ANTIBACTERIAL EFFECT OF WELSH ONION (*ALLIUM FISTULOSUM)* LEAF EXTRACT AGAINST *PSEUDOMONAS AERUGINOSA*

Nurhaliza D. Moya, Shaira J. Limpahong, Robert A. Sapungan
Bachelor of Science in Medical Technology Students
Saint Jude College PHINMA, Manila, Philippines

ABSTRACT

Bacterial infection are currently life-threatening. From the discovery of Penicillin, series of antibiotics were formulated to kill different pathogenic bacteria due to the constant evolving of bacterial resistance. But then, the use and study of medicinal plants as antibiotic are still in progress, hence this study focused on the antibacterial effect of *Allium fistulosum* against *Pseudomonas aeruginosa.* Quantitative experimental research design were used in this study. Antimicrobial Susceptibility Testing were utilized to analyse the minimum inhibitory effect of the *Allium fistulosum* extract in different concentration against Meropenem and distilled water. It found out that there is no significant difference in the zone of inhibition of *Pseudomonas aeruginosa* treated with 100%, 75%, and 50% concentration of *Allium fistulosum* extract and positive control. Hence *Allium fistulosum* extract is not effective as antibacterial agent against *Pseudomonas aeruginosa.* The researchers recommend to further study the effectiveness of the plant extract to other pathogenic bacteria.

Keywords*:* Welsh Onion, Pseudomonas aeruginosa, Meropenem

Introduction

Nowadays, the bacterial infection are most difficult to cure. Currently, the acquired infection are capable at least one antimicrobial drug and previously can be causative pathogen. The microorganism known for their virulence and capable to evolve their resistance are *Staphylococcus spp., Enterococcus spp., Pseudomonas aeruginosa and Acinetobacter spp.,* (Carmelli 2008). Human bodies has a variety of commensalism that create a critical part about human health. These microorganisms involve distinct territories. Some bacteria can be helpful as well as dangerous to human health. The skin and soft tissue infection oftentimes happen especially in wounds which can accommodate the host in clinical practice and at some point of bacterial infection. (Nivhols 1999; Eron et, al 2003; Dinubile and lipsky 2004). The *Pseudomonas aeruginosa* exist as a gram negative, rod shaped, opportunistic microbe that has the same infection to the hospital surroundings with its highest mortality rate. There are seventy five percent reported deaths due to sepsis because of transmitting the bacteria to the area of wound caused by burn (Barrow, Spies, Barrow, & Herndom, 2004).

There are some antibiotics that has usual treatment for these infectious bacteria. One of these known antibiotics is Meropenem also known as Carbanepems. The Meropenem (carbanepems) is an antibiotic drug that help the Pseudomonas infection cure with a combination of beta-lactam. An antibiotic namely Meropenem has great activity upon the wide range of microbes which involve some different types of gram positive and gram negative pathogen including many possible strains like Pseudomonas aeruginosa beside from anaerobic organism. (Wiseman et.al 1995; fish and singletary 1997).Welsh onion is also known as bunching onion or long green onion, with a Genus *Allium*, and a species of *fistulosum* and a family of alliaceae. This *Allium fistulosum* L. are recognized to be Scallion and it is popularized to be spice or vegetable and even for medicinal purposes all over the countries. It is rich in pollen and seed fertility. *Allium fistulosum* has its own benefits and uses for medicinal and therapeutic purposes in which it plays a role in preventing or treating cancer, it also contains a free radicals in which it can reduce of having gastric ulcer and bad cholesterols, and a phytochemicals together with its vitamin C that helps to improve their immunity. Those use of Welsh onion revealed medicinal benefits to human health as studies shows it also contains polyphenol molecules which involved flavonoids, tannins, as well as allicin that takes antioxidant and antimicrobial properties. Welsh Onion is a nutritious plant and it also provides an army of health benefits. *Allium fistulosum* have some advantages on health in which it acts as antimicrobial agents on anti- cancer, anti-cholesterol, anti-inflammatory and anti-oxidative properties that contains quercetin and allicin (Machavarapeut et al. 2005). Hence, the researcher aimed to conduct an experimental research to analyse the effectiveness of

Allium fistulosum leaf extract against *Pseudomonas aeruginosa.*

Statement of the Problem

This research aims to determine the antibacterial effect of *Allium fistulosum* extract against *Pseudomonas aeruginosa.* Specifically, this study aims to seek the answer for the following specific questions:

1.What is the zone of inhibition of *Pseudomonas aeruginosa* treated with:

a. 100% concentration of *Allium fistulosum* extract
b. 75% concentration of *Allium fistulosum* extract
c. 50% concentration of *Allium fistulosum* extract

2. What is the zone of inhibition of *Pseudomonas aeruginosa* against the positive control (MEROPENEM) at the:

a. 2.1 For the first trial
b. 2.2 For the second trial
c. 2.3 For the third trial

3. Is there a significant difference in the zone of inhibition of the *Pseudomonas aeruginosa* treated with 100%, 75%, 50% concentration of *Allium fistulosum* extract?

4. Is *Allium fistulosum* extract in different concentration effective in inhibiting the growth of *Pseudomonas aeruginosa*?

METHODOLOGY

Research Design

This study will use the experimental research design. An experimental type of research will be used a quantitative approach. This study used Two-way ANOVA with post-hoc test Bonferroni to compare the zone of inhibition extract in different concentrations together with the Meropenem (positive control) and distilled water (negative control).

Locale of the Study

The plant was purchased from the farm of Baguio City. The authentication of this plant was conducted at the Bureau of Plant and Industry at Quirino Manila City. Materials to be used were purchased at Bambang St. Metro Manila. The Phytochemical and extraction of Welsh Onion (*Allium fistulosum)* was conducted in Department of Science and Technology at Bicutan, Taguig City. The *Pseudomonas aeruginosa* was produced by the Fatima Medical Center, Antipolo Rizal. The experiment of this study was conducted at Fatima University Medical Center in Antipolo Rizal.

Samples of the Study

A fresh extract of Welsh Onion (*Allium fistulosum)* purchased on Baguio City. The *Pseudomonas aeruginosa*, Mueller Hinton Agar and the positive control (Meropenem) was purchased from Fatima Medical Center, Antipolo Rizal which were used in the determination in the zone of inhibition of different concentration was used for experimentation.

Ethical consideration

In this study it was assured that no human or animal experimentation involved. The researchers wore Personal Protective Equipment (PPE) and ensured to follow the rules inside the laboratory; and the protocol for the processes and procedures.

Data Gathering and Procedures

The Welsh Onion (*Allium fistulosum)* plant that will be used in the study were purchased from the Farm of Baguio City, Philippines .The plant was submitted in Bureau of plant and Industry for authentication and also to the Department of Science and Technology(DOST) for identification of phytochemical test and its specific extract to test for its affectivity.

Preparation of Materials/Apparatus and Sterilization of Instrument

The laboratory equipment used in this experiment were carefully prepared and sterilized. The equipment that was used was wrapped with a bond paper and placed in the autoclave basket, steamed under pressure at one hundred twenty one degree Celsius (121^{o}C) for thirty minutes (30) Sterilization was done to avoid inaccurate result.

Preparation of Culture Media

The researcher prepared 3.5g Mueller Hinton Agar Powder and was measured using the triple beam balance, 25ml of distilled water was added into the

Erlenmeyer flask for bacterial growth of *Pseudomonas aeruginosa* then was mixed well and brought to boil to dissolve completely the powder. Dispensed the required amount for each plate and let it cool before sterilizing by autoclave for 121ºC for 15-20 minutes. Next allowed to cool for few minutes. Lastly the plates were stored through refrigeration at 4-8C temperature in inverted position.

Preparation and Collection of *Allium fistulosum* Extract

The researchers used 3 kilogram of Welsh onion (*Allium fistulosum)* .The leaves were washed with Distilled water properly to remove dirt and chopped in fine particles and then delivered to Department of Science and Technology (DOST) at General Santos Avenue Bicutan, Taguig City for phytochemicals and extraction.The fresh 1.0 kilogram welsh onion were blended/osterized and soaked in 4.0 L of 95% ethyl alcohol for 48 hours. The mixture was filtered and the filtrate obtained was concentrated using rotary evaporator at 60ºC under vacuum for 3 hours. The concentrated extract was further evaporated using water bath at 60ºC to obtain a semi solid extract. The results of the crude extract of fresh 1kg welsh onion produced 3.5 L ethanol extract. The concentration of a filtrate yielded 65.0 gram of semisolid extract. Administration of different concentration of Welsh Onion. To administer the different concentration of Welsh Onion extract and commercially prepared antibiotic, disks diffusion method was used. To prepare 100% of Pure *Allium fistulosum* extract, 5 ml of pure extract will be measured. To prepare 75% of Pure *Allium fistulosum* extract, 3.75 ml of pure extract will be measured and will be diluted in 1.25 ml distilled water. To prepare 50% of Pure *Allium fistulosum* extract, 2.50 ml of pure extract will be measured and will be diluted 2.50 ml of distilled water. After preparing the concentration of Welsh Onion extract each sterile paper disks was impregnated with 30uL of diluted Welsh onion extract. The disks were allowed to dry. The researcher will be used four (4) trials in each petri dish. One petri dish for each was used for each 100%, 75%, 50% .Using the sterile forceps, the disks were placed on the inoculated blood agar medium. One paper disks on each plate was soaked in distilled water as negative control. The treated plates were labelled. The plates will be incubated at 37ºC for 24 hours. Diameter of the zone of inhibition was measured in millimetre with transparent ruler.

The results gathered were subjected to statistical analysis. The Administration of commercially prepared drug was used at the positive control. It is a semi synthetic Meropenem.

Data Analysis

The two way Analysis of Variant (ANOVA) was used in the analysis of the experimental data. It was used to compare the significance between the controlled grouped from *Allium fistulosum.* The two way Analysis of Variant (ANOVA) when there was one measurement variables. Bonferroni post-hoc Test are also applied in the Data analysis which means that it analysed the result of experimental data, and it is based on the probability of at least one type of error in set of comparison. It also involved statistical test at the same time.

RESULTS

In table 1 shows the zone of inhibition of positive control, negative control and different concentration of Welsh onion extract, the positive control which was Meropenem. The negative control was distilled water and the experimental group which was the Welsh onion extract in different concentrations of 100%, 75% and 50%. The experimentation had three trials. The Meropenem produced the highest level zone of inhibition which was 35 mm in first trial, 34 mm for second trial and 35 mm for the third trials respectively. The distilled water produced 6mm zone of inhibition in first trial, 6 mm for second trial and 6 mm for the third trials. In 100% extract produced 6 mm in first trial, 6 mm for second trial and 6 mm for the third trials. On the other hand 75% extract produced 6 mm in first trial, 6 mm for second trial and 6 mm for the third. Lastly, in 50% extract produced 6 mm in first trial, 6 mm for second trial and 6 mm for the third trials.

Meropenem	Distilled Water	50% Extract	75% Extract	100% Extract	
35	6	6	6	6	
34	6	6	6	6	0.4096
35	6	6	6	6	
		<0.0001			

Table 1 Zone of Inhibition of positive control, negative control and different concentration of Welsh onion extract

Table 2.0 Shows the Bonferroni post-hoc test measuring the level of significance of positive control which was Meropenem and the negative control which was distilled water. Based on the result the P-value is <0.001 which means it was significant for the result that p-value between 0 and 1 was significant results while <0.05 indicates that there was no significance.

Trial	Difference	T	P value	Summary
1	-29	79	P<0.001	S
2	-28	77	P<0.001	S
3	-29	79	P<0.001	S

Table 2.0 Meropenem vs. Distilled Water

Table 2.1 Shows the result of P-value of <0.001 which mean there was significant relationship between positive control which was Meropenem and the 50% of Welsh onion extract.

Trial	Difference	T	P value	Summary
1	-29	79	P<0.001	S
2	-28	77	P<0.001	S
3	-29	79	P<0.001	S

Table 2.1 Meropenem vs. 50% extract

Table 2.2 Shows the result of P-value of <0.001 which mean there was significant relationship between positive control which was Meropenem and the 75% of Welsh onion extract.

Trial	Difference	T	P value	Summary
1	-29	79	P<0.001	S
2	-28	77	P<0.001	S
3	-29	79	P<0.001	S

Table 2.2 Meropenem vs. 75% extract

Table 2.3 Shows the result of P-value of <0.001 which mean there was significant relationship between positive control which is Meropenem and the 100% of Welsh onion extract.

Trial	Difference	T	P value	Summary
1	-29	79	P<0.001	S
2	-28	77	P<0.001	S
3	-29	79	P<0.001	S

Table 2.3Meropenem vs. 100% extract

Table 3.0 Shown se result of P-value of <0.05 which mean there was no significant relationship between negative control which was distilled water and the 50% of welsh onion extract.

Trial	Difference	t	P value	Summary
1	0	0	P<0.05	NS
2	0	0	P<0.05	NS
3	0	0	P<0.05	NS

Table 3.0 Distilled water vs. 50% extract

Table 3.1 Shows the result of P-value of <0.05 which mean there was no significant relationship between negative control which was distilled water and the 75% of welsh onion extract.

Trial	Difference	t	P value	Summary
1	0	0	P<0.05	NS
2	0	0	P<0.05	NS
3	0	0	P<0.05	NS

Table 3.1 Distilled water vs. 75% extract

Table 3.2 Shows the result of P-value of <0.05 which mean there was no significant relationship between negative control which was distilled water and the 100% of welsh onion extract.

Trial	Difference	t	P value	Summary
1	0	0	P<0.05	NS
2	0	0	P<0.05	NS
3	0	0	P<0.05	NS

Table 3.2 Distilled water vs. 100% extract

Table 4.0 Shows the result of P-value of <0.05 which mean there was no significant relationship between 50% of Welsh onion extract and the 75% of Welsh onion extract.

Trial	Difference	t	P value	Summary
1	0	0	P<0.05	NS
2	0	0	P<0.05	NS
3	0	0	P<0.05	NS

Table 4.0 50% extract vs. 75% extract

Table 4.1 Shows the result of P-value of <0.05 which mean there was no significant relationship between 50% of Welsh onion extract and the 100% of Welsh onion extract.

Trial	Difference	t	P value	Summary
1	0	0	P<0.05	NS
2	0	0	P<0.05	NS
3	0	0	P<0.05	NS

Table 4.1 50% extract vs. 100% extract

Table 5.0 Shows the result of P-value of <0.05 which mean there was no significant relationship between

75% of Welsh onion extract and the 100% of Welsh onion extract.

Trial	Difference	t	P value	Summary
1	0	0	P<0.05	NS
2	0	0	P<0.05	NS
3	0	0	P<0.05	NS

Table 5.0 75% extract vs. 100% extract

Summary of Results

1. 100% concentration of *Allium fistulosum* extract produced 6 mm of zone inhibition 75% concentration of *Allium fistulosum* extract produced 6 mm of zone inhibition and for 50% concentration of *Allium fistulosum* extract produced 6 mm of zone inhibition.
2. The zone of inhibition of *Pseudomonas aeruginosa* against the positive control (MEROPENEM) produced 35 mm of zone inhibition for the first trial, 34 mm of zone inhibition for the second trial and 35 mm of zone inhibition for the third trial.
3. There is No Significant difference in the zone of inhibition of the *Pseudomonas aeruginosa* treated with 100%, 75%, 50% concentration of *Allium fistulosum* extract.
4. *Allium fistulosum* extract is not effective in inhibiting the growth of *Pseudomonas aeruginosa*.

CONCLUSIONS AND RECOMMENDATIONS

There is no significant difference between the zone of inhibition of *Pseudomonas aeruginosa* treated with 100%, 75%, and 50% concentration of *Allium fistulosum* leaf extract and distilled water as negative control but there is significant difference between the zone of inhibition of *Pseudomonas aeruginosa* treated with 100%, 75%, and 50% concentration of *Allium fistulosum* leaf extract and Meropenem as positive control. Therefore, the *Allium fistulosum* leaf extract is not effective as antibacterial agent against *Pseudomonas aeruginosa*.

Given that the result is ineffective, the researcher aims to use this research as an open source for experimental replication to further enhance the study, also, recommends the medical practitioners explore other herbal plants that are capable of exhibiting their effectiveness of antibacterial mechanism beyond this study.

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