetin, making it stronger as an antiangiogenic agent compared to quercetin in terms of numerical values. One irregularity in the data can be attributed to the fractal dimension of dimethyl sulfoxide being higher in value than that of the combination of anthocyanin and quercetin and almost equal to the dimension value of anthocyanin. Lastly, the data processed from the analysis was not considered significant.

Table 2: Kruskal - Wallis Test results from the fractal dimensional analysis of the skeletonized images of the samples

Treatment	N	Mean Rank
Treatment	N	Mean Rank
Quercetin	3	9.33
Anthocyanin + Quercetin	3	5
No treatment	3	10.33
DMSO	3	7.5
Total	15	

Discussion. – Flavonoids are recognized as chemopreventive agents to cancer and are the most abundant polyphenols in human diet. These molecules are especially known in inhibiting tumor growth by inducing apoptosis in multiple tumor cell lines and by halting cell-cycle [4, 12, 15, 19]. Multiple mechanisms have also been observed in which the flavonoids participate in that inhibit angiogenesis. In this study, the individual and combined performance of flavonoids anthocyanin and quercetin in inhibiting angiogenesis were assessed with the fractal dimension of blood vessels present on the CAM as parameter. The samples treated with flavonoids exhibited stronger anti-angiogenic effect than the negative control samples, but not to a statistically significant degree when the fractal dimension of the blood vessels were analyzed. In fact, no in-between group differences determined by this mode of assessment was statistically significant. Using the anti-angiogenic effect scoring method, however, significant in-between group differences were determined. For both methods, no synergistic effect was exhibited by the treatment with both anthocyanin and quercetin, but it is worth noting that the mean fractal dimension of all samples in this group is the greatest (when values from all replicates is averaged) based on fractal dimension means. This reveals that they manifested the mean fractal dimension of all samples in this group is the greatest (when values from all replicates is averaged) based on fractal dimension means. This reveals that they manifested the strongest anti-angiogenic effect, followed by the mean fractal dimension of samples treated with DMSO alone, albeit statisti-

Table 3: Test Statistics

Chi square	df	Asymp. Sig.
2.497	4	0.645

a: Kruskal Wallis Test
b: Grouping Variable: VAR0001

cally insignificant.

The possible mechanism behind the anti-angiogenic effect of the interaction between the two flavonoids is the antioxidant activity induced by both molecules in the chorioallantoic membrane. There have been reports that reactive oxygen species (ROS) stimulate the release of vascular endothelial growth factor (VEGF) signal protein [3] or mediate VEGF-induced signaling [1, 13, 25]. This is because ROS, specifically hydrogen peroxide (H2O2) which are produced by macrophages, enhance the expression of inducible nitric oxide synthase (iNOS), an enzyme responsible for the production of angiogenesis-mediating nitric oxide (NO) molecules (Han et al. 2001). Thus, antioxidants that scavenge H2O2 and superoxide down-regulate the expression of this enzyme and inhibit angiogenesis in vivo as a result (Polytarchou Papadimitriou 2004). Hidalgo et al. s (2010) study investigated the antioxidant activity of two interacting flavonoids by pairing all of the eleven (11) selected flavonoid groups, five of which are anthocyanins and two of which are quercetins. Each individual anthocyanin and quercetin has been found to have greater antioxidant capacity than when an anthocyanin is paired with a quercetin or vice versa . This indicates that the interaction has an antagonistic effect on the antioxidant capacities of the flavonoids. The suggested explanation for this phenomenon is that the hydrogen-bonding between flavonoids may have occurred, and reduced the availability of hydroxyl groups (-OH) in effect. Since the presence of hydroxyl groups is responsible for the neutralization of ROS, the antioxidant activity is reduced as a result of the interaction. The assay used in Hidalgo et al. s study, however, is the DPPH method which utilizes a radical distinct from hydrogen peroxide which is the one responsible for the stimulation of VEGF. Anthocyanin and Quercetin have both been shown to be able to scavenge H2O2 molecules, [26] making it possible that although hydrogen bonding may occur between the flavonoids, not the availability all hydroxyl groups is compromised, thus making the mixture still capable of neutralizing H2O2 molecules and inhibiting angiogenesis in the chorioallantoic membrane.

Following the strength of the observed anti-angiogenic effect of the mixture of anthocyanin and quercetin is that of DMSO had been confirmed to have anti-angiogenic effect on human aortic endothelial cells (HAECs) by decreasing the expression and activity of matrix metalloproteinase-2 (MMP-2) [10]. MMP-2 is an enzyme that breaks down the endothelial basement membrane to facilitate the growth of new capillaries. The down-regulation of these enzymes by DMSO had been found to be dose-dependent, causing significant anti-angiogenic effects at 2% and 3% DMSO concentration in the cell media DMEM-F12.

Conclusion. – The results of this study are not statistically significant in between fractal dimension means. However, the trends suggest that the combination of anthocyanin and quercetin exhibited the highest antiangiogenic effect in comparison to all the other treatments. Based on analysis, anthocyanin is more effective than quercetin in inhibiting angiogenesis. The three treatments were effective in inhibiting blood vessel growth. However, the mechanism of the treatments may not directly be about completely stopping the growth of blood vessels when a tumor grows. It could be that the treatments can only help in reducing the number of capillary branches or slowing down the rate of angiogenesis. The use of these treatments against angiogenesis can also be an indicator that foods that contain anthocyanin, quercetin, or the combination of both can also help in inhibiting angiogenesis for people suffering from angiogenesis-related diseases such as cancer and rheumatoid arthritis. In conclusion, the combination of anthocyanin and quercetin can control the growth and help in the process of inhibiting angiogenesis.

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Arijo: Location-Specific Data Crowdsourcing Web Application as a Curriculum Supplement

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Abstract – Smart devices are quickly becoming more accessible to the general public. With the proper tools, they can be used to supplement the work of educators. According to studies by Beeland Jr. and Roussou, learning through interaction has been considered to be effective by both students and teachers. This study aimed to develop an interactive curriculum supplement for smart devices in the form of a Location-specific Data Crowdsourcing Web Application (Arijo) which teaches students how to conduct experiments and upload their results to the internet for archival purposes. Arijo was developed with a combination of the Appsheet framework, Adobe Photoshop, and Google Maps. Three core functionalities were programmed: data input/output, data interpretation, and information dissemination. Arijo was able to perform its intended features such as recording and displaying data within specific locations, along with displaying guides on how to conduct an experiment. Arijo was able to fulfill its main objective, to be a curriculum supplement, through the aforementioned features. In the future, Arijo may be expanded to support more year levels and multiple curriculum, the Advanced Placement and International Baccalaureate systems for example, because of its modular nature.

Introduction. – Science, Technology, Engineering and Math or STEM, is an educational discipline focusing on the four aforementioned fields. Apart from promoting and exposing students to those fields, teaching STEM has been shown to have a positive impact on both students and educators alike. This includes teaching the efficiency and inquiry skills required to succeed in STEM-related endeavors[1]. A study by Estonanto[2] showed that there was a low acceptability of the new Philippine STEM curriculum among stakeholders. It revealed that the major problems were on the areas of the Facility and Instructional Materials, and the difficulty of coursework. He concluded that there was much to be improved about existing learning materials. New ways can be added to the area of the Facility and Instructional Materials to promote STEM, one of which is interactive learning. There exist studies that prove that interactive learning benefits both students and teachers. Beeland Jr.[3] in 2002 studied the effects of interactive whiteboards in classrooms, the purpose of which was to determine how student engagement was affected by using interactive tools throughout the learning process. The results showed that the aforementioned tool resonated with learners and lecturers alike while also increasing student engagement. Nowadays, the technology present in the interactive whiteboards of old can be found in everyday smart devices such as phones, laptops, and tablets. Smart devices are hardware that possess the ability to perform ubiquitous computing, which gives them the ability to run complex programs known as applications. These programs have multiple uses, one of which is data input and output. Using sensors commonly found in contemporary smart devices, some applications can find location-specific data such as GPS coordinates and ambient air temperature. Using the internet, some applications are able to disseminate information like news, weather forecasts and stock market statistics. Other applications even involve data crowdsourcing, the act of gathering data from the public. Data gathered via crowdsourcing is valuable for researchers, businesses, and the public alike, as they can help predict trends and identify potential solutions to problems. Location-specific data are among these, it being information related to a specific locale. An example of an application that primarily makes use of crowdsourced location-specific data is Waze, which uses traffic information sourced from public repositories to- For the full article, please visit https://bit.ly/2HS3u5L

HeroIn: An Assistive Medical Android Application for Common Diseases and Injuries

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Abstract – This study aimed to create an offline mobile application that assists users with disease or injury by recommending hospitals based on input of symptoms, providing contact information of these hospitals, and instructing first aid in case of injuries. The application was created using MIT App Inventor and runs on at least Android version 2.3.

Introduction. – At present, more and more Filipinos use smartphones. The International Data Corporation reports that among all the members of the Association of Southeast Asian Nations (ASEAN), the Philippines is the fastest-growing market for smartphones [1]. In 2016 alone, there were approximately 26.2 million smartphone users in the country; the number was predicted to rise to 30.4 million by the end of 2017 and 46.04 million by 2022 [2].

Along with the popularity of smartphones spawns the development of mobile applications, or apps, that are used in everyday life. These include social media applications, photo and video sharing applications, and music and video streaming applications. The most used smartphone apps include Youtube and numerous social media such as Facebook and its accessory app, Messenger [3]. The idea that people have many options for entertainment or interaction with other people via the internet means that there are already many applications dedicated to the social and leisure aspects of life; however, there are other aspects that may not usually receive enough attention from app creators and developers but can be used as ideas to make an application. This gives way for the potential of creating novel applications that usually do not share the same functions as other apps. An example of which can be an application that addresses referrals to hospitals or first-aid instructions.

Currently, there are existing online diagnostic websites that help users identify what illnesses they may have through an interactive symptom checker. One example is WebMD, where the user can input identified symptoms in which the site responds with possible diagnosis of diseases. WebMD can be accessed as a smartphone application; however, not all smartphone users have access to internet at all times, especially when travelling. This is why some mobile applications make use of features that do not require an internet connection. These applications make use of local storage so that they can be used with or without an internet connection.

Hence, the concept behind WebMD might be used as a basis in creating a novel application that has different features but shares a certain function.

It would be beneficial for Filipinos who are not feeling well or are injured to have immediate access to helpful information regarding the implications of their illness or injury. It would also be helpful if they had access to suggestions on what to do next depending on the urgency of their situation. The creation of an application for this might help attain that convenience for Filipino smartphone users.

It could be started off simply as a program in which users can input symptoms or injuries, for example. The app would then deduce possible diseases and recommend a hospital that may have a special facility for that possible disease, or the app would then provide first aid information if a user inputs an injury. The users may then choose a hospital to travel to for diagnosis and/or treatment, wherein the app would provide contact information of the hospital.

This study, therefore, was proposed to create a mobile application that performs mainly two functions: The first function is to direct users to appropriate hospitals in Iloilo City for diagnosis and treatment based on symptoms of

the disease. By receiving user input of symptoms, this application would function similar to an online diagnostic sites symptom checker but will not require an internet connection. Users would then be given contact information of the recommended hospitals as well as their locations. The second function is to provide first aid information for common injuries. Users may select from a list of common injuries in which each one has a set of instructions to be displayed once selected.

The conduct of this research may initiate similar studies, specifically those with further improvement in design, in the future.

The conceptualized name of the app is HeroIn. It stems from the expression that the doctor, or hero, is in as to represent a patient being addressed by the doctor. Also, the reference of the app to the drug 'heroin' might attract attention from potential users.

Methods. – Figure 1 and Table 1 depict the activities and the process involved in data-gathering.

	Activity	Predecessor	Duration (days)
A	Gathering of Information	*	14
B	Organizing of Information Gathered	A	2
C	Designing of Application Structure	B	1
D	Setting-Up of Web Database	*	5
E	Input of Information into the Web Database	A, D	2
F	Creating of Application Graphics (Assets)	B	5
G	Creating of Back-End Program of the Application	C	14
H	Adding of Web Database to the Back-End of the Mobile Application	G	2
I	Creating and Adding of Application Graphics (Layout)	H	3
J	Adding of Graphics (Assets) to the Mobile Application	F, I	2
K	Testing the Online Functionality of the Mobile Application	J	1
L	Testing the Offline Functionality of the Mobile Application	K	1

*no predecessor

Table 1: List of activities to have been accomplished, along with time duration in days.

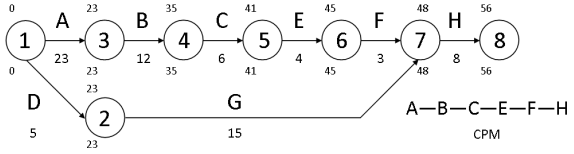


Fig. 1: PERT/CPM of the data gathering process.

Gathering and organizing of information. There were three different types of information that were gathered: 1.) common diseases and injuries, 2.) specialized clinics and hospitals, and 3.) first aid information.

First, information on common diseases along with their symptoms was gathered from the Philippine Department of Health's public website. The scope does not include all diseases in existence, but rather some of them that frequently occur in the Philippines. The symptoms of the diseases were categorized into lists to incorporate in the application.

Then, the contact information of the hospitals was gathered from their online profiles. Supposedly, the information was to be gathered from the hospital institutions themselves along with their permission for it to be included in the application. This was to ensure that the information is updated and reliable. However, due to unresponsiveness from the institutions in communication, the information used was taken instead from the hospitals' public websites.

Lastly, the first aid information was acquired from the website of the Philippine Red Cross - Iloilo Chapter, as well as from the website of the Department of Health. The information includes basic first aid dos and don'ts when handling common injuries.

Designing of application structure. Before the application could be programmed, its structure should be designed. The application structure should be designed based on the flow of events when using the application, as depicted in Figure 2.

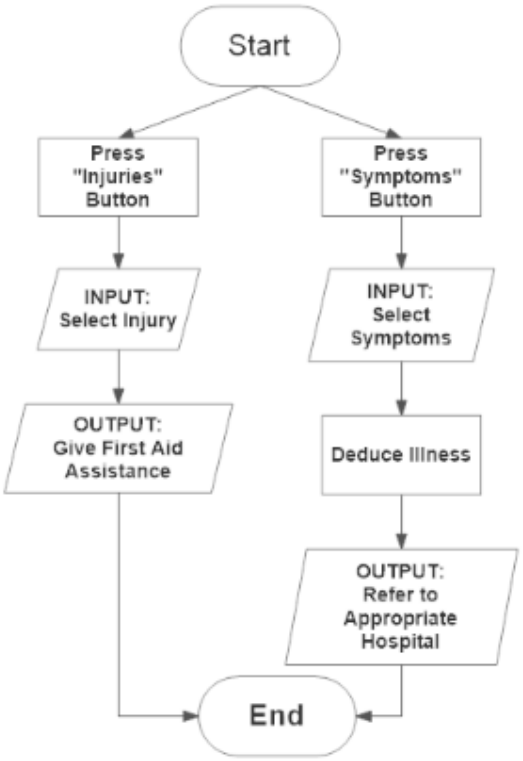


Fig. 2: Flow of events in the mobile application.

Creating of back-end of program. The functionality of the application was created using MIT App Inventor.

Home Screen. The home screen is the first screen the application shows after opening the application. It contains three buttons which will lead the user to either the symptoms screen, injuries screen, or about screen.

Symptoms Screen. The symptoms page contains a list of symptoms divided into two main categories: non-visual and visual symptoms. Users may check symptoms that they can identify then press the submit button. Doing this will take them to the hospital directory.

Hospital Directory Screen. The hospital directory displays a list of hospitals. The hospitals shown depend on the symptoms the user checked in the symptoms screen. Users may select a hospital then press the Hospital Details button which will lead them to the hospital details screen.

Hospital Details Screen. The hospital details screen provides contact information of the hospital previously selected by the user including phone number/s and email address.

Injuries Screen. The injuries screen displays a list of common injuries. Users may select an injury and press the Submit button in order to view first aid information for that injury.

About Screen. The about screen displays a short description of the application.

Creating and adding of graphics. The graphics were made using the software Microsoft Publisher 2010 and Photoshop CS6 Portable. The color scheme does not contain the color blue as it may cause eye strain due to its short wavelength and concentrated energy [4].

Testing. The functionality of the different parts of the application, such as the symptom checker, was tested. The hypothetical user would be provided a checklist for identifying symptoms of possible disease. Upon submission of the symptoms, the user would be shown a list of hospitals and, after a selection, the contact information. The user would also be provided an option to select an injury and be shown first aid information.

Results. – The Android application was coded using a personal laptop. It was coded and laid out using the online editor of MIT App Inventor. The application was tested through the use of personal laptops, through the use of emulators, and personal smartphones.

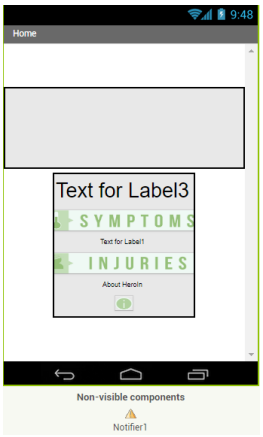
The graphics of the application were made using Microsoft Publisher and Photoshop CS6 Portable. The color scheme mostly consisted of different shades of green and yellow, and the texts in the application were all set to sans-serif.

The finalized application was capable of allowing the user to check the symptoms they are showing or experiencing. The application then uses the data acquired from to recommend appropriate hospitals that are capable of diagnosing the user based on their symptoms.

Discussion. – The application currently:

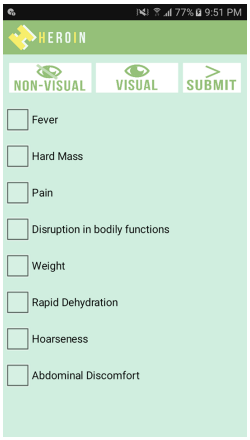
- allows users to identify their symptoms from a list of symptoms of common diseases
- suggests appropriate hospitals that could properly diagnose the user based on their identified symptoms

(a) Actual Screen

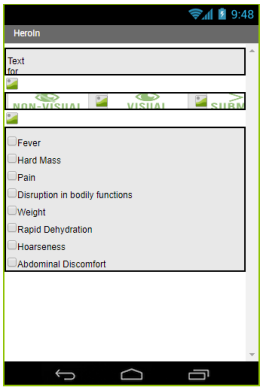


(b) Designer Screen

Fig. 3: Home Screen

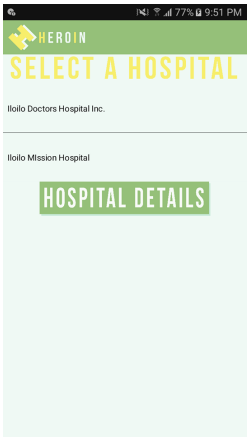


(a) Actual Screen



(b) Designer Screen

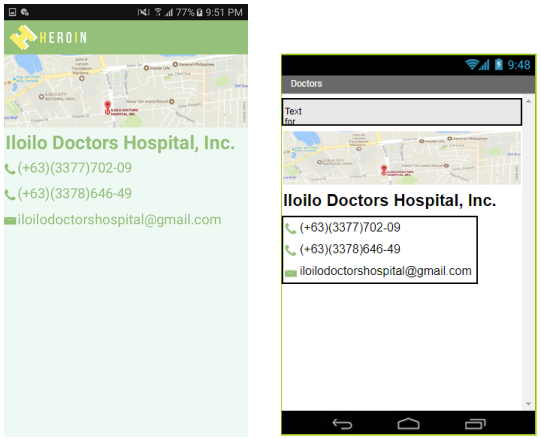
Fig. 4: Symptoms Screen



(a) Actual Screen

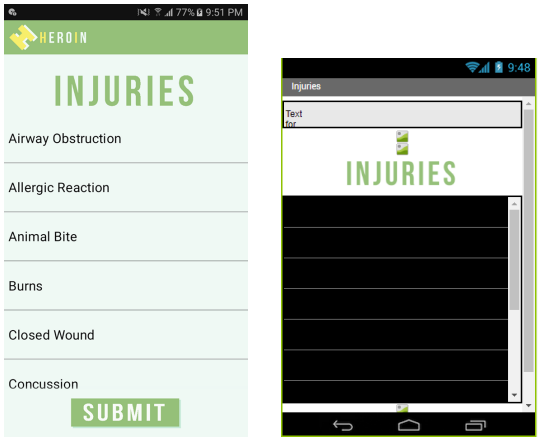
(b) Designer Screen

Fig. 5: List of Recommended Hospitals Screen



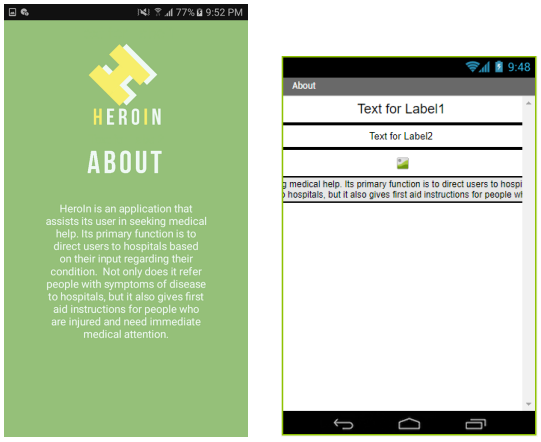
(a) Actual Screen (b) Designer Screen

Fig. 6: Contact Information Screen



(a) Actual Screen (b) Designer Screen

Fig. 7: Injuries Screen



(a) Actual Screen (b) Designer Screen

Fig. 8: About Screen

- provides contact information and location of given hospitals
- and provides first aid information for common injuries

WebMD, a similar app, also has a symptom checker; however, unlike WebMD, HeroIn does not inform the user of the possible disease/s they have based on the symptoms because a proper diagnosis can only be given by a medical professional. HeroIns hospital referral feature is similar to that of WebMDs with the main difference being the area where the hospitals are located. WebMD refers users to hospitals in the United States while HeroIn refers users to hospitals in Iloilo City. Finally, users are able to utilize all the features of HeroIn without an internet connection, which gives it an advantage over WebMD, an application that requires a stable internet connection whenever in use.

HeroIn	WebMD
Symptoms checker	Symptoms checker
Deduces illnesses	Diagnoses illnesses
Iloilo City	United States
No internet connection needed	Internet connection needed

Table 2: The features of HeroIn versus the features of WebMD.

Summary. – The application refers users to appropriate hospitals based on user-identified symptoms. Users can only identify their symptoms based on a list of symptoms of common diseases provided by the application. Users can also only select from a list of common injuries provided by the application, in which the application will display first-aid instructions for the selection.

Conclusion. – This application could allow users to use provided hospital contact information in order to be properly diagnosed at appropriate hospitals. It could also instruct dos and don'ts when performing first aid on injuries.

Recommendations. – Since the application was developed using App Inventor, its features are more limited than it could have been if it were developed using other Android application development software such as Android Studio. The coding style of App Inventor made it tedious to develop and organize because of its graphical style rather than the usual text-based style. A feature that could be added if a better software was used is one that would allow users to directly contact the hospital from the application. A more detailed 'about' page would also be an improvement. Some things that could be included

in the 'about' page are the scope and limitations of the application and disclaimers.

Another recommendation is to gather more reliable information. The information should be up to date and from a trustworthy source. In this study, the information incorporated into the application was merely from public online sites. A better action would be to directly consult institutions. This was not done in the study due to lack of time. Gathering contact information directly from the hospitals would provide users with contact methods that are more likely to result in a response from the hospital. Requesting of this information from the hospitals was attempted; however, none of the hospitals responded.

One more recommendation is to include more symptoms and hospitals in the application. This would broaden the scope of the application, thus benefiting more people.

* * *

This study would not have been accomplished without the help of our Research Unit Head, Mr. Harold Mediodia.

The following people have also assisted with the conduct of this study by serving as reviewers: Ms. Rubie Anne Bito-on, Engr. Raphael Eric Yturralde, Mr. Bernie Trompeta, and Mrs. Virna Jane Navarro.

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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.

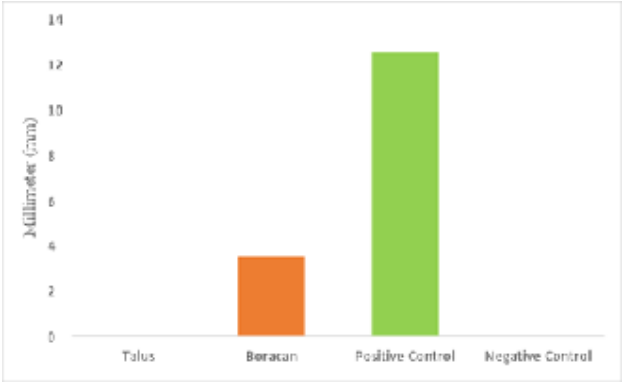

Fig. 1: Mean Zone of Inhibition of All the Treatments.

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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Alternatives Substrates for the Production of Pleurotus ostreatus (Oyster Mushroom)

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Abstract –This study aims to use agricultural wastes rice stalk, bran and husk as alternative substrates for Pleurotus ostreatus. The study was done at Western Visayas Integrated Agricultural Research Center, in ratios of 50:50 and 100:0, wastes to sawdust respectively. After harvest, the masses were weighed, the caps counted, and the biological efficiency was measured. Rice stalk treatments had the highest biological efficiency while rice bran and hull treatments did not produce any caps for the first flush. The dry mass post hoc results showed significant difference amongst all substrates, proving that rice stalk treatments have the highest biological efficiency and are most compatible with *P. ostreatus.*

Introduction. – Oyster mushrooms are commonly cultivated due to its rich nutrient content and various medicinal properties. It needs shorter growth time and utilizes industrial and agricultural wastes as substrates. It is low cost production as it does not require much resources [1].

Pleurotus ostreatus is the second most widely cultivated edible species worldwide. *P. ostreatus* takes a relatively shorter time to grow than other edible mushrooms. Along with it having little requirements for growing and a lesser chance of being diseased makes growing *P. ostreatus* easier and cheaper [2].

In most Asian countries rice is a major crop, and rice bran, rice husk and rice stalk are easy to purchase and are cheap as these are considered wastes. Mushrooms breakdown organic materials which other organisms cannot decompose. The mycelia produce enzymes that breakdown and use lignocellulosic wastes, usually used for biofuel, including agricultural wastes such as corn husks, rice stalks, wheat, and other organic matter [3].

This study aims to investigate alternative mushroom substrates using the agricultural wastes rice stalk, bran, and husk for mushroom production. The study aims to: (i) compare the growth of *Pleurotus ostreatus* based on fresh and dry mass, and number of caps and (ii) obtain the biological efficiency, of each alternative substrate.

Significance. This is a sustainable project that can be done at home, as rice production wastes rarely used for other than compost and feeds are utilized to reduce total waste and add to income by growing food. *Pleurotus* sp. are low-maintenance and require carbon sources provided by substrates that can be found in agricultural wastes.

Limitations. Limitations. This study will be limited to the use of alternative media from the agricultural wastes in the production of rice. The mushrooms to be grown will be *Pleurotus ostreatus*, the spawn obtained from West Visayas Agricultural Research Center.

Methods. – The study aims to use agricultural rice wastes as alternative substrates for production of *Pleurotus ostreatus* mushroom. This includes the procedures from preparing the substrates until the disposal of spent bags and statistical analysis.

Materials and equipment used. For the preparation of substrates, we have the wastes: rice stalks (RS), rice bran (RB), rice husk (RH), and sawdust (SD) with plastic bags as substrate bags and inoculated corn kernels.

Acquisition of mushroom spawn. The mushroom spawn was obtained from West Visayas Integrated Agricultural Research Center in Hamungaya, Jaro, Iloilo City.

Preparation of the area. The room was sprayed with disinfectant spray and the temperature was controlled using an air conditioning unit.

Preparation of substrates. For the preparation of substrate mixture, the total mass of each fruiting bags was 750 grams, comprised 98 percent of the alternative substrate with 1 percent molasses and 1 percent lime. Each treatment had five replicates to account for mushroom mortality.

There are 7 treatments: (i) the control, (ii) 100% rice stalk, (iii) 100% rice bran, (iv) 100% rice husk, (v) 50% rice stalk and 50% sawdust, (vi) 50% rice bran and 50% sawdust, and (vii) 50% rice husk and 50% sawdust. The control was based on substrate mixture ratio used by WESVIARC.

Bagging of substrates and sterilization. After mixing, the substrates were placed in plastics bags using a cylindrical metal mold. After filling the bag, the mold was removed and then a piece of polyvinyl chloride pipe was placed there to be a pathway for the spawn to go inside the bag. Cotton was then placed in order to avoid contamination, and another piece of plastic was used to cover it.

Inoculation of spawn. The spawn was divided equally among all the substrates and their trials and mixed thoroughly.

Observation period. The mushrooms are ready for harvest if their caps turn up from the outer corners. To remove the mushroom, it should be twisted from the base and pulled.

The parameters measured were the caps, fresh and dry mass. The biological efficiency was also calculated.

Caps. The number of caps in each fruiting bag was counted manually.

Fresh and dry mass. After harvesting, the mushroom was weighed using a weighing scale and the gram unit. The mushrooms were then air dried for 2 days and the dry mass was obtained.

Biological efficiency. Determined by the following equation [4]: BE =(dry weight of harvest / weight of dry substrate)x100

Disposal. After the data gathering, the substrates are disposed by vermicomposting.

Statistical analysis. The ANOVA test was used to compare all the means of the samples of each trial for every substrate to find out if there is a significant difference in terms of physical data gathered from the experiment.

Results. – This chapter contains the data obtained from the experiment, its statistical analysis, and significance. Using agricultural wastes such as rice stalk, bran, and hull as alternative substrates for *P. ostreatus* culture, the biological efficiency of each set-up was determined.

One reason that rice straw has the highest fresh mass is due to the number of substrate bags that fruited. There are 15 fruiting bags in each treatment and none of which were contaminated, resulting in more yield produced, compared to the other treatments wherein there are fewer substrate bags. Rice straw is the most effective among the three substrates since it has the greatest mushroom growth based on the masses of the harvest and the amount of fruiting bags that has growth in it.

Table 1: Mean Summary of Results from *P. ostreatus* Harvested from Treatments (N=15)

Treatment	Fresh Mass (in grams) $^{\alpha}$	Dry Mass (in grams)	Number of Caps $^{\alpha}$	Biological Efficiency (%)$^{\alpha}$
Control	5.7	2.5	2.7	22.5
Rice Straw	42.0 $^{\beta}$	9.7	18.4 $^{\beta}$	35.0 $^{\beta}$
Rice Hull	0	0	0	0
Rice Bran	0	0	0	0
Rice Straw and Sawdust	22.8	6.7	11.2	33.3
Rice Hull and Sawdust	0	0	0	0
Rice Bran and Sawdust	0	0	0	0

$^{\alpha}$ - ANOVA test significant at 0.05
$^{\beta}$ - LSD Post-Hoc test significant at 0.05

The ANOVA statistical test showed that the treatments have a significant difference with each other since the p-value is less than the alpha value 0.05, this means that the weight of the harvest per treatment differs and is not near equal with the others.

Since there is a significant difference between the treatments, a post hoc test was conducted. With the p-value of 0.03, rice straw was significantly different compared to the other treatments. It is the most effective among the three followed by sawdust and rice straw and then the control treatment.

Dry mass. The pure rice straw has the highest mean with 9.7 grams, followed by the rice straw and sawdust with 6.7 grams, and the control with the lowest mean of 2.5 grams.

The mean dry mass of the rice straw substrate is slightly higher than the other treatments since this treatment has the highest amount of fruiting bags that bloomed and most of the harvest outweighs the other mushrooms in the study. Based on the results in the fresh mass, the rice straw substrate will also have the highest dry mass since its fresh mass is also higher than others. It is then followed by the rice straw and sawdust substrate and

then the control treatment. Since the p-value is higher than the alpha value, there is no significant difference in the dry masses of the treatments, this means that the dry masses of the harvest are almost the same with each other.

Number of caps. The pure rice straw has the highest mean with 9.7 grams, followed by the rice straw and sawdust with 6.7 grams, and the control with the lowest mean of 2.5 grams.

The mean dry mass of the rice straw substrate is slightly higher than the other treatments since this treatment has the highest amount of fruiting bags that bloomed and most of the harvest outweighs the other mushrooms in the study. Based on the results in the fresh mass, the rice straw substrate will also have the highest dry mass since its fresh mass is also higher than others. It is then followed by the rice straw and sawdust substrate and then the control treatment.

The null hypothesis was accepted as the p-value is higher than the alpha value, there is no significant difference in the dry masses of the treatments. The dry masses of the harvest are almost the same with each other because they do not have a great difference.

Biological efficiency. The mass of the fresh harvest was divided over the mass of the dry substrate multiplied by 100 to calculate the biological efficiency of the the substrate treatment. Pure rice straw has the highest biological efficiency mean with 35%, followed by rice straw and sawdust with 33.3%, and the lowest with 22.5% from the control.

Based on the average biological efficiencies of the treatments, the rice straw treatment, which is the pure rice straw treatment, has the highest biological efficiency out of all the substrates, followed by the rice straw and sawdust treatment. Sawdust as part of a substrate is lacking since it is generally low in protein which is necessary in the cultivation of mushroom and should have undergone composting to effectively breakdown its cellulose and lignin components that aids in the releasing of essential nutrients for the mushroom [5].

This is also supported by the study of Girmay et al. (2016), which used sawdust as part of their treatment and garnered the lowest harvest weight and biological efficiency out of all their treatments [6].

The ANOVA results showed the p-value to be 0.032, which is less than the significant value of 0.05, meaning there is a significant difference in the biological efficiency of the treatments. This means that different substrates produce different biological efficiencies, therefore those with lower yields are less compatible in growing P. ostreatus and those with higher yields are more compatible with growing these species.

The post hoc test results showed that treatment B has a significance level of 0.009 which is lower than the alpha value, therefore the pure rice straw substrate has a significant difference from the other treatments. Comparing their compatibility, treatment B has the highest compatibility followed by treatment E and then the control.

Out of the 105 fruiting bags prepared, 50 of those were contaminated with green, yellow, and black molds. Trichoderma spp. is known to be the cause of the green mold disease in mushroom, mostly on the spawn running period. These molds usually affect the lignocellulosic substrates because it degrades cellulosic materials, examples of these are the substrates used in the study: rice stalk, rice bran, and rice hull [7].

Only three substrate treatments bore fruiting bodies: the control group, rice stalk and sawdust treatment, and the pure rice stalk treatment. The data shows that both rice stalk treatments have higher biological efficiency than the control.

Discussion. – After 2 days of air-drying, at least 70% of the total weight of the harvest was lost and this was the result of the drying up of water or moisture from the mushroom.

Treatment of rice hull and sawdust was fully wiped out by the mold with no visible signs of the inoculated mycelium spreading in the substrate, while treatments of pure rice hull, pure rice bran, and rice bran and sawdust showed no signs of primordial growth at all. The rice bran groups must have been too compact for the inoculated corn kernels to grow. The absence of growth can be possibly due to the rice bran and rice hull having too little cellulose in them for the mushrooms to feed on compared to the rice straw. Rice straw has been used in previous studies in growing P. ostreatus, such as in the studies of Kungu n.d.[7], Dundar et al 2008 [8], Girmay et al 2016 [6], Moonmoon et al 2010[1], and Yang et al 2013[9].

Rice hull has been used effectively as an additive when growing P. ostreatus in the study conducted by Frimpong-Manso et al 2011[10]. It was recommended to be used only as an additive as it was quick to dry up and lose moisture, and this is a possible reason as to why there was no observed growth in both the sawdust treatments.

According to the study of Starnets and Chilton 1983, airborne contaminants are the primary cause of culture that uses agar or grain by which corn kernels are considered grain culture [11]. In the case of the treatments, pure rice bran and rice bran and sawdust, that did not show any signs of growth, one of the possible causes could be the lack of airing. The fruiting bags contents were compact with the inoculated kernels not able to penetrate the material, and due to the brans powdery property, the mycelia did not survive. A study by Yildiz et al. (2002) stated that once the concentration of the rice bran exceeds 25%, the risk of contamination is increased [12]. This a possible reason for the causes of contamination of the substrates that used rice hull and why there was no growth.

Conclusion. – With the use of one-way ANOVA test, it was proven that there is a significant difference in the biological efficiency which measures the compatibility

of the substrates with the mushroom. The rice stalk groups (B and E) proved to have the highest biological efficiency and thus the best compatibility for growing *P. ostreatus*. Meanwhile, the rice bran treatments (D and G) and rice hull (C and F) barely went past the spreading of the mycelium and were unable to produce fruits in time with the first flush.

* * *

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Green Synthesis of Silver Nanoparticles Using Moringa oleifera sp. (Malunggay) Seed Aqueous Extract and Its Antibacterial Activity

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Abstract – Silver nanoparticles, in recent years have gained interest due to their applications in various fields, such as in medicine, as a result of its antibacterial properties. The synthesis of nanoparticles involves reduction and capping processes. The current processes, however, involve the use of toxic chemicals. A solution for this is the use of green synthesis, which is done through the utilization of biological components which acts as both a reducing and capping agent. The study used *M. oleifera sp.* seed extract to synthesize silver nanoparticles as they possess various biomolecules that make the process effective. Ultraviolet-Visible spectrophotometry was used to monitor the formation of the silver nanoparticles. The synthesized silver nanoparticles were viewed under a Transmission Electron Microscope and had a mean size of 12 nm and spherical shape. The silver nanoparticles also showed antibacterial activity against the *S. aureus* bacterial culture. The study concluded that silver nanoparticles can be synthesized using *M. oleifera sp.* seed extract.

Introduction. – Silver nanoparticles, in recent years have gained interest due to their applications in medicine, as a result of its antibacterial properties. It has also been acknowledged to have strong inhibitory and bactericidal effects along with antifungal, anti-inflammatory, and anti-angiogenesis activities[1].

It is required for silver nanoparticles to have a reducing and a capping agent for it to be synthesized and stabilized. Reduction takes place when silver ions (Ag^+) are reduced in aqueous or non-aqueous solutions and lead to the formation of metallic silver (Ag^0)[2]. The capping agent stabilizes the nanoparticle by limiting the size, controlling the nanoparticle morphology, and protecting the surface from aggregation of nanoparticles[3]. Both the reduction and the capping processes are essential for the synthesis of the silver nanoparticles; however, these processes would require a variety of chemical and physical methods which are potentially environmentally hazardous which involve use of toxic and perilous chemicals such as sodium borohydride or hydrazine that are responsible for various biological risks[4].

A solution for this is the use of an alternative method of synthesizing silver nanoparticles called green synthesis, a method that does not employ toxic chemicals but is done through the utilization of biological components which acts as both a reducing agent and a capping agent. This method cost effective, eco-friendly, and safer. It has been shown that among the candidates for green synthesis: plants, bacteria, and fungi; plants proved to be the most favorable because it contains effective biomolecules that enhance the synthesis rate[3].

The *Moringa oleifera sp.* tree is a candidate for the use of green synthesis, as it is highly nutritious and is a significant source of biomolecules that are necessary for the use reduction and capping of the silver nanoparticles[5]. A study by Prasad and Elumalai (2011)[6] was able to synthesize silver nanoparticles using *M. oleifera sp.* leaf extract and concluded that the leaf extract can demonstrate strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions. However, according to the phytochemical evaluation between the leaf and the seed, both of them had flavonoids and phenolics but only the seeds had alkaloids and proteins which are highly advantageous biomolecules that can act as capping agents[7]. According to the study of Jain et al. (2015)[8], the presence of a protein shell is highly

advantageous as it known to transmit solubility and stability in aqueous solution and is more effective when used against gram-positive bacteria. So far, *M. oleifera sp.* seed extract have not been used in synthesizing silver nanoparticles as a reducing and capping agent.

In this study, we will be utilizing *M. oleifera sp.* seeds because of the presence biomolecules such as alkaloids, flavonoids, phenolic compounds, and proteins which are favorable in synthesizing silver nanoparticles. This study aims to use a green method of synthesizing silver nanoparticles by using extract of *M. oleifera sp.* seed as both a reducing and capping agent and if it exhibits antibacterial activity.

Methods. – The conduct of the research experiment was divided in two phases: first, the initial state where the silver nanoparticles were synthesized with extract obtained from *Moringa oleifera sp.* seeds. The samples of silver nanoparticles were characterized using Ultraviolet-visible Spectrophotometer (Shimadzu 1800) and transmission electron microscope while the second phase was the testing of the silver nanoparticles antibacterial activity against *Staphylococcus aureus* culture.

In the first phase, the silver nanoparticles were synthesized using extract from *M. oleifera sp.* seeds which acted as both a reducing and a capping agent. The synthesis was done through three concentrations of *M. oleifera sp.* seed extract: a) 5 g b) 10 g c) 15 g in a 100 mL distilled water. The Ultraviolet-visible Spectrophotometer was used to monitor the formation of the silver nanoparticles during synthesis by measuring the absorbance levels. It was then characterized using a transmission electron microscope to know its average diameter and shape.

In the second phase, the silver nanoparticles antibacterial activity was checked against *Staphylococcus aureus*. This phase utilized the disk diffusion method through the four treatments, namely: a) water b) silver nitrate c) *M. oleifera sp.* seed extract solution and d) synthesized silver nanoparticles. The zone of inhibition in each treatment was then measured using a vernier caliper.

Seed extraction. M. oleifera sp. seeds were washed and dried to get rid of dirt. The seeds were then crushed and blended. The powdered seeds were weighed (5 g, 10 g, 15 g), and placed into three different 250 mL beakers containing 100 mL of distilled water, and were then heated at 250°C using a hotplate. The seed extracts were then filtered onto three different Erlenmeyer flasks using Whatman number six filter papers. The seed extracts were then stored in a refrigerator.

Synthesis of silver nanoparticles. 40 mL of 0.03 molar concentration (M) silver nitrate was measured. The silver nitrate was stirred at 400 rotations per minute (rpm). Then, ten mL of *M. oleifera sp.* seed extract was slowly dropped into the silver nitrate using a pipette. The same procedure was also done with the synthesis of the 10-g and 15-g *M. oleifera sp.* seed extract.

UV-visible absorbance spectroscopy. To monitor the synthesis of the silver nanoparticles quantitatively. Samples from the solution were taken at intervals 0, 15, 30, 45, 60 minutes, and 24 hours for Ultraviolet-visible Spectrophotometer analysis. One mL of the silver nanoparticles solution was taken using a micropipette and was transferred into a cuvette glass. The solution was then diluted with three mL of distilled water to allow the light to pass through during the UV-Vis spectrophotometer analysis. Two samples of cuvette were then placed inside the machine: one containing the silver nanoparticles solution and another one containing distilled water as the blank solution. The absorption spectra were then recorded from 300 to 700 nm.

Transmission electron microscopy. The 15-g solution of silver nanoparticles was brought and a small amount of it was placed in a 10 mL Erlenmeyer flask. The sample was then placed in a sonicator for 10 minutes in order for the aggregates to separate from one another. After the sample was sonicated, a small amount of the sample was transferred on the copper grid in a container. The container was then placed in a vacuum concentrator for 20 minutes in order to remove all moisture from the copper grid. The samples were then viewed on a transmission electron microscope at 25 000x, 40 000x, 200 000x, 400 000x, and 600 000x the original size.

Disk diffusion assay. Disk diffusion method was done to test the antimicrobial activity of the silver nanoparticles against *S. aureus.* The first agar plates, were divided into four quadrants each quadrant containing silver nanoparticles, silver nitrate, 15-g *M. oleifera sp.* seed extract, and, distilled water. Filter disks was then placed in each of the quadrant. The petri dish was then incubated for 18 hours to let the bacteria culture grow. After the incubation period, the standard zone of inhibition (ZOI) was measured using a vernier caliper.

Results and Discussion. – In order to monitor the stability and formation of the silver nanoparticles being synthesized, their absorption spectra were recorded using a UV-visible spectrophotometer while using distilled water as blank. Figures 1 to 3 show the UV-visible spectra of the silver nanoparticles formation using silver nitrate (0.03 M) as the precursor material with different *M. oleifera sp.* seed extract concentrations. All three silver nanoparticles solutions turned from colorless to pale yellow to brown while being synthesized which indicates the formation of the silver nanoparticles (AgNPs). The absorbance levels of the AgNPs synthesized by the 5-g seed extract as shown in Figure 1 increased with each passing time interval. Figure 2 of the AgNPs synthesized by the 10-g *M. oleifera sp.*

seed extract also showed the same trend with the exception of the decline of the samples absorbance levels at its 45th minute. The samples absorbance levels, however, increased during its 60th minute and after 24 hours. The AgNP synthesized by the 15-g *M. oleifera sp.* seed extract declined during its 60th minute otherwise its absorbance levels to increases over time. Overall, the absorbance levels of all the samples were observed to have increased particularly after 24 hours. Out of all the samples the AgNP synthesized by the 15-g *M. oleifera sp.* seed extract had the highest absorbance levels. This indicates great concentration of synthesized AgNPs which make the sample the better extract in order to produce AgNPs.

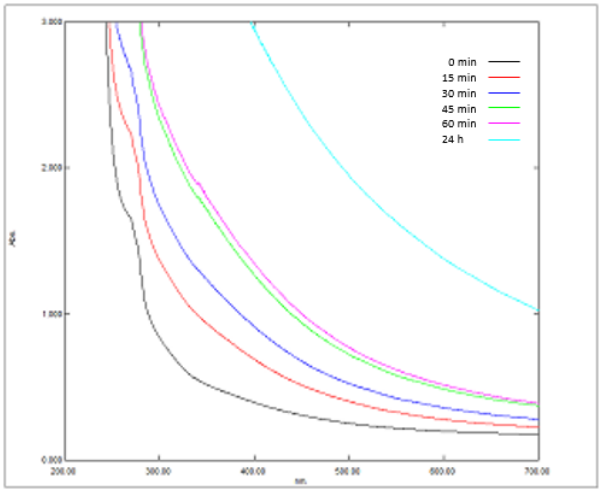

Fig. 1: Absorbance level of AgNPs solution using 5-g *Moringa oleifera sp.* seed extract.

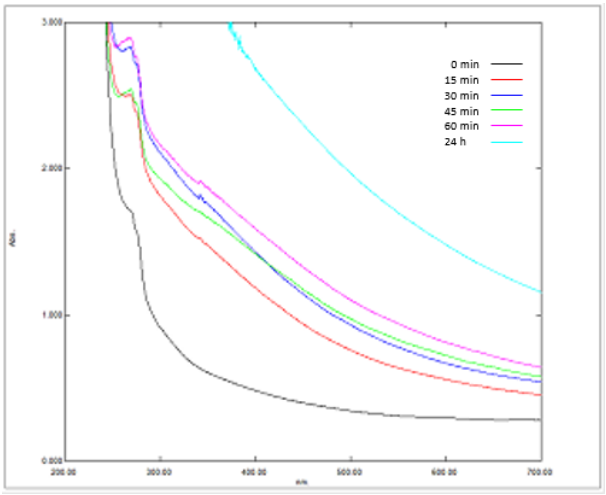

Fig. 2: Absorbance level of AgNPs solution using 10-g *Moringa oleifera sp.* seed extract.

Transmission electron microscopy indicated that the AgNPs synthesized with 15-g *M. oleifera sp.* seed extract

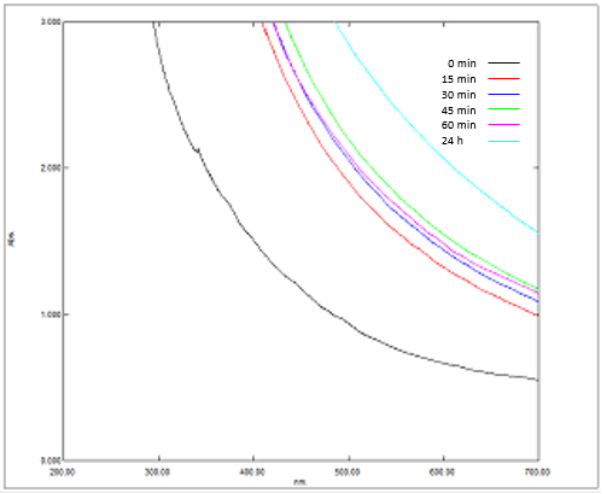

Fig. 3: Absorbance level of AgNPs solution using 15-g *Moringa oleifera sp.* seed extract.

were at a nanolevel, as shown in figure 4. Ultimately, the nanoparticles were mostly well dispersed and had a spherical shape while there were some to be found that were irregularly shaped. The diameter of the AgNPs had a mean size of 12 nm. Compared to the study of Sathyavathi et al. (2011)[9] which synthesized AgNPs with the mean size of 46 nm utilizing *Moringa oleifera sp.* leaf extract. This may be due to the fact that *Moringa oleifera sp.* seeds have more protein, that aids in the capping of AgNPs, content than the leaves[7]. It should be noted, however, that different concentrations of silver nitrate and plant extracts were used between these two studies which may have affected the size of the AgNPs. Typically AgNPs at smaller sizes exhibit more antibacterial property. In the study of Agnihotri et al. (2013)[10], it was concluded that AgNPs with the range sizes between 5-10 nm produced significant enhancement in killing bacteria. The study, however, used sodium borohydride and trisodium citrate as a reducing and capping agent respectively and did not utilize any biological compound. The shape of the synthesized nanoparticles was spherical which was very similar to studies that also synthesized using plant extracts. Shapes of nanoparticles usually have an effect on its performances including its antibacterial activity. Nanoparticles tend to usually be spherical since it minimizes energy in stable structures.

As shown in table 1, the AgNPs and silver nitrate exhibited antibacterial activity. The *M. oleifera sp.* seed extract and the distilled water treatments failed to inhibit the growth of the bacteria. The data from the disk diffusion assay were analyzed using Social Package for the Social Sciences Statistics software (SPSS). An independent samples t-test was conducted to compare the zone of inhibition in AgNPs and silver nitrate. There was no significant difference in the scores for AgNPs (M=0.90, SD=0.27) and silver nitrate (M=0.76, SD=0.27) condi-

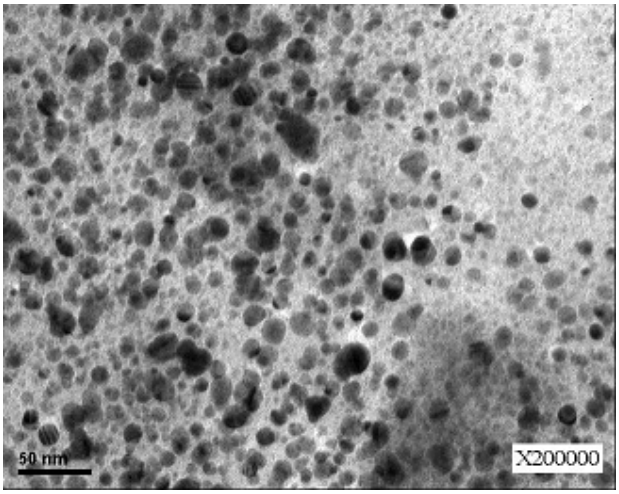

Fig. 4: Transmission electron microscope image of AgNPs synthesized with 15-g *Moringa oleifera sp.* seed extract.

tions; t (4)=0.622, p=0.568. The results suggest that both AgNPs and silver nitrate are comparable and can be both used as an agent against *S. aureus*.

Conclusion and Recommendation. – Silver nanoparticles were synthesized from the extract of the *M. oleifera sp.* seeds. The formation of the AgNPs was monitored via UV-Vis spectroscopy. Results showed that the AgNPs synthesized with 15-g *M. oleifera sp.* extract had greater absorbance levels than the AgNPs synthesized with the 5-g, and 10-g extracts. The synthesized silver nanoparticles were viewed under a transmission electron microscope and had a mean size of 12 nm and spherical shape. The silver nanoparticles also showed antibacterial activity against the *S. aureus* bacterial culture. It is still unknown whether this seed extract is only effective in synthesizing AgNPs; therefore, it is recommended in future studies to investigate the effectiveness of the *M. oleifera sp.* when used to synthesize other types of metal nanoparticles.

* * *

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Table 1: Susceptibility testing of *S. aureus* against AgNPs, silver nitrate, *M. oleifera sp.* seed extract, and distilled water.

Treatment	**Average ZOI** (mm)
Silver nanoparticles	9.0
Silver nitrate	7.6
M. oleifera sp. seed extract	0.0
Distilled water	0.0

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DNA Barcoding of Thiosulfate-Citrate-Bile Salts-Sucrose Agar-Selective Bacterial Species in the Mucus of Acropora millepora from Guimaras, Philippines

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Abstract – The purpose of this study was to identify Thiosulfate-citrate-bile salts-sucrose (TCBS) agar-selective bacterial species present in the mucus of *Acropora millepora* collected in Taklong-Island Marine Reserve, Nueva Valencia, Guimaras. Bacteria sample cultured in TCBS agar was sent to the Philippine Genome Center for the extraction and sequencing of the 16s rRNA gene. DNA Barcoding was done with the use of BioEdit software and BLAST. The bacteria sample cultured and subcultured in TCBS agar plates displayed green colonies, each having a diameter of 0.3 cm. The bacterial 16s rRNAgene was successfully extracted using Quick-DNA Fungal/Bacterial Miniprep Kit, amplified by PCR, and sequenced by Capillary Electrophoresis. The GenBank sequence database result displayed a 98 percent match with *Pseudomonas aeruginosa* 16S ribosomal RNA gene, partial sequence. This study will help assess the health of the *Acropora millepora* by further studying the implications of *Pseudomonas aeruginosa* found in its mucus.

Introduction. – Coral reefs support a high diversity of marine life, serving as home to 25 percent of allmarine lives on the planet, yet only taking up less than 0.25 percent of the entire marine environment. Seventy-five percent of the worlds coral reefs are threatened [13] and most are degrading [1]. Southeast Asias coral reefs are the most threatened in the world, and the Philippines, part of the Coral Triangle, has over 95 percent of reefs at risk. This makes the countrys coral reefs part of the most endangered coral reefs in the region [4]. In recent decades, there has been a dramatic increase on the part of threatened corals. Out of 845 reef building coral species, 32.8 percent of the 704 species assigned with conservation status are at an elevated risk of extinction [5].

The decline of coral cover results to the decrease in marine biodiversity. Unfortunately, even marine reserves are not enough to guarantee the survival of the organisms that greatly rely on corals [11]. A decline greater than 10 percent in coralcover will reduce the abundance of up to 62 percent fish species within three years [22]. Furthermore, a devastating decline reduces over 75 percent of reef fish species, of which 50 percent will halve their original numbers [11], putting rare species at a high risk of extinction [10].

Damage in coral reef systems are linked with coral bleaching where skeletal growth, reproductive activity, capacity to shed sediments, and resistance to competing species and diseases of the corals are reduced [8]. Coral reef bleaching has been observed on coral reefs such as the Great Barrier Reef [17]. Small scale bleaching events have been attributed with particular stressors such as increase in sea water temperatures [7] [3], whereas large scale bleaching cannot be explained by temperature alone [6]. Bleaching has been attributed to changes in ultraviolet radiation (UVR) and sometimes diseases such as Aspergillosis and Dark Spots [3] [14]. Some coral diseases and coral bleaching are caused by certain microorganisms, and findings show that pathogenic bacteria can cause coral bleaching to some corals [2] [12] [19] [20]. For example, bacteria under the genus of Vibrio have been observed to be pathogenic to coral *Oculina patagonica* [21]. These bacteria lives on the mucus of the host coral.

The aim of this research is to identify what TCBS agar-selective bacteria live in the mucus of *Acropora millepora*.

Methods. – *Overview.* The Methods is composed of three main parts, which is the culturing, extraction and sequencing, and dna barcoding.

Thiosulfate-Citrate-Bile Salts-Sucrose or TCBS Agar was prepared to be used for the bacterial culture. This

agar is a form of selective agar that selectively feeds only vibrio species.

For the preparation of the agar plate, the agar powder and water were poured in a media bottle, the agar powder and water having a ratio of 89.09 grams: 1 mL, respectively. After which, the mixture was set to boil and then poured into petri dishes for the bacterial culture.

The mucus samples were collected using sterilized syringes from two colonies of the hard coral, *Acropora millepora*, which was approximately a meter below the surface, from Taklong Island National Marine Reserve in Guimaras. The coral mucus were placed inside 1.5 mL microcentrifuge tubes and then transported to the laboratory in aseptic condition within two hours after collection.

Upon arrival at the laboratory, the mucus samples in the microcentrifuge tubes were then transferred to two centrifuge tubes. The centrifuge tubes were placed in a centrifuge, making sure that the two test tubes face each other to make sure that the centrifuge is balanced in the inside while it is rotating. The centrifuge was set at 2675 rpm for 3 minutes, causing the mucus to suspend on top of the liquid.

The samples then underwent a ten-fold serial dilution using 9mL of distilled water and 1mL of the mucus acquired through centrifugation.

To culture the bacteria, a triangular, glass hockey was used. Initially, the hockey has to be sterilized with the use of heat. After which, 0.1mL of the diluted coral mucus was placed on the agar plate, and then spread throughout the plate using the sterilized triangular, glass hockey. The agar plate was then closed and sealed, and then incubated at room temperature for three days.

After three days, the cultured bacteria were isolated using the Four Quadrant Streaking Method. A sterilized inoculating loop was used to streak the bacteria onto three new agar plates, after which they were then incubated for another three days at room temperature. This method was done once again after three days in order to ensure the purity of the samples. Morphological assessment was then conducted visually with the use of a vernier caliper, a compound microscope and an ultraviolet lamp.

After the assessment, an agar plate, containing the cultured bacteria with isolated colonies was packed tightly then secured to be delivered to the Philippine Genome Center in the National Institute of Molecular Biology and Biotechnology in the University of the Philippines Diliman for the 16S ribosomal RNA Extraction to Sequencing.

When the results arrived, the FASTA-formatted sequence was checked using the BioEdit software and was then inputted in the NCBI-GenBank. Basic Local Alignment Search Tool (BLAST) was used to find matching sequences in the NCBI databank. The highest matching sequence identifies the species of the bacteria.

Results. – This study aimed to culture and isolate TCBS agar-selective bacteria present in the mucus of *Acropora millepora* and extract and sequence their 16S rRNA for barcoding. Samples of *Acropora millepora*'s mucus was collected, prepared and plated on a TCBS agar plate for three days of incubation. Bacterial colonies were streaked on separate agar plates for subculturing after the third day. Bacterial morphology was assessed visually and with the use of a vernier caliper, compound microscope, and ultraviolet lamp. The petri dish containing the subcultured bacteria with isolated colonies was sent to the Philippine Genome Center for the bacterial 16S rRNA gene extraction to sequencing. The sequenced 16S rRNA was then analyzed using the BioEdit Version 7.1.9 and uploaded in NCBI-GenBank to identify the organism and gene using BLAST.

Culture TCBS agar-selective Bacteria. After three (3) days of incubation, all three petri dishes, A, B and C, plated with the coral mucus showed signs of bacterial colony growth. Only petri dish C showed isolated colonies numbering up to 12 distinct isolated colonies while the other two petridishes (A and B) had coalesced colonies. The colonies were green in color and around 0.3 cm in diameter. There was no bioluminescence observed after exposing the petri dishes to ultraviolet lamp. Under the microscope, there were no distinct differences observed; however, bacterial morphology was not thoroughly assessed. The subcultured petri dishes C1, C2, and C3 streaked from the isolated colonies of petri dish C also showed bacterial growth after three days of incubation. Subcultured petri dishes SC1, SC2, and SC3 streaked from petri dishes C1, C2, C3 respectively also showed bacterial growth after three days of incubation. SC2 and SC3 had isolated colonies.

DNA Extraction and Sequencing of 16s rRNA. The extraction to sequencing was done by experts of Philippine Genome Center. The sequencing result includes four files, two of which are of AB1 file and the other two of SEQ file. BioEdit shows a query length of 955 bases.

DNA Barcoding. The FASTA formatted sequence is a nucleic acid with query length of 955. The GenBank sequence database displayed *Pseudomonas aeruginosa* 16S ribosomal RNA gene, partial sequence. The description section shows a max and total score of 1555 and a 98 percent similarity, in terms of residues at the same positions in an alignment, with the FASTA formatted sequence emailed by the PGC. In the alignments section, the partial sequence of *Pseudomonas aeruginosa* displays 1555 bits(842) in score, 869/886(98 percent) in identities, and 4/886(0 percent) in gaps. Other alignments also shows *Pseudomonas aeruginosa* partial sequences with 98 percent identities.

Discussion. After the DNA Barcoding, the cultured bacteria was identified to be *Pseudomonas aeruginosa*. This indicates that the green colonies that grew in the TCBS agar are colonies of *Pseudomonas aeruginosa* and that no *Vibrio* bacteria is present in the mucus of *Acropora millepora* collected in Taklong Island National Marine Reserve. TCBS Agar is usually used to culture Vibrio species but inhibited only *Pseudomonas aeruginosa* which

could also grow in the TCBS agar [18] but with poor to no growth and green in colony color [16]. This result shows the presence of *Pseudomonas aeruginosa* in the mucus of *Acropora millepora* in TINMR.

Other studies conducted regarding coral-associated bacteria such as the study of Littman et. al (2009) [15] also received *Pseudomonas*-affiliated sequences from Magnetic and Orpheus Island clone libraries when they were studying the diversity of coral-associated bacteria in acroporid corals in the Great Barrier Reef. In a study conducted by Guimaraes et. al (1993) [9] on marine waters, they found out that as coliform, fecal streptococci, presumptive pathogenic yeast and heterotrophic bacteria count increase, there is also a notably increase in the occurrence of *Pseudomonas aeruginosa*. This could possibly explain the presence of *Pseudomonas aeruginosa* in the marine water; thus it is not impossible but that they could have possibly been present on the coral mucus too.

A correlation between the health of the water and the health of the coral cannot be made since our study focused on just identifying the bacterial species thriving on the coral mucus using a TCBS agar and not necessarily its abundance.

Conclusion. – Culturing of bacteria from the coral mucus in TCBS agar, extraction to sequencing of the bacterial 16S rRNA, and DNA Barcoding the result gives a 98 percent match with the bacteria *Pseudomonas aeurigonosa*.

* * *

This work would not be possible without the help of the people from Taklong Island National Marine Reserve, Guimaras for allowing us to gather samples from the marine reserve, as well as helping us throughout our sample gathering.

We are also grateful to the people whom we had the pleasure to work with. Each member of our strand namely, Lourde Frances Mye Almarza, Anna Karenina Dalabajan, Marc Angelico Elizalde, Suzielette Veve Hilay, Jimdel Ryu Mabaqiao, and Ma. Cailah Velasco, have helped and supported us, from the proposal presentation up to the final presentation.

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Effects of Collector Sea Urchin (Tripneustes gratilla) on the Reduction of Brown Seaweed (Dictyota spp. and Padina spp.) Cover in Post-Coral Bleached Systems

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Abstract – The purpose of this research is to determine the effects of *Tripneustes gratilla* in reducing the abundance of *Dictyota dichotoma* and *Padina spp.* in macroalgal-dominated marine ecosystems. Corals attached with macroalgae were exposed to the *Tripneustes gratilla* for two weeks. Measurement of the macroalgal density before and after the exposure of *T. gratilla* was administered. The Fleshy Macroalgal Index of the units with sea urchin and without sea urchin were also compared and analyzed. A software, PhotoQuad, was utilized to quantify the FMI of each unit. The results showed that the presence of *T. gratilla* did not significantly reduce (p=0.23) the macroalgal cover in comparison to the control group. Nevertheless, the compared FMI of the pre-exposure and post-exposure to the urchin showed a significant difference (p=0.023). In conclusion, the reduction of the macroalgal cover may be attributed to the presence of *Tripneustes gratilla*. Although it is only comparably different, the exposure to *Tripneustes gratilla* can potentially aid in the recovery of macroalgal-covered post coral bleached systems and in the control of invasive macroalgae population.

Introduction. – Coral reefs are known as the most biologically diverse and productive ecosystem on the planet [1]. Massive bleaching of corals has been observed over the past two decades in different parts of the worlds ocean [**?**]; Shenkar et al. 2005) and considered as the most serious threats for coral reefs [2]. It is caused by the disruption of the symbiotic interaction between the coral host and the symbiotic algae, Symbiodinium inducing a massive expulsion of these symbionts or loss of their pigments occur [2,3,14] . This phenomenon can be linked to global climate change and increasing ocean temperatures [4].

Coral mortality following bleaching events provides space on the reef for rapid colonization by turf-forming algae [5], which compete with corals for space, and prevent coral recover (Tanner 1995). Macroalgae produces allelochemicals which can influence the growth, survival and reproduction of other organisms within their vicinity [6]. They are known to suppress the settlement and fitness of corals which is why coral reefs that are dominated by macroalgae can lead to further decline [15] Haan J 2015). Results from previous studies showed that the coral-algal interaction have significant, species-specific, negative effects on the growth and survival of corals [7]. The dominant scleractinian group, Acropora spp., is found to be the most strongly impacted by contact with allelopathic macroalgae [15]. It is also highlighted that the outcome of the macroalgal-coral competition can have significant implications on the growth of corals [7].

The presence of sea urchins can influence the community structure of habitats containing marine plants and algae populations [8]. They are usually found in areas abundant in algae. The collector sea urchin (*Tripneustes gratilla*) is an herbivore widely found in tropical and subtropical areas [9]. Their abundance and distribution in coral reef areas suggest that they play an important role ecologically and is vital to the flow of matter and energy [10].

Methods. – This chapter discusses the methods used in conducting the study and in collecting the data. The research experiment is divided into four phases: collection of the samples needed, exposure of the macroalgae to

the coral fragments, exposure of the sea urchin to the macroalgal-coral setup and comparison of initial and final data of control and experimental setup.

Experimental Set-up. The experimental set-up of this study was based on the study of Rasher and Hay (2011) entitled Chemically rich seaweeds poison corals when not controlled by herbivores. The set-up was established in Piagau Island, Brgy. La Paz, Nueva Valencia, Guimaras, Philippines. The metal cage has a total of ten units. Five units of coral and macroalgae were prepared in the cage for the control group. As for the treatment group, five units of coral, macroalgae, and sea urchin were prepared. Randomized Complete Block Design (RCBD) was used as the experimental design. In RCBD, replicates are separated randomly and each treatment has an equal probability of being assigned to a given experimental unit.

Collection of Samples. The researchers, with the assistance of professional divers, gathered bleached coral fragments of *Acropora spp.* found in Taklong Island National Marine Reserve, Guimaras, Philippines. These fragments are approximately the same length, five to six cm, for the standardization of the macroalgae-coral contact. It was collected using rubber gloves to avoid direct contact with the corals. The macroalgae were collected in Brgy. Lapaz, Nueva Valencia Guimaras having the same weight of five grams per unit. Lastly, the *Tripneustes gratilla* were collected in Brgy. Canhawan, Nueva Valencia, Guimaras with the assistance of authorized personnel. The researchers were assisted by experts in the field of identifying the organisms stated above to validate the identification of the organisms used in the study.

Coral Planting. The branches were glued individually into cement cones with a diameter of 8 cm using an underwater epoxy. Marine epoxy was used because there are no recorded negative effects as coral adhesives and it is effective in attachment and transplant survival (Dizon et al. 2008). It is essential to plant the corals in order to simulate the natural conditions of the macroalgal-coral competition. See fig. 1

Placement of Corals in the Caged Racks. The treatments and controls replicates (n=15 coral branches each) have separate metal racks. The metal racks were covered with 1x1 cm grid metal screen to avoid other herbivores from entering. The racks were placed on designated areas around the island. Each of the coral branches within the cement cones were interspersed 15-cm apart across the metal rack. As a means to minimize the extraneous variables and for easier measurement, there is a divider between each of the coral branches. The measurement of the whole metal rack is 115x46x50 cm (LxWxH). Three metal racks were used so that there would be 15 organisms for each experimental group for the statistical analysis.

Fig. 1: Coral branch planted into a cement cone

Exposure of D. dichotoma and Padina spp. to Acropora spp. The macroalgae species *D. dichotoma* and *Padina spp.* were collected around the site. As seen in Figure 2 (appendix b), nails were embedded on the opposite side of each cement cone in order for the rope attached with the D. dichotoma and Padina spp. to be positioned over each nail head. In this way, the D. dichotoma and Padina spp. were in stable contact with the coral. The macroalgae used in the coral-macroalgal contact are found in the site. In order to avoid stress compounds that might be emitted from the deployed *D. dichotoma* and *Padina spp.*, the whole thalli was used. The coral branches were exposed to the macroalgal species for 14 days. Initial data will be collected before the exposure of *T. gratilla* to the treatments.

Exposure of T. gratilla to the treatments. The *T. gratilla* were collected in Brgy. Canhawan, Nueva Valencia, Guimaras. Only one *T. gratilla* will be exposed per unit. The exposure will last for 14 days.

Measurement of the Fleshy Macroalgal Index (FMI). The FMI will be measured using the photoquad method. Photoquad method provides visual estimation but in a digital version. This method is usually used in coral monitoring programs in field surveys [**?**]. The remaining macroalgae attached to the rope were photographed. After taking a picture, the photo quadrat analysis software, photoQuad , quantified the FMI using the grid cell count option. A direct estimate of the species cover was automatically performed. The software offers a more versatile, quick and accurate result. The FMI of each unit will be measured before and after the exposure *T. gratilla* for comparison.

Data Analysis. In order to compare results, statistical software PAST will be used. The statistical test T-test will be used in order to compare the means of FMI of each unit. The initial and final FMI of the controls were also compared and analyzed. The statistical software used in computing the paired sample test was IBM SPSS

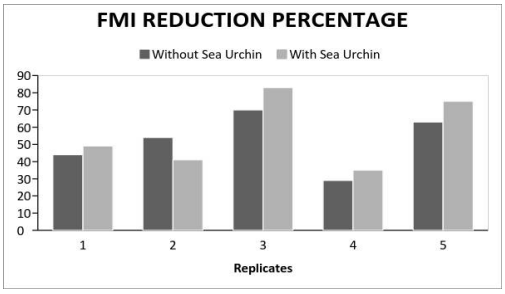

Fig. 2: Comparison of the Fleshy Macroalgal Index reduction values between units with and without sea urchin

Statistics 22.

Proper Disposal Tripneustes gratilla were returned to its natural habitat, *Acropora spp.* were replanted around the area of Piagau Island, and the remaining *Dictyota dichotoma* and *Padina spp* were disposed. The metal cages were left in their original locations to serve as a fish nursery, as prescribed by the Department of Environment and Natural Resources.

Results and Discussion. – There is a general reduction in percent coverage in all the experimental groups. Despite having a preference in consuming *Padina sanctae-crucis* compared to any other macroalgal species (Stimson et al. 2007). The presence of *T. gratilla* in post-coral bleached systems did not significantly reduce the macroalgal cover in comparison to the control group (See fig.2, refer to table 1). Although, it can be observed that the FMI reduction percentage of the treatments, in general, were greater compared to that of the controls. There was only a comparable difference. Nevertheless, the compared FMI of the pre- and post-exposure to the urchin showed a significant difference (See fig.3, refer to Table 2). This suggests that certain factors have probably affected the reduction of macroalgal cover of both experimental groups which has led to some discrepancies in the data acquired.

First probable factor may be the failure to control all possible existence of extraneous variables, such as the presence of other organism inside the cages. The unwanted organisms found in the cage were school of juvenile reef fishes and sea cucumbers. The cages were intentionally designed to restrict the entrance of these organisms, yet their natural biomechanisms has allowed their entrance. The presence of these organisms was beyond the control of the researchers. The small size of juvenile reef fishes and the capability of sea cucumbers (*Synapta maculata*) to congest themselves has enabled them to penetrate the cages (Elder Trueman, 1980). It is possible that these organisms have consumed the macroalgae in each unit, since these organisms are known herbivores (Hay, 1997; Martinez-Diaz and Perez-Espana, 1999). Second probable factor is the unstable weather

Table 1: Computed t-value between units with and wthout sea urchin based on the FMI

Fleshy Macroalgal Index (FMI)	
Variable	t-value
Cell count	0.3625
Area(cm2)	0.41675
Coverage percentage	0.23299

conditions. In a study conducted by Sinnot (2009), it concludes that water quality has a significant effect on the growth of macroalgae at certain levels. It has been shown that turbidity severely limits the capacity of macroalgae to photosynthesize and grow (Hay 1981). The varying parameters due to the unstable weather conditions during the data gathering might have greatly affected the macroalgal growth. Inconsistent environmental conditions could lower the metabolic rate [10] Third, the weight of the sea urchin might also have affected the percentage reduced because of the varying capacity of consumption. Because of the variance in the weight and sizes of the sea urchin, the rate of consumption also differs. The smaller the size of the sea urchin corresponds to a higher ingestion rate of the organism [10]. Lastly, it may also be caused the deduction of replicates of both experimental groups due to the factors aforementioned. Originally, there were three cages, and each cage contains five replicates of each control and treatment groups, but due to these uncontrolled variables, some of the replicates have been contaminated. The researchers disregarded the contaminated units in order to avoid unreliable and inaccurate data.

The significant reduction of the macroalgae from pre- to post-exposure may be attributed to the general herbivory of *Tripneustes gratilla*. Studies suggest that broad-spectrum preference and fast feeding mechanism can be utilized as a potential biocontrol agent, particularly to invasive macroalgae (Stimson et. al., 2007; Conklin and Smith, 2005). Given that the one of the main food preference of T. gratilla is a *Padina sp.* and it is also known to feed on *Dictyota sp.*, there is a higher possibility that this is the reason behind the obtained data.

A slight gain of color in some of the corals was visually observed after the exposure to *T. gratilla.* The bleached structures of the corals when pre-exposed to the macroalgae turned to a faint brown color. This suggests that there is a possible recovery from bleaching. However, in order for this statement to be verified, further studies need to be done. The corals which recovered were returned to their natural habitat for them to further recuperate.

Table 2: Computed t-value and p-value between the pre- and post-exposure FMI values

Fleshy Macroalgal Index (FMI)			
Variable	t-value	Df	Significant
Cell count	7.039	14	0.017
Area(cm2)	7.114	14	0.014
Coverage percentage	6.787	14	0.023

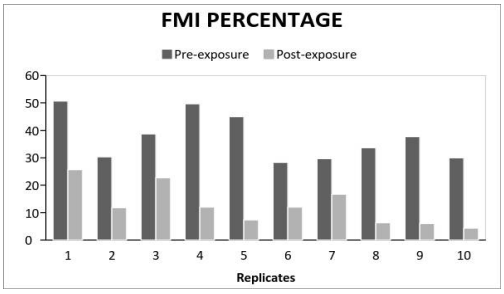

Fig. 3: Comparison of the Fleshy Macroalgal Index percentage values between units before and after exposure to sea urchin

Conclusion. – The reduction of the macroalgal cover may be attributed to the presence of *Tripneustes gratilla*. Although it is only comparably different, the exposure to *Tripneustes gratilla* can potentially aid in the recovery of macroalgal-covered post coral bleached systems and in the control of invasive macroalgae population.

* * *

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Synthesis of Chitosan-Hydroxyapatite Composite Gel from Waste Crab Shells for Oil Adsorption

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Abstract – Composites consisting of oil adsorbing materials such as chitosan and hydroxyapatite has been proven to be a viable oil adsorbent in treating oil spills. As a waste product, crab shells can be utilized as a source of raw materials for an oil adsorbent. In this study, the oil adsorption capacity of synthesized chitosan-hydroxyapatite composite were investigated and compared. Chitosan and hydroxyapatite were simultaneously extracted from crab shells. The extracted samples were then verified using the Fourier Transform Infrared Spectroscopy. 1:1 ratio of chitosan and hydroxyapatite were prepared in a form of a gel composite.

Introduction. – Oil is highly essential to man specifically to the energy industry because it is one of the main sources of electricity. However, oil possesses a great threat to the environment if not properly observed. In 2015, records show that approximately 7000 tonnes of oil is spilled globally with portions happening here in the Philippines [1]. The known oil spill in the Philippines happened in the year 2006 where an oil tanker sank just off the coast of the Guimaras and Negros islands. Although oil spills may also happen in land, marine spillage tend to be more common as offshore drilling is better in terms of investment [2].

Oil spills cause pollution to the environment which greatly affects both plants and animals living near the affected area. Surface animals like seabirds and otters tend to be the ones greatly affected since oil floats in water. Not only does the pollution slowly kill the animals but also their floating corpses when they die will lead to more pollution yet less in scale [3].

Methods in cleaning up the oil takes a lot of time and resources which slows down the treatment of the water. Some of the solutions are not advisable due to the consequential effects it withholds. One solution is burning the oil, however, it may increase air pollution due to the combustion process. In addition, oil tends to spread on the surface in a thin film thus having a difficulty in burning. Another solution involves emulsifying detergent, but it does not remove oil from the water as it only separates the oil to fine particles [4]. Leaving the oil alone itself is already a solution as it will naturally break down [5], however in terms of long-term effects, this option is not advisable. Other solutions also include sucking up the oil, containing the spilled area within a large dome and even using bacteria that eat up the oil itself [6].Solutions vary from being industrially-made to eco-friendly alternatives, however majority of these require a huge amount of funding.

Cleaning up oil spillages is difficult to perform as it requires more sophisticated valuable resources and machinery. Although cheap alternatives of cleaning up oil have been found. One of these alternatives are chitosan-hydroxyapatite (chitosan-HAp) composites which can be used as a sorbent for oil cleanup [7] [8]. From related studies, chitosan-HAp composites could solve the problem of water pollution as it can also remove other pollutants [9]. However, it is preferred to utilize natural waste products as a source for composite synthesis not only effective in oil adsorption but also cheap and reduces waste products.

Natural waste products such as waste crab shells can be a possible source for chitosan and hydroxyapatite. Both can be found at the exoskeletons of crabs [10] [11]. In addition, 1,354 tonnes of crab shells is wasted every year which can be utilized for chitin production [12]. By utilizing waste crab shells for the synthesis of the chitosan-HAp composite, not only oil spills can be treated but also levels of crab shells can be reduced. Thus, this study aims to

create a chitosan-HAp composite from waste crab shells that could be effective for oil adsorption.

Chitosan and hydroxyapatite were extracted from the crab shells. A composite in gel form was produced from these two materials with a ratio of 1:1 chitosan-hydroxyapatite. It was then tested for oil adsorption capacity. The chitosan and hydroxyapatite were verified using Fourier Transform Infrared Spectroscopy instrument. Furthermore, personal protective equipment was worn at all times and the chemicals were properly disposed in accordance with the Materials Safety Data Sheet.

There were two (2) batches of the composite with eight (8) samples per batch. Composite A had an adsorption capacity of 0.107 0.177 while composite B had an absorption capacity of 0.049 0.019. Both had the capacity to adsorb oil however, there was no significant difference p-value at 0.065.

Methods. – This study is composed of four phases: extraction, synthesis of composite, testing, and data analysis.

Extraction of chitosan. Five hundred (500) grams of crab shells were cleaned and crushed. The crab shells were put in a 1L beaker with 4% NaOH to boil at 100C for one (1) hour to deproteinize the samples [10]. The samples were cooled down at room temperature. The samples were then pulverised. The pulverised crab shells were sieved using a 0.2 mm mesh sieve to acquire finer particles of crab shell powder. One percent (1%) HCl was added to the sample powder in a ratio of 1:4 w/v powder to HCl for 24 hours. The HCl was drained from the mixture. Two percent (2%) NaOH solution was added to the powder in a 1:2 w/v ratio powder to NaOH solution. This treatment was done for one hour to remove the albumen. The treated powder was washed using distilled water [13] [14]. The sample was then soaked in boiling 50% NaOH for two (2) hours. The powder was filtered from the beaker and cooled down at room temperature for one (1) hour. The powder was washed carefully with 500 mL 50% NaOH. The powder was oven dried at temperature of 120C for 24 hours [15].

Extraction of hydroxyapatite. Five hundred (500) grams of crab shells were cleaned and crushed. The crab shells were boiled in distilled water for two (2) hours to remove adherent meat. The crab shells were filtered from the beaker and air dried. The crab shells were pulverised and then sieved using 0.2 mm sieve to acquire fine particles. The acquired powder was heated in a furnace at 1000C for five (5) hours to form calcium oxide powder. It was cooled down at room temperature. The powder was then mixed with distilled water in a 1:1 w/v ratio solution

The phosphoric solution in 0.6M was added dropwise resulting a 5:3 m_{powder}/v_{H3PO4} solution. The solution was kept at room temperature for 24 hours to precipitate. The precipitate was filtered using a Whatman No. 40 filter paper and washed carefully with distilled water. The precipitate was oven dried at 120C for three (3) hours and sintered by heating it in a furnace at 900C for four (4) hours [16].

Drying. The collected samples were sundried for 6 hours under sunlight and air dried in the laboratory at night for two days until no more moisture was present in the sponge. They were then minced into the finest possible particle that can be obtained.

Synthesis of composite. A ratio of 1:1 chitosan and hydroxyapatite was followed. Twelve (12) grams of chitosan was dissolved with the use of a magnetic stirrer at 600 rev/min in a 250 mL distilled water with 1% v/v acetic acid. Same amount of hydroxyapatite was added slowly to the mixture while stirring. Followed by the addition of 12.5 mL of 2.5% glutaraldehyde solution to the mixture. After the 90 minutes of stirring, 15 g of gelatin powder was added to the mixture while continuously stirred at 40C. While warm, the mixture was poured into eight (8) molds which were divided into two (2) batches, batch A and B. Both batches of the composite were air dried to form completely for 48 hours [17] [18].

Testing. The initial weight of the samples were recorded with the use of an analytical balance. The samples were submerged in crude oil for 30 minutes then removed with a strainer and were weighed. The amount of oil adsorbed was calculated using the following formula [19]:

$$qe = (\frac{w_o - w_e}{M})$$

Where:
$q_e = amountofoiladsorbedperunitweightofadsorbent$
$w_o = initialweightofoil(g)$
$w_e = weightofoilsampleaftertheadsorptionprocess(g)$
$M = massofadsorbent(g)$

Results. – This study uses Fourier Transform Infrared Spectroscopy Instrument. In figure 1, an infrared spectrum of extracted chitosan is shown. A wide peak is located in 3200-3400 cm^{-1} wavelength attributed by the vibration of the stretching of O-H and N-H bonds. Additionally, sharp peaks are found at around 1400 cm^{-1} and 900 cm^{-1} wavelengths caused by CH-OH and C-O-C bonds respectively.

In figure 2, the infrared spectrum of the extracted hydroxyapatite is shown. Sharp peaks are located at around 900, 1100, and 3600 cm^{-1} wavelengths due to P-O, P-O, and O-H bonds respectively. Another peak is located at

around 1400 cm^{-1} wavelength attributed by the C-O bond.

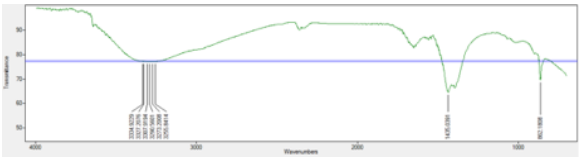

Fig. 1: Infrared spectra of extracted chitosan

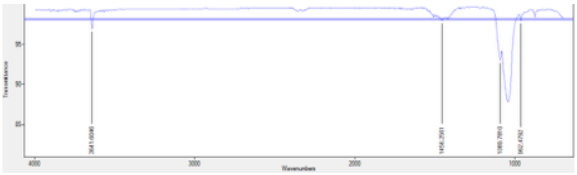

Fig. 2: Infrared spectra of extracted hydroxyapatite

The adsorption capacity of the composite was measured by weighing the molded composite before and after it was introduced to the crude oil. To identify the significant differences, the Independent Samples t Test statistical tool was used. Table 1 shows the difference between composite A and B. Composite A had an adsorption capacity of 0.107 0.177 while composite B had an absorption capacity of 0.049 0.019. Although composite A had higher adsorption capacity, there is no significant difference between composite A and B with a p-value of 0.065 shown in table 2.

	Composite	N	Mean	Std. Deviation	Std. Error Mean
Weight	A	8	.107200	.1767566	.0624929
	B	8	.048775	.0190642	.0067402

Table 1: Group Statistics

		F	Sig.	t	df
Weight	Equal variances assumed	4.011	.065	.930	14
	Equal variances not assumed			.930	7.163
	Equal variances not assumed			.930	7.163

Table 2: Independent Samples t-Test

Discussion. – In figure 1, a wide peak was located at 3200-3400 cm^{-1} which confirmed the presence of O-H bonds. However, the significant peaks were located at 1400 and 900 cm-1 contrary to 1150 and 1317 cm^{-1} [20]. One possible reason was that the chitosan did not fully convert from the deacetylation of chitin. The C-O bond from the sample maybe from the C=O bond from the chitin which may not have fully converted [21].

In figure 2, sharp peaks were located at 962, 1090 and 3641 cm^{-1} . This was almost identical to the standard hydroxyapatite IR spectrum [22]. Two distinguishable peaks (962 and 1090 cm^{-1}) were located close to peaks 964 and 1093 cm^{-1} from the reference spectrum which confirmed the presence of P-O bonds. Moreover, another peak at 3641 cm-1 attributed to O-H bond present in hydroxyapatite. From the similarities of peaks, it can be implied that the sample was hydroxyapatite. However, an extra peak was located on wavelength 1456 cm^{-1} . Broad bands at 3432 and 1642 cm^{-1} attributed to the adsorbed water [22].

The composite produced was in a form of a soft gel. There were two (2) batches of the composite in order for the data to be more reliable. Each batch was composed of eight (8) small composites. The adsorption capacity of the composite was measured by weighing the molded composite before and after it was introduced to the crude oil. Composite A had an adsorption capacity of 0.107 0.177 while composite B had an absorption capacity of 0.049 0.019. From the results, it was shown that the chitosan-HAp mixture between the two (2) batches was insignificant with p-value at 0.065 yet both had the capacity to adsorb oil.

Conclusion.. – It was found out that chitosan-HAp composite gel was viable as an oil adsorbent due to its effective capacity to adsorb oil. In addition, there was no significant difference between composite A and B with a p-value of 0.065. For further studies, it is recommended to test various ratios of chitosan-HAp to determine the most effective adsorption capacity of the composite.

Recommendations. – To further improve this study, the use of equipment such as freeze-dryer and other cross-linking chemicals are recommended for the synthesis of the composite for it to form a more stable gel. It is recommended to test various ratios of chitosan-HAp to determine the most effective adsorption capacity of the composite. Additionally, using other forms possible for the composite such as sheets, microbeads and is also recommended. If certain equipment or chemicals are unavailable, it is recommended to use alternative methods for the synthesis.

* * *

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Fabrication of Sugarcane Bagasse Based Mesh as an Adsorbent of Copper (Cu^{2+}) Metal Contaminant for Wastewater Treatment

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Abstract –Water pollution is one of the main problems faced in the present due to contaminants from industrial areas discharged to the environment such as toxic heavy metals. Sugarcane bagasse as a raw waste material can be utilized to adsorb known metal contaminants such as copper. In this study, the percent reduction of the fabricated bagasse mesh was investigated. The testing of the product involved exposure of the mesh to known concentrations of synthesized wastewater. The final concentrations were then analyzed using the UV-Vis spectrophotometer. Results show that the mean of percent reduction amongst 5 replicates is 37.2 31.5 percent. Certain parameters, such as contact time and pore size, affect the adsorption rate of the mesh yielding low in value. From these results, it is concluded that sugarcane bagasse based mesh is viable for copper adsorption. Modifications is recommended to further increase the adsorption rate of the product.

Introduction. – Water is an essential resource due to its importance to human survival. Industrial use and commercial use are examples of water usage that rely on large quantities of water. Different industries may be a great source of income in some developing countries; however, it brings negative consequences to aqueous environments near industrial sites. Industrial applications such as processing, rinsing, scrubbing, and cooling of water cause industrial wastewater, a source of harmful contaminants, to increase that may be discharged to the environment if not properly observed [3]. Textile factories, an example of an industrial site, can be a major cause of water pollution due to its discharge of contaminants such as harmful chemicals and dyes. Over 2,000 types of chemicals and 7,000 types of dyes from textile production were identified and could potentially contaminate the water near its site [13]. These chemicals and dyes that are exposed to water systems cause water pollution, which is a serious concern that needs to be addressed. These chemicals include heavy metals, such as copper, cobalt, and zinc, which are considered the most harmful pollutants that pose a serious threat to human health [6]. Copper is a toxic heavy metal, which causes death if ingested in high concentrations. Copper in its dissolved state is unfilterable, thus difficult to remove in the water system. There are numerous ways to remove copper from contaminated water. One of these methods is chemical precipitation, which removes copper via hydroxide and sulfide; however, it may pose negative effects to the environment from the ionic exchange process [8]. Another method is the use of bio filtration systems that accumulate contaminants from urban storm water runoff, preventing discharge to the water; however, this method is expensive since mechanical parts being used in this system are costly [5]. Aerobic biodegradation, the breaking down of organic contaminants when oxygen is present, is a method for conventional wastewater treatment. This can also decrease the concentration of contaminants; however, it possesses low removal efficiency due to low biodegradation of these contaminants such as dyes [11]. Aside from the methods stated, there are other alternatives that can be used for removing contaminants. There is an increase of popularity in using agricultural wastes to treat wastewater since it is low-cost, efficient and practical to use for water clean-up [9]. One of these wastes is sugarcane bagasse, or bagasse, which are fiber residues after sugar extraction from sugarcane [2]. In the Philippines, the largest sugar output is located in the Visayas region, the Negros Island particularly has the biggest hectarage of bagasse [12]. Bagasse can be utilized for product making and as an energy resource; however, a significant portion of it is stockpiled and may be considered waste if not used in the long run. Using bagasse for wastewater treatment could be a possible application. There have been several studies such as the study by Rana et al. [10] that uses bagasse as an adsorbent for copper. Using raw agricultural waste material as an adsorbent to remove metal contaminants is a solution that is both low-cost and time-efficient. From this advantage, bagasse can

be utilized to create a product that could increase the adsorption efficiency of the material. Possible modifications such as mesh structure could increase adsorption efficiency due to increase of surface area and also contribute in filtering unwanted filterable solids in the water. There are no studies or methods regarding the synthesis of mesh made from bagasse fibers to treat wastewater, thus, this study aims to create a mesh from bagasse fibers to adsorb Cu^{2+} ions from copper contaminated water. This study specifically aims to fabricate a mesh from sugarcane bagasse, test the mesh against contaminated water, and determine the percentage of bagasse mesh. As more industries rely on water usage for production, greater amounts of contaminated water are discharged and cause water pollution to the environment. Creating alternative technologies to remove unwanted toxic particles, such as copper, is preferred to decrease the pollution rate of environmental waters. Studies of copper removal in drinking water have been conducted to minimize the casualties of copper intoxication that may affect negatively to human health. In addition, implementing mesh based design using raw materials could also solve the problem of suspended solids in water through filtration. Using wastes such as sugarcane bagasse to adsorb harmful chemicals in contaminated water will not only help in wastewater treatment but will also promote the use of wastes as low-cost adsorbents to remove pollutants in the environment.

Methods. – This study aims to create a mesh from bagasse that can adsorb Cu^{2+} ions from synthetic copper contaminated water. Five meshes were coated with raw sugarcane bagasse with epoxy. The mesh made from the bagasse was treated with sulguric acid for thirty (30) minutes and then dried in an oven for 24 hours at 100C. Testing of the products involved using polyvinyl chloride (PVC) pipe with the attached fabricated mesh as a makeshift adsorbing column and let the synthetic wastewater flow through the pipe three times. The concentrations were then measured using the ultraviolet visible spectrophotometer (UV-Vis).

Preparation. One kilogram of bagasse was acquired from Central Hawaiian, Silay City, Negros Occidental. It was pre-washed and dried in an oven at 150C for 2 hours. Glasswares used were acquired from the SRA Chemistry at PSHS-WVC and were pre-washed, dried and calibrated. For the copper stock solution, 0.200g of solid $CuSO_4$ was diluted with 25.0 mL of distilled water to achieve a concentration of approximately 0.0500M. The solution was then further diluted by mixing 15.0 mL of the 0.05M copper solution with 735.0 mL distilled water in a 1L beaker to acquire a 750.0 mL of 1.00 x 10^{-3} M copper solution. 500.0 mL of the copper stock solution were used for the testing of the mesh while the remaining 250.0 mL were stored and further diluted to be used as standards for analysis.

Mesh fabrication and treatment. The mesh was created by using sterile gauze as base. The base was used because of the bagasse fibers short length. Then, the epoxy was beforehand diluted with 100 percent denatured alcohol to effectively spread the epoxy between the fibers. The solution was then added to the mesh to hold the fibers. The mesh is then left to dry overnight. The bagasse meshes were treated with concentrated sulfuric acid for 30 minutes and then were kept in an oven at 150C for 24 hrs to activate the adsorbent.

Fig. 1: Treatment of the bagasse

Testing. The fabricated mesh was attached to one end of the PVC pipe using a rubber band. The set-up served as a makeshift adsorbing column. The synthetic wastewater, 100mL of the copper stock solution, was then poured to the other end of the pipe. This process was then repeated thrice to acquire maximum percent adsorption of copper [1]. Afterwards, the synthetic wastewater will be stored in a container for UV-Vis spectroscopy. This was repeated five times for a total of five replicates.

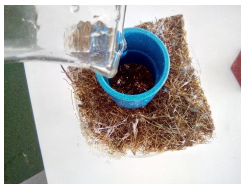

Fig. 2: Mesh testing

This process was then repeated thrice to achieve a much higher adsorption capacity for five mesh replicates. Five varying concentrations were prepared from the copper stock solution.

Fig. 3: Five concentrations

Then, two drops of aqueous ammonia were added to each test tube. Then these concentrations were analysed using the UV-Vis spectrophotometer to acquire the standard curve.

Analysis. Five unknown concentrations from the testing were also analysed. Again, two drops of ammonia were

added to each flask and the concentrations of the tested wastewater were analysed using the UV-Vis spectrophotometer. After acquiring the data, percent reduction of copper was calculated using this equation.

$$\%\ Reduction = 100 - \left(\frac{Concentration\ of\ adsorbate}{Initial\ concentration\ of\ adsorbing\ solution}\ x\ 100\right)$$

Disposal. Proper disposal was then observed. Remaining bagasse wastes were put in a separate bag and was properly disposed. Materials and chemicals used during testing were kept in secured waste containers and was disposed in hazardous bins.

Results. – These are the results of the study. Before presenting the numerical results, the characterization of the mesh was discussed. The figures show the bagasse mesh before and after chemical treatment. After treatment, bagasse mesh increased in thickness and there is partial discoloration of the material.

Fig. 4: Bagasse mesh before testing

Fig. 5: Bagasse mesh after testing

Figure six shows the percent reduction of copper among the five mesh replicates. It is evident in the figure that the mesh can adsorb variable amounts of copper. However, values of copper reduction are spread out due to high standard deviation.

Discussion. – It is shown that Replicate 3 had the lowest percent reduction rate of 21.5 percent while Replicate 5 has the highest with 58.9 percent. The mean of the reduction values of five replicates is 37.2 percent with a standard deviation of 31.5 percent.

Effects of contact time. Certain parameters such as contact time, initial concentration, chemical treatment, epoxy, pore size affected the adsorption rate of the bagasse mesh. The contact time was the major parameter that affected the adsorption of the material. Due to small contact

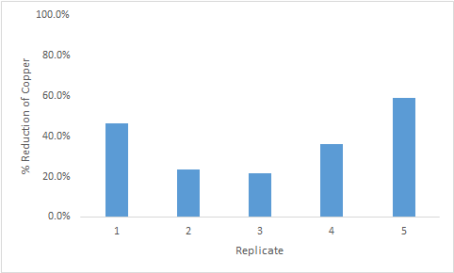

Fig. 6: Percent reduction of copper

periods of the product to the water, the bagasse could not properly form complexes with the copper.

Effects of sulfuric acid. Sulfuric acid treatment to bagasse also increase the adsorption rate of the product. From a study by Mesfin Yeneneh et al. [7], sulfuric acid contributes to the essential stretching of functional groups such as hydroxyl, carboxylic acid, carbonyl, aromatic and phenolic groups which binds the heavy metals. The mechanism behind involves sulfuric acid removing lignin compounds in the bagasse which increases the concentration of hydroxyl and carboxylic acid groups.

Effects of epoxy. The bagasse incorporated with epoxy resin as an adhesive formed a composite. However, the epoxy resin may block the fibers from adsorbing the copper ions which may contributed to the low adsorption rate of the bagasse fibers alone but epoxy is also shown to adsorb metal contaminants [4]. It is not known on what extent the epoxy adsorbed the copper ions during testing.

Conclusion. – To conclude, sugarcane bagasse based mesh is viable to remove copper in wastewater. However, due to its low adsorption rate compared to the average percent copper reduction, the product needs further improvements. This study introduces a new approach of wastewater treatment and this product is open for further development that could potentially increase its efficiency in removing not only copper, but contaminants in general.

Recommendations. – To further improve this study, it is recommended to:

- Apply modifications of the mesh. Increasing the mass of bagasse or increasing layers of bagasse mesh is recommended.
- Create alterations of product such as sponges or cotton made out of bagasse.
- Utilize a smaller pore-sized mesh to decrease the flow time of the water. Decreasing the pore size of the material could possibly increase the adsorption rate of the product due to longer exposure times.
- Use a more precise analysis test for copper concentration, such as AAS (Atomic Absorption Spectroscopy)

in order to test the product in much lower concentrations of copper

- Test the product on other metal contaminants or dyes to know the extent of adsorption.
- Further study the adsorption kinetics of the product as certain parameters, such as pH and temperature, to understand how it affects the adsorption rate of the sugarcane bagasse as a mesh product.
- Use other chemicals, such as succinic anhydride and oxalic acid, as treatment of the bagasse. Comparative studies amongst different chemical treatments can be further studied.
- Characterize the porous structure of treated bagasse mesh to confirm signs of adsorption rate increase.
- Compare copper reduction of raw bagasse and bagasse-epoxy composite to know the effect of epoxy to the adsorption rate of the composite.

* * *

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Eradication of Staphylococcus aureus Biofilms By Synerigistic Action of Basil Oil and Vancomycin

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Abstract – The purpose of this study is to quantify the in vitro synergistic activity of basil oil and Vancomycin. A microtiter plate assay was used to determine the anti-microbial activity of the combination of both compounds against *Staphylococcus aureus* biofilms. The pre-formed biofilms were exposed to three different treatments: (1) Vancomycin, (2) basil oil, (3) and the combination of both. Water was used as the negative control. The three treatments were all active in eradicating the pre-formed biofilms with Vancomycin being the most effective followed by the combination of the two compounds. However, total biofilm removal was not achieved in any of the three treatments. The resulting action of the combination is less than that of vancomycin alone, but higher than that of basil oil alone, suggesting that there is an antagonistic interaction between the two compounds.

Introduction. – Staphylococcus aureus is a gram-positive cell that may be observed as grape-like clusters. By 1961, a short time after penicillin was introduced, certain Staphylococcal strains have evolved and developed resistance to most common antibiotics (i.e., Vancomycin, Linezolid and Daptomycin). Staphylococcus aureus can cause skin infections in addition to many other types of infections. Spread of this organism to the bloodstream is known as bacteremia or sepsis. This can result to serious complications like pneumonia, endocarditis which can lead to heart failure, and osteomyelitis [1]. Aside from the serious effects of S. aureus, data gathered in 2013 [2] suggested that Methicillin-Susceptible Staphylococcus aureus, or just S. aureus was common in the Philippines. Out of the 637 patients of with S. aureus growth, Methicillin-Susceptible Staphylococcus aureus accounted for 61.4% of it.

The problem of S. aureus not only lies with its prevalence but also with its decreased susceptibility to antibiotics because of the bacterial biofilm it forms. Bacterial biofilms are sticky mass of bacteria embedded in an extracellular matrix. Bacteria in a biofilm are 101000 times more resistant to the effects of antimicrobial agents and account for more than 80% of all microbial infections in humans [3]. Biofilms cause certain antibiotics and other external agents ineffective in killing this bacterium [4]. Thus, several alternatives have been studied by researchers as novel solutions against S. aureus biofilms.

One study in particular is the use of essential oils to eradicate these biofilms. An essential oil or a volatile oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. An oil is "essential" in the sense that it contains the "essence of the plant's fragrancethe characteristic fragrance of the plant from which it is derived. Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing. They are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products.

Essential oils have been used medicinally in history. Medical applications range from skin treatments to reme-

dies for cancer and even to antimicrobial effects. Additionally, many essential oils are relatively easy to obtain, have low mammalian toxicity and degrade quickly in water and soil making them environmentally friendly. However, essential oils contain very concentrated properties of the herb or plant from which they are derived from. So, essential oils must be used with care, with proper education and in safe amounts. Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking for antimicrobial properties of extracts from these compounds in aromatic plants particularly essential oil. The physical barrier of biofilms composed of exopolymeric substances (EPS) can account for their increased antibiotic resistance. Bacterial EPS are molecules released in response to the physiological stress encountered in the natural environment. Eugenol is a phenylpropanoid, a group of plant secondary metabolites with a wide variety of functions both as structural and signaling molecules. Eugenol is found in Ocimum Basilicum basil oil and other essential oil and plays a significant role in dental and oral hygiene preparations [5].

Synergism is a correlated activity of two or more structures so that the combined action is greater than when each structure is working independently. The study of Yadav et al. [6] suggests that Eugenol exhibits significant anti-biofilm activity against Methicillin-Resistant Staphylococcus aureus biofilms when it was synergised with the antibiotic Carvacrol. Eugenol concentration of 0.5Minimum Inhibitory Concentration (lowest concentration of an antimicrobial that will inhibit the growth of a microorganism after overnight incubation) decreased the biomass of biofilm by more than 50%. Eugenol was also synergistic with Fluconazole, a cell-wall synthesis inhibitor antibiotic against Candida albicans biofilm. Since a synergistic activity was observed when Eugenol was combined with some antibiotic, it is also possible that it will exhibit synergism when tested with other antibiotics.

A potential antibiotic can be a cell-wall synthesis inhibitor. Cell-wall synthesis inhibitor antibiotics showed high ratios between the susceptibility of free flowing bacteria in suspension (planktonic cells) and biofilm cells. The ratios were: 7.0 for Cefazolin, 6.4 for Vancomycin and 5.6 for Dicloxacillin [7]. Cefazolin showed the highest ratio but it has poor stability and in the presence of a mutated S. aureus that synthesizes -lactamase and possesses mecA to facilitate its survival [8]. The next potential antibiotic would be vancomycin. Vancomycin resistance is higher in the biofilm mode of growth than in the planktonic mode of growth [9]. In the study of Singh et al. [10], Vancomycin penetration was also significantly reduced (P¡0.05) through S. aureus biofilms. This study aims to eradicate S. aureus biofilms by synergizing the compounds basil oil and Vancomycin.

Methods. – The methods is composed of five main parts; Acquisition, Media preparation, Growing, Exposure, and Analysis. Different combinations were used to treat the bacterial biofilms. Minimum inhibitory concentrations were used for the exposure of the pre-formed biofilms to the different combination treatments, the bacterial biofilms were treated in 96-well microtiter plates and was analyzed using a microplate reader.

Acquisition of S. aureus bacterial strains and compounds. A strain of S. aureus pure bacterial culture was obtained from University of the Philippines - The National Institute of Molecular Biology and Biotechnology, Los Baos, Laguna. Basil oil was purchased from an herbal store in a mall. Vancomycin was bought from a local drug store. The culture was stored in a refrigerator (Condura CSD230SA) at 20C. S. aureus was streaked directly to a petri plate with Nutrient Agar in order to obtain a subculture. The plate was wrapped and incubated at 30C for 24 hours. The subculture was then assessed for purity by examining the similarities in cell morphology of the bacterial colonies that formed.

Media Preparation. The glasswares (petri plates, test tubes, micropipette tips, stirring rods) were autoclaved (Delixi LS-B35L) for 15 minutes at the optimal pressure of 15 psi. Using a 500 mL media bottle, 20 grams of Tryptic Soy Agar was mixed with 500 mL distilled water. The mixture was cooked in the hot plate (LMS HTS-1003) and continually stirred until the mixture cleared up. Using a separate 500 mL media bottle 2.4 grams of Nutrient Broth was suspended in 500 mL of distilled water. The solution was mixed using a sterile stirring rod until the solid was dissolved. Peptone water was prepared with 0.5 grams of Bacteriological Peptone, and 4.8 grams of Sodium Chloride. It was then suspended in 500 mL of distilled water in a 500 mL media bottle. The media was autoclaved for 15 minutes at 15 psi and was cooled down afterwards. In four sterile test tubes, 15 mL of Nutrient Broth mixture was pipetted. Peptone water of 10 mL was pipetted into two test tubes. Nutrient agar (20-30 ml) was poured to each agar plate. After the agar has solidified it was stored in the refrigerator at 20C.

Growing of S. aureus. An inoculating loop was used to transfer an isolated colony from the agar plate to a test tube filled with 15 mL of Tryptic Soy Broth with 0.4 grams of glucose to create a subculture. The test tube containing the subculture was incubated for 18 to 24 hours at 30C. A McFarland procedure was conducted to compare the optical densities of the cultures, using a bond paper with black and white stripes.

Growing of S. aureus biofilm. A 96-well microtiter plate was used to grow the biofilms of S. aureus. 200 L of the

previously diluted liquid media was transferred to columns 2, 4, 6, 8 and 10 of the plate using a micropipette. The microtiter plate was sealed and placed in the incubator for 24 hours at 30C. (Yadav, 2015). This was conducted inside the laminar flow hood.

Exposure of biofilm to Basil Oil and Vancomycin. The microtiter plates seeded with S. aureus was removed from the incubator and was placed in the laminar flow hood. The biofilms formed in the microtiter plates was exposed to 5% v/v basil oil, 128 g per mL Vancomycin, and a combination of 12 g per mL Vancomycin and 5% v/v Basil oil. water and broth for controls. Since the oil wouldnt mix completely with water, a 100 L of emulsifier Tween 20 was used. 150 L of each treatment was transferred to each well of columns 2, 4, and 6 and rows B-G. Column 8 acte was pipetted with water for a negative control. After incubation, the plates were gently washed two times with sterile Phosphate Buffer Saline. Thereafter, the plates were stained with 125 L of crystal violet (0.1%) for 15 minutes. Excess stain was removed by washing with Phosphate Buffer Saline. The crystal violet (CV) attached to the biofilm samples was dissolved with 125 L of ethanol. The absorbance at 600 nm (OD600) was measured using a Microplate Reader.

Evaluation and Analysis. The wells of the microtiter plate were checked for absorbance using a Microplate Reader. The absorbance values are the measure of the cells of S. aureus biofilm. An ANOVA statistical analysis with a level of confidence of 0.05 was performed in order to compare the means of the absorbance values of the untreated cells in different treatments.

Disposal of Used Media. Concentrated Lysol was diluted in a beaker containing water and be poured into the used agar plates, and test tubes. At optimal pressure for autoclaving, 15 psi, the plates were autoclaved to ensure the killing of the bacterial cells. After 15 minutes of autoclaving, the plates were cooled down for disposal of used media. All the glasswares that were used were washed using an antimicrobial liquid dishwashing soap and were properly and safely stored. Used microtiter plates were soaked in 70% ethanol for 5 minutes for continued sterilization and disposed into the hazardous/used media waste can.

Results. – The anti-biofilm activity of basil oil and cell-wall synthesis inhibitor Vancomycin was determined against gram-positive Staphylococcus aureus with water as the negative control. 128g/mL Vancomycin and 5% (v/v) basil oil and the combination of both showed antimicrobial activity against S. aureus biofilms.

In microplate reading, the absorbance value of each well corresponds to the un-eradicated biofilm (biofilm mass) in it. The absorbance mean values (see Fig 1) for the biofilms treated with Vancomycin and basil oil and their combination were lower than that of the controls. Among the three treatments, Vancomycin had the lowest biofilm mass followed by the combination and basil oil alone.

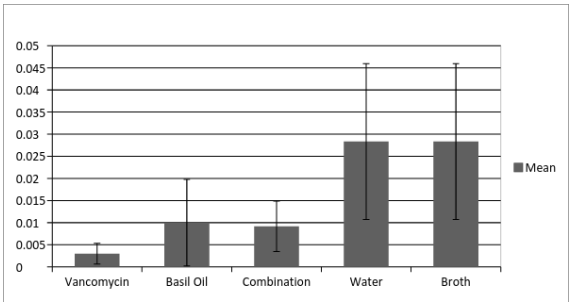

Fig. 1: Absorbance mean values of *S. aureus* after exposure to treatments

	Sum of Squares	DF	Mean Square	F	p(same)
Between Groups	0.001813	3	0.000604333	20.13	2.953E-06
Within Groups	0.000600333	20	3.00167E-05		

Fig. 2: ANOVA table

In the One-Way ANOVA, the calculated p value ($p = 6.56x10^{-7}$) of the absorbance values of the biofilms is less than 0.05 which means that the three treatments have significant differences in eradicating biofilm when compared to the control. This indicates that all treatments were effective in eradicating S. aureus biofilm. The three treatments, however, had no significant difference when compared to each other.

Discussion. – The absorbance values represent the amount of uneradicated biofilms left on the surface of the wells of the microtiter plate. The three treatments, Vancomycin, basil oil and the combination of both showed no significant difference amongst each other but they were significantly lower than that of the control.

Statistically speaking, all of the treatments were effective in eradicating biofilms. The insignificant difference between the three treatments states that there is no synergism between Vancomycin and basil oil. Although there is no significant difference, the absorbance value of Vancomycin was lesser than that of the other two treatments, making it the most effective treatment. The Minimum Biofilm Eradication Concentration (MBEC) used was the highest that was found and may be one of the probable extraneous variable that led to the penetration of the antibiotic.

In vitro susceptibility of a microorganism to a particular treatment does not guarantee the success of the clinical use of the therapeutic agent. The main disadvantage of the results of in vitro studies is that it is difficult to carry out comparison among each of the studies because of the different test methods, test assays, and variation in chemical phytoconstituents in essential oils (Yap et al 2013).

Basil oil constituents include phenylpropanoids that are potential biofilm treatments. Three of the major components are eugenol, geraniol, and linalool [11]. The bactericidal effect of eugenol on Streptococcus agalacticae seems to be dependent on changes in cell envelope, as judged by alterations in the morphology and structure observed in treated cells [12]. Studies report that eugenol induce cell lysis through protein and lipid leakage leading to the disruption of cytoplasmic contents in membranes. The anti-bactericidal component of Eugenol wasnt identified yet. In this study, the absorbance value of basil oil was low, which means that it effectively eradicated biofilm.

In a study conducted by Yap et al. [13], out of the 35 essential oils-antibiotic pairs, only four of them showed synergistic effect. Another study also shows no synergism between essential oils, namely; cinnamon and oregano essential oils and antibiotics (not mentioned specifically) indicating that not all combinations of antibiotic and essential oils will show synergistic effects. The interaction between Essential oils and antibiotics can produce four possible types of effects: indifferent, additive, antagonistic, or synergistic effects. An additive effect is observed when the combined effect is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less when they are applied together than when individually applied. Synergism is observed when the effect of the combined substances is greater than the sum of the individual effects while the absence of interaction is defined as indifference [14].

The release of cellular content in the treated bacteria led to the hypothesis that the first effect of an essential oil is membrane disruption [15]. Although, it should not be ignored that interaction with other targets in the bacterial cell might play a key role with the observed antibacterial effects. Essential oils are composed of a large number of chemical constituents and these constituents rely on the genetic make-up of the plant where it came from. Due to the complex composition of essential oils, it is likely that their antibacterial activity is because of the different mechanisms of action that implies to target the cell. It is because of this reason that bacteria rarely develop resistance mechanisms for essential oils.

Chemical polymorphism is also a characteristic of essential oils. The genus Ocimum, in which basil oil belongs to, is known to have a high degree of chemical polymorphism [16]. Genetic factors, as well as environmental factors can be attributed to such influence on the chemical composition of the essential oil, it was established that the production of phenolic compounds is favoured in warmer and drier climatic zones, while the other, Nonphenolic compounds usually accumulate in higher quantities in cooler and damper areas [15]. Thus, this volatility exhibited by the essential oil can be a cause for variations in its composition, functional groups, and active components that can affect their synergistic interactions with the antibiotic. Most of the antimicrobial activity in EOs is found in the oxygenated terpenoids (e.g., alcohols and phenolic terpenes), Different terpenoid components of EOs can interact to either reduce or increase antimicrobial efficacy [14].

A study conducted by van Vuuren et al. [17], suggests that a combination of an essential oil, M. alternifolia, with ciprofloxacin against a gram-positive bacteria S. aureus showed antagonistic effect. Antagonism is a phenomenon where the effect of the combinations of two or more substance is less compared to their effect individually. The antagonistic effect was postulated to be due to the combination of the essential oil and the antibiotic raising the dose to an unacceptable therapeutic level. Thus, when combining the two it is also important to take note of the ratio at which the two components exist [17]. This antagonistic interaction recommends that natural treatments using essential oils must be monitored carefully when combined with antibiotics.

Conclusion. – Vancomycin and basil oil are both effective in eradicating biofilms but there is no synergism between the two agents.

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Microwave Copolymerized Carboxymethyl Cellulose-Chitosan Hydrogel

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Abstract – Copolymerization is a method used to connect different chains of polymers to form a hybrid macromolecule with modified properties of both polymers. Copolymerization can be done with different methods, one of which is microwave irradiation. Some naturally-occurring polymers are known to have excellent biocompatibility, biodegradability, and absorption. They are modified to become hydrogels or superabsorbent polymers that retain a huge amount of water. Microwave radiation initiates copolymerization. This study copolymerized carboxymethyl cellulose with chitosan using microwave radiation for 3, 6, and 9 minutes with carboxymethyl cellulose microwaved for 3 minutes as control. The findings of this study showed that the CMC-Chitosan hydrogel yielded lower gel content compared to the control group which is the CMC hydrogel. The polymer irradiated for 9 minutes is the most efficient in terms of absorbency. All in all, the three experimental set-ups had exhibited ideal absorption values, with a slower de-swelling ratio.

Introduction. – Hydrogels or superabsorbent polymers are materials that exist in gel state in the presence of water and absorb large amounts of liquid. They are used in the agricultural field to reduce irrigation water consumption and to increase the water retention of soil [1–3]. Hydrogels can also be used for contact lenses and wound dressings [4].

Cellulose forms the cell wall of plants and is an abundant superabsorbent material that is biodegradable and renewable from which carboxymethyl cellulose (CMC) can be synthesized. The synthesis of this derivative is necessary to overcome the poor reactivity of cellulose that makes it difficult to transform into other materials [5, 3]. According to a study[6], the presence of CMC in a hydrogel provides the hydrogel itself with electrostatic charges anchored to the network, which doubles its swelling capacity.

Copolymerization is a common method for the modification of surfaces of polymers and important to improve the physical or chemical properties of polymers [7]. Chitin is commonly synthesized from crustacean shells from which chitosan can be derived. Chitosan a highly-hydrophilic polymer is non-toxic, biocompatible, biodegradable that makes it a good choice for hydrogel preparation. Ultraviolet (UV) radiation can be used to crosslink or link the polymer chains of cellulose-based polymers to improve the properties of the molecule, however UV crosslinker machines are difficult to acquire. Microwave, on the other hand, is present in almost every household and establishes fast, cost effective, and environmentally friendly way of copolymerization [8].

Carboxymethyl cellulose can be copolymerized with chitosan via microwave radiation to produce a hydrogel efficient in retaining water for agricultural purposes. The efficiency of the synthesized polymer will be based on its gel content, absorbency, and de-swelling ratio or rate of water release [9].

Methods. – This study aimed to synthesize a superabsorbent polymer from carboxymethyl cellulose crosslinked with chitosan via microwave radiation. The gel content, water absorbency, and de-swelling ratio of the polymer were tested. The CMC microwaved for 3 minutes served as the control whereas the different durations of microwave irradiation of CMC and Chitosan (3, 6, and 9 minutes) served as the independent variables.

Materials. Commercially available Carboxymethyl Cellulose of edible grade was obtained. Chitosan was also bought. Since chitosan is water insoluble, 2 lactic acid aqueous solution was added.

Microwave Copolymerization of Chitosan. This copolymerization method was taken from the Microwave Initiated Synthesis and Application of Polyacrylic Acid

Grafted Carboxymethyl Cellulose of Mishra et al.[10] . Fives grams of CMC was dissolved in 100 ml distilled water. Fifteen grams of Chitosan dissolved in a 100 mL solution before it was added to the CMC solution. Constituents were mixed in the reaction vessel (500 mL beaker). The reaction vessel was subsequently placed on the turntable of a microwave oven. Microwave irradiation using American Home AMW-6510W with operation frequency of 2450 MHz at a power of 700 W was performed for 3, 6, and 9 minutes. Periodically, the microwave irradiation was paused (as the reaction mixture started to boil, i.e. at 65 C) and was cooled by placing the reaction vessel in cold water. This was to avoid competing homopolymer formation reaction or the reaction between CMC-CMC molecules and chitosan-chitosan to the minimum and also to prevent any thermal damage to the backbone polymer chain. The reaction vessel and its contents were cooled and kept undisturbed for 12 h to complete the polymerization.

Evaluation of Superabsorbent Polymer. To determine the gel content of the hydrogel, the polymers were cut and any non-copolymerized Chitosan or CMC formed by competing homopolymer formation reaction were removed from the copolymer synthesized by washing it with a methanolwater mixture (36 mL:4 mL). The hydrogel was dried at 100C for 6 hours. The remaining part or the insoluble and crosslinked parts were dried and weighed [11]. The equation to determine the gel content is shown in Equation 1

$$Gelcontent = (W_o/W_1) * 100 \quad (1)$$

where W1 is the weight of the insoluble part of the sample (after the extraction) and W0 is the weight of dried hydrogel before extraction.

The absorbency of the hydrogel or the volume of water the polymer can absorb was determined as follows: 1 g of hydrogel was immersed in 200 mL distilled water at room temperature (30 2C) and the weight was measured every hour for five hours. The tea bag was allowed to drain for 10 min or until the excess water stopped dripping. The equation for the swelling capacity or absorbency is shown in Equation 2

$$Absorbency = (W_2 - W_1/W_1) \quad (2)$$

where W1 and W2 represent the weight of the dry polymer and the swollen gel, respectively. The rate of absorption was obtained by determining the absorbance at consecutive time intervals.

The hydrogel was swollen until it reached its equilibrium absorption capacity or steady uptake of water which will be taken in a beaker placed in a tray with holes and kept at room temperature (30 2C), which resulted to the spontaneous escape of the absorbed water or the de-swelling of the polymer. The weight of the polymer was taken every five hours for 30 hours. The equation for the de-swelling

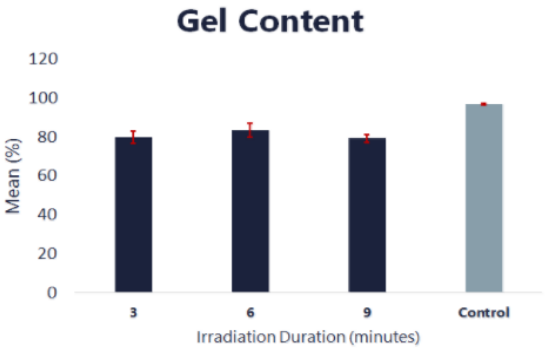

Fig. 1: Gel Content

ratio is shown in Equation 3

$$Deswelling = W_t/W_to \quad (3)$$

where Wt and Wto are the weight of the sample at deswelling time (t) and initial weight of the fully swollen hydrogel, respectively.

Chemicals used were put in closed containers before disposal. Used glasswares and equipments were washed with dishwashing liquid and rinsed thoroughly. The polymers were placed in a sealed container and disposed and all wastes were segregated.

An ANOVA statistical analysis using PAST (Paleontological Statistics Software) with a level of confidence of 0.05 was performed to compare the means of the gel-content, absorbency, and de-swelling ratio of the polymers crosslinked at varying durations.

Results. – The gel content of CMC-Chitosan hydrogels microwaved for different periods were determined with CMC microwaved for three minutes as control. The gel content mean values in Fig. 1 of the CMC-Chitosan hydrogels were adjacent to each other. Using Tukeys pairwise comparison, it showed that there is no significant difference between the CMC-Chitosan hydrogels. However, in One-Way ANOVA, the calculated p value of the gel content (p=2.28x10-8)is less than 0.05, indicating that there is a significant difference between the CMC-Chitosan hydrogel groups and the CMC hydrogel group.

The absorbance values of CMC-Chitosan hydrogels microwaved for different periods were determined every hour with the CMC microwaved for three minutes as control. This was done hourly for five hours to depict a trend of the absorbency. Fig. 2 shows that as the time of irradiation increases, the absorbency also increases. The highest absorbency is the hydrogel irradiated for nine minutes, and the least absorbency is the control. This shows that the combination of CMC and Chitosan is more effective than CMC alone.

Using One-way ANOVA, the calculated p value (p=2.08x10-8) shows that there is a significant difference between all the variables including the control.

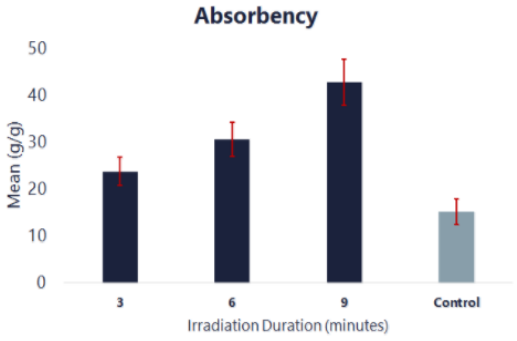

Fig. 2: Absorbency

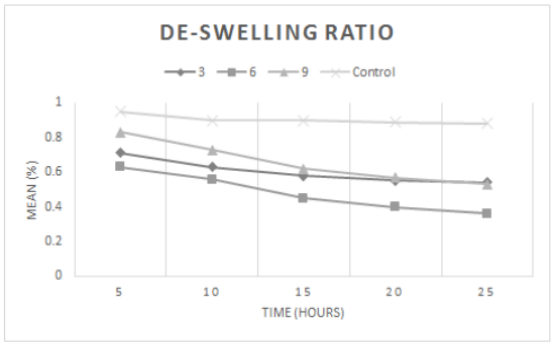

Fig. 3: De-Swelling Ratio

Fig. 3 is the trend of the de-swelling ratio of the hydrogels with different irradiation durations. The control and the hydrogel irradiated for three minutes are slow to release adsorbed water and the hydrogels with high absorbances, those irradiated for 9 minutes and 6 minutes, have fast de-swelling rates. The two slowest de-swelling rates have almost parallel trends and so do the two fastest de-swelling rates.

Discussion. – Copolymerization is the process of modifying the properties of hydrogel. In this study, microwave irradiation was used to copolymerize CMC with chitosan. When small polar molecules like water are microwaved, the whole molecule rotates and this rotation produces heat 10. There is no breakage of bonds because the whole molecule is rotating. On the other hand, if macromolecules like polysaccharides are copolymerized using microwave radiation, the polar bonds (O-H bonds) along with the other bonds (e.g. C-C bond) show rotation. The partial rotation of the molecules lead to breakage of the polar bonds. This process leads to free radical sites formation where copolymerization takes place 12. Connecting another polymer on CMC results to a copolymer that is mostly 2-O- and 6-O- linked 10. This is explained by the neighboring side chains on the cellulose backbone. Because of lack in free space, the mobility of the segments are greatly reduced and molecules are inhibited to penetrate this layer. This was consistent with the study of El-Mohdy (2014) in which the Fourier Transform Infrared Spectroscopy proved the copolymerization of 2-Acrylamidoglycolic Acid with CMC on the CMCs hydroxyl group -CH2COO in the C2 position. Chitosan has a primary amino group (C2) and a primary (C3) and a secondary free hydroxyl groups (C6) that gives it a considerable opportunity for chemical modification. Depending on the reaction conditions (temperature and pH) the reaction can take place on the amino group, on the hydroxyl groups or on both 13. Depicts the chemical modification of chitosan in a neutral media wherein the only modification happens at the primary amino group. In the study of Jayakumar et al., thioglycolic acid crosslinked with chitosan via amide bond formation mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. The result is consistent with the illustration above that showed that copolymerization chitosan in an acidic media results to a major substitution on the amino group 14. The pH value of the CMC-Chitosan copolymer was 7.12 which means that the crosslink happened at CMCs hydroxyl group -CH2COO at C2 and at chitosans primary amino group (C2). To determine the percentage of the copolymerized hydrogel, gel content was evaluated. Gel content is the measure of the degree of copolymerization between polymers, which means that the higher the gel content, the more flexible and durable the hydrogel. In the study by Sutradhar et al. (2015)12, the gel content of hydrogel prepared from CMC-acrylamide increases as the irradiation dose increases, however, its absorbency decreased with the increasing dose. This is due to the cross-linked density in the polymer. The more dense the copolymerization the lesser vacant space there is in the network for free solvent to enter 12. In contrast to UV irradiation and other thermal curing techniques, microwave induced copolymerization is more effective in terms of lesser reaction time and does not need an additional radical source to induce copolymerization. Compared to UV irradiation it is also more effective in an ambient oxygen atmosphere, where the inhibitory effect of oxygen could affect the recombination of free radicals. In this study, the gel content of the control is significantly higher than the hydrogels with CMC-Chitosan. This means the copolymerization worked better with CMC alone because the resulting product managed to have significantly higher absorbance values compared to that treated with Chitosan.. This may be because of the numerous polar molecules along the CMC chain compared to the Chitosan chain. These polar molecules will be candidates for hydrogen bonding when microwave irradiation takes place. The absorbency of the hydrogel determines its swelling property. Absorbency denotes the amount of fluid which a given amount of hydrogel can absorb. The absorption of the combination of CMC and Chitosan is significantly higher than the absorption of hydrogel-forming microneedle were evaluated. It shows that the longer the hy-

drogel is exposed to the radiation, the greater its swelling capacity, but for every material, there is a limitation. A Hydrogel with high absorption are efficient for agricultural use since it retains the moisture in the soil for a longer period of time, preventing the plants from drying. High absorptivity is also valued for its use in diapers because it will allow more urine to be stored. If the synthesized hydrogel will be used for the aforementioned examples, it is optimal to use a CMC-Chitosan hydrogel irradiated for nine minutes. De-swelling ratio is the percentage of the remaining hydrogel after releasing the absorbed water for a time interval. In this study, the control had the slowest de-swelling rate. The de-swelling behavior of the swollen hydrogels might be affected by various environmental factors such as temperature, humidity and also by other factors such as size of the polymer, chemical composition, crosslinking density of the polymer, thickness of the hydrogel, mechanical strength of the hydrogel, gel content 15. In this study, the de-swelling of the control has the slowest rate. This could be because the control has the highest gel content meaning the crosslink inside the polymer is dense. These small spaces make the diffusion of water molecules out of the hydrogel surface slower leading to a high de-swelling percentage. Hydrogels are used in personal hygiene products, one of which are diapers. Controlling content leakage is crucial to diapers because it reduces the risk of fecal contamination and thus the potential for the spread of illness. The de-swelling ratio should be adjusted to the hydrogels minimum for it to be efficient for reducing leakage in diapers. Therefore, hydrogels irradiated for six and nine minutes are not optimum for diapers because of their high de-swelling ratios. De-swelling ratio is also important for the use of hydrogels in drug delivery systems since it determines the rate of release of the drug from the hydrogel that encapsulates it. Copolymerizing chitosan unto CMC can increase the efficiency of the hydrogel for drug-release due to the Chitosans hydrosolubility. It is a useful polymer for mucosal drug delivery because it adheres to mucosal surfaces. It also has other properties such as pH sensitivity, biocompatibility and low toxicity 16 . Moreover, chitosan is metabolized by certain human enzymes, especially lysozyme, and is biodegradable 17. If the drug placed within the hydrogel needs to be quickly diffused, it is optimum to the CMC-Chitosan hydrogels irradiated for six and nine minutes since they have the highest de-swelling ratios. If the drug needs to be released in slow amounts, the CMC hydrogel or the CMC-Chitosan hydrogel irradiated for three minutes may be chosen. These are all based on the irradiation duration alone but further variations such as changing the CMC-Chitosan ratio can be done to the hydrogels to modify their de-swelling properties to fit the application. CMC-Chitosan hydrogels copolymerized through microwave irradiation have lower gel content than the CMC hydrogel. Longer irradiation time results to a polymer with higher absorbency and faster de-swelling rate. In terms of absorbency, the hydrogel microwaved for nine minutes is the most efficient.

* * *

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Effect of Potassium Polyacrylate in Soil to Growth of Zea mays in Polyethylene Glycol-4000 Simulated Drought

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Abstract – Zea mays L. (corn) is a staple food for about 20% of the Philippine population, making it one of the most important food crops in the country, but are threatened to have a decline in production due to El Nio and water shortage in the Philippines. This study aimed to investigate if the addition of potassium polyacrylate to the soil will improve soil water retention to help optimize the drought tolerance of Zea mays (maize). This can help develop a new method in assisting plant survival during drought conditions and increase crop yield and growth. Since drought is an impending problem that greatly affects agricultural industries, this study can help address the problem of hunger in some areas, especially those which rely greatly on corn produce.Thirty corn seeds were planted and divided among six different groups, five under PEG-induced drought stress with varying concentrations of potassium polyacrylate incorporated to the soil, and one under normal conditions. Height, number of standing leaves, and soil moisture content were determined weekly for four weeks. Twenty-five plants germinated after five to six days. By the fourth week, only 11 plants were left. The results of the different parameters were insignificantly different, indicating that potassium polyacrylate was ineffective in improving soil water retention and does not help in the optimization of drought tolerance of maize. Increasing sample size, measuring daily osmotic potential, increasing frequency of measurements, observing until the reproductive stage and using PEG 6000 or 8000 instead of PEG 4000 are recommended for further studies.

Introduction. – Zea mays L. (corn) is a staple food for about 20% of the Philippine population, making it one of the most important food crops in the country [1]. The largest corn producer in the country is Mindanao, which contributes 50% of the total national production. Due to the severe drought conditions brought about by the El Nio phenomenon however, corn prospects in Mindanao for 2016 were expected to decline relative to the five-year average and that of 2015 [2, 3]. The Philippine Statistics Authority reported the volume of corn production in 2015 to be 7,518.75 metric tons and in 2016 to be 7,218.81 metric tons, yielding a production growth rate of -3.99% for years 2015-2016 [4]. Moreover, the World Resources Institute (WRI) conducted a study which predicted that by the year 2040 the Philippines will have a high degree of water shortage. The study went further and predicted that the agricultural sector of the country would receive the highest degree of water stress among three sectors, the other two being the industrial and domestic sectors [5]. Currently, farmers would use various irrigation techniques or water at a certain schedule for optimal efficiency to address the effects of drought on their agriculture [6]. Essentially, drought refers to the fragile balance between water supply and demand and is not necessarily just a physical occurrence that is defined by the weather. Agricultural drought happens when there is insufficient moisture for average crop production on farms or average grass production on rangeland [7]. During this period, decreased rainfall and increased sunlight leads to soil moisture depletion. The quality of soil degrades because of lack of organic activity and increased wind erosion. Wind erosion causes dust storms and sand deposition which could kill even more vegetation [8,9]. Superabsorbent Polymers(SAP) are materials that are able to absorb and retain large volumes of liquid and aqueous substances up to hundreds of times its own weight and is made from partially neutralised, lightly cross-linked polyacrylic acid. SAPs are used ideally for diapers and adult incontinence pads for the absorption of

medical dressings and controlled release medium. Potassium polyacrylate is a SAP that is helpful for plants and can improve its structure [10,11]. Potassium is important in numerous processes such as photosynthesis, photosynthate translocation, protein synthesis, control of ionic balance, regulation of plant stomata and water use, and activation of plant enzymes [12]. Potassium is also a primary osmoticum in the maintenance of low water potential of plant tissues, aside from being an essential macronutrient for plant growth and development. Therefore, the accumulation of abundant K+ in plant tissues may play a vital role in water uptake for plants under drought conditions [13]. Given the ability of Superabsorbent Polymers to retain water, an agricultural concern for soil under drought conditions, this study proposes to investigate if the incorporation of potassium polyacrylate to the soil will enhance its water retention and reinforce the drought tolerance of Zea mays. This study aimed to investigate if the addition of potassium polyacrylate to the soil will improve soil water retention to help optimize the drought tolerance of Zea mays (maize). Specifically it aimed to measure and compare the germination time, number of standing leaves, soil moisture content, height growth rate of Zea mays plants grown in drought and normal conditions with and without potassium polyacrylate as well as identify the relationship between the soil moisture content rate and height growth rate between the aforementioned conditions.

Methods. – The study was conducted in a room in the Iloilo Sports Complex Hostel. There were six test groups with five maize plants each. One group was under normal environmental conditions, without potassium polyacrylate applications. Five other test groups were subjected to drought stress through the addition of PEG, and had five different concentrations of potassium polyacrylate applications in percentage ratio to the soil, specifically, 0, 0.08, 0.2, 0.5 and 1. The mean germination time, mean number of standing leaves, mean height and mean soil moisture content of all the plants in all the setups were determined and compared. Results were compared weekly to determine if there was a significant effect on the growth of plants in terms of the aforementioned parameters given the different treatments during each of the various stages of growth. The corn seeds were obtained from the Provincial Agriculture Office, Region 6. The polyethylene bags in which the samples were placed were bought from Janiuay Market in Iloilo. The loamy soil was obtained from Brgy. Danao, Iloilo City. PEG - 4000 and apparatuses were obtained from Patagonian Enterprises, Iloilo City. Potassium polyacrylate was obtained from Shanghai iChemical Technology Co., Ltd. Fluorescent lights were used as they are one of the best sources of artificial light for plant growth. The study utilized 20-watt fluorescent bulbs with a distance of 6ft and 9 inches from the pots. A daily photoperiod of 14 hours was used as most facilities growing corn in a greenhouse use this duration of artificial light [14]. One corn seed per pot with dimensions of 6 x 6 x 8 was planted 2 inches deep into 2500 grams of loamy soil. The plants were watered every day with the specified amount of water they need depending on which stage of growth they undergo as shown in Table 1. The area was kept at 25 C. PEG - 4000 solution was used to simulate drought conditions. The solutions were prepared at 20% concentration to be added at four-day intervals by quantities depending on the daily water requirement.The potassium polyacrylate was incorporated 15cm below the topsoil at a dose of 0.23g per square foot as shown below [15]. This is done with the purpose of improving soil structure and water penetration, increasing retention capacity, decreasing water runoff and avoiding erosion [16]. Potassium Polyacrylate is not mixed with the soil in order to keep the polymers together, since single polymers are unlikely to absorb water to their maximum potential [17]. The number of days lapsed after planting when the seed has sprouted were noted for the germination time. Physical parameters such as plant height and number of standing leaves were recorded weekly. A ruler was initially used to measure the height of the plants. When plant height became higher than 12 inches, a meter stick was used. Height growth rate was calculated based on the change of standing height within the span of one week. Measurement of soil water content was also done weekly at the PSHS laboratory by gravimetric method. Three out of five plants per test group were taken soil samples of one teaspoon or approximately 5 cc or 5 mL. The wet weight of soil samples was measured. Soil samples were oven dried at a temperature of 100-110C for an hour. Samples were weighed after oven drying and are to be dried again for 30 minutes. This process was repeated until the weight of the sample becomes constant to get the dry weight measurement. One-way ANOVA was conducted for three parameters to determine if the addition of potassium polyacrylate had a significant effect on germination time, number of standing leaves, height, soil water content among treatment groups. An alpha of 0.05 was used to compute for the significance of the data. Kruskal-Wallis was conducted for the number of standing leaves for the same purpose.

Results. – *Germination Time* All plants with potassium polyacrylate had a germination time of about five days with group 1 and 0.08 having mean germination times of 5.5 days. All plants without potassium polyacrylate had a germination time of about six days. The plants which were put under drought conditions had the longest time to germinate among all the plants with a mean germination time of 6.25 days (Figure 1). Differences among the values were not significant at an alpha of 0.05. The P-value for the germination time was 0.235, signifying that any of the different concentrations of potassium polyacrylate treatments would not significantly affect the outcome of the germination time of maize plants.

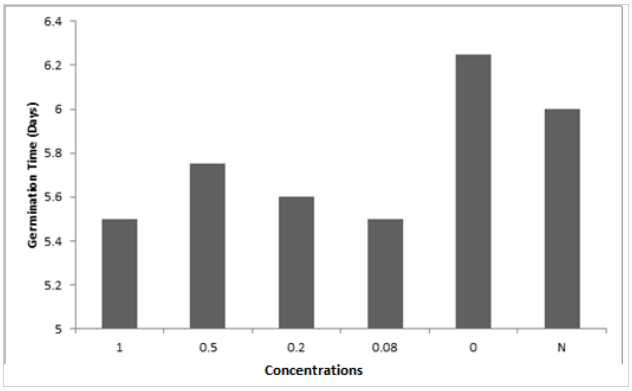

Fig. 1:

Number of Standing Leaves At week one, groups 1 and 0.08 had two standing leaves in all plants, while group 0.5 and group 0 had zero to two leaves and group 0.2 had one to two leaves. At week two, all groups had three leaves in all plants except for groups 1 and 0.2 with one plant in each of the groups having four leaves. At week three, groups 1 and 0.2 still had three to four leaves, group 0.5 still had three leaves in all plants, group 0.08 had only one standing leaf left in the only plant that was still alive and group 0 had two plants without any standing leaves and one plant with three leaves. At week four, group 1 had zero to one leaf, group 0.5 had zero to two leaves, group 0.2 had one to two leaves, group 0.08 plants were all dead and group 0 had one plant remaining without any standing leaf (Figure 2). Results were not significant at =0.05 for all weeks, indicating that the number of leaves among the plants were generally not affected by the potassium polyacrylate treatments and the different concentrations of those treatments in different stages of growth.

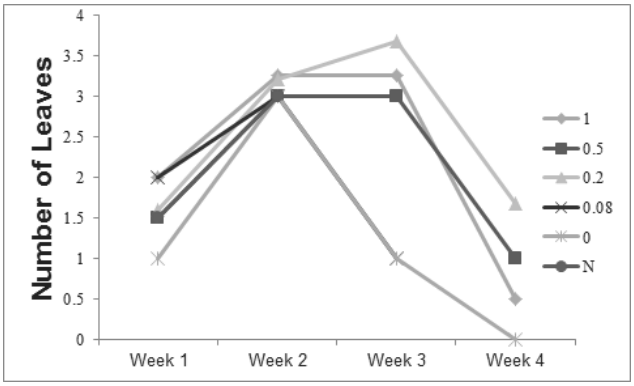

Fig. 2:

Moisture Content Groups 1, 0.5 and 0.08 had approximately 23% mean moisture content, group group 0.2 had approximately 24% and group 0 had approximately 21% (Figure 3). Moisture content for all groups, except for groups 0.2 and 0.08, generally increased on the second week. All groups decreased in soil moisture content on the third week. Moisture content among groups was only significantly different at an alpha of 0.05 in the second week with a p-value of 0.03. The moisture content in the third week yielded a p-value of 0.540, the greatest deviation to the critical value among the weekly results.

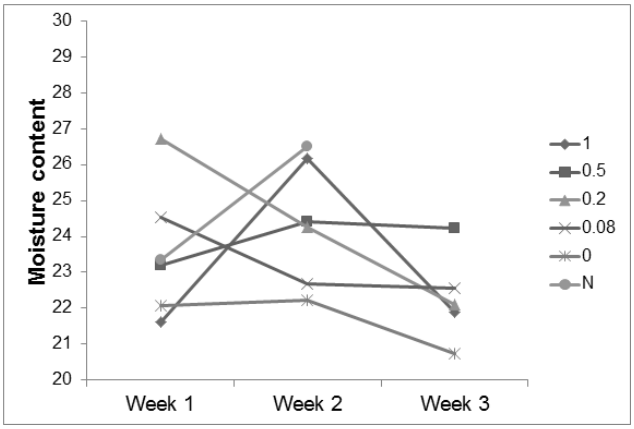

Fig. 3:

Height Growth Rate Between the second and first week, group 0 had the highest growth rate mean of approximately 400%, groups 1, 0.08 and N had the higher growth rate means at around 300%, while the other groups had around 200%. Between the third and second week, group 0.08 had the highest growth rate mean while group 0 had a negative growth rate mean. For the last and third week, group 1 had a negative growth rate mean, while group 0 was able to regain a positive growth rate mean (Figure 4). Group 0.08 continued to have the highest growth rate mean. Growth rates were only significantly different at an alpha of 0.05 in the last week, with only 11 plants left alive.

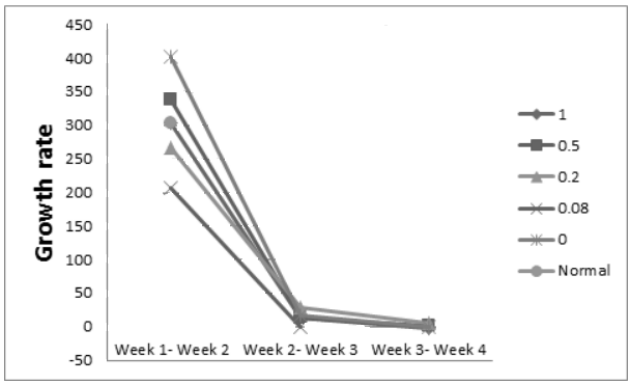

Fig. 4:

Mortality Rate By the end of the span of four weeks only the group with 1% Potassium Polyacrylate had sustained all of the plants that germinated. Groups with 0.5% and

0.2% had one and two deaths respectively. The group without reinforcement under drought conditions only had one plant left at the end of the study. The group under drought conditions with 0.08% and the group under normal conditions had no plants left at the end of the study (Figure 5).

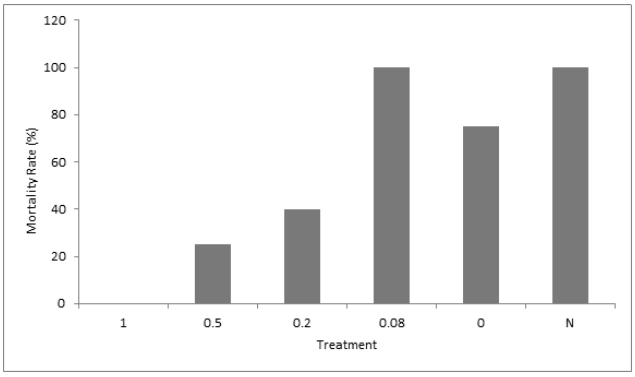

Fig. 5:

Discussion. – Since the results were generally not significantly different, three possibilities may be inferred. First, that drought conditions were not properly stimulated with PEG-4000. In the study that was used as basis for the application of PEG in this study, the effects of PEG were only investigated within a 12-day period [18]. At applications every four days for four weeks, osmotic potential may no longer be of similar value through time or may not have a damaging effect on plant growth.The means of the plant height and number of standing leaves were all less in group 0 than in group N, and germination time was longer in group 0 than in group N, indicating that growth was slightly stunted for group 0. However, in Table 8, these factors, although quantitatively still less in group 0 than in group N, increased at a greater rate in group 0 than in group N. The possible reason for this is that the plants could have developed coping mechanisms or acclimatized to the conditions. Plant acclimation is the process of adjusting to a gradual change in environmental conditions in order for a plant to maintain its performance. This is done by the development of tolerance, resistance or avoidance mechanisms [19]. Second, that potassium polyacrylate was not able to execute the mechanism involving osmosis. No study was found indicating the specific requirements for osmosis by potassium polyacrylate, such as extracellular and intracellular water volumes. Without the release of the absorbed water in dry conditions, potassium polyacrylate gives little to no effect on the growth of maize. Since moisture content was not significant between the different setups shown in Table 8, it is highly probable that osmosis was not in optimal conditions. Third, that the plants were not able to receive the requirements for optimal growth. Maize prefers pH in the range of 6.0-7.2 in order to maximize growth and ensure nutrients. However, the aging of PEG was found to have effects on its chemical properties, including a reduction in pH [20]. Furthermore, aging is accelerated by warm temperature or room temperature, light and the presence of oxygen, all of which are present in our set-up [20]. This means that the PEG that was watered on the plants might have affected the pH of the soil. Thus, since a regular soil test was not taken over the course of this study, it is possible that this requirement was not met, yet the issue was unidentified and contributed to the stunted growth of the plants. Moreover, the plants were watered at night time, past 6:00 pm. This may be a factor that could have affected the growth of the plants in two ways. First, because transpiration occurs during the day, moisture from watering at night would not have been expelled by the stomata and made the plant vulnerable to pathogen infiltration, which might have then caused rot or damaging injuries to the foliage. Second, because the soil is compacted, water moves slowly into the topsoil from the surface, and without the sun to evaporate the puddle of water on the surface, roots may have received less air and moisture. The plant stem and crown may have also been harmed by pathogens or may have rotted from the concentration of water in the plant base [21].

Conclusion. – In conclusion, the addition of Potassium polyacrylate to soil does not improve soil water retention and consequently does not help optimize the drought tolerance of Zea mays.The incorporation of Potassium polyacrylate to soil for plants under drought conditions had no significant difference to plants in both drought and normal conditions. All parameters namely germination time, number of standing leaves, soil moisture content, and height growth rate displayed no significant difference among all set-ups.

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Occurrence of Intestinal Helminth Parasites in Domestic Dogs (Canis familiaris domesticus) in Arevalo, Iloilo City, Philippines Using the Parasep Fecal Parasite Concentration Tenchnique

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Abstract – This study tested domestic dogs in Arevalo Iloilo City Philippines for the prevalence and intensity of helminth parasites using the parasep fecal parasite concentration technique.The parasites were identified along with the risk factors that accompanied it. Results showed that 21 out 30 dogs were positive for infection with Ancylostoma sp. as the most prevalent species with a prevalence of 30 percent and intensity of 93 eggs per gram. This study concluded that there is an occurrence of intestinal parasitic infection of domestic dogs in Arevalo, Iloilo City, Philippines.

Introduction. – The domestic dog (Canis familiaris domesticus) is generally considered as the first domesticated animal and has been mans companion since the start of animal domestication (Gharekhani 2014). Furthermore, it is also considered as one of the most common household pets in the world. Its constant interaction with human beings made it one of the few organisms who are adapted to the human niche, thus dogs can also be affected by the diseases human beings may acquire. However government actions such as providing information to the citizens about the risks of disease transmission, control of zoonoses transmitted by domestic animals and control of stray dogs are practically non-existent here in the Philippines, resulting in an increasing risk of exposure to zoonoses transmitted by these animals. Dogs serve as reservoir for an array of protozoan and helminth parasites shedding off oocysts/ cysts and eggs in their feces, which could be a cause of soil and water contamination thus increasing the spread of diseases (Santarm et al 2004). There are approximately 60 species of parasites known to infest dogs, some of which are Taenia sp., Echinococcus sp. (hydatidosis), Dipylidium caninum, Toxocara canis (visceral larval migrans), Ancylostoma sp., (cutaneous larval migrans), Giardia sp., and Cryptosporidium sp.. A majority are considered zoonotic, which means that it is capable of human infestation. Additionally, humans are usually the final or definitive host of these parasites where it reaches sexual maturity (Gharekhani 2014). Some of the main causes of morbidity in dogs are intestinal parasites such as Ancylostoma sp., Toxocara sp., Trichuris sp., especially for newly whelped or for puppies (Martinez-Moreno et al.2007). Stray dogs, which are prevalent in almost every industrial area in the Philippines, could be considered as vulnerable targets of parasitic infection. Taking account that helminth eggs remain viable for months in contaminated soil where these dogs scavenge for food, it is most likely that they would ingest these eggs thus becoming the reservoir hosts for future infestations. Symptoms of the presence of parasites in dogs include vomiting, diarrhea, dermatitis, and may sometimes be asymptomatic (Getahun 2012). Specifically, hookworm infestations (Ancylostoma sp.) could develop a severe, sometimes fatal anemia (Bowman 1999). Studies have shown that the prevalence of intestinal parasites in dogs ranges from 4-40 percent both in Japan and Canada, with a higher prevalence in developing countries such as Nigeria and Ethiopia, which reaches to 60 percent (Amissah-Reynolds et al. 2016). Additionally, since dogs live in close association to humans, zoonotic transmission or the transfer of a disease directly from animals to humans could lead to infestation by accidental ingestion of the oocysts. Hookworm larvae (Ancylostoma duodenale) could also penetrate the skin and may travel to organs like the respiratory tract before proceeding to the intestines to develop into maturity (Institute for International Cooperation in Animal Biologics 2013). If not treated, it may cause malnutrition, diarrhea and/or excessive weight loss. Fatal cases include morbidity and mortality particularly in undeveloped areas where autoinfection is rampant

(Kuciket al. 2004). The awareness whether a certain community contains a population of infected dogs is important given the fact that parasitic infection covers a much larger scope than the regular household. Since soil and water contamination could take place through improper fecal management and open defecation of dogs, the prevalence of parasitic infestation in stray and leashed dogs as well as its intensity could provide an image of how rampant the infestation is. Being able to determine the presence and the scope of infection could offer help especially in looking for possible solutions to this problem especially when the Philippines doesnt have a any data or statistics that shows this.

Methods. – *Selection of Study Site.* Iloilo is one of the four (4) provinces in Panay Island located in Region-VI Western Visayas of the Philippines to which its capital is Iloilo City. Currently, there are seven (7) districts in Iloilo City which are Arevalo, Iloilo City Proper, Jaro, La Paz, La Puz, Mandurriao, and Molo. Based on the 2010 Census on Population and Housing (CPH), the current population of Iloilo City is 424,169. According to a study conducted by Robinson et al. 1996, the human to dog ratio in the Philippines is one is to three (1:3). This means that for every dog, there are three (3) humans accompanying it. Based on this data, there should be about 142,000 dogs in Iloilo City. Among these districts only one (1) district was selected. The number of barangays was considered due to the limited time frame given for the study. Added with safety reasons, the district of Arevalo was selected as the district to be the study site. It is located 10.6859 N, 122.5118 E having 13 barangays which are Bonifacio, Calaparan, Dulonan, Mohon, Quezon, San Jose, Santa Cruz, Santa Filomina, Santo Domingo, Santo Nio Norte, Santo Nio Sur, Sooc, and Yulo Drive.

Selection of Domestic Dogs. Selection criteria for the dogs included the presence of leashed dogs in households and consent from the owners to collect samples with a criteria of if and only if the fecal sample was approximately one (1) to three (3) days old. Information such as the age, breed, diet, and medical treatment such as vaccination and deworming of the dogs were also taken. After one household was selected we selected the next household approximately 10 households away from the first one to avoid the auto infections of parasites in dogs.

Collection of Fecal Sample. One mini parasep fecal sample container was given to each of the chosen households. Instructions on how to collect the samples were given and told to collect one gram of stool. They were visited again one (1) day after the distribution in order to obtain the fecal samples. One (1) gram of stool samples from each dog was obtained and was placed in the fecal container and then placed in a storage box until the collection of samples was completed. The obtained specimens were then brought to the Philippine Science High School Western Visayas Campus Laboratory for processing.

Preparation of Fecal Sample. The Parasep Fecal Parasite Concentration Technique (PFPCT) was used in order to process the fecal samples and to detect the presence of helminth eggs. Using the scoop of the Mini Parasep, one (1) gram of stool sample was collected and placed inside the container that contained the mixture of 10 percent Formalin and Triton X.

Microscopy. When all of the samples have undergone processing, each sample was examined using a microscope. Using a pipette, about one (1) ml from each test tube was dropped on a glass slide and then covered using a glass slip. Under the low power objective, each slide was examined using the traditional S-direction scanning. All eggs found were counted and recorded. Parasite Identification In order to identify the genus of the parasite, the morphology of each suspected egg was compared to actual parasitic eggs. See Fig.1

Parasitic Egg	Morphology
Dipylidium spp.	Refer to Appendix B.1
Taenia spp.	Refer to Appendix B.2
Toxocara spp.	Refer to Appendix B.3
Trichuris spp.	Refer to Appendix B.4
Ancylostoma spp.	Refer to Appendix B.5

Figure 1: Standard Morphology of Eggs

Data Analysis. The terms prevalence and mean intensity of infection were used to denote the percentage of infected hosts in the sample and the number of parasite that was recovered from each infected host. The intensity of helminth eggs present was in eggs/g. The prevalence and mean intensity was be determined using the following. See Fig 2.

$$Prevalence = \frac{Number\ of\ Samples\ Positive}{Total\ Number\ of\ Samples}$$

Figure 3.1

$$Mean\ Intensity = \frac{Number\ of\ Eggs\ per\ Sample}{Number\ of\ Positive\ Samples}$$

Figure 3.2

Figure 2: Prevalence and Intensity

Safety and Precautions. Segregation of waste materials were followed thoroughly. All materials that were used were labelled. Ideal proper laboratory attire were worn. Laboratory gown, pair of gloves, and masks were used during the entire duration of the study. All glassware were handled with care. All glassware were rinsed three times into the waste container before they were cleaned. Extreme caution was followed when handling the chemicals.

Glassware were stored in a safe place to avoid scratches and breakage. After every experiment, the glassware were cleaned and dried as soon as possible prior to storing. All the glassware used were placed on a pot full of water and were left for boiling after using them. The glassware were

then rinsed with tap water twice, they were first rinsed with liquid detergent and then rinsed for the second time with distilled water. Glassware were then placed upside down in a tray for drying. Uncontaminated materials and contaminated materials were labeled. Then when dry, the materials were ready for storage or for disposal. Glasswares contaminated with biological material were emptied and decontaminated by boiling. They were rinsed thoroughly with tap water, drained and air dried.

PARASITE SPECIES	EXAMINED	INFECTED	PREVALENCE [%]	MEAN INTENSITY [epg]
Ancylostoma sp.	30	9	30.0	93
Trichuris sp.		8	26.7	91
Toxocara sp.		3	10.0	21
Larvae		1	3.3	39

Figure 3: Prevalence and mean intensity of gastrointestinal helminth in Domestic Dogs in Arevalo, Iloilo City, Philippines

Comparison of Dog Ages. Higher prevalence of Ancylostoma sp. is associated with puppies with a prevalence of 80 percent compared to those of adult dogs which is around 65 percent. This result is also similar to the study of Esquivel et al. (2015), Savilla et al. (2011), and Little et al. (2009) to which their studies showed that puppies whose age were below 12 months were more susceptible to parasitic infection. A possible inference to this is that since puppies tend to stay where their mother stays, especially leashed dogs, the tendency is that the puppies will acquire the parasites through auto-infection. This is possible because eggs can thrive on soil or a certain area for a long time which is why proper cleaning of a dogs home is encouraged. Another reason is that some parasitic worms such as Trichuris canis can be transferred directly from parent to offspring during birth. Comparison of Diet. A higher prevalence in dogs who ate table food was also associated with a prevalence of 85.7 percent compared to those who were fed with dog food and left-over food with prevalences of 60 percent and 69.2 percent respectively. However, this result is in contrast with the study conducted by Coman (1974) where wild dogs who ate unclean food or those found in the wild had a higher prevalence in terms of occurrence of parasites. The reason behind the relationship between dogs who ate table food having a higher prevalence compared with those who did not is deemed unclear.

Results and Discussion. – This study aimed to determine the presence of helminthic infection among dogs in Arevalo, Iloilo City, Philippines. The method Parasep Fecal Concentration Technique was used in order to isolate the helminthic eggs from the fecal debris. Each sample was viewed under a light microscope using the High Power Objective. It also aimed to associate some risk factors to the prevalence and intensity of parasitic infection such as age, breed, diet, and previous medications specifically deworming. Data were recorded and analyzed. Domestic dogs are possible reservoir hosts of helminth parasites. This could pose public health hazards as dog parasites usually are zoonotic, or if not, could cause visceral larva migrans and may cause complications to to the human body (Lim 2010). By determining the presence and by identifying the prevalence, intensity, and risk factors associated with helminth parasitic infection, further studies could be made to minimize this (Paul et al. 2010 and Traversa et al. 2014). Originally, this study aimed to have at least 10 samples per barangay, but in the middle of the conduct of the study, a change in the method of isolating parasitic eggs, which was Formalin-Ether Concentration Technique (F.E.C.T.) originally, was made due to a restriction in the distribution of the chemical diethyl ether of the government. The head of the medical laboratory of University of San Agustin Iloilo, advised that PFPCT should be used as an alternative to F.E.C.T. due to the fact that it is easier and faster to conduct compared to the latter. Also, only 40 paraseps were procured and were distributed to the barangays found in Arevalo, Iloilo City because the said laboratory only had one (1) box which contained 40 paraseps. An additional waiting time of four (4) weeks would be needed for the supply to arrive and due to the time restriction during the conduct of the study, it was advised that the data gathering should be continued. Instructions were given to the dog owners on how to use the parasep to obtain one (1) gram of fecal sample, but some did not follow the directions which lead to the destruction of the paraseps filter. Also, some homeowners discarded the paraseps thinking that it would not be taken again by the researchers which finally lead the researchers to obtain 30 fecal samples. A positive occurrence was found for helminth infestation in Arevalo, Iloilo City, Philippines which was around 70 percent of the total sample. Therefore, out of 30 samples examined, 21 were positive for helminth infection while nine (9) were negative. Results also showed that the parasite with the highest mean intensity was Ancylostoma sp. (93 eggs/gram), followed by Trichuris sp. (91 eggs/gram), and Toxocara sp. (21 eggs/gram). See Fig. 3

Comparison of Sex. Male dogs were seen to be more susceptible to parasitic infection compared to females with prevalences 68.8 percent and 57.1 percent respectively.

Literatures in the correlation of dogs sex to its susceptibility to parasitic infection shows no consistency. Studies conducted by Katagiri et al. (2008) showed that there is no significant difference in the vulnerability of both sexes. However, another study showed that parasitic infection was more prominent in female dogs (Sowenimo 2009). In contrast, Venturini et al. (2008) reported that males have a higher prevalence in terms of helminthic infection. *Comparison of Medication In-take.* Certain associations cannot be made in this study especially since the col-

lected fecal samples only came from dogs who were not dewormed. Although studies showed that dogs who were dewormed showed less susceptibility to parasitic infection compared to those who were not (Asano 2004).

RISK FACTORS	CATEGORIES	EXAMINED	INFECTED	PREVALENCE [%]
Age	Puppies	10	8	80.0
	Adults	20	13	65.0
Diet	Dog Food	10	6	60.0
	Leftovers	13	9	69.2
	Table Food	7	6	85.7
Medication	Dewormed	0	0	0
	Not	30	21	76.7
Sex	Male	16	11	68.8
	Female	14	8	57.1

Figure 4: Overall prevalence of gastrointestinal helminths based on different risk factors Common factors can be associated to the high overall prevalence. One of these is autoinfection in dogs due to improper cleaning of cages. The dogs were placed in the same area of the house where they both ate and defecated. Most helminth parasites can be acquired through the fecal-oral route, while some can infect through skin penetration. When areas where feces with parasitic eggs are not cleaned thoroughly, the eggs can hatch into larvae and penetrate the skin of the dogs, or the eggs can stay dormant until they are ingested by the dogs. This is supported by the study conducted by Esquivel et al. (2015) on dogs who lived in a cage in a dog shelter showed that since no disinfectant were used when cleaning the dogs cages, a higher prevalence rate of parasitic infection was seen compared to their counterparts who were taken care of properly and were not leashed. The dogs could have acquired the infection long before the study was

conducted, and since they were not able to receive deworming, this could have highly increased the prevalence

rate. The dogs could have acquired the parasites in other dogs with direct contact it may be during long walks or stray dogs entering the property. It may be also due to ticks, mosquitoes, and other parasites from other dogs.

* * *

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Characterization of the Physical Properties of Bacillus thuringiensis Corn husk Fibers through Alkalization

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Abstract –*Bacillus thurigiensis* (Bt) corn husk bers were characterized for possible usage in textile manufacture. The ber yield, diameter and length were determined and compared to that of native sweet corn husks bers Bt corn husks were subjected to alkalization for 60 minutes at 5 g/L and 10 g/L NaOH concentrations. Fibers subjected to 5 g/L and 10 g/L NaOH concentration treatment had a ber yield of 14.69% and 16.74% respectively. The ber diameter and the ber length was greater in the 5 g/L (817.7 m and 11.7 cm) than in the 10 g/L (723.7 m and 5.3 cm) NaOH concentration treatment. Fiber yield was measured using a standard analytical balance. Measurement showed no signicant dierence between the two corn species. Diameter of bers was also measured using the Laser Diraction method and also no signicant dierence can be observed. The ber length, measured using a Vernier Caliper had a signicant dierence. Bt corn husk bers do not have an observable advantage over native sweet corn.

Introduction. – For the past several years, due to the rapid changes in climate, awareness towards these environmental issues accelerated industries to start utilizing sustainable and renewable resources instead of conventional synthetic materials [1]. For a material to be classied as sustainable, it should be biodegradable or recyclable, made from renewable resources, should in any way not cause any harm to the environment and must be on par with conventional materials in terms of quality and price. Agricultural byproducts are amongst one of the most common renewable resources which are usually secondary or residual products from common drops such as cotton, rice, corn and wheat. Residues such as husks, bagasse, and stalks are usually discarded in most countries. Research regarding these residues are commonly studied in the Philippines since its an agricultural country and majority of the practices within the land include burning of these residues or turning them into fertilizer. Some are also utilized as alternative fuel however proper knowledge and procedures are yet to be released and may even pose a threat to the environment as well as the people. This however can help farmers since it reduces their expenditures in the aspect of farming. These agricultural byproducts are cheap source of cellulosic bers. The composition and structure makes them suitable to be used as composites, textile, and paper manufacturing [2]. Bacillus thuringiensis (Bt) corn is a genetically modied species of corn which includes the Bt protein capable of giving protection from pests. Bt-corn hybrids are dierent from nonBt corn not only only in the genetic code but also in some additional genetic material (Bessin 2010). The Bt protein comes from a soil bacterium, Bacillus thuringiensis, which has the ability to produce protein toxins which are harmful to larvae of the pests [44]. Plant based bers contain cellulosic materials that provides strength and rigidity to the bers. Aside from cellulose, non-cellulosic materials are also present such as pectin which holds the bers together and lignin which acts as a binder for cellulose bers adding strength and stiness to the cell walls [4].

According to the Department of Agriculture (DA), corn is the second most important crop in the Philippines in par with being the second largest agricultural crop grown in the world according [5]. Approximately 14 million Filipinos regard corn as their main source of food. Besides being food for humans, nearly 50% of livestock feeds contain corn. Besides the corn itself, other parts can also be utilized for biofuel from corn oil and as stated beforehand, forage and silage for livestock feeds. The numerous amounts of agricultural byproducts such as cob, husk and silk produced by the massive production of corn, especially the Bt corn, is under-utilized or considered as waste.

These agricultural byproducts can be used as a source of raw material for ber. Majority of the parts besides the corn itself such as the cob, husk and silk is under-utilized and considered as a waste by many. These byproducts can be used as a source of ber for many other uses [6].

Methods. – The conduct of the research experiment is divided in six phases: rst, the collection and Bt corn husks; second, the ber extraction; third, calculating the ber yield; fourth, the calculation of the ber diameter; fth, the measurement of the ber length and; sixth, analysis of the data using statistical tests.

Materials. Bt corn was acquired from a sweet corn vendor near the Jaro Public Market. These Bt corn were from a plantation San Miguel, Iloilo. The husks were transported to the Philippine Science High School - Western Visayas Research Laboratory for storage prior to ber extraction. Chemicals and laboratory equipment such as glasswares and measuring devices were borrowed from the Chemistry Lab of the school. Other basic materials such as distilled water were bought from the local supermarket.

Procedure. To extract the bers three set-ups containing 50 grams of corn husks were placed in a container with a 5 g/L NaOH concentration and another three set-ups containing 50 grams of corn husks were submerged in a one liter distilled water with 10 g/L NaOH concentration placed in a two-liter beaker. These beakers were placed on a hot plate set to 450 for 90 minutes. After alkalization, the bers were rinsed in a beaker lled with 50 mL distilled water for ve times. They were then placed on another beaker containing a 50 mL of a 10% acetic acid solution, and then rinsed again until pH 7 is reached. The pH level was measured using a pH meter. After extraction the bers were air dried for 48 hours, each setup was weighed using a top loading balance. A total of 15 samples were descriptively and inferentially measured. The diameters of the samples were measured using the laser diraction method. Each strand was placed on a cardboard with a 4cmx1cm slit. A 532 nm green laser pointer was situated 30cm from the strand. The distance between the strand and the wall was 4m. As the laser pointer hit the strand, the bright bands were measured from the center of the bright central band to the starting edge of the rst bright band to the left. For each measured distance, the diameter of the ber was solved using the formula

$$\frac{\lambda}{\frac{D_m}{D_w}} \tag{1}$$

where = 532 nm, d_{wall} = 4m since it is the distance between the wall and the strand and dis the distance between the center of the bright band of the laser and the starting edge of the rst bright band to the left. The samples were measured from end to end using a Vernier caliper to get its length. The data acquired were analyzed using the IBM Statistical Package for the Social Science (SPSS) Software. The mean and standard deviation were determined for descriptive analysis. The t-test was used to determine signicant dierences between two independent samples in the study.

Results. – This study specically aimed to extract bers from Bt corn husks and determine and compare the ber yield, diameter, and length of extracted Bt corn husk bers when subjected to alkalization treatment with NaOH concentration of 5 g/L and 10 g/L. Bt corn husks were boiled per each concentration and 150 g of the husks were divided into three setups with 50 g each. The three set-ups were boiled in a 2 L solution of water and NaOH. The solution of NaOH and water followed a concentration of 5 g/L and 10 g/L. The three set-ups were boiled for one hour. Each setup was then rinsed with 100 mL 10% acetic acid solution. After rinsing, the bers were again rinsed with distilled water until pH 7 was reached. The pH was measured using a digital standard pH meter. Fifteen samples that were randomly picked per setup were measured for ber diameter and length.

Fiber Length, Fiber Diameter, Fiber Yield. The ber yield after alkalization is higher in the 10 g/L (16.74%) than in the 5 g/L (14.69%) NaOH concentration. Table 1 shows the data.

Table 1: Fiber Yield

NaOH Conc	Mean Fiber Yield	Std. Dvt.
5g/L	14.69	±5.36859
10g/L	16.74	±2.09001

The fiberr diameter is greater in the 5 g/L (817.7 μm) than in the 10 g/L (723.7 μm) NaOH concentration treatment. See table

Table 2: Fiber Diameter

NaOH Conc	Mean Fiber Diameter(cm)	Std. Dvt.
5g/L	817.7	±156.34684
10g/L	723.7	±83.76536

The ber length is greater in the 5 g/L (11.8067 cm) than in the 10 g/L (5.3333 cm) NaOH concentration treatment.

Table 3: Fiber Length

NaOH Conc	Mean Fiber Diameter(cm)	Std. Dvt.
5g/L	11.8	±2.93873
10g/L	5.3	±1.85831

t-test Results on Fiber Yield, Diameter, and Length. The t-test results shows that there is no signicant difference between the ber yield of Bt corn husks treated with 5 g/L and 10 g/L NaOH concentration as reected by the

t(4) = -0.547, p=0.613. Additionally, there is no signicant dierence between the ber diameter of Bt corn husks treated with 5 g/L and 10 g/L NaOH concentration as reected by the t(4) =0.918, p=0.411. However, there is a signicant dierence between the ber length of Bt corn husks treated with 5 g/L and 10 g/L NaOH concentration as reected by the t (4) =3.225, p=0.032. See table.

Table 4: T-test Results

Fiber Property	df	t-value	p-value
Yield	4	-0.547	0.613
Diameter	4	0.918	0.411
Length	4	3.225	0.032

Discussion. – Fibers subjected to 5 g/L and 10 g/L NaOH concentration treatment had a ber yield of 14.69% and 16.74 % respectively. The ber diameter diameter in the 5 g/L is 817.7 m and 723.7 m in the 10 g/L NaOH concentration treatment. The ber length in the 5 g/L is 11.7 cm and 5.3 cm in the 10 g/L NaOH concentration treatment. There is very little dierence with the ber yield of Bt corn husks treated with 5 g/L and 10 g/L NaOH concentration. With the dierence of only 2%, there is no substantial eect in the ber yield of Bt corn husks with 5 g/L and 10 g/L NaOH concentration alkalization treatment. The results of Yilmaz in his study in 2013 regarding the chemical extraction parameters of corn husks showed that corn husks treated with 5 g/L NaOH concentration has higher yield (6%) than the husks treated with 10 g/L NaOH concentration (3%). The results obtained by Yilmaz were lower than the results acquired by the researchers which are 14.69% for the 5 g/L and 16.74% for the 10 g/L NaOH concentration treatment. Similar to the ber yield, there is no signicant dierence in the diameter of bers extracted from Bt corn husks. In the study of Ekhuemelo and Tor in 2013 regarding the assessment of corn stalk and husk characteristics, the corn husk ber diameter is 30.19 m. This value is lower than the ber diameter value acquired by the researchers. The dierence in the results acquired compared to the other studies mentioned above is due to the dierence in the components of Bt and non-Bt corn husks; Bt corn husks have higher content of lignin than non-Bt corn hybrids [7]. Lignin, together with cellulose, is the most important structural component of natural bers. Lignin assists in the water transportation and rigidity of plants by adding strength and stiness to the cell walls [8]. The basic components of bers such as cellulose, hemicelluloses and lignin govern the physical properties of bers [9]. The 5 g/L and 10 g/L NaOH concentrations of alkalization treatments have substantial eect on the ber length. The harshness or higher concentrations of alkalization treatments have aected the extracted ber length negatively. NaOH bounds to cellulose molecules into aqueous solution to a certain extent, and prevents macromolecules from associating, so greater amount of NaOH has harsher eect to the bers [10]. The length of corn husk bers extracted by Yilmaz in his aforementioned study is 15.5 cm for the bers treated with 5 g/L NaOH concentration and 9.3 cm for the bers treated with 10 g/L NaOH concentration. This is consistent with the results acquired by the researchers in which bers treated with higher NaOH concentrations have shorter length. Physical properties of natural bers are important in determining the suitability of bers in dierent industrial materials [9]. The length and diameter of Bt corn husk bers extracted from both 5 g/L and 10 g/L NaOH concentrations are within the range of bers extracted from date palm. The similar physical properties shared by date palm and Bt corn husk bers have potential for a wide range of applications [11,12]. These applications include natural ber composites (NFC) and modern industrial applications such as materials for the automotive industry [11]).Factors that may aect the data acquired by the researchers are the following: dierent models of hot plates used by each setup, the rinsing done separately by the three researchers, and the other factors such as climate, weather conditions and soil quality [13]. Furthermore, various other physical properties were not characterized by the researchers due to the time constraint and lack of budget and expertise. Compared to the industrial properties of ber, bt corn husk bers are on par in terms of basic properties such as ber length. In terms of ber length distribution, bt corn bers are not equal to that of industrial ber. This is due to the processes done in order to retrieve the ber. Fiber length distribution indicates the strength, length and yield of a ber. The higher the value, the higher its potential in the industry. Bt corn husk bers are not equal to industrial bers however it still has potential in the industry due to the fact that it comes from agricultural byproducts. In summary, there is no signicant dierence in the ber yield and diameter of Bt corn husks bers, while there is a signicant dierence in ber length. Bt corn husk bers dier in native corn husk bers due to the abundance of lignin in Bt corn husk bers. Lignin is one of the most important structural components of natural bers. These physical properties of Bt corn husks are within the range of date palm bers. Consequently, Bt corn husk bers have a potential wide range of applications.

Conclusion. – The ber yield and diameter of bers treated with 5 g/L and 10 g/L NaOH concentration have no signicant dierence while there is signicance in ber length. As compared to industrial ber, bt corn husk bers are comparable only in terms of mechanical properties such as length and cannot stand alone as enough evidence for it to be in the same level as industrial bers.

Recommendations. – It is recommended to conduct the boiling of Bt corn husks in 5 g/L and 10 g/L concentration at the same time since factors such as air temperature and humidity has an eect in the boiling time and cooking of Bt corn husks[14]. The use of fully-functional hot plates with similar models is also recommended as this, too, can aect the boiling time and cooking. It is also recommended to use Bt corn husks with the same moisture content, age,

and condition. Using dierent NaOH concentrations and cooking time is also recommended since this can aect the physical properties of Bt corn husk bers[15]. Expansion or additional parameters to be acquired is also advised not only to gain more supporting data but also to expand the range of uses of the ber and not limit it to textile industries only.

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Verification of antidiabetic potential of Aloe vera: α -amylase inhibitory assay of crude aloe gel and aloe latex with rind extracts

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Abstract – Aloe vera has been known to have antidiabetic potential. Some studies state that aloe vera is antidiabetic because of the aloe latex while some studies state that its antidiabetic property is exhibited by the aloe gel. This study aims to verify whether aloe vera latex with rind and aloe vera gel indeed have antidiabetic potential. This study used the -amylase assay in which the inhibition of maltose was measured using a spectrophotometer. The results show that both aloe gel and aloe latex with rind extracts have antidiabetic property. It is recommended that active antidiabetic constituents be isolated and undergo further analyses to determine which constituents have better potential to be used for drug development.

Introduction. – Aloe vera is widely grown worldwide and has many uses. It is used to treat burns, inflamed skin condition, diabetes, rheumatic arthritis, rheumatic fever, ulcers and indigestion. It is also used in treating inflamed internal organ conditions such as the inflammation of stomach, small intestines, liver, pancreas and kidneys (Joseph and Raj 2010). Diabetes, on the other hand, is a condition in which the process of food for use as energy is not properly done by the body. The body obtains its energy from consumed food which is processed into glucose or sugar. The hormone that helps glucose enter into cells of the body is known as insulin, a hormone which is produced by the pancreas. When one has diabetes, the body is either incapable of using its own insulin properly or it doesnt make enough insulin. This causes sugar to build up in the blood and increase the blood glucose level. Increased blood glucose level gives higher risk of getting diabetes; lowering blood glucose levels is one method to treat diabetes.

Aloe vera has a potential to treat diabetes according to a study of Kim et. al. (2009) as aloe vera showed significant results in decreasing insulin resistance and thereby lowering blood glucose levels of diabetic mice. Previous study by Yongchaiyudha et. al (1996) also shows that aloe vera has a potential to be a treatment for diabetes. This study gave oral administration of aloe vera juice to patients with diabetes for two weeks and results show that blood glucose levels and triglyceride levels fell.

In a study of Okyar et. al. (2001), they tested the effects of aloe gel extract and aloe leaf pulp extract on diabetic mice. The results show that aloe vera leaf pulp extract results to an increase in blood glucose levels of mice and only aloe vera gel showed a decrease in the blood glucose levels of the test samples. Thus, according to Okyar's et. al. (2001) study, aloe vera leaf gel has a potential to treat diabetes while aloe vera leaf pulp extract does not.

Ajabnoor (1990) was able to identify the active constituents of aloe vera barbaloin and isobarbaloin (collectively known as aloin) and resin. Tanaka et. al. (2006) also identified that the active diabetic constituents in aloe vera are the five phytosterols - lophenol, 24 - methyllophenol, 24-ethyl-lophenol, cycloartanol and 24- methylene cycloartanol. Phytosterols are found in the aloe gel while the aloins and the resin are found in the aloe latex. Thus, according to Ajabnoor (1990) the antidiabetic potential of aloe vera is due to substances in aloe latex, a yellowish substance between the aloe gel and aloe rind; and according to Tanaka et. al. (2006), the antidiabetic potential of aloe vera is due to its aloe gel.

Various studies state different results on the antidiabetic part of aloe vera; some studies show that it is due to aloe gel while some studies show that it is due to aloe latex or aloe leaf (rind). There is inconsistency in the results of various studies. Thus, there is a need to determine whether the aloe vera parts indeed antidiabetic potential.

This study was able to verify the capability of aloe vera parts - aloe gel and aloe latex with rind - to have antidiabetic potential. An in-vitro test,α-amylase inhibition assay was used to determine whether the aloe vera part positively or negatively inhibited sugar breakdown.

Materials and Methods. – *General Extraction of Aloe Vera Plant.* The leaves were separately plucked from the aloe plant. The leaves were then washed and tap dried. The aloe gel was separated from the latex and rind by scraping using a stainless steel spoon. The aloe gel extracts were immediately subjected to further processes while the aloe latex with rind extract were set aside and air dried for around 48 hours.

Antidiabetic Aloe Vera Constituents. The target constituents from the aloe gel are aloins and aloeresins while the target constituents from the aloe latex and rind are phytosterols. The following procedures intended on optimizing the crude aloe vera extracts to obtain refined concentrations of the target constituents.

Solvent-solvent Extraction. Aloe gel and aloe latex with rind extracts used methanol as the extracting solvent as target constituents are polar molecules. Nonpolar solvent used was hexane.

Optimization of Aloe Gel. The fresh leaf gel was homogenized using an electric blender. The aloe gel was mixed with 100 mL hexane and 100 mL methanol and placed in the separatory funnel. The mixture was shaken three times and the inverted stopper was removed to release air. The system was closed again and the shaking procedure was repeated 3-5 times. The mixture resulted to two immiscible solutions of methane extract (bottom layer) and hexane extract (top layer). Methanolic extract was retrieved and placed in a rotary evaporator. A more concentrated aloe gel and methanolic mixture was retrieved from the rotary evaporator.

Optimization of Aloe Latex with Rind. The latex and the rind was sun/shade dried for two days. The dried portions were manually broken to smaller pieces using a mortar and pestle and were further powderized using an electric blender. The aloe latex solutes were dissolved in 100 mL hexane. The solution was mixed with 100 mL methanol and placed in the separatory funnel. The mixture was shaken three times and the inverted stopper was removed to release air. The system was closed again and the shaking procedure was repeated 3-5 times. The mixture resulted to two immiscible solutions of methane extract (bottom layer) and hexane extract (top layer). The methanolic solution containing the aloe latex and rind was placed in a rotary evaporator. More concentrated aloe latex, rind, and methanolic mixture was retrieved from the rotary evaporator.

The aloe gel was tested by the assay first, then the latex and rind were tested by the alpha amylase test two days after. The same aloe plant was the source of the aloe gel and aloe latex with rind.

α-Amylase Inhibitory Assay. Aloe vera extracts with different concentrations were first prepared in test tubes we had 0%, 20%, 40%, 60%, 80%, and 100% by volume. The diluting agent used is distilled water. The 0% sample had no aloe vera extract and only distilled water. Total volume of the different concentrations was 2 mL. 200μL of 0.02 M sodium phosphate buffer, and 20μL of α-Amylase was added in the assay. In a concentration range of 10-100μL/ml, this solution was incubated at room temperature for 10 minutes. 20μL of 1% starch solution was added on each test tubes. 400μL of 3,5 - dinitro salicylic acid (DNSA) reagent was added. The tubes were incubated in boiling water for five minutes and cooled at room temperature. The reaction mixture was diluted with 5 mL distilled water. The absorbance was measured at 540 nm using a UV-spectrophotometer. The control used is the blank sample and reference concentration is Acarbose, an α-amylase inhibitor.

Statistical Analysis. The α-amylase inhibitory activity was calculated. The formula for percent inhibition was used:

$$\%Inhibition = \frac{(A_{540}control - A_{540}test)}{A_{540}control} * 100 \qquad (1)$$

The percent inhibition values were plotted versus log inhibitor concentration and was evaluated by nonlinear regression analysis from the mean inhibitory values using Microsoft Excel. The IC50 values were then determined from the plot.

Results and discussion. – α-Amylase inhibitory activity of *Aloe vera* spp. were investigated in this study. Methanolic extracts of aloe gel and aloe latex with rind were prepared and five different concentrations of each methanolic extract were tested for the percent inhibition of α-amylase. The percent inhibition of the samples and the IC50 values of the extracts were calculated.

Aloe latex with rind extract at 60% concentration showed highest inhibition activity (67.61%) while the aloe gel extract at 20% showed the least inhibition activity (-5.85%). The IC50 values are 39.12% and 155.82% for aloe latex with rind and aloe gel extracts respectively.

Since both the extracts were generally able to get positive percent inhibition results, it can be said that: in aloe vera, both the aloe gel extract and aloe latex and rind extract has the potential to inhibit the -amylase enzyme.

Table 1: Percent Inhibition and IC50 values of different concentrations of aloins and phytosterols.

conc. of extract (%)	**% inhibition of Aloins** (%)	**% inhibition of Phytosterols** (%)
20	33.62	-5.85
40	57.51	11.29
60	67.61	9.65
80	-	19.67
100	-	38.14

Table 2: IC50 values of aloins and phytosterols.

IC50 of of Aloins (%)	**IC50 of Phytosterols** (%)
39.12	155.82

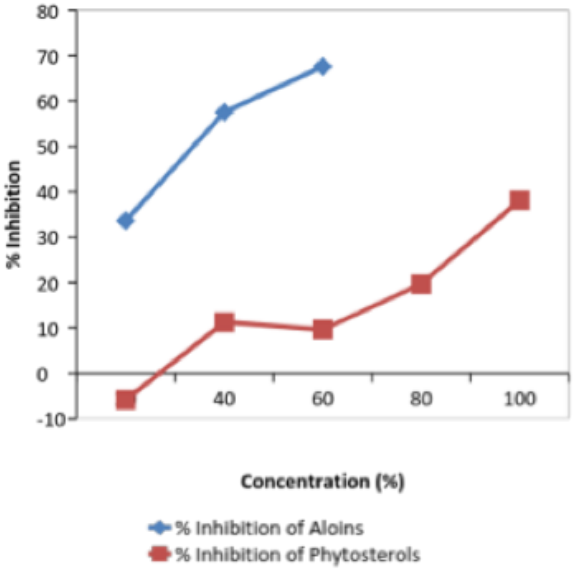

Fig. 1: The percent inhibition of aloins and phytosterols at different concentrations

It can also be observed in Figure 2 that as the concentration increases, the inhibition activity increases. This implies that the more the aloe extract, the greater the inhibition of the amylase enzyme.

However, the results are inconsistent as there are fluctuations in the percent inhibition as seen on the 20% and 40% aloe gel extract concentration. This might be due to an unhomogenized test samples, difference in optimization rates, and inconsistencies in the spectrophotometer.

The percent inhibition was calculated from the absorbance of samples in the spectrophotometer. The absorbance at 540 nm measures the amount of maltose in the sample. Each of the samples contains αamylase enzyme, sodium phosphate buffer, starch solution, dinitrosalicylic acid (DNSA), and the extract concentrations. The enzyme is responsible for breaking down the starch in the solution and the buffer maintains the pH of the solution so as to prevent any disruptions in the reaction. The broken down starch is maltose and some other substituents. The spectrophotometer measures the amount of light for a certain colour; however, the samples have vague colours. So, DNSA is needed for colouring the samples so that the target constituent could be read by the spectrophotometer.

The goal of this assay is to inhibit the enzyme activity of the α-amylase so the samples should have smaller amounts of broken down starch or maltose. Our reference sample is acarbose, a known α amylase inhibitor. Since acarbose is a known αamylase inhibitor, it would mean that the amount of maltose in the acarbose sample is zero or minimal because it was able to inhibit the αamylase enzyme, the responsible for making maltose. In the negative control sample, the extract was replaced with distilled water so there is no extract that could inhibit the activity of the enzyme and so the maltose level should be higher than the inhibitor. Expected absorbance values, output by spectrophotometer, should be lower than the negative control and at par with the positive control (or the reference sample) to say that the extract has a potential to inhibit the αamylase enzyme activity.

To be able to simplify the data, percent inhibition of each sample is calculated. Percent inhibition is a ratio of the difference between negative control absorbance value and sample value and the negative control absorbance value multiplied to 100%.

Antidiabetic activity is present once percent inhibition is positive. Higher percent inhibition means lesser amount of maltose present comparing it to the negative control. Positive percent inhibition is already an indication that the extract was able to inhibit antidiabetic activity being the one to lessen the amount of maltose in the sample.

The highest percent inhibition calculated is the aloe latex with rind extract at 60% concentration and the value is 67.61%. This would mean that it was able to inhibit 67.61% of enzyme activity compared to the negative control or the amount of maltose it had is 67.61% less compared to the negative control. The least inhibition calculated is the aloe gel extract at 20% concentration and the value is - 5.85%. This would mean that enzyme activity was not inhibited by the extract and that the amount of

maltose it had was 5.85% higher than that of the negative control.

Through the positive percent inhibition, this study was able to verify the antidiabetic potential of the two extracts aloe gel and aloe latex (and rind). The gel extract contains the phytosterols while the latex and rind extract contains the aloins. This study can be supported by studies of Ajabnoor (1990) and Tanaka et.al. (2006) that states that aloins and phytosterols found in aloe vera have antidiabetic potential.

To be able to represent the antidiabetic activity of the extracts, the IC50 values were calculated. The IC50 value is the concentration needed to be able to get a 50% percent inhibition. The IC50 values are 39.12% and 155.82% for aloins and phytosterols respectively. Positive IC50 values indicate that both aloins and phytosterols inhibit antidiabetic activity.

To be able to do this study or this method again, there are some key points that the researchers in this study learned. (1) The aloe gel and aloe latex should come from the same aloe vera plant to have more reliable results. (2) In the extraction of the extracts in the rotary evaporator, aim for the powdery extract and make sure to retrieve all of the solvent.

This study retrieved liquid extracts at the rotary evaporator so it had variable concentrations of methanol and aloe extracts. This study used the volume/volume percent concentration with extract from the rotary evaporator as the initial liquid and distilled water as the diluting agent. The ideal concentration units, basing Beer-Lamberts law, is mg/ml or μg/ml, so that the results will be more reliable. (3) Reference cell is the acarbose and this is needed when using the spectrophotometer; and control is the negative sample or the blank sample without any extracts.

The data gathering of this study was repeated twice because the first data gathering did not have the acarbose and the aloe extracts were collected from different plant sources. (4) Assay should have three replicates with three triplicates each to be able to have more reliable results. In this study, only one replicate with three triplicates was performed because of insufficient extracts. However, there were also inconsistencies with the triplicates. In the aloe gel methanolic extract, trial 1 was inconsistent with trials 2 and 3, and therefore it had been omitted in calculating the average of the percent inhibition of the phytosterols. Inconsistencies of these trials could be attributed to the samples which easily become unhomogenized even after shaking.

If ever this study will be repeated for verification, certain aspects can be improved especially on the data gathering procedure. More plots or more concentrations of samples can be done to increase validity and reliability of results. Replication of samples can be done for the assay to see if absorbance results vary and also get more reliable results. In performing the assay, one should note the proper storage conditions and concentrations of the chemicals. One should also note the placement and distribution of the chemicals during the assay and make sure that the solutions to be placed in the spectrophotometer are homogenous.

In summary, this study was able to show that both the latex and rind extract was able to inhibit the alpha amylase activity and that the extracts have a potential to be antidiabetic.

Conclusion. – Both aloe latex with rind extracts and aloe gel extracts, which are crude extracts of antidiabetic constituents aloins and phytosterols, respectively, have antidiabetic potential.

* * *

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Comparison of Sodium Alginate-Based Slow-Release Beads with Varying Calcium Chloride Concentrations

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Abstract – Calcium alginate-based slow-release beads have properties which allow them to be used as fertilizer reservoirs. This study aimed to determine whether the varied concentrations of the cross-linkin agent, calcium chloride, has any effect on the slow-release rate of the beads in soil application and in water. Three concentrations at (3, 2, and 1 percent w/v) of calcium chloride were tested for UV-vis absorption and soil application, and their slow-release rates were determined. No significant difference was found among the various calcium chloride concentrations in both water and soil application, however the UV-vis spectrophotometry test showed that the 1 percent w/v calcium chloride concentration set-up had the slowest release rate in water.

Introduction. – Fertilizers also known as agrochemicals are readily available materials that provide plants the necessary elements that they need in order to grow and develop. Elements such as nitrogen, phosphorus, and potassium which are present in fertilizers help in hastening vegetative growth, increasing disease resistance, improving water intake, and stimulating root, flower, and seed development.

Despite the advantageous effects fertilizers have on plants, they can also be harmful as they may cause fertilizer burn. Fertilizer burn is caused by the overabundance of soluble salts in the soil due to excess application of fertilizer on plants [1]. It is characterized by scorching-like formations along roots or leaves, yellowish or brownish discoloration in the leaves, root damage, and stunting. Plant health such as low fruit quality, weak stem, fewer flowers, and the wilting of a plant can also be affected or influenced by fertilizer burns [1].

In addition, fertilizers can also cause harm in the environment through bioaccumulations in the food chain and contamination of various ecosystems [2]. One example of environment harm would be the occurrence of algal bloom, a result of eutrophication wherein large amounts of nutrients are accumulated in large bodies of water [3]. Despite being beneficial to the marine ecosystem for producing huge amounts of phytoplankton and cyanobacteria, intense occurrence of algal bloom can also be detrimental.

The adverse effects of fertilizers on plants and the ecosystem motivated researchers to find solutions in mitigating these effects. One solution they have developed is the use of superabsorbent polymers to control the release of fertilizer. In this way, the loss and use of fertilizers, and its adverse environmental effects can be mitigated. Slow-release fertilizers have the ability to reduce leaching, volatilization, and degradation of fertilizers. Furthermore, these materials can also cause soil compaction and prevent erosion and water run-off. These effects of slow-release fertilizers can help mitigate detrimental effects fertilizers have on the environment. These materials also improve the water-holding capacity of the soil. Moreover, ionic plant nutrients such as nitrogen, phosphorus and potassium are also stored, thus, slow-release formulations are better than conventional fertilizers.

For the development of slow-release fertilizers, polysaccharides are often being used as matrix since they are hydrophilic and biodegradable. Polysaccharides include cellulose, cyclodextrine, alginate, starch, dextran, guar gum, pectin, and chitosan. Alginate, a natural polysaccharide form algae, is one of the most commonly used materials for slow-release bead production [4]. Various studies have shown the use and application of the said polymer in the development of slow-release fertilizers.

For one project, a nanoreservoir made of alginate was designed in order to gradually release water from the bead [4]. Another study developed a similar product and it was found to have food slow-release capacity, and it was also able to improve the water-holding and water-retention capacity of the soul [5]. Furthermore, microbeads formed using sodium alginate, especially those who cross-linked with calcium chloride, are being highly studied for various medical and agricultural applications.

As shown, there is an abundance of studies which utilize sodium alginate for microbead production. This is because its viscosity and gelling, film-forming, thickening, and stabilizing properties [6]. However, there is lacking information regarding other microbead composition which can further improve its slow-release ability. Due to this, slow-release beads, with varying concentrations of its cross-linking material, calcium chloride, were made in this study in order to assess the effect of its varied concentrations on the slow-release ability of the beads.

Methods. – Calcium alginate-based slow-release beads were developed using sodium algiante and calcium chloride through the process of ionotropic gelation. These beads were varied in the calcium chloride concentration applied in order to assess which calcium chloride concentration is the most efficient in promoting the slow-release ability of the developed beads. The beads were applied and tested in water as well as in the soil. In the water setup, using UV-Vis spectroscopy, the rate of absorbance levels of the released content of the beads int he release medium was recorded and calculated. on the other hand, the rate of weight loss of the beads for seven days was determined for the soil setup.

Materials. In making the calcium chloride solution sodium alginate solution, and alginate beads, glassware such as conical tubes, beakers, and graduated cylinders were used. In addition, laboratory equipment such as the vortex mixer, UV-Vis spectrophotometer, and top-loading balance were aso utilized. Specimen cups and syringes at 1cc, 2cc, and 3cc were also utilized in this study. All laboratory glassware and equipment were available in the Philippine Science High School- Western Visayas Campus laboratory.

Preparation of calcium chloride solutions. Three varying calcium chloride concentrations were made at 1, 2, and 3 percent w/v concentrations. Calcium chloride weighing 2.5, 5.0, and 7.5 grams were separatelt mixed in distilled water in three 250mL volumetric flasks.

Preparation of sodium alginate solutions. Three percent of sodium alginate solution was made by putting 1.5 grams of sodium alginate in a 50mL conical tube. It was then initially added with a small amount of distilled water and was mixed using the vortex mixer and was diluted to mark. Continuous mixing was done after every addition of distilled water until it was fully mixed. The mixture was then left to settle overnight in order to allow it to dissolve completely.

Synthesis of calcium alginate-based beads. 3mL of the 3 percent w/v sodium alginate solution, 1mL red food coloring, and 0.15mL glycerol were mixed in a separate conical tube using a mixer. 125mL of the calcium chloride solution was placed in a beaker. The homogenous solution was then added drop-wise using a 10mL syringe with a 0.32mm needle from a height of 6cm into the calcium chloride solution, as shown in fig. 1. The dropped beads were allowed to settle in the calcium chloride solution for 40 minutes. After settling, the beads were removed from the calcium chloride solution and were washed with distilled water. The beads were then allowed to drain. After draining the beads, they were weighed and prepared for the soil and water testing.

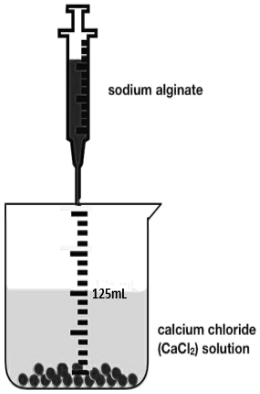

Fig. 1: Ionotropic gelation.

Testing of slow-release beads in water. 0.2 grams of the developed slow-release beads were taken from every treatment in order to be used for water testing. Three set-ups of 400mL distilled water placed in 400mL beakers were prepared to serve as the release medium. The beads were immersed in the prepared release medium. At every ten-minute interval, 5mL of the solution was taken out from the release medium in order to detect the content of the red food coloring using an ultraviolet spectrophotometer. The absorbance of the sample solution was measured.

Testing of slow-release beads in soil. For each set-up, beads weighing 0.5 grams were prepared. These beads were then placed inside a mesh and were buried into 150g of soil at a depth of 10cm from the soil surface, as shown in fig. 2. The weight of the beads were checked at a 24-hour interval. For the weighing, the beads were

removed from the soil, washed with distilled water, drained for five minutes, and weighed. After weighing, the beads were placed back inside the mesh and were buried into the soil again. This was done every 24 hours for a period of seven days.

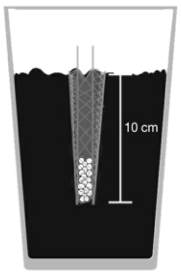

Fig. 2: Setup of slow-release beads in soil.

Results. – In terms of the weight loss of the slow-release beads over time, the beads with a three percent w/v calcium chloride concentration had he slowest rate of release, whereas those with a one percent w/v calcium chloride concentration had the fastest rate of release.
Contrary to this, the hydrogel beads which had a two percent w/v calcium chloride concentration has the highest absorbance for the wavelength range of 590 to 470nm, at all time intervals. It is followed by the slow-release beads which contain three percent w/v calcium chloride concentration. Lastly, the beads which contained a one percent w/v calcium chloride concentration had the fastest rate of release had the least absorbance at all the aforementioned wavelengths.

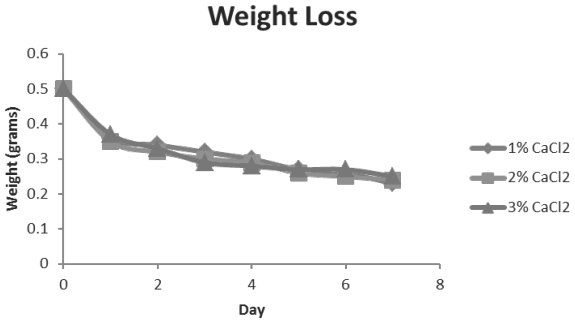

Fig. 3: Weight loss of slow-release beads for seven days.

The beads in the treatment which have a three percent w/v calcium chloride concentration had the slowest release rate among them. This is consistent with the study of Badwan et. al [8], where the calcium alginate

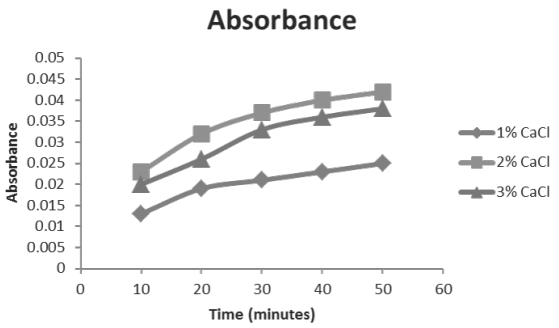

Fig. 4: Absorbance of water throughout the 50-minute duration.

beads for drug delivery that were made from the highest concentration of calcium chloride (15 percent w/v calcium chloride concentration) had the slowest rate of release. According to Badwan et. al [7], this may be attributed to the water content present in the beads. The beads with the lower concentration of water and a higher concentration of calcium chloride have resulted to a lower swelling effect on the beads, thus the slower its release. This is also similar to the study of Tavakol et. al [8] on alginate NO-carboxymethyl chitosan beads for drug delivery. The NO-carboxymethyl chitosan beads prepared from the higher concentrations of calcium chloride had a lower swelling degree due to the increased cross-linked density of the beads. Despite having the slowest release rate among the three treatments, the treatment with a three percent w/v calcium chloride concentration had no significant difference among the other two treatments at the alpha level of 0.05, having a p-value of 0.909.

Contrary to the results of the soil setup, it was the treatment with a one percent w/v calcium chloride concentration which had the slowest rate but there is also no significant difference among the three treatments. This is not consistent with previous studies on the mechanism of slow-release beads. Supposedly, it is the treatment with beads having a higher calcium chloride concentration which will have a slower release rate. There must be a difference in the behaviour of the slow-release beads in water and in soil due to the great number of impurities in the soil setup. Therefore further studies regarding the mechanism of slow-release beads in both the soil and water setups must be conducted.

Conclusion. – There is no significant difference among the treatments in both the soil and water setups. Therefore, the beads with a one percent w/v calcium chloride concentration can be used to produce slow-release beads in a large scale without utilizing huge amounts of cross-linking material for there is no significant difference on the slow-release rates of the three treatments with a

varying calcium chloride concentration.

Recommendation. – Since the data for the water set-up is anomalous, further studies on the mechanism of slow-release beads should be conducted. It would also be better if the actual fertilizer material will be loaded to the bead itself to see whether there is a difference on the beads' mechanism.

* * *

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The Effect of Plant Spacing on the Voltage Performance of a Shared-Anolyte Plant Microbial Fuel Cell Utilizing Ipomoea aquatica

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Abstract –Plant Microbial Fuel Cells (P-MFCs) are bio-electrical chemical devices that utilize bacteria in plant rhizodeposits to generate electricity. In order to determine the effect of plant spacing on the voltage output of P-MFCs, shared-anolyte P-MFC system utilizing *I. aquatica* were designed and constructed. A single shared-anolyte P-MFC contains three P-MFCs connected in series, with all three plants in the same soil. They were three shared-anolyte P-MFC setups, with each setup having a triplicate. The plant spacing of the setups were 5, 7, and 9 cm respectively. According to the results, shared-anolyte P-MFCs with shorter plant spacing produced a greater voltage output. This shows that P-MFCs can be a potential candidate for future power generation, because shorter plant spacing is needed, which means that the space they will take up will be lesser as well. The actual voltage output of the shared-anolyte P-MFCs is only 20.8%, 19.6%, and 20.9% for the 5, 7, and 9 cm plant spacing setups respectively.

Introduction. – A microbial fuel cell (MFC) is a bio-electrical chemical device that generates electricity from organic substrates using the respiration of microbes. Due to their ability to generate electricity while treating wastewater [13], MFCs show great potential for sustainable bio-energy production. MFCs can also be used as biosensors [7] and small power production systems like sediment batteries [19]. The basic components of MFCs include an anode and a cathode separated by a proton exchange membrane. The microbes break down the substrates in the anaerobic anode chamber, which produces carbon dioxide, protons and electrons. The protons produced cross the proton exchange membrane to combine with the oxygen in the cathode chamber, which results in water. The electrons then travel from the anode to an external circuit and generate an electric current [23]. The sediment MFC is a type of MFC where one electrode is placed into a sediment rich organic matter while the other is placed in overlying oxic water [13].

One of the challenges that MFCs face is their low performance for commercial use [13]. The materials used for MFC construction are also expensive, thus limiting mass production [17]. The reactor or container of the MFC alone accounts for 68.5% of its cost. These two factors greatly limit the ability of MFCs to function on a wide-scale, since their high cost yields little performance. There have been several methods developed to potentially increase the voltage performance of an MFC. One proposed solution is to install multiple unit cells in a single reactor [11], however, a serious potential drop is observed when connecting unit cells in series. This serious potential drop is caused by the ions from the anode traveling through the electrolyte to reach the cathode. One way to avoid or lessen the potential drop phenomenon is to increase the resistance between the unit cells [9]. When the distance between unit cells was increased to increase resistance, a significant improvement can be seen in the performance of the MFCs with shared anolytes. MFCs with low density will not be ideal for large scale applications because they will take up a greater amount of space.

Plant MFCs (P-MFCs) are a type of sediment MFC that has a plant introduced into the MFC system. P-MFCs can also avoid the expensive costs of building an MFC, since P-MFCs can be operated by installing anodes and cathodes in-situ, without a need for expensive reactors or proton exchange membranes. Most plants used in P-MFCs have been aquatic plants [22]. Ipomoea aquatica, more commonly known as water spinach or kangkong, is an

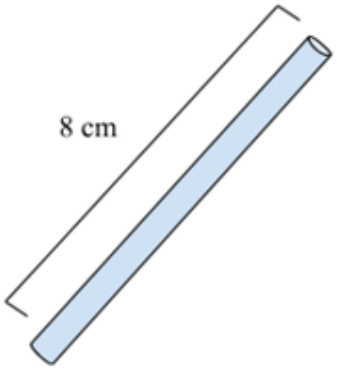

Fig. 1: Graphite Rod

aquatic plant that is also harvested as a crop [10]. Water spinach also has other uses, such as ethnomedical uses in India [3].

Due to the need of *I. aquatica* to be spaced at a certain distance from each other for ideal growth, P-MFCs can be a possible method to scaling up MFCs. They do not require a high density unlike other types of MFCs, because plants need spacing. This allows them to become a possible solution to the potential drop phenomenon observed when connecting multiple unit cells in series. Increasing the distance between unit cells has already shown to decrease the effect of potential drop. The need of proper spacing for plant growth can be used to offset the distance needed to overcome the potential drop phenomenon.

Using a shared-anolyte system for P-MFCs provides several advantages, such as being able to generate electricity while being able to grow crops at the same time. The growing plants can also assist in the removal of carbon dioxide in the atmosphere.

Methods. – Three rectangular plywood containers were constructed for use as *I. aquatica* containers. The containers were separated into three rows. The containers were then filled with soil along with the anodes. Three *I. aquatica* were then planted on every row. A fourth pot without any *I. aquatica* plants planted was also used as the control. The distance between the *I. aquatica* was 5 cm, 7 cm, and 9 cm for each of the rectangular pots respectively. The cathodes were then added at the base of the plants. The plants were given 1 week to acclimatize. After acclimatization, the voltages were measured using a multitester.

Materials and Equipment. – *I. aquatica* was acquired from Western Visayas Agriculture and Research Consortium. Soil was acquired from the institution. Steel mesh, pencils, insulated copper wires, epoxy, breadboard, and soldering iron are acquired from local hardware stores.

Electrode Construction. – Graphite was extracted from pencils through burning. The graphite were then cut into lengths of 8 cm. The graphite were then soldered to copper wires to produce the anodes and cathodes.

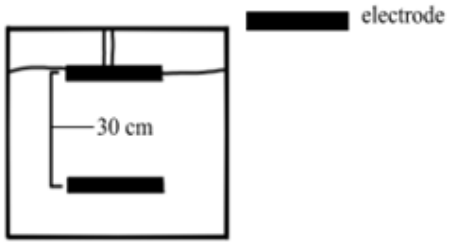

Fig. 2: Individual P-MFC Setup. The lower electrode is the anode.

P-MFC Construction. – The anode was placed in the bottom of the plywood container. The plywood container was filled with 30 cm depth of soil. The *I. aquatica* was then planted on top of the soil. The cathode was placed at the base of the plant.

Shared-anolyte P-MFC Construction. – There were three setups for the shared anolyte P-MFCs, and every setup had a triplicate. The different setups have the respective plant spacings of 5, 7, and 9 cm. A shared anolyte P-MFC contains three individual P-MFCs connected in series. The shared-anolyte P-MFCs were acclimatized for one week.

Cultivating I. aquatica. – Stem cuttings of I. aquatica were acquired from WESVIARC. The I. aquatica were planted on the rectangular plywood pots. Each pot is separated into three rows, and every row has three I. aquatica planted. The distance between the plants are 5 cm, 7 cm, and 9 cm for each of the rectangular plywood pots respectively. In addition, I. aquatica are watered using a watering can [16].

Data Gathering. – The measuring of the voltage began after the acclimatization period from the connected P-MFCs and individual P-MFCs were analyzed using a digital multimeter every hour from 10:30 am to 3:30 pm, for three days. These times were chosen so that the plants are able to receive sunlight. Each individual P-MFC had their individual voltages measured. Their collective voltage in series were then measured by connecting three P-MFCs in a row

Disposal. – The wires were first disconnected from the anode and the cathode. They were then separated from each other. The plants were individually taken out of the setup. The anodes and cathodes were washed with tap water while the graphite was removed by washing it away and was thrown in a trash receptacle. The soil was returned to the site where it was gathered. The I. aquatica plants were disposed of into a biodegradable trash receptacle.

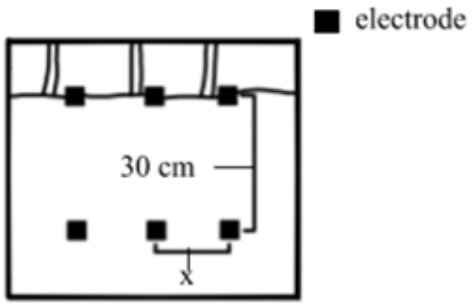

Fig. 3: Shared-anolyte P-MFC. The x represents the plant spacing for the different setups which are 5, 7, 9 cm.

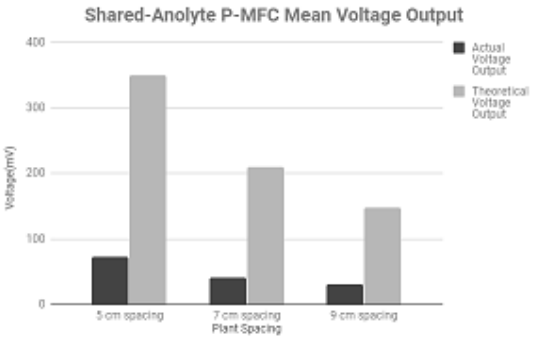

Fig. 4: Shared-anolyte P-MFC Mean Voltage Output

Results. – Shared-anolyte P-MFCs utilizing I. aquatica were constructed using a rectangular plywood box as the container and pencil graphite as the electrodes. The P-MFCs were given a one week acclimatization period. There were three setups for the shared anolyte P-MFCs, each setup having 5, 7, and 9 cm plant spacing respectively. Every setup also had a triplicate. The voltage output of the shared-anolyte P-MFCs were measured every hour from 10:30 am to 3:30 pm, for three days. The voltage output was measured by connecting the three P-MFCs in the shared anolyte system in series. The following graph shows the overall mean voltage output for every setup. The actual voltage output refers to the mean voltage output.

Discussion. – The results show that the lesser the plant spacing, the greater the voltage output of the shared-anolyte P-MFC. According to statistical analysis, the difference between the results are statistically significant at a confidence level of .95. This is contrary to the results of a previous study regarding the shared-anolyte designs of MFCs, in which the voltage outputs were greater when the distance between the cells were greater [9]. This might be due to the substrates used in the study. The previous study used sludge as the substrates for the bacteria, while this study used soil rhizodeposits as its substrates. It is possible that since the plants were closer to each other, their rhizodeposits may have combined. This may have provided the P-MFC more nutrients and more bacteria, which resulted in its greater voltage output, since the rate of cellular respiration is also higher. Despite being connected in series, the voltages of the individual P-MFCs in the shared-anolyte system did not stack as they should, theoretically. This may be due to electrons not moving to the cathodes when the P-MFCs are connected in series. The electrons may have been attracted to the ground in the shared-anolyte PMFC system, causing them to stray from their intended paths. The actual voltage output of the shared-anolyte P-MFCs is only 20.8%, 19.6%, and 20.9% for the 5, 7, and 9 cm plant spacing setups respectively. This design is also inefficient, because the voltage output of an individual P-MFC in the system almost always higher than the shared-anolyte P-MFCs voltage output. This means that voltage does not stack in this design, just as observed in other stacked MFC systems [1]. A possible explanation as to why the voltage outputs did not stack might be due to This technology is still not viable for wide-scale and commercial use, because it does not produce enough power. This design did not use oxic water as a separation between cathodes and anodes. including oxic water in the design could significantly change the results as this produces another layer of separation between the anode and the cathode.

Conclusion. – A shared-anolyte P-MFC was successfully created using I. aquatica plants. It can be concluded that a decrease in distance between plants increases the voltages produced by the P-MFC system. The voltage output of the P-MFC is greater the shorter the plant spacing, which may be due to the rhizodeposits combining when the plants are in close vicinity to each other. The voltage produced by the P-MFC system was lower than the voltages produced by the individual P-MFC which makes this design impractical. P-MFC of this design should be kept as individual cells for usage as this would provide higher voltages.

Recommendations. – It is recommended to have more setups with varying distances. This would provide information regarding the limit of distances between P-MFC. Other plants should also be taken into consideration as different plants could have varying effects on the voltages provided by the P-MFC. Electrodes made of more efficient materials would be an improvement as these provide more reliable data.

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T.E.R.R.A. (The Economical Response to Reduce Air Pollution): A New Device for Facilitating the Sequestration and Mineralization of (CO_2)

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Abstract – The atmospheric level of carbon dioxide (CO_2) has exceeded the 400 parts per million (ppm) threshold in 2015, making global warming a concerning cause for alarm. To lessen carbon emissions, Nickel Nanoparticles (NiNPs) have been proven to catalyse mineral carbonation in a Calcium Hydroxide ($Ca(OH)_2$) solution (Bhaduri Siller, 2012). This is seen as a viable method to reduce CO_2 in the atmosphere. However, this technology has not yet been applied in an actual device. T.E.R.R.A. (The Economical Response to Reduce Air Pollution) is a device which utilizes the catalytic effect of NiNPs and uses it to lessen the carbon emissions of vehicles. When tested in a laboratory setting, it was found that T.E.R.R.A. was able to sequester 82.5 percent of CO_2 bubbled through. With lower pressure and improvements in the design, it is projected that higher amounts of CO_2 may be sequestered by T.E.R.R.A., deeming it a feasible device for the mitigation of carbon emissions.

Introduction. – Carbon dioxide (CO_2) is a colorless and odorless gas that is composed of two oxygen atoms linked to a single carbon atom through a covalent bond. It is a greenhouse gas (GHG), or an atmospheric trace gas that allows solar radiation from the sun to pass through towards the Earth, but is partially opaque to the thermal radiation emitted by the Earth outwards. These properties allow naturally occuring GHGs to regulate the temperature of the Earth. In fact, without these gaseous compounds, the average global temperature would be around 34 °C lower than it is today [1]. However, human activity has been altering the carbon cycle through increased emissions, thereby altering the regulation of global temperatures.

According to the Intergovernmental Panel on Climate Change (IPCC), CO_2 has been the biggest contributor to climate change in terms of radiative forcing RF, or the net increase or decrease in the thermal energy that reaches the Earths surface. This is the because it is the most abundant GHG, constituting 72 percent of total emissions. In addition, CO_2 lasts longer in the atmosphere compared to other GHGs. When a pulse of CO_2 is emitted into the atmosphere, 40 percent will remain in the atmosphere for 100 years, 20 percent will remain for 1,000 years, and the final 10 percent will remain for 10,000 years[2]. These reasons make CO_2 perhaps the most threatening GHG.

Due to its adverse effects on the environment, scientists have been looking to reduce the concentration of CO_2 in the atmosphere. Research facilities such as the Mauna Loa Observatory in Hawaii have been monitoring the long-term activity of atmospheric CO_2 [3]. In September 2016, the Observatory showed that the atmospheric CO_2 level permanently surpassed the 400 parts per million (ppm) threshold. This is the highest concentration of atmospheric CO_2 in the last five million years (Scripps Institution of Oceanography, 2016). Additionally, the number is projected to reach 450 ppm by 2034, which is considered a high risk level [4].

An atmospheric CO_2 level below 450 ppm is needed in order to have a 50 percent chance to stabilize global temperatures at less than two degrees celsius above the preindustrial period. The biggest human activity that emits CO_2 is the burning of fossil fuels for energy and transportation. When fossil fuels burn, carbon and hydrogen

react with oxygen in the air to produce CO_2 and water. In particular, the burning of fossil fuels for transportation is the second largest CO_2 contributor. In the U.S., 32 percent of total CO_2 emissions in 2015 came from the transportation sector. Despite the fuel economy of modern day vehicles reaching a record low, an average passenger vehicle still emits about 4.7 metric tons of CO_2 per year [5]. Aside from this, reliance on vehicles for transportation is increasing as industrialization and modernization reach far flung areas.

The intake of CO_2 contributes to air pollution and promotes the spread of respiratory diseases. According to the World Health Organization, approximately seven million people worldwide die annually from air pollution, over six million of which were recorded in Asia in 2016. Aside from this, CO_2 contributes to global warming, the phenomenon that causes the gradual increase in the Earths temperature. It is caused primarily by GHGs which ultimately cause the greenhouse effect: the trapping and redirecting of heat back into Earth.

Thus, carbon capture and storage (CCS) is considered a crucial strategy for meeting CO_2 emission reduction targets, such as that set by the UNFCCC. Present methods include catalytic converters, scrubbing, and cleaning of fuels before combustion. Another method of sequestration is through mineral carbonation, which involves bubbling the gas into an aqueous solution of divalent metal. The end product of this process is inert solid carbonates. Although mineral carbonation is a permanent and safe option for sequestration, many claim it is unfeasible because of its slow reaction rate.

In order to hasten the reaction involved in sequestration, a catalyst has been identified by scientists Baduri and Siller [6]. These are Nickel Nanoparticles, which range below 1,000 nanometers. The use of NiNPs as a catalyst in the rate limiting step of reversible hydration in the process mineral carbonation has been proven to hasten the sequestration of CO_2 by threefolds. This study aims to design and evaluate a device using NiNPs which sequesters and mineralizes CO_2 . Thus, a device is proposed which utilizes NiNPs in the mineral carbonation of CO_2. The proposed device shall be known as T.E.R.R.A. or The Economical Response to Reduce Air Pollution.

Methods. – The project involved two phases: Phase 1: The Design Phase, and Phase 2: The Construction and Evaluation Phase. The first phase is the preparation phase which involves theoretically optimizing the device, and later constructing it. The second phase will be the evaluation phase which will evaluate the efficiency of the device through laboratory experimentation.

Materials and Equipment. All glasswares borrowed such as volumetric flasks, erlenmeyer flask, graduated cylinders, beakers, and etc are readily available at the Philippine Science High School Chemistry Stockroom. All reagents used for the reactions are of analytical gradeThe synthesis of NiNPs necessitated nickel chloride hexahydrate ($NiCl_26H_2O$) as precursor material, sodium borohydride ($NaBH_4$) as reducing agent and sodium citrate dihydrate ($C_6H_5NaO_72H_2O$) as capping agent. For the sequestration and mineralization process, calcium hydroxide ($Ca(OH)_2$), H_2SO_4, and nitric acid (HNO_3) was utilized. All these chemicals are also readily available at the Philippine Science High School Chemistry Stockroom except for the $NiCl_26H_2O$ and H_2SO_4 which was purchased from Patagonian Enterprises in Jaro, Iloilo City. Materials which include distilled water, hoses, hose clamps, spare parts, and CO_2 tanks were bought from local stores. Equipment such as analytical balance, pH meter, hot plate, magnetic stirrer, filter paper, iron stand and ring, cork borer, oven and centrifuge, were readily available at the PSHS Laboratory Stockroom. The Transmission Electron Microscope (TEM) was also available at the Southeast Asian Fisheries Development Center (SEAFDEC), Tigbauan, Iloilo.

Device Design. A design was conceived through the aid of a professional mechanical engineer, Edgar Allan Vargas. The engineer was able to contribute mostly to the practicality of the device design rather than the chemical design behind the device. The extent of his contribution was mostly centered with regards to its feasibility to manufacture, its practicality, and changes in dimensions to facilitate the welding of stainless steel tube. Furthermore, he also gave valuable information in, the drafting of the device design, the purchasing of materials and the construction of the device. Numerous revisions in the design have been submitted for review and for further more changes. These are all listed and explained in the results and discussion section.

This design involves the utilization of 3 bubbling chambers. As CO_2 is bubbled through the solution, it reacts in the first chamber with $Ca(OH)_2$ to initiate the carbon mineralization process and is then converted in $CaCO_3$. However, since not all CO_2 is expected to react, additional chambers have been added to allow the escaping CO_2 to be sequestered and react again with the $Ca(OH)_2$ filled chambers. To further understand this process, an airflow diagram visualizing the movement of CO_2 can be seen.

A rectangular chamber was chosen for the sake of fabrication. Stainless steel is a relatively hard metal to bend and weld. Also, allowing for other geometrical shapes would have also meant an irregularly shaped device and would have made it more bulkier. Rectangular shapes decreases the total space the device occupies. Hence, for our device a rectangular chamber was chosen. To empty the $Ca(OH)_2$ solution in the device, a removable drain has been placed under the bottom of the device. The design also includes input and output tubes to facilitate the flow of air. Some padding was placed between the roof of the device and the chamber so that it may be sealed sealed airtight. The roof of the device is removable with nuts and bolts holding it together.

Construction of Device. The device was constructed

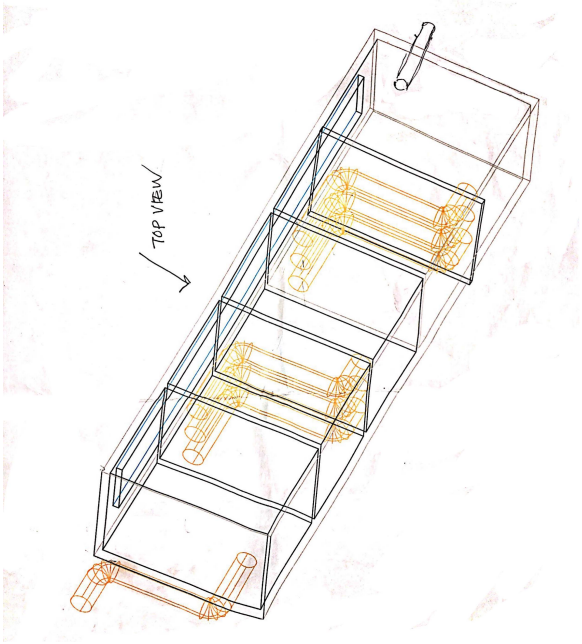

Fig. 1: Interior of the proposed design of T.E.R.R.A.

through local stainless steel fabricators. Negotiations with M.Estomo Fabrication Stainless Works was set with the total cost for fabrication (labor and materials) is P8,000. The final image of the device can be seen below.

Synthesis of Nanoparticles. Nickel nanoparticles was synthesized simultaneously during the construction of the device. This step is in preparation for experimentally evaluating the device. The chemical reduction method will be applied in the synthesis of NiNPs. First, 7.132 g of nickel chloride hexahydrate ($NiCl_26H_2O$) was dissolved in 30 mL distilled water in a 250mL beaker. After which, 16 g of sodium citrate dihydrate ($C_6H_5NaO_72H_2O$) was added to the solution to act as a capping agent. The prepared solution was then placed in a water bath where it was heated (at 40oC) and magnetically stirred (at 400 rpm) for one hour.

During the one hour stirring time, a separate solution of 2.27 g sodium borohydride ($NaBH_4$) was prepared to act as a reducing agent. This concentration corresponds to 2:1 molar ratio of $NaBH_4$ to $NiCl_26H_2O$ (Nayak et al.). The $NaBH_4$ solution was kept for temporary storage in a sealed 50 mL volumetric flask at room temperature while waiting for the $C_6H_5NaO_7$ and $NiCl_26H_2O$.

After an hour of stirring, the $NaBH_2$ solution was then added dropwise to the $NiCl_26H_2O$ and sodium citrate solution for 10 minutes while continuously stirring at 400 rpm (at 180o°C). The solution turned black, which indicated the reduction of the nickel ions. After the addition of $NaBH_4$, the temperature of the hot plate was decreased to 80°C and left constant for two hours to allow the reaction to complete.

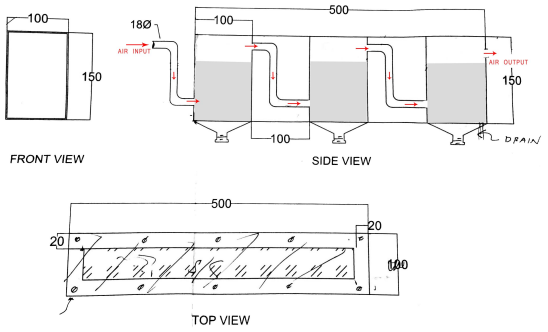

Fig. 2: Interior of the proposed design of T.E.R.R.A.

The resulting solution was filtered using filter paper and alternately washed thrice with distilled water and ethanol. To prevent excess moisture that would contaminate the nickel nanoparticles, it was dried using a hot air oven at 80° Celsius for one hour. The weight of the produced nickel nanoparticles was recorded. Characterization techniques will not be done for the synthesis of the NiNPs since this method has already been confirmed to work from the researchers previous studies.

*Production of CO_2.*To evaluate the device, CO_2 was necessary for the reaction to take place and hence the purchasing of a CO_2 tank. A makeshift regulator was made by using adapters, tees, hose, and hose clamps. An -in brass adapter for the CO_2 tank was fitted. This was then connected to a -in stainless steel tee. The other 2 junctions of the tee was connected to a pressure gauge and a customized hose nozzle. The customized hose nozzle was made by welding a to -in adapter and -in to -in hose nozzle. Thus, the resulting customized hose nozzle had -in socket and -in hose. A -in hose was then connected in the hose nozzle and was fastened by the hose clamp. Tefflon was also wrapped between the adapters to ensure the no gas would leakout.

Using the setup shown below, a water displacement method was done to bubble in 20L or 39.29g of CO_2. 20L of water was sealed in a large plastic bag. The water level plastic bag was then marked, signifying that this was 20L of water bubbled in. Then, a similar volume of CO_2 corresponding to the marked 20L was pumped for a period of time of 14.56 seconds. Hence, opening the valve for 14.56 seconds translates to 20L of CO_2. A firm plastic bag was chosen so that their will not be any stretch marks which may compromise the volume of the container.

Evaluation of Device. For added information, the fluid dynamics of the bubbling $CO2_2$ may also be calculated based on the data (calculations shown in appendix). Utilizing, 20L of CO_2 over 14.56s, this translates to 1.37L/s of CO_2 being bubbled into the solution. Using the bernoulli equation one can also derive its velocity which is equal to 4.84m/s and its pressure which is 23.24Pa or 0.003 PSI. This means that only as much as 0.003 PSI back pressure

Fig. 3: Interior of the proposed design of T.E.R.R.A.

from cars is necessary to facilitate the mineral carbonation process. This shows that 1.37L/s of gas is very doable in practical as well as experimental situations since it generates only minimal back pressure.

Assuming, a 1:1 ratio (i.e. a 100percent efficiency) approximately 39.32g of CO_2 will necessitate 68.87g of Ca(OH)2. $Ca(OH)_2$ was set as the excess reactant and spread evenly amongst the 3 chambers and 24g of CO_2 was added per chamber for a total of 72g. Furthermore, based on previous studies, an optimal concentration of 30 ppm or 0.09g of NINP was also added into the solution, evenly spread amongst the 3 chambers.

As a result of reaction of $Ca(OH)_2$ and CO_2, $CaCO_3$ was expected to precipitate at the bottom of the chambers. The contents of each chamber was then drained, filtered and washed, oven-dried, and weighed. The precipitates were washed with distilled water while being filtered to ensure the purity of the $CaCO_3$. This was then oven-dried at 60°C overnight. These were done to determine the amount of $CaCO_3$ precipitate present in the solution.

Results. – *NiNPs Characterization.* To determine the the size distribution of the synthesized NiNPs, a Transmission Electron Microscope (TEM) from the Southeast Asian Fisheries Development Center (SEAFDEC) was utilized. The majority of the NiNPs were seen to be well below 50 nanometers (Fig. 22) at 200,000x magnification. Using, 600,000x magnification, select samples of the NiNP reach lengths of below 10 nanometers (Fig. 23).

*Various Designs of Prototype.*The first objective of this project was to come up with a feasible design for the fabrication which utilizes NiNPs. Numerous designs have been conceptualized and all of which have one thing in common, which is the utilization of water and $Ca(OH)_2$ for mineralization of the bubbled CO_2. The very first few designs include the use of a cyclone separator attached to a device with one bubbling chamber (Figure 37). This was to remove the particulate matter in the effluent gas using centrifugal force before proceeding to the bubbling

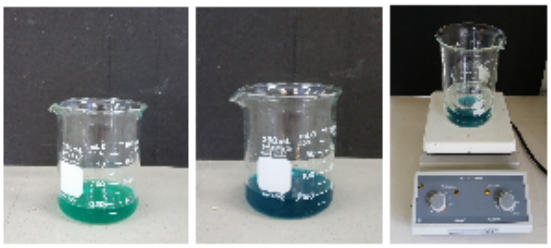

Fig. 4: Interior of the proposed design of T.E.R.R.A.

process.

In the preliminaries, a prototype of this design was constructed using 1.5L PET bottles and tubings and gas containing particulate matter was blown into the device using a vacuum. Through ocular observation the device was able to remove the particulate matter from the gas. For the mineralization phase, a large surface area of the bubbling area would be ideal in a small and compact container for it to be a feasible device to be attached on vehicles. However, upon consideration, it was deemed that the design was bulky and due to the addition of a cyclone separator in that design. Hence, this was removed from the design and the project was limited to dealing with CO_2 only. The removal of particulate matter was deemed to be out of the scope of this study and a newer one has been conceived to adjust for a more ergonomic device.

The second design included a smaller but more complex system. It was designed to be attached to the tailpipes of cars. It was also self-sufficient on its own. It basically involves 2 components: an aerator to draw in ambient air and bubble this through a solution of $Ca(OH)_2$ and NiNP. This is then released outside. It uses air stones to diffuse air through the $Ca(OH)_2$ solution and have more surface area for the reaction leading to an increased reaction rate. However, another consideration in this design is the power source for the aerator.

This design was further evolved and instead of an aerator, a customized air pump was fitted to sequester and aerate more air. Hence, the development of the third design (Figure X). This was introduced together with a piston and wheel to push and pull air, one way valves, and a DC power supply (battery) powers the said pump.

Shown below is the overview of the third design together with an air flow diagram of how air was supposed to navigate through the device. More components such as the tailpipe clamp was also added in this newer design.

However, upon the advice of the research adviser, research panelists, and the advice of an engineer. The design was too complex since the device was only an initial prototype. As much as possible, only the main component (bubbling vessel) should be functional since it was only an initial prototype. Another rationale as to why it had to be simplified was because sequential components leads to an increased risk of dysfunctionality in the de-

vice. In this case, for example, as suggested by a research panelist, when the device tailpipe clamp is loose, or when the battery runs out, or when the air pump is jammed, the device would not work. Hence, the design was simplified and ultimately the most recent design was conceived. These involved using the pressure of the gas coming from the tailpipe itself to bubble through the device, thereby, eliminating the need for extra components but increasing back pressure in tailpipes (listed in methods; 23.24 Pa or 0.003 PSI). The design was further improved by adding two extra chambers for a total of three chambers so that the efficiency of sequestration may be more efficient. The rationale for this addition was because the gas which the first chamber could not sequester would be passed on into the second chamber and then to the third.

There were also other designs which was conceived however, they did not utilize NiNPs and were not included because these were not included in the scope of this study.

The next objective of this study was to determine the amount of CO_2 sequestered by utilizing $CaCO_3$ as a measurement of success. The amount of CO_2 sequestered per chamber was taken from the recovered precipitate. The weight of precipitate on each chamber was measured individually. The total precipitate in each tank was then measured. 67.6g of $CaCO_3$ on the first tank, 84.66g of $CaCO_3$ on the second tank, and 69.02g of $CaCO_3$ on the third tank were recovered. Finally, a blank trial (without NiNPs) was also tested and it had a total of 66.76g of $CaCO_3$ recovered.

The amount of CO_2 sequestered was derived through dimensional analysis of the amount of $CaCO_3$ formed. Of the 39.32 g of CO_2 released, an average of 32.44g of CO_2 was recovered, approximately, making the average efficiency of the device 82.5 percent. So far, there have been no researches utilizing mineral carbonation as its method of sequestering CO_2 hence, this cannot be compared to other developmental projects. Through the given data one may also calculate the individual efficiencies of the device. By starting with the $CaCO_3$ sequestered of each tank, one may also calculate the individual efficiencies of each tank.

Chamber 3 had a higher efficiency as compared to the other two. Although chamber 3 may have had the a high efficiency (65.5 percent as compared to the other two), the total efficiency is not the sum of individual efficiencies in chambers 1 to 3.

Interpretation of Results and Findings. Aside from $Ca(OH)_2$, $CaCO_3$ precipitate was also used as a measurement of efficiency, as its production indicates the mineralization of CO_2. Other measurements of the mineralization of CO_2 would be the formation of water, and the depletion of $Ca(OH)_2$. The formation of water was not utilized to measure the mineralization of CO_2 because it would have been more accurate to measure the formation of $CaCO_3$ instead. $Ca(OH)_2$ could not have also been measured since excess amount of $Ca(OH)_2$ was added into the solution. Ideally, when the ratios are 1:1 $Ca(OH)_2$ could have been measured through the pH of the solution. However, this could still lead to imprecise calculations, because as long as there is still more than enough $Ca(OH)_2$ than its solubility (1g/L), it will still have effectively the same pH before and after bubbling. This makes calculations imprecise with the use of the pH meter. Hence the only viable choice was the measurement of $CaCO_3$

$Ca(OH)_2 + CO_2 \;\; H_2O + CaCO_3$ [1]

The product efficiency of the device in sequestering CO_2 was 82.5 percent. There are several explanations as to how the sequestration did not reach 100 percent ideal. One possibility is a physical flaw in the construction of the device. The device may possibly not have a completely airtight container and CO_2 may have escaped through the gaps. Another reason may be caused by the speed of the CO_2 gas that was bubbled (1.37 L/s). This airflow is might have been too fast and gave the CO_2 lesser time to dissolve in the solution. Remembering the basics of chemistry, the surface area of 2 reactants is a key factor and is directly proportional the reaction speed of both reactants. In conjunction with this, a faster bubbling of CO_2 translates to more CO_2 aerated in a short period of time and hence, the consolidation of the bubbles in the solution, forming one big bubble instead and decrease surface area:volume ratio. This gives lesser surface area for the CO_2 to react and mineralize. Instead, when CO_2, is aerated slowly, little bubbles are made and surface area:volume ratio of these miniscule but numerous bubbles are definitely greater than the big bubble. This theory is also supported by the data when comparing the individual efficiencies of the chamber. As it can be seen, there is an increasing efficiency from chamber 1 to chamber 3. Since all other variables (pressure, temperature, size of chamber, weight of $Ca(OH)_2$, amount of NiNPs and etc) were kept constant, only the amount of input CO_2 per chamber changed as this entered the solution. This implies that it is the amount of CO_2 over a period of time which could have been responsible for the increasing efficiencies of the chambers. A slower aeration of CO_2 translates to a more efficient mineralization process.

Conclusion. – *Summary of Findings* A device design was conceived and constructed. It involved the use of $Ca(OH)_2$ to react with CO_2. This process was hasted by the addition of NiNPs. A total of 73.76g of $CaCO_3$ was formed during the course of the experiment as a result of the reaction between CO_2 and $Ca(OH)_2$. This converts to a total of 32.44g of CO_2 mineralized out of the 39.32g or 20L of CO_2 bubbled. The entire device efficiency was calculated to be 82.5 percent efficiency, with the three individual chambers having 27.39 percent, 38.20 percent, and 65.5 percent efficiency. This study reports the successful development and the construction of a prototype of T.E.R.R.A and the mineralization of CO_2 using Nickel nanoparticles as catalyst. The efficiency of the device was calculated to be 82.5 percent based on the amount of CO_2 sequestered from $CaCO_3$.

Recommendations This project is only the initiation

phase of the development of T.E.R.R.A and will serve as an assessment of its potential as a viable commercial product.It recommended to experimentally determine the amount if CO_2 sequestered at varying CO_2 pressures to simulate the exhaust pressures of vehicles. Through this, a trend can be observed and a mathematical model can be generated from the relationship of the two variables. For improvements on the device, the effect of other variables such as type of gas (NOx and SOx), exhaust temperature and back pressure should be taken into account. Future additions to the feature may also include a particulate matter filter powered by a sustainable power source generated from the vibrations of the vehicle and CaCO3 and NiNPs retrieval apparatus.

* * *

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Designing of storage units and spider web collectors for Pholcus phalangiodes for the mass production of spider silk

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Abstract –Spider silk has been a subject of interest among many researches due to its remarkable mechanical properties. However, existing methods for the extraction of spider silk can exhaust the silk production of spiders and it takes about a week for some spiders to recover lost silk. The purpose of this study is to design and construct storage units for P. phalangioides which allows the spiders to naturally spin their webs for collection. The storage units are composed of 25 units (five by five), painted black on the interior with proper ventilation and spider silk collectors for each of the units on the base. The spiders were properly fed and hydrated, and the collection of silk was done every five days. The percentages of produced spider silk and collected spider silk were obtained and were evaluated using arbitrary levels: from very low (0 percent) to very high (100 percent). A high production of spider silk with a mean average of 82.00 percent was obtained. The spider silk collectors with a very high efficiency value of 98.083 percent, collected a mean average of 80.25 percent of the total area covered by the silk. Additionally, there was no significant difference between experimental groups as determined by one-way ANOVA (p=0 .121); it proves that every storage unit has equal chance of enabling the spider to yield high production of spider silk. The storage units were conducive environments for the spiders to spin their silk. Mimicking the natural habitat of spiders in order for them to yield high production of spider silk was achieved.

Introduction. – Spiders belong to the class of arachnids and they are one of the few animals that can produce silk naturally. A spider can spin silk when it is landing, building its web, catching its prey, making protective nests and cocoons, and suspending itself [9]. Spider silk has been a subject of interest among many researches mostly because of its toughness, ductility, elasticity, antimicrobial properties, hypoallergenicity, biodegradability [10] and even its refractive index in optics [8]. It has been used by humans for thousands of years when hunting, fishing, and as well as for bandages [10]. Studies have also shown that it is tougher than Kevlar which is a synthetic fiber of high tensile strength. Spider silk also can possibly be used as a scaffolding material for growing organ tissues and as a fiber optic cable for embedded devices in tissues [8].However, the artificial production of spider silk as a long, strong fiber for human use has been difficult. There have been developments that procure artificial silk that is similar to that of a spiders by inserting the gene code for silk production of an *Araneus ventricosus* spider to the silkworm, *Bombyx mori*. However, the results show that the silk produced by the genetically modified silkworms are not as tough as that of the *A. ventricosus*. Another way to produce actual spider silk without harming the spider is the use of a hand-driven reeling machine which involves hooking up its spinneret on the machine. However, this can exhaust and completely use up the spiders silk material and it takes the spider approximately a week before it can fully recover its silk [7]. In a project headed by Godley Peers, it took them more than one million golden orb spiders, 82 people, and four years to produce a cloth made entirely of spider silk that measures four feet by eleven feet. The process was impractical in the sense that it required more time, manpower, and resources to produce an amount of cloth made entirely of spider silk. Considering the time it takes for the spider to recover its silk, and their safety in terms of mortality, spiders subject for milking must have storage units

that can keep them away from environmental factors that can affect their behavior and risk their life, and/or must be allowed to spin their silk naturally. *P. phalangioides* (longbodied cellar spider or daddy long legs) are common house spiders which belong to the group of web-building spiders. They usually make webs that are tangled and loose to easily catch their prey in dark undisturbed places like basements, under stones and ledges, or in caves [5]. Due to its pervasiveness, *P. phalangioides* can easily be captured and used for spider silk farming.

Materials and Methods. – The methodology consists of three main parts: the designing process and construction, web production and collection, and assessment of the product. The cover has two layers of clear acrylic sheet; the first one is the top layer with squares of 13 cm by 13 cm cut out from it. The cut-outs which were used as the cover slips and were then bored with 9 holes that serve as ventilation holes. The second is the bottom layer with squares of 11 cm by 11 cm cut out from it, but the cut-outs were discarded. The second layer was glued together with the first layer so that it acts as the holder for the cover slips. In order to ensure accuracy, the design was rendered on CorelDRAW, and the cutting process was done using a laser cutter. One end of the cover slips was taped to form a tab which granted easier access into the storage units during assessment and feeding.

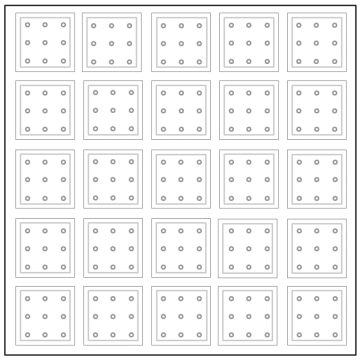

Fig. 1: Cover Lids Composed of the Bottom Layer and the Upper Layer With Ventilation Holes

The whole storage unit, which is a grid of five by five square units (a total of 25 storage units), is consisted of four outer walls, four inner long panels and 20 smaller panels which were glued together to form storage units, each having interior measurements of 15 cm by 15 cm by 15 cm. It provides just enough leg room for our *P. phalangioides* as recommended (three times its leg length).

For the base, the area was carefully calculated considering the average body length of the spiders, such that the spiders would not be crushed during spider web collection. We then bored 25 holes which were placed right at the center of each of the 25 storage units on the base. The wooden rods of 3mm-diameter, which served as the web collectors, were inserted into the holes. The walls around

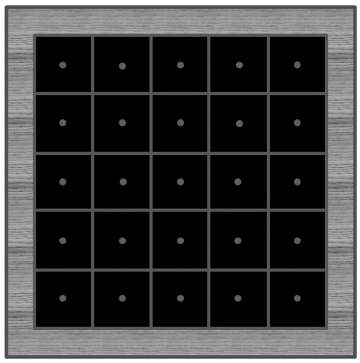

Fig. 2: Base with 25 holes for the collector and margin for the space allowance during collection

the base limits the movement of the storage units during web collection.

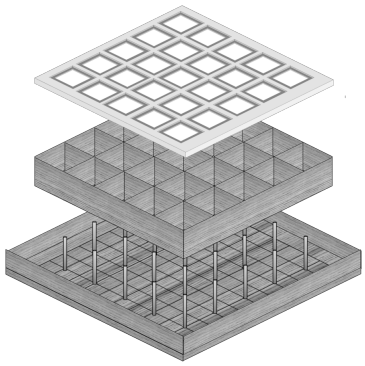

Fig. 3: Assembly of Cover Lids, Storage Units and Web Collectors

The product was assembled, without gluing them altogether, by placing the base with web collectors at the bottom most, the storage units in the middle, and the acrylic cover and slips at the topmost. The interior of the storage units including a part of the base below the storage units were then painted black which provided a darker environment that is suitable for the spiders. The spiders were left to produce their webs for five days. The web collection process was done by moving the storage units in a rotary motion. With the use of a mobile application called Seconds, the rhythm or pacing of movement was controlled. The spiders were fed and hydrated with live nymph lats and dampened cotton balls once every 3 days. For the assessment of the collection, the design was evaluated before and after the web collection process. With the use of quadrants marked on each of the cover of the storage units, the coverage of spider web in the storage units were estimated. If the web covered the entire quadrant, that is considered as 100 percent, if half, then 50 percent, if one fourth, then 25 percent, and so on. To get the percentage of the web collected in terms of surface area, we formulated this equation:

$$P = \frac{Precollection - Postcollection}{Precollection} x100$$

Pre-collection values determined the web coverage of the web produced by the spiders, and to obtain the collected spider web, the post-collection values were subtracted from the pre-collection values.

Results and Discussion. – The spiders were able to produce very high amounts of web with a mean average of 82.00 percent web coverage (Table 1). Most of the storage units have very high levels of web production. Having live lymph lats as food source might have stimulated the spiders to produce high levels of web since more web production helps them in catching their prey. Aside from that, other conditions such as adequate leg space, proper lighting, ideal temperature, and minimal human intervention were properly met.

Table 1. Average Percent of Web Production

Group	A	B	C	D	E	Mean
Web Production	95	92.50	77.50	66.25	78.75	82.00

In web collection, an average of 98.083 percent efficiency was obtained from the spider web collector marking its efficiency level as very high (Table 2). 80.25 percent of the area covered by web was collected by the web collector and only 1.75 percent remained uncollected. All the web collectors exhibited very high levels of collection as shown in this figure. Most of the uncollected web were clinging to the topmost part of the storage units (cover slips) or to edges where the collectors can no longer reach. Furthermore, some of the wooden rods were slightly bent or did not reach the proper dimensions to encompass the whole height of the storage units.

Table 2. Average Percent of Web Collection

Group	A	B	C	D	E	Mean
Web Collection	90	92.50	75	66.25	77.50	98.083

No spiders were killed in the data gathering. However, one spider escaped and another got injured. The escaped spider was replaced by a new spider on the last two data collections. Whenever there is a collection on-going, the spiders would attached themselves to the cover lids to protect themselves from being squashed by the collector. The spaces in the storage units allowed the spiders to move freely and to find areas where the collector could no longer reach (i.e. uppermost portion and corners). The injury of the spider was not caused by the movement of the base in the collection but due to the entanglement of its legs to a cotton ball. One possible explanation for the escape of the spider was that it was hard to notice its presence in the storage unit due to the dark interior. The spider might have camouflaged and disappeared during feeding or assessment. As determined by one-way ANOVA, there was no significant difference between the means of the sample groups. Having the p value of 0.121 (Table 3), the spider can produce the same amount of web regardless of the storage unit it is placed in. The experimental units were uniform and unvaried all throughout the data gathering and all of the spiders were under the same conditions and treatments.

Table 3. One-way Anova of the Means of the Sample Groups

0	Sum of Sqr	Mean Sqr	df	F-ratio	p
Between groups	2790.625	697.656	4	2.086	1 1.21
Within groups	6687.500	334.375	20		
Total	9478.125		24		

Conclusion and Recommendations. – The storage units were conducive environments for the spiders to produce their web. Mimicking the natural habitat of spiders in order for them to yield high production of spider web was achieved. Conditions such as adequate leg space, proper lighting, adequate food supply, and minimal human intervention were met. The spider web collector was able to collect very high levels of spider web without killing any spiders. For future developments, it is recommended to use an electro-mechanically operated spider web collectors or storage units. Additionally, using a metallic rods as web collectors would be more efficient since it has a smooth surface and spider web would be easier to harvest, and finally, strictly following the measurements of the conceptual design is essential in order to avoid discrepancies.

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Potential for Biodiesel Production of Selected Seaweed Species from Taklong Island, Guimaras

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Abstract – The total lipid content and fatty acid composition of *Dictyota dichotoma, Padina minor*, and *Sargassum cristaefolium* found in Taklong Island, Guimaras were evaluated for their biodiesel potential. The total lipid contents were determined using the Bligh and Dyer method while the fatty acid compositions were analyzed using Gas Chromatography-Mass Spectrometry. *Padina minor* (11.00% of its weight(w)) showed the highest total lipid content, followed by *Dictyota dichotoma* (8.00% w), and the lowest is *Sargassum cristaefolium* (5.67% w). This study evaluated the lipid content of the species and supported established researches regarding conspecific variations between species. With a total lipid content exceeding 10%, *Padina minor* may be considered as a suitable candidate for biodiesel production in terms of total lipid content. The assessment of the biodiesel properties of the three seaweed species also passed the European biodiesel standards: EN 14214 and ASTM D6751-02.

Introduction. – The world currently relies heavily on fossil fuels as an energy source providing for more than 85% of the worlds energy needs [1]. However, fossil fuels are finite and can cause harmful effects to the environment due to its excessive greenhouse gas emissions [2],hence the demand to develop alternative ways to minimize our dependency to fossil fuels has become of great importance [3].

In an effort to answer this problem, recent researches focus on the production of sustainable bioenergy resources such as biofuels. Unlike the conventional burning of fossil fuels, biofuels get its energy from biological sources, such as vegetable oil and animal fats, that can be used for heat, electricity and fuel, which, overall, produces less greenhouse gases [4]. Additionally, biofuels produce renewable energy from biological feedstocks [5]. For this reason, its feedstocks can be stored until needed and provide a liquid fuel alternative to various energy-related crisis, including fuel for transportation, which is perceived to be dependent on biofuel in the coming decades [6].

In the Philippines, climate change has posted a major concern and led to the ratification of various acts pertaining the use of biofuels as an alternative to petroleum and other crude oils [7]. The Philippines, being an agricultural country, uses and develops land crops as feedstocks for biofuel, thus raising the conflict between food security and energy sustainability [8]. Moreover, due to its geographical location, the country is oftentimes struck by typhoons and other natural disasters that impose a detrimental impact on its agricultural resources [9], such as coconut, corn, cassava and sugarcane, which are the main sources of biofuels in the Philippines [10] .

Nonetheless, the archipelagic country has a diverse set of marine resources that is left untapped. The potential of marine organisms, especially of macroalgae, to serve as feedstocks for biofuel has received global attention for its easy and low-maintenance growth and relatively high oil yield [11]. At the present time, there about 820 species of seaweeds known in the Philippines and only a few has been studied for its biofuel potential [12]. However, the main focus of research for macroalgae is their cultivation in production of oil-based products, most especially the liquid biofuel-derived biodiesel, which is used as fuel for transportation vehicles [13].

Unfortunately, the ability of an organism to produce lipid does not necessarily make it a good candidate to be used as feedstock for biodiesel. To assess the qualification of an organism for efficient biodiesel production, there are two factors that must be considered: its total lipid content and its physical and chemical properties based primarily on its fatty acid-methyl ester composition [14].

In order to determine the quality of the lipid to be extracted and its suitability for biodiesel use, transesterification, or the process of preparing the extracted lipid to

biodiesel, is used to produce fatty acids methyl esters [15], wherein compositions are identified to be able to assess the number of different carbon chains [16]. This is going to be compared from the provisions of biodiesel standards, such as American Society for Testing and Materials ASTM D6751 and EN 14214 in Europe, which contain the appropriate range of value for different properties [17]. If the calculated values are within the range set by the standards based on the properties tested, the subject lipid is suitable for commercial use.

This research aims to identify the biodiesel potential of seaweed species from Taklong Island, Guimaras, Philippines by quantifying their total lipid content and determining the physical and chemical properties of seaweeds through the quantification and evaluation of the fatty acid methyl esters compositions of the lipid extract in order to assess their biodiesel potential [18].

Method. – *Acquisition of Seaweed.* The three most abundant seaweed samples were collected at the coast of Taklong Island, Guimaras at around 15:00 to 16:00 in three different sampling sites. The seaweed species were handpicked via snorkeling at varying depths within the range of one (1) to two (2) meters. They were placed inside one-gallon Ziploc bags filled with seawater and were transported to the laboratory inside an icebox. They were then put inside a freezer with a temperature of 4°C for storage.

Species Identification. Small representative portions of each individual samples were preserved in 70% ethanol for verification purposes. The species were identified by comparing the morphological characteristics of the samples to existing photographs of the species found on Taklong Island, Guimaras. The identified species were *Dictyota dichotoma*, *Sargassum cristaefolium*, and *Padina minor*.

Cleaning of Seaweed Samples. The seaweed samples were washed with tap water to remove attached coral parts, stones, and epiphytes, and to thaw ice bits that formed around the samples from being stored in the freezer. The samples were then sun-dried for at least an hour to remove any excess moisture.

Water Quality Test. The water quality of the three sampling sites, where each seaweed species was collected, were tested using PASCO Advanced Water Quality Sensor PS-2230 package borrowed from the SRA equipment unit of Philippine Science High School - Western Visayas Campus. The parameters quantified were temperature (with Stainless Steel Temperature Probe PS-2153), conductivity (with Conductivity Electrode 10x PS-2571), pH level (with pH electrode PS-2573), and dissolved oxygen (with Optical Dissolved Oxygen Probe 003-14185). They were measured by their corresponding sensors and were done so as per the instructions written on the handbook that went with the equipment. The values were displayed on the software that was included in the equipment package. The values of the temperature for all sample sites were specifically quantified instantaneously in order to yield more accurate data, while the values of other parameters were measured, not necessarily instantaneously, from the water samples that were acquired and stored inside Ziploc bags. All the components were calibrated before each usage with the use of distilled water to clean the sensors. Another parameter, the salinity of the seawater, was also determined as a derivation from two tested components, conductivity and temperature.

Total Lipid Analysis. The procedure followed in this study was based on the total lipid extraction method of Bligh and Dyer (1959).

Homogenization. The sun-dried samples were minced using a pair of scissors as a means to pulverize them. A triple balance beam was used to measure one-gram of minced seaweeds, which was prepared in triplicates. The weighed seaweeds were placed inside test tubes and were added with one mL of chloroform and two mL of methanol resulting in a 1:2 ratio. The mixtures were vortexed for six minutes and an additional one mL of chloroform was added again and vortexed for another 30 seconds. Finally, one mL of distilled water was added and vortexed for 30 seconds resulting in a final ratio of 2:2:1 of chloroform:methanol:distilled water mixture.

Separation of Phases. The mixtures were filtered using a glass funnel and a Whatman no. 3 filter paper setup in order to obtain their liquid components. The filtrates were then transferred into another set of test tubes where the separation of phases was going to take place. In order to do that, the samples were centrifuged at 1000 rpm for five minutes at room temperature. The upper phases (methanol layer) were siphoned out using a Pasteur pipette. The lower phases (chloroform layer) were then transferred to their respective preweighed test tubes.

Evaporation. The filtrates were treated to a hot water bath using a hot plate with the temperature set at around 185°C until the remaining chloroform contents of the solutions were removed completely, leaving the surface of the base of the test tubes clad with lipids only.

Measurement of Lipids. The total weight of the remaining lipids was measured through gravimetric analysis using an electronic balance. The values obtained from the quantification of the total weight were subtracted from the weights of their respective preweighed test tubes. The differences were the corresponding total lipid contents (TLC) of the replicates. The total lipid content percentage (TLPC) in relation to its weight was calculated with the formula:

$$TLPC(\%) = \frac{TLC(g)}{Weight of Seaweed Species(g)} \qquad (1)$$

Fatty Acid Analysis- Derivatization. The samples for lipid analysis were stored in an icebox and transported to the laboratory of the College of Fisheries and Ocean Sciences department of University of the Philippines - Visayas

(UPV) in Miag-ao, Iloilo where it was subjected to pretreatment for Chromatographic analysis. The lipids undergone derivation using 14% BF_3 as reagent in order to produce Fatty Acid Methyl Esters, afterwards these lipids were to be injected in the Gas Chromatography with Mass Spectrometry (GC-MS). The process was done with the aid of the laboratory technician of UPV.

Chromatographic Analysis. The pretreated samples were injected in the Gas Chromatography with Mass Spectrometry (GC-MS) equipment for analysis of FAMEs. The complete procedure was done using GC: Clarus 600 Gas Chromatograph; MS: Clarus 600 T-Mass Spectrometer of UPV.

Total Lipid Content. Since each seaweed species was done in triplicates, the mean of the three values, as well as the standard error of mean, of the total lipid content was calculated. The obtained results served as the final total lipid content of the seaweeds (mg g-1 sd).

Biodiesel properties. Sixteen biodiesel properties were calculated using the FAME composition of each species. Fuel properties derived from FAME profiles are the following: degree of unsaturation (DU), long chain saturation factor (LCSF), cold filter plugging point (CFPP), iodine value (IV), saponification value (SV), cetane numbers 1 and 2 (CN1 and CN2), saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), kinematic viscosity, density, higher heating value (HHV), amount of C18:3, number of double bonds (Db), and oxidation stability.

Results. – *Water Characteristics.* The seawater parameters, including conductivity, dissolved oxygen, salinity, pH level, and temperature, did not vary much across different sampling sites given their close proximity among one another. Site 2, where *Padina minor* was collected, showed the highest temperature (29.6 C°) and conductivity (52978.33 uS/cm), followed by Site 1, where Dicytota dichotoma was gathered, (28.1 C°)(50641.73 uS/cm), and the lowest is Site 3, where *Sargassum cristaefolium* was collected, with values 28.0 C° and 42344.38 uS/cm for the temperature and conductivity, respectively. The same order applies to the salinity as the values were derived from the two parameters aforementioned. In terms of pH level, Site 1 (7.3) displayed the highest value, followed by Site 2 (7.1), and the lowest was Site 3 (7.0). On the other hand, Sites 1 and 3 showed the highest amount of dissolved oxygen (6.22 mg/L) followed by Site 2 (6.18 mg/L). (See Table 1)

Total Lipids. The total lipid content as the mean of three replicates with respect to the dry weight of *Dictyota dichotoma* (DD), *Sargassum cristaefolium*(SC), and *Padina minor*(PM) are shown in Table 2. *Padina minor* (110 mg g-1 w 1.00 SE) showed the highest lipid content, followed by *Dictyota dichotoma* (80 mg g-1 w 1.00 SE), and the lowest is was *Sargassum cristaefolium* (56.7 mg g-1 w 0.88 SE). (See Table 2).

Discussion. – This study initially aimed to discover whether the species found on Taklong Island, Guimaras on the arbitrary date of its collection would serve as good feedstocks for biodiesel production by quantitatively and qualitatively assessing their total lipid content and fatty acid methyl ester profile. In addition to that, the quality of water was also examined to create a basis for cultivation technologies in the future if the species were to be proven to be viable and efficient sources of biodiesel when applied in practice. Another reason for water testing was to plot out environmental and external factors behind conspecific variations by comparing the data with other published works.

This study provides evidence that despite having the reputation of being unsuitable for oil-based byproducts due to its presumable low lipid yield, some seaweed species exceed other microalgae species [19], which are in theory viable candidates for biodiesel feedstock for its high lipid content, although their cultivation proves to be economically inefficient. In terms of lipid content alone, two of the three species can be prospected as candidates for cultivation for its relatively high lipid content: *Padina minor* (11% w) being the highest, and *Dictyota dichotoma* (8% w).

There have been no study yet done specifically on the lipid content of *Padina minor*; however, the genus Padina generally has lower lipid content, with values averaging from 1-5% and has been found to contain higher level of polyunsaturated fatty acid composition in comparison to terrestrial crops [21], which results in more unstable and less quality biofuel but fit for pharmaceutical functions and human consumption [22]. These differences between the species and genus scale of the organism therefore suggests that, rather than being of a unique genus group, *Padina minor* is more of a unique individual species by having an unusually high oil yield.

Almost contrastingly, the genus Dictyota has relatively high lipid yield, even exceeding 20% w, a value that can compete with most microalgal species [23]. Specifically, *Dictyota dichotoma* had been studied for its lipid content before, with results varying between species of different locations, but more than 10% w, still, [24], although the results do not deviate dramatically from this studys (8% w). This anomaly called conspecific variable is a natural characteristic of organisms living in different environmental conditions. This rests the case on the importance of measuring environmental factors (in our study, the water quality) that may affect the chemical compositions of an organism. Also, by studying this, and proving the relationship between an organisms genotype and its environment, people may be able to develop optimal cultivation technologies and conditions to increase lipid yield among seaweeds, which have relatively low lipid content but are low-maintenance and fast-growing. Thereby, spending less on the production of biodiesel, or any other oil-based byproducts of seaweeds for that matter. The possible effects of water quality to the species will be fur-

Site Num.	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Salinity (PSU)	pH Level	Temperature (C°)
1	50641.73	6.22	31.06	7.3	28.1
2	52978.33	6.18	31.66	7.1	29.6
3	42344.38	6.22	25.49	7.0	28.0
Average	48654.81	6.21	29.40	7.1	28.6

Table 1: Value of seaweed parameters measured at each sample site.

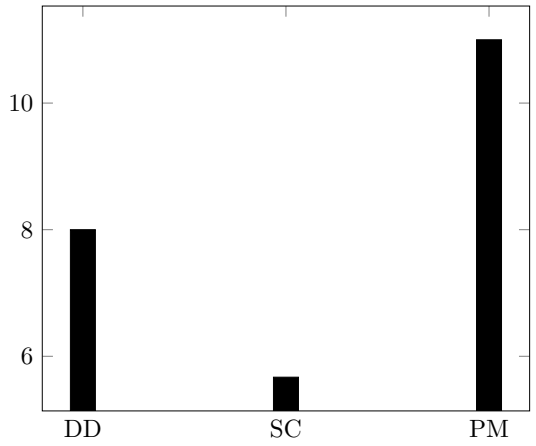

Table 2: Total lipid content (%) of the three samples

ther discussed in detail later on.

Like *Padina minor*, there is little study conducted on the lipid content of *Sargassum cristaefolium*, but a patent on the use of the species yielded slightly conclusive results with the present study [25]). In the patent, the species only contained 2% w, which suggests that *Sargassum cristaefolium* has low lipid content. However, the difference between the lipid percentage (almost 4%) is worth taking notes and implies that there could be something that had affected the species in our study to produce such a difference between the same species. This also suggests a case of conspecific variance due to difference in location. Although there does not have plenty of studies pertaining the lipid content of the genus Sargassum in general, the use of it to produce biofuel is not foreign [26].

On the other hand, several research studies observed environmental conditions to be some of the factors that influence the production of lipid in organisms. In the review study of Juneja et al [27], they noted that the factors affecting the increase in lipid growth as a survival mechanism or biological comfortability included lower pH level, lower temperature, and higher salinity. For the other two parameters, dissolved oxygen and conductivity, there are no studies focusing on their influence on the production of lipid in seaweeds; however, conductivity is related to salinity as the latter parameter is derived from the latter. In the present study, organisms of the same species were not subjected to different environmental conditions, rather the water quality of each species were gathered and assessed whether it affected the amount of lipid content or not, in comparison to other studies. As the direct mechanism with which each species practiced most could not be observed, and the values did not vary dramatically, no solid connection can be established. Also, the values are conclusive to the normal values of seawater parameters. Despite the claims of other studies regarding the relationship between the genotype of an organism and its environmental location, this study does not have enough comparisons to support the claim.

Conclusion. – This study evaluated the total lipid content and fatty acid composition of *Dictyota dichotoma*, *Sargassum cristaefolium*, and *Padina minor* for biodiesel production. Of the three species screened, *Padina minor* showed the highest potential in terms of its lipid content and it may be a suitable candidate for biodiesel production.

To further improve this study, the lipid and fatty acid content of the remaining seaweed species must be explored. To add to this, the lipid and fatty acid content should be documented throughout the year, since the environmental conditions of different seasons might affect their lipid content. By extension, other external factors should also be considered and studied, in order to attain optimal cultivation technology for higher lipid yield from seaweeds. The lipid and fatty acid content at different seaweed parts should also be taken into account. The freshly gathered seaweed samples should also be cleaned prior to storage. In determining the lipid weight, the Folch method is the better procedure since the Bligh and Dyer method loses its accuracy when extracting tissues with high lipid contents. To further increase the accuracy of the results, the sample sizes and solvent volumes should be increased to limit the possibility of anomalous data. It lowers the difficulty in separating the methanol and chloroform phases therefore decreasing the chances of having leftover methanol in your filtrate. To maximize the extract gathered, the leftover tissues should be re-extracted again using chloroform and methanol. In the evaporation of the chloroform phase, it should be noted that it should not be exposed to air as it will cause oxidation to the fatty acid content thereby affecting the data. To remedy this, it is recommended that nitrogen should be used to evaporate the chloroform phase as it limits its contact with air.

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Antioxidant activity and phytochemical screening of the methanol, ethyl acetate, and hexane extracts of Lansium domesticum seeds

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Abstract – This study intended to conduct a qualitative phytochemical test and an antioxidant assay on *Lansium domesticum* seeds grown in the Philippines. The seeds were extracted through maceration in methanol, ethyl acetate, and hexane. The antioxidant activity of the seeds were tested by measuring the absorbance of the free radical, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) or ABTS through the use of a UV/Vis spectrophotometer. The results were measured in % free radical scavenging activity, using 2% [g/ml] Vitamin C in distilled water as the positive control.

Introduction. – Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. These can be found in various foods including fruits and vegetables. These certain types of cell damages are caused by free radicals and is also thought to be a factor in some diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration amongst others. [1]

Generally, there are two types of antioxidants: synthetic antioxidants and natural antioxidants. Most antioxidants in commercial use are synthetic, for example tert-butyl hydroxyquinone (TBHQ) butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These have been prohibited by governmental policy because they are carcinogenic when given in vivo. [2]

Plants are potential sources of naturally occurring antioxidants and have garnered interest as possible sources of antioxidants and other biological active substances. These include ascorbic acid, benzoic acids, carotenoids, cinnamic acids, flavonoids, folic acid, tocotrienols and tocopherols [3].These antioxidants prevent free radical damage and reduce the risk of chronic diseases. The search for newer natural antioxidants has been increasing, namely those belonging to plant origin. [4]

Lansium domesticum, otherwise locally known as lanzones is a tropical fruit indigenous to throughout the entire Southeast Asia region. According to several studies, lanzones have been known to exhibit many medical properties but it is said to be underutilized here in the Philippines [5]. Its skin and leaf extracts are antimalarial [6], anti-melanogenic which is for the regulation of skin pigmentation [7], and antipyretic which is used in the prevention of fever [8].

A study by Klungsupya et al (2015) made use of *Lansium domesticum* grown in Thailand which underwent ethyl acetate fractionation of the peel and maceration extraction using 50% aqueous ethanol. The total phenolic content and total flavonoid content was then solved for. The results showed a high potential for antioxidants. This study aims to determine the antioxidant activity and the phytochemicals found in the methanol, ethyl acetate and hexane extracts from the seeds of *Lansium domesticum* found in the Philippines.

Methods. – The Methods is composed of two main parts; During the phytochemical testing a change in color to brownish- black indicated the presence of phenols [10], a colorless sample indicate the presence of flavonoids, and a foam layer indicates the presence of saponins [10, 11].

The subjects of the study are limited to extracts from the seeds of a variety of *Lansium domesticum* found in the Philippines. The seeds will be used instead of the fruit itself because these fruits are edible while these seeds are considered as wastes. Additionally, the samples used for the 10% concentration was prepared separately from the other concentrations using a different batch of samples. This was done due to time constraints. The different batches of lanzones were assumed to be of similar species and grown at similar conditions.

Chemicals. Ferric chloride(5%), sodium hydroxide(10%), hydrochloric acid (5N), Wagner's reagent, manganese dioxide (70%), and phosphate buffer (pH 7; 01.M) were obtained from Philippine Science High School- Western Visayas chemistry stockroom. Ascorbic acid was purchased from a local drug store and 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) diammonium salt (ABTS) was purchased from iChemical. Methanol, hexane, and ethyl acetate were purchased from Patagonian Enterprises. All chemicals were stored at room temperature away from direct sunlight.

Sample Preparation. Two batches of lanzones weighed eight kilograms in total. *Lansium domesticum* seeds were dried at 40-45C for 12-16 hours in an oven. The dried samples were then ground, crushed and then sifted. 38g of the sample was added to three identical amber reagent bottles with 190mL of solvent in each bottle- one with hexane, another with methanol and the last with ethyl acetate- making the solvent to dry weight ratio 5:1.

Evaluation of phytochemical screening. A phytochemical analysis was conducted to test for the presence of selected phytochemicals that are related to this activity. The samples were allowed to soak for approximately 24 hours while being frequently agitated before being subjected to the phytochemical analysis.

The phytochemical screening was done in accordance to the methods indicated by Tiwari et al (2011) with slight modifications. Phenols were tested by preparing a stock solution (1 ml) which was pipetted into a test tube and 3-4 drops of 5% ferric chloride solution was added. A change in color to brownish- black will indicate the presence of phenols.

Flavonoids was tested using a stock solution (1 mL) which was pipetted into a test tube. Using a dropper, 10% sodium hydroxide solution was added drop by drop until an intense yellow colour appears in the test tube. 5N hydrochloric acid was then added into the mixture drop by drop until the liquid turns colorless to indicate the presence of flavonoids.

Saponin test was done with a stock solution (1 mL) that was placed in a graduated cylinder and diluted with 20 mL of distilled water. It was then shaken by hand for 15 min. If a foam layer of roughly 1 cm appeared on the top of the test tube, which indicates the presence of saponins.

Alkaloid test was done with a stock solution (1 mL) which was placed in a test tube and 5-15 drops of Wagner's reagent was added. The presence of a brown-colored precipitate indicated the presence of alkaloids

Evaluation of antioxidant capacity. The various extracts were tested on their ability to scavenge the 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic-acid),or ABTS+ radical cation. The assay will be conducted as stated in studies by Bayoumi et al. and El-Sherbeni et al. Prior to the the ABTS+, a 7.0 pH 0.1 M phosphate buffer was made. The ABTS+ was prepared by mixing of ABTS solution (0.1g/100mL) with of manganese dioxide (25mg/mL) in a 2:3 ratio both prepared in the phosphate buffer (pH 7; 0.1M) and mixed in an amber reagent bottle. The mixture was then centrifuged at a little above 2000 RCF for 10 mins. The supernatant was collected and it was allowed to stand in the dark inside an amber reagent bottle wrapped in tin foil in until the absorbance was stable.

Prior to the assay, the remaining mixture of solvent and sample after the phytochemical analysis was filtered out and then allowed to evaporate under a fume hood until each solvent was at the same volume. The 120 mL mixture was reduced by 83.33% to give a 20mL solution. Different concentrations (50%, 25%, and 10%) of the reduced mixture was prepared by making 1:1 extract to buffer mixture and served as the stock solution. The extracts were diluted with methanol instead of the mother solvent, this is to allow the more nonpolar solvents, hexane and ethyl acetate, to mix with the buffer.

A 0.2 mL stock solution was pipetted into 1.8 mL of the ABTS+ solution in a the cuvette. Measurements were be taken as soon as the extract is placed, after 1min, 5min, 15min, and 30min. A blank sample was run without ABTS, using only the phosphate buffer. 0.2 mL of 1:1 solvent to buffer ratio was used instead of the stock solution for the negative control. All measurements were done in triplicate and the means of the measurements were used in the final equation.

The antioxidative activity of the tested samples will be calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$E = ((Ac - At)/Ac)100 \quad (1)$$

where: At and Ac are the respective absorbance of tested samples and control. ABTS+ radical scavenging activity will be expressed in percent scavenged.

Results. – This study aims to determine the antioxidant activity and the phytochemicals found in the methanol, ethyl acetate, and hexane extracts from the discarded seeds of *Lansium domesticum*.

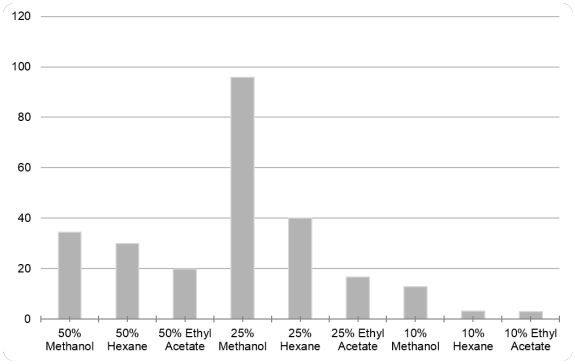

Figure 1: Summary of antioxidant activity in every concentrations and solvents

		Phenols	Flavonoids	Saponins	Alkaloids
First Batch Samples	Methanol	-	+	+	-
	Ethyl Acetate	-	+	-	-
	Hexane	-	-	+	-
Second Batch Samples	Methanol	-	-	+	-
	Ethyl Acetate	-	-	-	-
	Hexane	-	-	+	+

Figure 2: Results of the phytochemical tests performed on the seed samples collected in September-October (First Batch Samples) and March/April (Second Batch Samples). + = positive; - = negative

Discussion. – *Phytochemical tests.* The results are in contrast to the study of Solidum which conducted a phytochemical screening on lanzones found in Manila. The study showed that lanzones was positive for alkaloids for all the samples which was only present in the hexane crude extracts in this study. Saponins were also not present in the samples of the study.

There was no color change to brownish-black in any of the samples which indicates an insignificant amount of phenols found in the sample. This may have been due to the prolonged soaking period of the first batch and the short soaking period of the second bath.

The concentrations used in this study are not based on the concentration of the sample in the solvent. Rather, the remaining mixture of solvent and sample after the phytochemical analysis was filtered out and then allowed to evaporate to 16.67% of the original amount of solvent. The concentrations use this mixture as the basis of the 100% concentration.

ABTS assay. The radical scavenging activity of ethyl acetate yielded the smallest percentages out of all 3 extracts reaching 20% free radical scavenging activity on both 25% and 50% concentrations and 3% free radical scavenging activity at 10% concentration after 30 minutes of reacting with ABTS+. Hexane on the other hand, had the second largest yield with 30% free radical scavenging activity at 25%, and 50% concentrations and 3% free radical scavenging activity at 10% after 30 minutes. The methanol extracts had the strongest free radical scavenging activity in all three concentrations. For the 25% concentration, free radical scavenging activity reached 90% at 15 minutes and stabilizing there until the 30 minute mark. Free radical scavenging activity reached 34% for the 50% concentration and 13% for the 10% concentration by the 30 minute mark (refer to Figures 1, 2, and 3).

Throughout the conducting of this study, there has been much room for error. The solvents ethyl acetate and hexane are very volatile and this study was conducted in small amounts so some of these results may have been affected by constant error especially the results from the 10% concentrations.

Dead End. – More comprehensive testing is required in order to verify the potency of lanzones as potential source of antioxidants. Based on the results of the study, the following courses of action are recommended:

- To measure the strength of each phytochemical in each extract.
- Focus on either measuring different concentrations at one time or one concentration at different times.
- Investigate the difference of saponins found in the methanol and hexane extracts or why saponins were not found in the ethyl acetate extract.
- Investigate the difference of phytochemical content and antioxidant activity based on the different seasons of lanzones.
- Produce a crude extract before conducting the assays.
- Try smaller concentrations to check for optimal absorbance.

Conclusion. – More compounds with antioxidant properties can be found in the methanol extracts of *Lansium domesticum* seeds than in the hexane and ethyl acetate extracts and that Lanzones can be a viable source of antioxidants. More comprehensive testing is required in order to verify the effectiveness of lanzones as potential source of antioxidants.

* * *

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Comparative Analysis of Sun Protection Factor (SPF) of Randomly Selected Branded Sunscreens Manufactured in the Philippines

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Abstract –UV radiation is a type of radiation emitted by the sun, alongside infrared and visible light. It is used by the body to help mediate natural synthesis of vitamin D and endorphins used by the body, but excessive UV radiation has adverse effects to the skin, like sunburns (erythema), premature skin aging, photosentivity, suppression of the immune syste, and even skin cancers. With the rise of temperatures due to global warming, demand for sunscreens - cosmetic products used to reduce the amount of UV radiation that reaches the skin - has also risen. Some studies have experimentally measured discrepancies between labeled and measured SPF values for the sunscreens. This study compares the labeled and measured SPF of some of the sunscreens manufactured in the Philippines, and has found major differences between the labeled and measured SPF values of the sunscreens.

Introduction. – UV radiation is a type of radiation emitted by the sun, alongside infrared and visible light. UV radiation is used by the body to help mediate natural synthesis of vitamin D and endorphins in the skin, but excessive UV radiation has adverse effects to the skin. Overexposure to UV rays may cause many skin defects, like sunburns (erythema), premature skin aging (wrinkles), photosensitivity, suppression of the immune system or even skin cancers.. For many years, sunscreens have been recommended by dermatologists, not only as a protective measure against excessive amounts of sunlight, but also because of their contribution to the prevention of skin photodamage. All of cosmetic products with sunscreen properties are designed to absorb or reflect the suns UV radiation in order to protect the skin cells from damage [1, 2].

Sunscreen companies use advertisements that show the SPF values of their products. But other SPF analysis studies done in other countries using their marketed local products show that the true SPF values are inconsistent with the SPF values advertised by sunscreen companies [1–4]. Similar studies are done in other countries and regions such as Brazil [1] and the Kurdistan region of Iraq [2]. However, their regulations for sunscreen products are different in comparison to the sunscreen regulations followed by the Philippine companies [5]. To date, there has been no published studies done in SPF analysis of sunscreens in the Philippines. This paper thus seeks to verify the labelled SPF values in common brands of sunscreens manufactured in the Philippines.

Adulteration refers to a product failing to meet state standards. Conventional cosmetics such as personal care products are typically provided to consumers as predetermined formulations on a basis that they either purchase the product from the inventory or they forgo the purchase. A drawback of this method of doing business is that the cosmetic product may not be optimized to accommodate a consumer's needs and preferences. The formulation may have a fragrance that is either too strong, excessive or insufficient moisturizers, allergens or some other problematic ingredient, concentration or deficiency [6]. It could also provide insufficient protection from the intended cause of problem such as a sunscreen failing to provide enough SPF values as marketed. If a product wasnt able to deliver on their promised performance, the company that created the product is liable to be sued for product adulteration.

Thus the researchers want to determine if the selected sunscreen lotions are able to meet the required standards that is the SPF levels that are indicated in their labels.

This study aims to determine and compare the labeled

SPF values of five sunscreen lotions manufactured in the Philippines to the SPF values measured using UV-Vis spectrophotometry. Specifically, this study aims to:

- Determine the absorbance of the randomly selected five white-colored sunscreen body lotions using UV-Vis spectrophotometry
- Solve for the SPF values of the lotions using their absorbance properties; and
- Compare the experimental SPF values of the sunscreen lotions to the SPF values listed in their labels.

Significance. This study could be used to provide information to the consumers and serve as basis for the Food and Drug Administration to initiate proper regulations and provide correct labeling of sunscreen products.

Limitations. This study was limited to five sunscreen lotion brands locally produced in the Philippines which is available in the countrys local commercial outlets. Only five brands were used to conserve cost in the study. The samples used in the research were white body lotions with SPF of 30 to ensure uniformity.

Materials and Methods. – The study was conducted in Philippine Science High School- Western Visayas Campus Chemical Instrument Lab and in West Visayas State University's University Research and Development Center.

Materials and Equipment. Five different brands of sunscreen, all of which were white sunscreen lotions with SPF of 30, were bought from various stores in Iloilo City; they were labeled A to E. The absolute-grade ethanol required was partly bought by the researchers and partly supplied by the laboratories. The research facilities lent the researchers the necessary laboratory equipment: test tubes, pipettes, parafilm for sealing the test tubes, the centrifuge, the vortex mixer, and the UV-Vis Spectrophotometer.

Preparation of samples for UV-Vis spectrophotometry. 0.05g of sunscreen was dissolved in 5mL of ethanol in a test tube with the assistance of a vortex mixer. The solution was allowed to stand for 5 minutes to let any insoluble compounds settle, before being spun at 6,000 RPM for 5 minutes in a centrifuge. Transferring the solutions from the centrifuge required great care so as not to disturb the settled particulates at the bottom. Serial dilution was then carried out; 0.5mL of stock solution was taken and poured to a test tube that contained 4.5mL of ethanol, making this the first dilution. Succeeding dilutions were performed by taking 0.5mL of the previous dilution and mixing it with 4.5mL of ethanol in another test tube; two such additional dilutions were performed, with mixing done in between dilutions using a vortex mixer.

Analysis of samples. The absorbance characteristics of each sunscreen sample in the 320-290nm wavelengths were analyzed at every 5nm intervals using UV-Vis spectrophotometry. SPF was calculated using the Mansur equation:

$$SPF_{spectrophotometric} = CF * \sum_{290}^{320} EE(\lambda)I(\lambda)Abs(\lambda) \quad (1)$$

Where:

- EE(λ) = Erythemal effect spectrum
- I(λ) = Solar intensity spectrum
- Abs(λ) = Absorbance of sunscreen product
- CF = Correction factor of 10

Note: EE(λ)xI(λ) are constants [7] (see Table **??**)

Data analysis. The determined SPF values of each sunscreen lotion were compared to its labeled SPF values by determining their percent difference. The percent difference will show if the determined SPF values are consistent with their respective SPF values if they fall within 10% of the labelled SPF values.

Results and Discussion. – All of the tested sunscreens had SPF values that fell short of their labeled SPF values, with sample E (75.09%) having the largest difference between the labeled and measured SPF values, and sample C with the smallest percentage of difference (55.17%). Other studies [1,2] have also measured discrepancies between the labeled and measured SPF values; percentage differences of 97.89% have been reported [2].

Table 1: Labeled and measured SPF of sunscreens used in the study

Sample	Active Ingredients	Labeled SPF	Measured SPF	Percentage Difference
A	TiO_2	30	10.00	66.65%
B	Octisalate, Ensulizole Avobenzone	30	7.31	75.64%
C	Oxybenzone, Homosalate	30	13.45	55.17%
D	TiO_2	30	10.79	64.02%
E	TiO_2	30	7.47	75.09%

Data variation and validity can be affected by the use of non-validated spectrophotometric methodology used to determine the absorption characteristics of the sunscreens; of note would be readings of negative absorbance for the sunscreen samples. One possible source of problems in the data gathering is the addition of other additives in

the sunscreens, the most readily known being whitening agents, compounds used to lighten skin tone, in the sunscreen, since 4 of the 5 of the sunscreens in the study had whitening agents and these may have caused significant interference in the measurement of absorbance. Another possible source would be the nature of the active ingredients, specifically how they work as an active ingredient it is important to note that in 3 of the 5 tested sunscreens, titanium dioxide, TiO2, was used as an active ingredient; TiO2 as a sunscreen active ingredient works by reflecting and scattering sunlight [8], and the reflection and scattering of UV rays due to the TiO2 particles may have affected the variation of data. Octisalate, ensulizole, avobenzone, octibenzone, and homosalate are examples of chemical absorbers where they work by absorbing the UVA and/or UVB radiation. Sunscreen B in particular is a broad-spectrum sunscreen since octisalate and ensulizole absorb UVB radiation while avobenzone absorbs UVA radiation. Avobenzone may have affected the data since it exclusively absorbs UVA radiation. The combination and concentration of ingredients in the sunscreens can affect absorption data [1].

Other factors that may have affected data variation and validity would be the use of different concentration of ethanol that is used as the solvent to dissolve the sunscreens; the combination and concentration of ingredients in the sunscreens; the effect of various components of the vehicle; and the pH system of the sunscreen and emulsion rheological properties. This effect is reflected in a finished formulation, especially for lotions with an SPF greater than 15. The effect of a solvent is only readily apparent at higher percentage [1]. While the method has been confirmed to be accurate [9], the spectrophotometric in-vitro method has its own limitations: it is challenging to extrapolate from the results of in-vitro back to the biology of the intact organism - in layperson's terms, it is challenging to know or determine whether or not the results of the in-vitro test will also apply when the same treatment is tested on the intact organism.

Summary of Findings. – There was a large inconsistency between the labeled and measured SPF values, and this may be due to the interactions between the ingredients in the sunscreens.

Conclusion. – This study concludes that the selected sunscreens manufactured in the Philippines that were selected for the study have their measured SPF values inconsistent with their labeled SPF values. This however does not conclude that the selected sunscreens did not deliver on the labeled SPF level since the ingredients in the sunscreens may have affected the measured SPF.

Recommendation. – This study recommends the following:

- Alterations in the sample preparation; examples of alterations would be different solvents, different dilution factors, and using additional steps;
- Further in-depth studies about manufacturing practices in the Philippines with respect to sunscreens;
- Further in-depth studies about the interactions between ingredients in sunscreens and how these interactions affect the sunscreens' protective ability;
- The effect of emulsion type (such as water-based or oil-based) on the sunscreens' protective ability;
- Other methods of testing can be tried, such as spreading the sunscreen samples on a substrate, ex. a quartz plate [3], or an epidermal membrane [4].
- Larger sample size.
- Usage of only one cuvette, as individual defects unique to every cuvette may have contributed to the variance of data.

* * *

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