WHITE ONION (Allium cepa) BULB BASED AGAR CONCENTRATIONS ON THE GROWTH RATE OF GRAM NEGATIVE BACTERIA

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ABSTRACT

Clinical or bacterial isolation and identification are the first steps to a more successful bacteriological study. The discovery of culture media enabled microbiologist and medical technologist in the field of bacteriology to easily identify and isolate bacterial microorganisms. As most commercially available culture media are expensive, there is a need to find alternative media or to reduce the amount of agar added during the preparation of culture media in laboratories especially those with less facility. The present research therefore was carried out to test the efficacy of white onion (*Allium cepa*) bulb extracts for its ability to support the growth of the selected bacteria; namely: *Escherichia coli, Serratia marcescens,* and *Klebsiella pneumoniae*. The extracts were prepared using aqueous suspensions. The aqueous suspensions were tested at concentrations of 25%, 50%, and 100%. All suspensions showed a positive growth of the tested bacteria. Among Gram Negative bacteria tested, *Klebsiella pneumoniae*, with a mean colony count (in cfu) of 651.67 which has (p-value=1.0> α =0.05) exhibited heavy growth by all prepared suspensions followed by *Serratia marcescens* which has the value of 558.67 cfu (p-value=0.006< α =0.05) while *Escherichia coli* with a value of 325.11 cfu (p-value=0.025< α =0.05) exhibited light growth. The growth of the tested bacteria was comparable to that obtained on MacConkey agar which is used as routine commercial media in isolating Gram Negative bacteria. Hence, the bulb extracts of *Allium cepa* (white onion) could be used as an economically available source of nutrients which possess natural antioxidants and anti-fungal agents to support the growth of the tested Gram Negative bacteria.

Key words: *Allium cepa (white onion), bulb based agar, growth rate, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae*

INTRODUCTION

Clinical or Bacterial isolation and identification are the first steps to a more successful bacteriological study. Isolation is done to obtain pure bacterial cultures from different sources. Bacterial isolation plays a major step in the diagnosis and management of the illness. Bacteria are usually isolated from the environmental sources. Pure culture is very essential in the morphological, physiological, biochemical characteristics and the susceptibility studies of a particular bacterial strain to an antimicrobial agent (Ruangpan & Tendencia, 2004).

A culture media is a special medium used in different microbiological laboratories for the culture and growth of different kinds of microorganisms. Culture medium is composed of different nutrients that are essential for microbial growth. There are different culture media that are being used in the laboratories. They are categorized according to their Composition (Synthetic and non-Synthetic), Consistency (solid, liquid, and semi solid), Dispensing or Formation (tubed or plated), and to their Function/Application (general, enriched, selective, and differential) (Paul, 2013).

One of the main advantages of the discovery of culture media was that it enabled microbiologist and medical technologist in the field of bacteriology to easily identify and isolate bacterial microorganisms. With the use of different types of culture medium, they can easily glimpse of what is the possible isolated bacterial strain that has grown in the medium. But the usage of culture media has also its disadvantage, one of which is the high cost of culture media ingredients. As most commercially available culture media are expensive, there is a need to find alternative media or to reduce the amount of agar added during the preparation of culture media in laboratories with less facility (Arulanantham, Pathmanathan, Ravimannan & Niranjan, 2012).

Onions have been known to contain many medicinal qualities by different cultures around the world. Numerous health benefits have been attributed to the vegetable. This has led many researchers to test whether the proposed medicinal attributes of onions are valid. Onions are one of the richest sources of compounds such as flavonoids and organosulfurs. The presence of the Flavonoid and Organosulfur compounds may explain the anti- microbial activity of *Allium cepa* plant (National Onion Association, 2011). In 2013, The Valuable chain analysis and competitiveness strategy showed that Philippines registered a total production of 134,169.92 metric tons with an average yield of 8.70 metric tons per hectare. Central Luzon accounting for 55% of the total production is considered as the largest onion producing region followed by the Ilocos Region that covered 30% of the national production and then Cagayan Valley accounted for 5% of the production. In the Central Luzon, the province of Nueva Ecija is the leading producer of onions, accounting for 99.9% of the region's production. In Ilocos Region, it is Ilocos Norte who is accounted for 51% of the region's production in 2013 and considered as the main producing province of the region. Municipalities of Badoc, Vintar, and Pasuquin are the major producers of onion in Ilocos Norte. In Cagayan Valley Region, Nueva Vizcaya produced 99% of the region's onion production in 2013. Most of the onions produced in Nueva Vizcaya are white onions which are grown in the municipality of Aritao.

Hence, the study was aimed to determine the concentration of White Onion (*Allium cepa*) Based Agar that was most supportive on the growth of the Gram Negative Bacteria. Onions serve as a good nutrient source and they are locally available cheap materials. Onion bulbs selected as a natural protein source to

formulate the media became the driving source for the researchers to maximize its use for the benefit of mankind.

Research Questions

The study aimed to discuss the effectiveness of White Onion (*Allium cepa*) as an alternative medium for the growth of *Escherichia coli, Klebsiella pneumoniae* and *Serratia marcescens*. Specifically, the research answered the following questions:

1. What is the rate of growth of the following bacteria on White Onion (*Allium cepa*) Based agar after 24 hours?
    a. *Escherichia coli*
    b. *Klebsiella pneumoniae*
    c. *Serratia marcescens*
2. What is the colony forming unit (cfu) of the Gram Negative Bacteria on the different concentrations of White Onion (*Allium cepa*) Based agar?
    a. 25% concentration
    b. 50% concentration
    c. 100% concentration
3. What is the mean growth rate score of the Gram Negative Bacteria on the different concentrations of White Onion (*Allium cepa*) Based agar?
    a. 25% concentration
    b. 50% concentration
    c. 100% concentration
4. Is there a significant difference on the mean growth rate score of the Gram Negative Bacteria on the different concentrations of White Onion (*Allium cepa*) Based agar after 24 hours?

Hypotheses

1. There is no significant difference on the growth of *Escherichia coli, Klebsiella pneumoniae,* and *Serratia marcescens* on White Onion (*Allium cepa*) Based agar.
2. There is no significant difference on the colony count of *Escherichia coli, Klebsiella pneumoniae,* and *Serratia marcescens on* White Onion (*Allium cepa*) Based agar.
3. There is no significant difference between the growth of *Escherichia coli, Klebsiella pneumoniae,* and *Serratia marcescens* on White Onion (*Allium cepa*) Based Agar & MacConkey Agar (positive control).

Significance of the Study

Culture media were used for growing and counting microbial cells, selecting microorganisms and obtaining pure cultures. A culture medium contains numerous

nutrients, minerals, energy sources, growth promoting factors, metals, buffer salts and gelling agents (for solid media) to support bacterial growth and survival.

Readily made culture media are of high-cost the reason why there is a need to find alternative media or reduce the amount of agar added during the preparation of culture media in various laboratories with less facility. The ingredients of a culture media especially the agar that is used to solidify the media, adds to the cost of the readily available media. Moreover, this alternative media will be made in accordance to the standard criterion of a media that will not cause difficulty.

This research study determined the concentration of White Onion (*Allium cepa*) based agar that is most supportive on the growth of Gram Negative Bacteria. This study can also benefit medical technologists for easier preparation, isolation and identification of the said species.

Literature Review

White Onion (*Allium cepa*)

Bacteria are one-celled or unicellular microorganisms. They reproduce when one cell splits into two cells through a process called binary fission. Microbial culture is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions which is used to determine the type of organism, its abundance in the sample being tested, or both. It is one of the primary diagnostic methods of microbiology and used as a tool to determine the cause of infectious disease by letting the agent multiply in a predetermined medium. In microbial culture we need first to obtain bacterial samples by inoculating the organism. Second is, isolating the bacterial culture by streaking it on the agar and incubate it for 24 hours. Lastly, is evaluating the growth of the organism after 24 hours of incubation (Khalid 2011).

Types of Agar Plates

A culture media is used in microbiological laboratories to grow different kinds of microorganisms. It is composed of different nutrients that are essential for microbial growth. Since there are many types of microorganisms, each having unique properties and requiring specific nutrients for growth, there are many types based on what nutrients they contain and what function they play in the growth of microorganisms.

A culture media may be solid or liquid. The solid culture media is composed of a brown jelly like substance known as agar. It also contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating

bacteria or for determining the colony characteristics of the isolate. Semisolid media are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility. And Liquid (Broth) medium contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth**.** Different nutrients and chemicals are added to it to allow the growth of different microorganisms (Hassam, 2017).

Laboratory uses of all types of agar plates

Culture medium or growth medium is a liquid or gel designed to support the growth of microorganisms. There are different types of media suitable for growing different types of cells. Some organisms, termed fastidious organisms, need specialized environments due to complex nutritional requirements. There are many different types of media that can be used to grow specific microbes, and even promote certain cellular processes. Some media contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory-culture collections. Other media are used to select for or against the growth of specific microbes. Some are also used to distinguish one microorganism type from another growing on the same media.

Culture media are really helpful for the growth and identification of microorganisms. It is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible and repeatable microbiological test results is reduced (Todar, 2012).

Types of Culture Media

There is a range of different culture media available. Different types of culture media are typically divided. There are types of important culture or growth media used in microbiological laboratories: one is the preservation media in which its basic purpose is to let these microorganisms grow safely in an ensured environment that has all the important nutrients. Second is the enrichment medium which usually composed of bacteria taken from a liquid source and allows the microorganisms to multiply and has the essential nutrients that are required for it. Third is the selective culture media which allows the growth of certain microorganisms while inhibits the growth of the others. One example of selective media is the Mannitol Salt Agar which only allows the growth of Gram Positive bacteria. Another one is MacConkey agar which inhibits the growth of gram positive bacteria and allows the growth of gram negative bacteria. Also the White Onion Based Agar is an example of a selective media which supports the growth of gram

negative bacteria. Fourth is the differential media which allows the growth of certain microorganisms while inhibits the growth of the others. Example is MacConkey agar which differentiates the gram negative bacteria depending on their ability to ferment lactose. Fifth is the Resuscitation Culture Media which is used for growing microorganisms that are damaged and have lost the ability to produce due to certain harmful environmental factors. Last is the general purpose media which is a media that has a multiple effect (Hassam, 2017).

Onion as Culture Media

The microorganisms are grown for many purposes. Culture media used in the laboratory cultivation of microorganism supply the nutrients required for the growth and maintenance. Generally the cheap locally available materials such as cereals, legumes, and onion may serve as alternative nutrient media to grow bacteria and fungi (Uthayasooriyan, Pathmanathan, & Ravimannan, 2016). Onion contains nutrients that can effectively support the growth of microorganisms. As the readily available culture media are expensive, there is a need to find alternative media.

Distribution of Plant

Among Northern Africa, Europe, North America, and in Asia it has been reported that the over 600 members of *Allium* family has been distributed. It is cultivated across different countries like Thailand, Taiwan, Pakistan, Korea, the main islands of Japan, United Kingdom, Indonesia, Malaysia, India and other regions of the world. (Abbasi, 2011) Each of the *Allium* families have difference in terms of color, form, and taste. They are all, to some extent dissimilar in terms of nutraceutical content, phytochemical and biochemical (Benkeblia & Lanzotti, 2007).

In 2013, The Valuable chain analysis and competitiveness strategy showed that, Philippines registered a total production of 134,169.92 metric tons with an average yield of 8.70 metric tons per hectare. Central Luzon accounting for 55% of the total production is considered as the largest onion producing region followed by the Ilocos Region that covered 30% of the national production and then Cagayan Valley accounted for 5% of the production. In the Central Luzon, the province of Nueva Ecija is the leading producer of onions, accounting for 99.9% of the region's production. In Ilocos Region, it is Ilocos Norte who is accounted for 51% of the region's production in 2013 and considered as the main producing province of the region. Municipalities of Badoc, Vintar, and Pasuquin are the major producers of onion in Ilocos Norte. In Cagayan Valley Region, Nueva Vizcaya produced 99% of the region's onion production in 2013. Most of the onion in Nueva Vizcaya is grown in the municipality of Aritao.

Phytoconstituent and Chemistry of *Allium cepa*

Onions are one of the richest sources of compounds such as flavonoids and organosulfurs. Flavonoids which contain substances (quercetin, kaempferol and myricetin) are one of the active compounds found in great amounts. The antioxidant activity which has a high level is influenced by quercetin and its derivatives. Furthermore, other substances such as vinyl 10 dithiins, trisulfides, disulfides, and cepaene, are also some of the phytochemicals found in *Allium cepa* (National Onion Association, 2011). Due to its nutritional values, the use of *Allium cepa* as an alternative treatment for several diseases have been greatly appreciated (Szalay, 2014). It is also used as an essential source of phytoconstituents and food flavour. The presence of the Flavonoid and Organosulfur compounds may explain the anti- microbial activity of *Allium cepa*plant. A wide spectrum of biological activities makes onion a potential therapeutic agent.

Bacterial pathogens related to Onion

Enterobacter cloacae, Pantoea ananatis, and Burkholderia cepacia, are some of the significant pathogenic microorganisms affecting onion bulbs. *Pseudomonas cepacia* (Burkholder, 1950), formerly known as *Burkholderia cepacia*, causes Sour skin which serves as the most common and important bulb disease that affects onions near harvest. The harvesting equipment, tillage, prolonged air exposure and moist conditions of onions may cause the bacteria to generally penetrate the plant (Schwartz & Mohan, 2008).

Escherichia coli

Onion *(Allium cepa)* is known to be a rich source of quercetin and sulfur-containing compounds which combines to provide one-two punch to bacteria. Onion extract has been found to be against some pathogens such *as Escherichia coli, Salmonella,* and *Helicobacter pylori*. At J. Nihon University School of Dentistry, researchers conducted a study and discovered that onion exhibits an antibacterial action against *Streptococcus*, a gram positive bacterium and two other microorganisms that cause gingivitis. Researchers have discovered that fresh, raw and recently chopped onions exhibits strong antibacterial effect, but lost their antibacterial effect after being left for 48 hours.

Klebsiella pneumoniae

In Guangzhou city, Guangdong Province, China of January 2015, five decayed onion bulbs were collected; externally, they were asymptomatic, no signs of being infected and decayed but when they were cut in half they released a stinky odor. After six weeks of storing in a room temperature, the undamaged onion bulbs became soft, watery and they eventually became decayed. Five diseased bulbs

were picked and were surface-sterilized in 70% ethanol for 30 seconds, rinsed 3 times in sterilized water, and cut into small pieces approximately 1 to 5 mm in length. The small pieces of onion were then soaked in sterilized water for 5 min, and the suspension was streaked onto Luria-Bertani (LB) agar plates, and incubated at 28°C for 24 h (Gao, 2014). The morphology of the colonies exhibited by the onion in Luria-Bertani was consistent, non-pigmented, convex, round and smooth with entire margins of the medium. The cells were nonmotile in Luria-Bertani medium with 0.3% agar. It was then identified through 16S rRNA sequencing that the isolated bacteria from the diseased tissue were *Klebsiella pneumoniae*. (Liu, Lv, Gu, & Zhou, 2015).

Serratia marcescens

One of the most important bacteria reported to promote growth of agronomically valuable plants is the *Serratia marcescens* but it also is known as one of the opportunistic pathogen that is responsible for an increasing number of serious nosocomial infections and colonization of hospital wards. The antibiotic resistance that is present in *Serratia marcescens* is coded by genes located in plasmids and integrons. The isolated from onion pathogenic bacterium *Serratia marcescens* IMBG291 exhibited some peculiarities. This bacterium demonstrated both phytopathogenic and plant growth promotion activities in the same ecological niche (onion), depending on environmental factors. A reason for the bacterium to become pathogenic for field-grown onions remains unclear yet, but it is not excluded that a pathogenic phenotype was provoked with an elevated season temperature. The pathogenicity process is regulated with the environmental stimuli and probably with mobile genetic elements. The integron is a mobile genetic element that can be found in an onion-derived bacterium. They play a role in the arrangement of genes via site-specific recombination of the gene cassettes. Integrons were first discovered as a result of investigations into the phenomenon of multiple antimicrobial agents' resistance. Integrons and their associated gene cassettes are present in at least 10% of bacteria. Integrons are often located in plasmids or transposons, thus enabling the rapid spread of the gene cassettes among a wide variety of bacterial species. The clinical isolates of *Serratia marcescens* were shown to harbor integrons on conjugative plasmids. The predominant class of integron that carry resistance cassette is the Class 1 integrons. The isolate from onion *Serratia marcescens* IMBG291 identified as plasmidless, and the integron is located on the bacterial chromosome or it is an element of a genomic island. The isolate exhibited multiple antibiotics resistance, and apparently the determinants encoding these resistances were located on the bacterial chromosome because no plasmid DNA had been detected. PCR was carried out to demonstrate relatedness of the isolate IMBG291 to pathogenic or non-pathogenic *Serratia* (Kozyrovska, Ovcharenko, Voznyuk, & Zaetz, 2010).

Research Paradigm

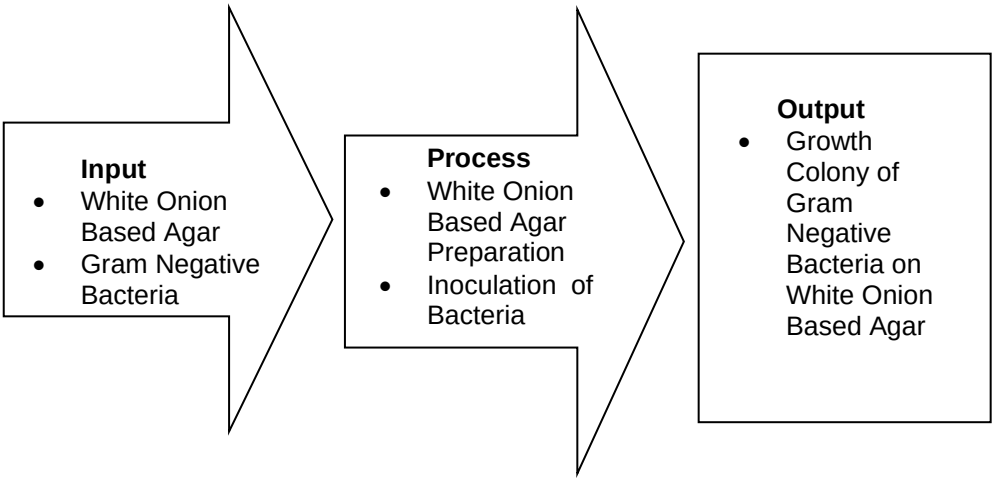


Figure 1. *Research Paradigm*

The illustration above shows the concept of the study. Onions are high in protein which enables to provide the needed nutrient to support the growth of the selected gram negative bacteria. The researchers utilized different concentrations of White onion and used the preparation of White Onion Based agar based from a study entitled "OEM- A new medium for rapid isolation of onion-pathogenic and onion-associated bacteria" by Zaid, Bonasera, and Beer (2012). Kruskal-Wallis test was utilized to determine the growth rate of the selected gram negative bacteria under different concentrations of White Onion Based Agar.

METHODS

Research Design

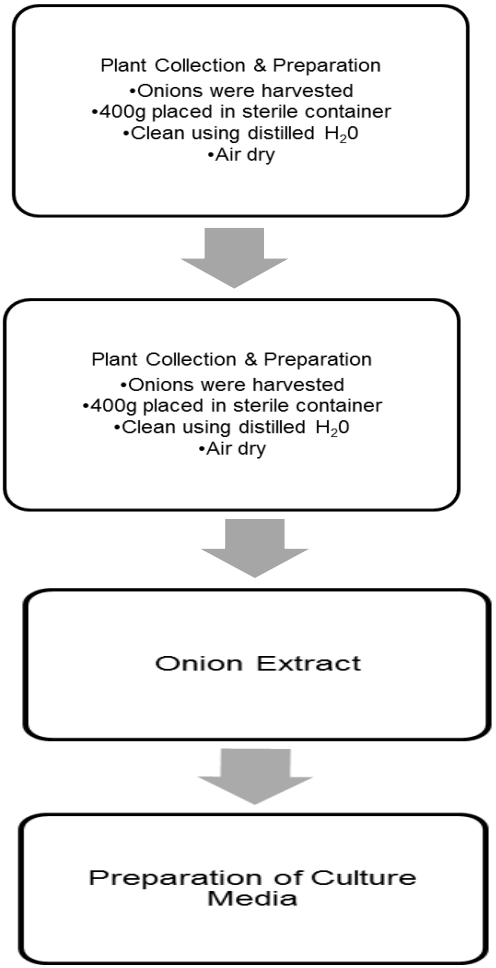
The research study used experimental design to determine the concentration of White Onion (*Allium cepa*) Based agar that was most supportive on the growth of Gram Negative Bacteria such as *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens*. The study was concerned and limited into assessing the in vitro antibacterial activity of white onion (*Allium cepa*). The other bacterial strains are beyond the researchers' concern. The researchers had only limited their study on the efficacy of the selected plant to examine the growth of *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens*.

Locale of the Study

The study was conducted and completed at University of Saint Louis Tuguegarao City, Medical Technology Laboratory. The collection of the white onions was at Aritao, Nueva Vizcaya.

Subjects of the Study

This research study utilized three Gram Negative Bacteria specifically *Escherichia coli, Klebsiella pneumoniae,* and *Serratia marcescens* which were given certification of authenticity, purity, and viability by National Institute of Molecular Biology and Biotechnology in the University of the Philippines Los Baños.



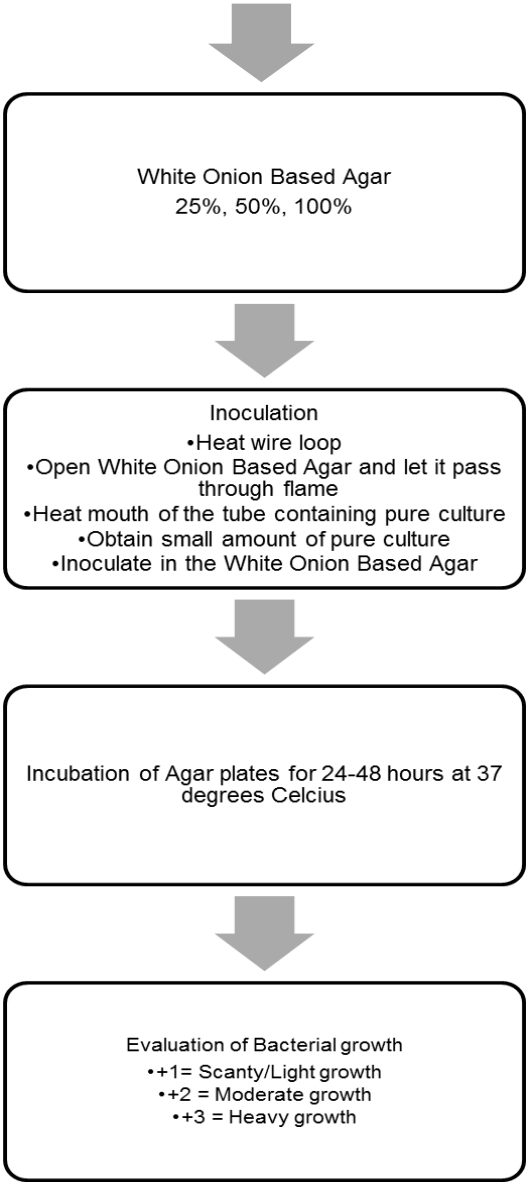


Figure 2. *Methodological Workflow*

Data Gathering Procedure

1. Plant Collection and Preparation

1.1 Fresh white onions were harvested at Aritao, Nueva Vizcaya manually.
1.2 400g of white onion bulbs were collected randomly and placed in a clean sterile container.
1.3 The researchers cleaned the onions using distilled water, and air-dried for one hour to remove the excess water.
1.4 Then the onions were macerated before it was put in the blender.

2. Onion Based Agar Preparation: Extraction

The following procedures were based on Zaid, Bonasera and Beerthat (2012).

2.1. The onions were washed using distilled water and allowed to air dry for one hour.
2.2. The outer covering of the onion were manually peeled off.
2.3. Exactly 400g of fresh onion bulbs were blended and the raw juice was extracted after standing in a clean glass contained for 24hrs.
2.4. The pulp obtained was left in a clean, sterile glass container and shaken vigorously to allow for proper extraction.
2.5. The pulp was filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored below ambient temperature until required.

3. Onion Based Agar Preparation

3.1. Onion Based Agar (25%)

3.1.1. Measure 6 grams base agar and 375 mL of distilled water.
3.1.2. Heat to boiling to dissolve the medium completely.
3.1.3. Mix 125 mL of onion extract on 500 mL beaker containing base agar.
3.1.4. Sterilize by autoclaving at 121 degrees Celsius at 15 pounds pressure for 15 minutes.
3.1.5. Cool at 45-50 degrees Celsius.
3.1.6. Mix well and pour into sterile petri dish
3.1.7. Incubate a control medium for 24 hours to check for contamination.
3.1.8. Refrigerate for storage.

3.2. Onion Based Agar (50%)

3.2.1. Measure 6 grams base agar and 250 mL of distilled water.
3.2.2. Heat to boiling to dissolve the medium completely.

3.2.3. Mix 250 mL of onion extract on 500 mL beaker containing base agar.
3.2.4. Sterilize by autoclaving at 121 degrees Celsius at 15 pounds pressure for 15 minutes.
3.2.5. Cool at 45-50 degrees Celsius.
3.2.6. Mix well and pour into sterile petri dish.
3.2.7. Incubate a control medium for 24 hours to check for contamination
3.2.8. Refrigerate for storage.

3.3. **Onion Based Agar (100%)**

3.3.1. Measure 6 grams base agar and 500mL of onion extract.
3.3.2. Heat to boiling to dissolve the medium completely.
3.3.3. Sterilize by autoclaving at 121 degrees Celsius at 15 pounds pressure for 15 minutes.
3.3.4. Cool at 45-50 degrees Celsius.
3.3.5. Mix well and pour into sterile petri dish.
3.3.6. Incubate a control medium for 24 hours to check for contamination.
3.3.7. Refrigerate for storage.

4. Inoculation of Gram Negative Bacteria

The following procedures were adopted and based on the Virtual Amrita Laboratories Universalizing Education (2011).

4.1. This process was done inside a biosafety cabinet.
4.2. The wire was heated using the Bunsen burner then let it cooled.
4.3. White Onion Based Agar was opened and passed through the flame.
4.4. MacConkey Agar was opened and passed through the flame
4.5. The tube that contains the pure culture of gram-negative bacteria was opened.
4.6. The mouth of the tube was heated.
4.7. The sterilized loop was inserted and obtained small amount of pure culture.
4.8. The pure culture that was inoculated was streaked on the White Onion Based Agar.
4.9. The pure culture that was inoculated was streaked on the MacConkey Agar

5. Incubation Procedure

The following procedures were adopted and based on the Virtual Amrita Laboratories Universalizing Education (2011).

5.1. The agar plates used including the MacConkey Agar which is the positive control was incubated.

5.2. It was placed inside the incubator with the temperature of 37 degrees Celsius.
5.3. The bacterial samples were allowed to grow for about 24 – 48 hours of incubation.

6. Evaluation of Bacterial Growth

The researchers evaluated the growth through colony counting to determine the effectiveness of Onion based agar in isolating *Escherichia coli, Klebsiella pneumoniae*, and *Serratia marcescens*. The general purpose of isolation streak is useful for most specimens. The relative numbers of organisms were estimated based on the extent of growth beyond the original area of inoculum.

6.1. Growth in the first quadrant were graded as 1+, or light or scanty growth
6.2. Growth in the second or third quadrant were graded as 2+ to 3+, or moderate growth
6.3. Growths in the third and in the fourth quadrant were graded as 4+, or heavy growth. (Mahon, 2011)

Data Analysis

This research utilized Kruskal-Wallis test to determine the significant difference on growth rate between treatments on each strain of bacteria. Post-hoc tests were also used for pair wise comparison on each treatment against the control. Simple means and standard deviations were also computed.

Waste Management Disposal

Materials that were used and those that needed to be discarded such as culture tubes and plates were placed in a biohazard-marked autoclave bag and were autoclaved at temperature of 121°C with a pressure of 15psi for duration of15-30 minutes. The autoclaving process was performed and completed at University of Saint Louis Tuguegarao Medical Technology Laboratory.

Ethical Considerations

The researchers sought permission from the Associate Dean for Health and Allied Sciences, Academic Dean for School of Education, Arts, Sciences and Health, University Research Ethics Board (UREB), Vice President for Academics and University President for the conduct of the study. This research study was given an ethical clearance number 51520.

The researchers also gave letter to the authorized personnel of the Department of Agriculture Bureau of Plant Industry for statistical information and

authentication of onion (*Allium cepa*) plants used in the study which was signed by the research adviser.

A letter was also given to the Director of National Institute of Molecular Biology & Biotechnology (BIOTECH) in the University of the Philippines Los Baños to permit the researchers in the purchasing and processing of *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens.*

The researchers sought permission from the Laboratory Technician in requesting for the materials used to conduct the study.

RESULTS

Table 1. *The Mean Growth Rate of Escherichia coli, Klebsiella pneumoniae, and Serratia marcescens isolated on White onion (Allium cepa) Bulb Based Agar and Positiv Control after 24 hours incubation*

Bacteria Cultured	Treatments	Mean Growth Rate Score	Qualitative Description
Escherichia coli	25% *A. cepa* Extract	2	Scanty
	50% *A. cepa* Extract	3	Moderate
	100% *A. cepa* Extract	2	Scanty
	Positive Control (McConkey)	2	Scanty
Klebsiella pneumoniae	25% *A. cepa* Extract	3.33	Moderate
	50% *A. cepa* Extract	4	Heavy
	100% *A. cepa* Extract	4	Heavy
	Positive Control (McConkey)	3	Moderate
Serratia marsescens	25% *A. cepa* Extract	2.33	Scanty
	50% *A. cepa* Extract	4	Heavy
	100% *A. cepa* Extract	3	Moderate
	Positive Control (McConkey)	4	Heavy

Table 1 shows the growth rate of the following Gram-negative bacteria: *E. coli*, *K. pneumoniae*, and *S.marcescens* on White Onion (*Allium cepa*) Based Agar using 25%, 50% and 100% concentrations.

Table 1 shows that after 24 hours incubation, 50% concentration of White onion *(Allium cepa)* Bulb Based Agar supports moderate growth of *Escherichia coli*. Also, 50% and 100% concentrations support heavy growth of *Klebsiella pneumoniae*.

Whereas under 100% concentration, varying results were obtained with *K. pneumoniae* showing heavy growth followed by *S. marcescens* exhibiting moderate growth and *E. coli* showing the least rate of growth.

Nevertheless, the table also shows that White Onion(*Allium cepa*) based agar with 25% concentration wasn't capable to produce promising results to support the growth *of E. coli and S. marcescens*, except for *K. pneumonia*e which showed moderate growth.

Hence, these observations indicate that the 50% concentration of White Onion (*Allium cepa*) based agar have the highest efficiency to support the growth of *E. coli*, *K. pneumonia, and S. marcescens*. However this doesn't mean that the 25% and 100% concentrations were not effective to support the growth of the bacteria being tested.

Table 2. *The Mean colony count (in cfu) of E. coli, K. Pneumonia, S. marcescens isolated on Onion (Allium cepa) Based Agar and Positive Control after 24 hours incubation*

Bacteria Cultured	Treatments	Mean Colony Count (cfu)
Escherichia coli	25% *A. cepa* Extract	277.33
	50% *A. cepa* Extract	320.67
	100% *A. cepa* Extract	377.33
	Positive Control (McConkey)	417.00
Klebsiella pneumoniae	25% *A. cepa* Extract	582.33
	50% *A. cepa* Extract	677.00
	100% *A. cepa* Extract	695.67
	Positive Control (McConkey)	589.00
Serratia marsescens	25% *A. cepa* Extract	472.33
	50% *A. cepa* Extract	629.00
	100% *A. cepa* Extract	574.67
	Positive Control (McConkey)	602.00

After 24 hours of incubation, bacterial growths were observed in all concentrations of white onion based agar. Evidently, the table also shows that *Klebsiella pneumoniae* has the highest growth with a mean colony count of 651.67. Hence, it is evident that the different concentrations (25%, 50% and 100% concentrations) can effectively support the bacterial growths of the three microorganisms tested.

Table 3.1 *Statistical Test for Difference between Treatments, Grouped by Strain of Bacteria using Kruskal-Wallis H test*

Bacteria Tested	$\chi^2(2)$-value	p-value	Decision
Escherichia coli	9.000	0.029	Reject Ho
Klebsiella pneumoniae	6.143	0.105	Accept Ho
Serratia marsescens	8.250	0.041	Reject Ho

The table above shows that the growth rate for *E. coli* and *S marsescens* showed significant differences among the different treatment groups while the growth rate of *K. pneumonae* showed no significant difference among the different concentrations of white onion based agar and the positive control.

Table 3.2. *Post Hoc Analysis for the Significant Difference in Growth Rate of Escherichia coli per Treatment Group*

	25% *A. cepa* Extract	50% *A. cepa* Extract	100% *A. cepa* Extract	Positive Control
25% *A. cepa* Extract				
50% *A. cepa* Extract	0.011*			
100% *A. cepa* Extract	1.000	0.011*		
Positive Control	1.000	0.073	1.000	

It can be gleaned from the table above that *E. coli* cultured in different concentrations of white onion based agar showed significantly the same growth rate as those cultured in the positive control. Moreover, the 50% concentration showed greater (t=-2.535) growth rate compared to the 25% concentration of white onion based agar; and the 100% concentration (t=2.535) showed significantly better growth rate than the 50% concentration.

Table 3.3. *Post Hoc Analysis for the Significant Difference in Growth Rate of Serratia marsescens per Treatment Group*

	25% *A. cepa* Extract	50% *A. cepa* Extract	100% *A. cepa* Extract	Positive Control
25% *A. cepa*				

Extract				
50% *A. cepa* Extract	0.009*			
100% *A. cepa* Extract	0.386	0.083		
Positive Control	0.066	1.000	0.221	

It can be gleaned from the table above that *S. marsescens* cultured in different concentrations of white onion based agar showed significantly the same growth rate as those cultured in the positive control. Moreover, the 50% concentration showed greater (t=-2.598) growth rate compared to the 25% concentration of white onion based agar.

DISCUSSION

A number of studies have been carried out to find alternative source of culture media to replace Macconkey Agar. In a study, sago was effectively used to replace nutrient source as well as agar for the growth of selected bacteria (Arulanantham et. al., 2012). The researchers utilized onion because of the rich sources of compounds such as flavonoids and organosulfurs which are needed by the microorganisms in order to grow. Onion bulb extract constituted the only source of nutrients for the bacterial stain. In 2008, it is reported that most of isolates were present in a detached onion bulb scale assay (Jacobs et al., 2008).

As shown in the results, when the extracts were tested on *Escherichia coli*, *Klebsiella pneumoniae*, and *Serratia marcescens*, the bacteria exhibited growth with 25%, 50%, and 100% concentrations of the onion extract. The growth on the 25% is not favorable due to the varying results that are shown in the study. The same can be said to the 100% concentration for having inconstant results. However, the growth on the 50% concentration showed promising results which to support the growth of the Gram Negative Bacteria that are used in the study. The colony count of the bacterial strain although there have been growth on the different concentrations of the Onion Agar and that it can support the growth of the bacterial strain result shows that 50% concentration of White Onion Based Agar has the highest mean colony count. The possible weak antibacterial effect of onion is due to high water content of the plant and less concentration of sulfur compounds which accumulate during the onion maturation (Doycheva & Satchanska, 2014)

The formulated media concentrations supported the growth of all test organisms and shows that there is concentration dependent increase in bacterial growth with 50% as the most efficient in growing the three Gram Negative bacteria. Therefore it was clear from this study that the onion extracts media with different concentrations of 25%, 50% and 100% affected the growth of the three microorganisms (Arulanantham et. al., 2012).

CONCLUSION

This research study concludes that under 25% and 100% concentrations of White onion *(Allium cepa)* Bulb Based Agar support the growth of *Escherichia coli, Klebsiella pneumoniae, and Serratia marcescens.* However, the 50% concentration exhibited optimum support to the growth of *Klebsiella pneumoniae, and Serratia marcescens.* Furthermore, it is concluded that 50% concentration of the same agar is comparable with the positive control, specifically Mac Conkey Agar.

RECOMMENDATIONS

From the results of the study, the following recommendations were made:

1. The same study can be performed by using Gram Positive Bacteria to make sure that only Gram Negative bacteria will grow on the white onion based agar.
2. A similar study should be made which will utilize other Gram Negative Bacteria.
3. The same study can also be performed by using onion scallions instead of onion bulbs to test its ability to support bacterial growth.
4. A similar study should be made which formulates 75% concentration of white onion (*Allium cepa*) bulb based agar.
5. Further studies should also be conducted to compare 4, 8, 12, 16, 20, and 24 hours of incubation period of white onion (Allium cepa) bulb based agar.

REFERENCES

Abd-Alla, M. H., Bashandy, S. R., Ratering, S., & Schnell, S. (2011). First report of soft rot of onion bulbs in storage caused by Pseudomonas aeruginosa in Egypt. *Journal of plant interactions*, *6*(4), 229-238. Retrieved on September 26, 2017 from http://dx.doi.org/10.1080/17429145.2010.535618

AliDabighian, M. (2016). The impact of onions and figs extracts on Streptococcus pyogenes bacteria. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *6*(4), 128.Retrieved on October 10, 2017 from www.iaees.org

Arulanantham, R., Pathmanathan, S., Ravimannan, N., & Niranjan, K. (2012). Alternative culture media for bacterial growth using different formulation of protein sources. *J Nat Prod Plant Resour*, *2*(6), 697-700. Retrieved on July 22, 2018 from file:///C:/Users/hp/Downloads/Ad-and-dis%20(1).pdf.

Bakht, J., Khan, S., & Shafi, M. (2014). In vitro antimicrobial activity of Allium cepa (dry bulbs) against Gram positive and Gram-negative bacteria and fungi. *Pak J Pharm Sci*, *27*(1), 139-145. Retrived on September 26, 2017 from https://www.pakbs.org/pjbot/PDFs/45(S1)/01.pdf

Bora, K., & Sharma, A. (2009). Phytoconstituents and therapeutic potential of Allium cepa Linn.-A Review. *Pharmacognosy Reviews*, *3*(5), 170. Retrieved on October 26, 2017 from www.phcogrev.com

Corzo-Martínez, M., & Villamiel, M. (2012). An overview on bioactivity of onion. *Onion Consumption and Health. 1ª Ed. Nueva York: Nova Science Publishers, Inc*, 1-48. Retrieved on September 25, 2017 from https://www.researchgate.net/publication/269332973

Degirmencioglu, N., & Irkin, R. (2009). Survival of some microorganisms in the presence of onion (Allium cepa l.) extracts in vitro. *Polish journal of food and nutrition sciences*, *59*(1). Retrieved on October 10, 2017 from http://www.pan.olsztyn.pl/journal/

El-Nagerabi, S. A., Ahmed, A. H., & Elshafie, A. E. (2016). In vitro evaluation of selected plant extracts as biocontrol agents against black mold (Aspergillus niger Van Tieghem) of onion bulbs (Allium cepa L.). *Science and Technology*, *5*(1), 147-152. Retrieved on September 25, 2017 from https://www.researchgate.net/publication/291808134

Eltaweel, M. (2013). Assessment of Antimicrobial Activity of Onion Extract (Allium cepa) on Staphylococcus aureus; in vitro study. In *International Conference on Chemical, Agricultural and Medical Sciences (CAMS-2013) Dec* (pp. 29-30). Retrieved October 19, 2017 on from http://dx.doi.org/10.15242/IICBE.C1213068.

Goodarzi, M., Nanekarani, S., & Landy, N. (2014). Effect of dietary supplementation with onion (Allium cepa L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. *Asian Pac. J. Trop. Dis*, *4*(Suppl 1), S297-S301. Retrieved on September 26, 2017 from http://www.wpsa.com/index.php/wpsa-proceedings/2013/19th-european-symposium-on-poultry-nutrition/1462-effect-of-dietary-supplementation-with-onion-allium-cepa-l-on-performance-and-intestinal-microflora-composition-in-broiler-chickens/file

Hannan, A., Humayun, T., Hussain, M. B., Yasir, M., & Sikandar, S. (2010). In vitro antibacterial activity of onion (Allium cepa) against clinical isolates of Vibrio cholerae. *Journal of Ayub Medical College Abbottabad*, *22*(2), 160-163. Retrieved on October 8, 2017 from http://www.ayubmed.edu.pk/JAMC/PAST/22-2/Hannan.pdf

Kirilov, A., Doycheva, A., & Satchanska, G. (2014). Antibacterial activity of mature and green Allium cepa. *Ecol Eng Environ Prot*, *1*, 12-17. Retrieved on July 25, 2018 from http://ecoleng.org/2014/12-17.pdf.

Lieberman, V. M., Zhao, I. Y., Schaffner, D. W., Danyluk, M. D., & Harris, L. J. (2015). Survival or growth of inoculated Escherichia coli O157: H7 and Salmonella on yellow onions (Allium cepa) under conditions simulating food service and consumer handling and storage. *Journal of food protection*, *78*(1), 42-50. Retrieved on November 5, 2017 from http:10.4315/0362-028X.JFP-14-281

Mnayer, D., Fabiano-Tixier, A. S., Petitcolas, E., Hamieh, T., Nehme, N., Ferrant, C., ... & Chemat, F. (2014). Chemical composition, antibacterial and

antioxidant activities of six essentials oils from the Alliaceae family. *Molecules*, *19*(12), 20034-20053. Retrieved on October 19, 2017 from www.mdpi.com/journal/molecules 191220034.

Murugaiyah, P. (2015). *Preliminary screening of Allium cepa linn for its antioxidant activity, cytotoxicity on human chronic myelogenous leukaemia cell line (K562) and antibacterial properties* (Doctoral dissertation, UTAR). Retrieved on November 5, 2017 from http://eprints.utar.edu.my/1819/1/Thesis_-Parthiban-.pdf.

Ovcharenko, L. P., Voznyuk, T. M., Zaetz, I. E., Potopalsky, A. I., Reva, O. N., & Kozyrovska, N. O. (2010). A mobile genetic element in Serratia marcescens, a causative agent of onion disease. Retrieved on October 19, 2017 from https://repository.up.ac.za/bitstream/handle/2263/15477/Ovcharenko_Mobile%282010%29.pdf?sequence=1&isAllowed=y

Packia Lekshmi, N. C. J., Viveka, S., Jeeva, S., & Raja Brindha, J. (2015). Antimicrobial Spectrum of Allium Species–A Review. *History*, *15*(44), 1-5. Retrieved on October 26, 2017 from http://jeffreydachmd.com/wp-content/uploads/2015/06/Antimicrobial-Spectrum-Allium-Garlic-Packia-Lekshmi-2015.pdf

Patil, S., Sivanandhan, G., & Thakare, D. (2015). Effect of physical and chemical parameters on the production of Red exopigment from Penicillium purpurogenum isolated from spoilt onion and study of its antimicrobial activity. *Int. J. Curr. Microbiol. Appl. Sci*, *4*, 599-609. Retrieved on November 10, 2017 from http://ijcmas.com.

Ponnulakshmi, R. (2013). Efficacy of bulb extracts of Allium cepa varieties (red, white and small onion): An in vitro antifungal and antioxidant activity. Retrieved on October 10, 2018 from www.ijpbs.net

Ruangpan, L., & Tendencia, E. A. (2004). *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment*. Aquaculture Department, Southeast Asian Fisheries Development Center. Retrieved on July 22, 2018 from http://hdl.handle.net/10862/1616.

Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *JoVE (Journal of Visualized Experiments)*, (63), e3064. Retrieved on November 15, 2017 from vlab.amrita.edu/index.php?sub=3&brch=73&sim=212&cnt=1

Shahverdi, S., Khaki, A., & Jafari, B. (2017). Investigating the antioxidant effect of Allium cepa after exposure to escherichia coli on biochemical factors, the blood antioxidants, and testis tissue in rats. *Crescent J. Med. Boil. Sci*, *4*, 32-38. Retrieved on November 5, 2017 from http://www.cjmb.org

Stoyanova, M., Moncheva, P., & Bogatzevska, N. (2012). Occurrence of phytopathogenic bacteria of Enterobacteriaceae family in bulbs of cultural and ornamental plants. *Sci. Technol*, *2*(6), 39-44. Retrieved on November 5, 2017 from http://www.sustz.com/journal/VolumeII/Number6/Papers/MariyaStoyanova2.pdf

Todar, K. (2004). Nutrition and growth of bacteria. Todar's Online Textbook of Bacteriology. Retrieved on July 23, 2018 from http://textbookofbacteriology.net/nutgro_3.html.

Uthayasooriyan, M., Pathmanathan, S., Ravimannan, N., & Sathyaruban, S. (2016). Formulation of alternative culture media for bacterial and fungal growth. *Der Pharmacia Lettre*, *8*(1), 431-436. Retrieved on July 23, 2018 from http://www.scholarsresearchlibrary.com/abstract/formulation-of-alternative-culture-media-for-bacterial-and-fungal-growth-6090.htmlZohri, A. N., Abdel-Gawad, K., & Saber, S. (1995). Antibacterial, antidermatophytic and antitoxigenic activities of onion (Allium cepa L.) oil. *Microbiological research*, *150*(2), 167-172. Retrieved on October 8, 2017 from https://www.researchgate.net/publication/15599151.