ejpmr, 2023,10(6), 56-67

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

AN IN-VITRO DETERMINATION OF ANTIBACTERIAL PROPERTY OF MALATUNGAW FRUIT ETHANOLIC EXTRACT (*MELASTOMA MALABATHRICUM*, FAMILY: MELASTOMATACEAE) AGAINST *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA*

Audrey Nicole C. Aquino*, Camila D. Barzaga, Sarah Faye D. Dualan and Juan Nelson T. Limbo

College of Pharmacy, School of Medical Laboratory Sciences, St. Dominic College of Asia, Bacoor City, Cavite, Philippines.

***Corresponding Author: Audrey Nicole C. Aquino**

College of Pharmacy, School of Medical Laboratory Sciences, St. Dominic College of Asia, Bacoor City, Cavite, Philippines.

Article Received on 31/03/2023 **Article Revised on 20/04/2023** **Article Accepted on 11/05/2023**

ABSTRACT

Melastoma malabathricum, a member of the Melastomataceae family, contains a variety of phytochemicals, including alkaloids, flavonoids, and saponins, that could induce antibacterial properties. However, there have been few scientific studies conducted on *M. malabathricum*, and more investigation is required to establish the basis for its ethnomedicinal properties. Consequently, the purpose of this study is to examine the antibacterial potential of the ethanolic extract of Malatungaw fruit against specific Gram-negative and Gram-positive bacteria, namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, the objective of this research is to compare the antibacterial activity of Malatungaw fruit ethanolic extract to that of standard antibacterial compounds against the selected pathogens. This study employs a quantitative experimental design to answer the research question via antimicrobial assay and minimum inhibitory determination. One-way analysis of variance (ANOVA) was used to analyze the results, and $P < 0.05$ was considered statistically significant. In comparison to other concentrations, Extract E, which is 100% pure ethanolic Malatungaw fruit extract, produced the most significant inhibitory zone against *Staphylococcus aureus*. *Staphylococcus aureus* was more susceptible to the antibacterial effects of the Malatungaw ethanolic fruit extract. Consequently, this plant species may serve as a future resource for the development of alternative medicines.

KEYWORDS: *In-vitro, Malatungaw, Phytochemical, Antibiotic resistance, Pathogen.*

INTRODUCTION

Melastoma malabathricum is a large angiosperm flowering plant species from the Melastomataceae family, and it is a monoecious plant. This species is commonly found in the Philippines and is known as Busisi and Malatungaw plant (Pulhin et al., 2020; Bayas et al., 2018). Malatungaw is a well-known herb used to cure various ailments and conditions, including skin issues, according to Reduann et al. (2020). The plant's leaves are used to prevent scarring and treat conditions such as dysentery, diarrhea, and hemorrhoids. Additionally, according to Zheng et al. (2021), this plant is used to treat high blood pressure, and its leaves have anti-inflammatory, antipyretic, antioxidant, cytotoxic, antidiarrheal, anti-ulcer, and anti-cancer properties. While the chemical constituents of this plant species have been identified, more research is needed to establish their biological functions.

The efficacy of previously discovered essential antibiotic groups, including tetracyclines, cephalosporins, aminoglycosides, and macrolides, is at risk due to the growing incidence of antimicrobial resistance. Antibiotic resistance has emerged as a critical challenge in the healthcare system. Drug resistance can impact health outcomes, treatment duration, and cost. Therefore, the pursuit of discovering novel antibiotics would be a crucial aim. In line with this, natural products have undergone partial pre-screening by nature and have been designed for specific biological interactions, often resulting in the discovery of novel mechanisms of action and demonstrating pharmacological potential. The structural and mechanistic uniqueness of molecules found in natural plant sources, which encompass a vast range of complex and diverse compounds, holds promise for the discovery of novel drugs.

In the past few years, various researchers have explored the potential of plant and microbial extracts, as well as synthetic compounds, as antibacterial agents. Assessing the comparability of outcomes poses a challenge due to the varying methodologies and techniques utilized. The significance of a plant extract exhibiting antimicrobial properties is noteworthy. However, it is crucial to gain a

deeper understanding of the compound's antimicrobial effects prior to its implementation in human healthcare. Therefore, the aim of this study is to investigate the following queries:

1. What is the percentage yield of the extracted Malatungaw fruit *(Melastoma malabathricum)?*
2. What are the phytochemical constituents of Malatungaw fruit extract *(Melastoma malabathricum)*?
3. What are the average zone of inhibition in *Pseudomonas aeruginosa* and *Staphylococcus aureus* when data will be grouped according to:

3.1) *Extract A*: 7.5 mL of Malatungaw fruit ethanolic extract dissolved in 15 mL water solvent

3.2) Extract *B*: 15 mL of Malatungaw fruit ethanolic extract dissolved in 15 mL water solvent

3.3) Extract *C*: 30 mL of Malatungaw fruit ethanolic extract dissolved in 15 mL water solvent

3.4) *Extract D*: 45 mL of Malatungaw fruit ethanolic extract dissolved in 15 mL water solvent

3.5) *Extract E*: 100% Concentration of Malatungaw fruit ethanolic extract

3.6) Control for both bacteria: Ciprofloxacin

3.7) Positive Control for *Pseudomonas aeruginos*a: Amikacin

3.8) Positive Control for *Staphylococcus aureus*: Oxacillin

3.9) Negative Control Sample free-disc

4. Is there a significant difference in antibacterial activity within the treatments against bacterial strains tested?

5. Is there a significant difference in the zone of inhibition between *Staphylococcus aureus* and *Pseudomonas aeruginosa* when they will be observed per treatment?

METHODOLOGY

Research Design

The research paper utilized a quantitative experimental methodology to assess the antibacterial properties of natural plant crude extract derived from Malatungaw fruits. The study aimed to determine the concentration of the sample that exhibits antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Sample Collection

Fruits of Malatungaw were gathered from Sibuyan Island, Romblon. Plastic sacks were utilized as provisional containers for the fruit samples throughout the shipment phase. The fruit samples were rinsed with tap water as a means of removing potential contaminants that could compromise the integrity of the crude extract. The process involved pulverizing 350 grams of air-dried Malatungaw fruits for extraction.

Plant Authentication

The Malatungaw plant specimen was acquired from Sibuyan Island, Romblon. The sample was sent to the Department of Agriculture, Bureau of Plant Industry, Malate, Manila for species-level identification, authentication, and verification.

Extraction

The fruit was detached from its stem prior to extraction, followed by crushing to remove the pulp. The air-drying process was carried out for around one week until the fruit became brittle. The fruit of Malatungaw was extracted through the maceration process. To extract the compounds from Malatungaw fruit, 100 grams of its powder was soaked in 500 mL of 80% ethanol for 24 hours while shaking intermittently. The extract was filtered using Whatman filter paper No. 1. The drying process of the filtered extract was performed using a rotary evaporator under reduced pressure at 40°C and 50 rotations per minute. The extracts were stored in amber bottles and refrigerated prior to usage. To determine the yield percentage of the crude sample, the formula described by Boonsong et al. (2011) was utilized. The methodology involved the preparation of a stock solution of crude extracts at different concentrations for the purpose of conducting antimicrobial tests.

% yield (v/w) = volume of crude extract (mL)/weight of dry plant material (g) x 100

Phytochemical Screening

Using standard techniques, the freshly prepared crude extracts were subjected to chemical testing to identify several groups of chemical compounds included in the extracts. Numerous phytoconstituents, including tannin, flavonoids, terpenoids, alkaloids, steroids, glycosides, phenol, and saponin were identified in the extracts of the dry powdered fruit, according to Evans's Trease and Evans Pharmacognosy (2009).

Test for Saponins

To test for saponins, 3 mL of distilled water was added to the crude extract. The test tube containing the mixture was subjected to vigorous shaking for a duration of 15 minutes. The presence of saponins can be detected by observing the layer of foam.

Test for Alkaloids

The sample (15 mL) was subjected to evaporation to dryness at 55 °C, followed by dissolution of the residue in a 10% v/v hydrochloric acid solution (10 mL). The alkaloids were extracted using a precipitation method with 10 mL of a 10% v/v ammonia solution, followed by an ether extraction using 15 mL of ether. The ether is evaporated to dryness and then 1.5 mL of hydrochloric acid is introduced. Mayer's reagents were added to 0.5

mL of the acidic solution in the amount of two to three drops, resulting in the formation of an opalescence precipitate.

Test for Tannins
To prepare the sample, 50 mg of extract was mixed with 3 ml of a 10% lead acetate solution in distilled water. The methodology involved in determining the presence of tannins was based on the observation of a significant white precipitate formation.

Test for Steroids
The dry extract (100 mg) was dissolved in chloroform (2 mL). The lower layer was created by cautiously introducing sulfuric acid. The technique involves identifying the presence of a steroidal ring at the interface through the observation of a reddish-brown hue.

Test for Phenols
To analyze the sample's alcoholic solution, a mixture was prepared by adding two milliliters of distilled water and a few drops of a 10% aqueous ferric chloride solution to one milliliter of the solution. The approach used indicates that the observation of a blue or green hue is indicative of the presence of phenols.

Test for Terpenoid
Two milliliters of chloroform and one milliliter of saturated hydrochloric acid were added to one milligram of extract. A terpenoid can be identified by its characteristic reddish-brown color.

Test for Fixed Oils
Oil stains appear on the paper when a small amount of the dried extract is squeezed between two filter sheets, indicating the presence of fixed oils.

Test for Flavonoids
To perform the test for flavonoids, 0.5ml of the sample's alcoholic extract was mixed with diluted HCl (1%) and a small amount of Zn in a test tube. The resulting mixture was then heated for several minutes. Flavonoid presence is determined by observing the development of a pink or brown coloration.

Test for Glycosides
The sample alcoholic extract was dissolved in 1 mL of water, followed by the addition of sodium hydroxide. The methodology involves identifying the presence of glycosides through the observation of yellow color production.

Test for Anthocyanins
Anthocyanins were detected by combining 2 mL of plant extract with 2 mL of 2 N HCl. Anthocyanins were detected by observing a color change from pinkish-red to violet-blue upon the addition of ammonia.

Antimicrobial Assay

A. Preparation of Test Organisms
The bacterial strain was grown in 10 milliliters of tryptic soy broth (TSB) at a temperature of 35 degrees Celsius for a period of 24 hours.

B. Preparation of Antimicrobial Assay Plates
1. The culture was adjusted to a turbidity level equivalent to 0.5 McFarland Standard after being incubated overnight.
2. A sterile petri dish or plate of standard size was utilized to add 200 µL of the adjusted bacterial suspension.
3. Mueller-Hinton agar of 15 to 20 ml was added and stirred onto plates, which were then allowed to congeal and dry as per the manufacturer's instructions.
4. The plates were first incubated at 35°C for an hour prior to the addition of samples onto filter paper discs.

C. Addition of samples to filter paper discs (10-mm)
A volume of 100 µL of the liquid sample was transferred onto sterile filter paper discs measuring 10-mm. The discs were subsequently arranged on the assay plates prepared in the previous step.

D. Incubation and Interpretation of Results
1. The inverted plates were incubated at 35°C overnight.
2. Following incubation, the observation of zones of inhibition was conducted. The measurement of the zones of inhibition was conducted using a caliper. The aseptic lifting of the paper is performed to examine the area beneath the sample in the absence of any surrounding zones around the paper discs.

Reactivity Rating
0 - None (No detectable zone around or under specimen)
1 - Slight (Some malformed or degenerated cells under the specimen)
2- Mild (zone limited under the specimen)
3 - Moderate (zone extends 5 to 10 mm beyond specimen)
4 - Severe (zone extends greater than 10 mm beyond specimen)
Inhibitory Activity Rating: (+++) complete; (++) partial; (+) slight, and (-) negative

Statistical Analysis
The statistical approach employed in this study involved the use of one-way analysis of variance (ANOVA) to evaluate the means of inhibitory zone of M. malabathricum ethanol extract on pathogenic bacteria. Statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1: The weight and percentage yield of crude extracts from the samples of Malatungaw.

Plant Sample	Extract	Amount	% Yield (v/w)
Malatungaw Fruit	Ethanolic Crude Extract	130 mL	37.14
	Dried Samples	350 grams	

The percentage of crude ethanol extract yields computed based on the weight of dried and powdered fruit samples and the ethanolic crude extract is presented in Table 1. From the table, 37.14 % (v/w) was computed. The calculated percentage yield exceeds the study by Dharmaraj et al., 2015, in which the highest percentage yield for *M. malabathricum* leaf extracts from seven different locations was 18.29%. In contrast, Lestari et al. (2022) investigation of *M. malabathricum* leaves produced extraction yields ranging from 5.11 to 21.37 % under nine distinct extraction conditions. Consequently, the fruit extract yield obtained in this study is greater than the leaf extract yield observed in a previously mentioned study. Therefore, it may be concluded that the fruits (pulp, seed) of *M. malabathricum* contained a higher concentration of active ingredients.

Table 2: Phytochemical Screening Results of Malatungaw Fruit Ethanolic Extract.

Active Compound	Result
Anthocyanins	+
Saponins	-
Alkaloids	+
Tannins	+
Steroids	-
Phenols	-
Terpenoids	-
Flavonoids	+
Glycosides	-
Fixed Oils	-

In this study, the ethanolic extract of Malatungaw fruit contained phytochemicals such as anthocyanins, flavonoids, alkaloids, and tannins, as indicated in Table 2. In the study of Sari et al. (2018), the ethanolic extract of *M. malabathricum* fruit contains alkaloids, flavonoids, saponin, tannins, triterpenoids, and carbohydrates. However, the findings of this study were not similar to those found in the literature as saponin, triterpenoids, and carbohydrates were not present in the phytochemical analysis of this study.

Table 3: Zone of Inhibition Mean Score for *Pseudomonas aeruginosa* per Treatment.

Treatment	Mean	N	SD
A	10.00	3	0.00
B	10.00	3	0.00
C	10.00	3	0.00
D	10.00	3	0.00
E	10.00	3	0.00
F	37.09	3	1.69
G	26.63	3	0.50
H	0.00	3	0.00

Legend: *Treatment A= 7.5ml extract dissolved in 15 ml water solvent | Treatment B= 15 ml extract dissolved in 15 ml water solvent | Treatment C= 30 ml extract dissolved in 15 ml water solvent | Treatment D= 45 ml extract dissolved in 15 ml water solvent | Treatment E= Melastoma malabathricum Extract E (10 mm), F= 10 mm Ciprofloxacin | Treatment G= Positive Control Amikacin 30 ug (6 mm) | Treatment H= Negative Control Sample- free disc (10 mm)*

The table above shows the mean Zone of Inhibition Mean Score for *Pseudomonas aeruginosa* per Treatment. Each treatment has three trials. The zone of inhibition is indicated by the creation of a clear zone surrounding the well, indicating the absence of bacterial growth.

For Treatment A, the Zone of Inhibition mean score is 10.00. For Treatment B, the zone of Inhibition mean is 10.00. For Treatment C, the zone of Inhibition mean is 10.00. For Treatment D, the zone of Inhibition mean is 10.00. For Treatment E, the zone of Inhibition mean is 10.00. For Treatment F, the zone of Inhibition mean is 37.09. For Treatment G, the zone of Inhibition mean is 26.63. For Treatment H, the zone of Inhibition mean is 0.00.

The present study's results are similar to Elmido, C., Balangcod, K., and Balangcod, T.'s (2018) investigation on the antibacterial efficacy of plant extracts against

Pseudomonas aeruginosa. This paper reports on the consistent measurement of 10 mm antibacterial activity of Melastoma malabathricum leaves. The present study reveals a larger zone of inhibition as compared to the study conducted by Apridamayanti et al (2021). The inhibitory effects of *Melastoma malabathricum* leaf ethanol extract against *P. aeruginosa* were investigated in a related study. Results showed that at a dosage of 6.25 mg/mL, the extract had an inhibitory zone diameter of 6.40 ± 0.26, while at a dosage of 12.5 mg/mL, it had a maximum inhibitory zone diameter of 10.40 ± 0.36 mm.

Table 4: Zone of Inhibition Mean Score for *Staphylococcus aureus* per Treatment.

Treatment	Mean	N	SD
A	10.90	3	0.72
B	11.14	3	0.15
C	12.35	3	0.39
D	11.81	3	0.58
E	13.68	3	0.45
F	31.24	3	0.36
G	18.32	3	0.94
H	0.00	3	0.00
Legend: *Treatment A= 7.5ml extract dissolved in 15 ml water solvent | Treatment B= 15 ml extract dissolved in 15 ml water solvent | Treatment C= 30 ml extract dissolved in 15 ml water solvent | Treatment D= 45 ml extract dissolved in 15 ml water solvent | Treatment E= Melastoma malabathricum Extract E (10 mm), F= 10 mm Ciprofloxacin | Treatment G= Positive Control Oxacillin 1 ug (6 mm) | Treatment H= Negative Control Sample- free disc (10 mm)*			

The average Zone of Inhibition Mean Score for *Staphylococcus aureus* per Treatment is shown in the table above. Each treatment undergoes three trials. The zone of inhibition is denoted by the formation of a clear zone around the well, which indicates the absence of bacterial growth.

For Treatment A, the zone of inhibition mean score is 10.90. For Treatment B, the zone of inhibition mean is 11.14. For Treatment C, the zone of inhibition mean is 12.35. For Treatment D, the zone of inhibition mean is 11.81. For Treatment E, the zone of inhibition mean is 13.68. For Treatment F, the zone of inhibition mean is 31.24. For Treatment G, the zone of inhibition mean is 18.32. For Treatment H, the zone of inhibition mean is 0.00.

Results showed that Treatment A, B, C, D, and E displayed complete inhibitory activity and slight reactivity, evidenced by the presence of malformed cells in the test specimen. On the other hand, Treatment F and G exhibited complete inhibitory activity and severe reactivity, as indicated by the zone extending beyond 10 mm from the specimen. On the other hand, Treatment H did not produce any visible or detectable zone around or under the specimen.

The present study's zone of inhibition surpasses that of Apridamayanti et al's (2021) findings. The present study reports the diameter of the inhibition zone for *Melastoma malabathricum* leaf ethanol extract against *S. aureus*. At a concentration of 6.25 mg/mL, the inhibition zone diameter was found to be 7.23±0.275 mm, while the maximum diameter of the inhibition zone was observed to be 10.77±0.236 mm at a dose of 25 mg/mL.

Table 5: Test for Significant Difference for *Pseudomonas aeruginosa* Among Treatments.

Compared Group		p-value	Significance	Ho Decision
A	B	1.000	Not Significant	Accept
	C	1.000	Not Significant	Accept
	D	1.000	Not Significant	Accept
	E	1.000	Not Significant	Accept
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
B	C	1.000	Not Significant	Accept
	D	1.000	Not Significant	Accept
	E	1.000	Not Significant	Accept
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
C	D	1.000	Not Significant	Accept

	E	1.000	Not Significant	Accept
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
D	E	1.000	Not Significant	Accept
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
E	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
F	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
G	H	0.000	Significant	Reject
Legend: Treatment A= Approx 20ml; 7.5ml extract dissolved in 15 ml water solvent | Treatment B= Approx 20ml; 15 ml extract dissolved in 15 ml water solvent | Treatment C= Approx 20ml; 30 ml extract dissolved in 15 ml water solvent | Treatment D= Approx 20ml; 45 ml extract dissolved in 15 ml water solvent | Treatment E= Melastoma malabathricum Extract E (10 mm), F= 10 mm Ciprofloxacin | Treatment G= Positive Control Amikacin 30 ug (6 mm) | Treatment H= Negative Control Sample- free disc (10 mm)				
**Significant at .05 alpha level*				

The table above shows the Test for Significant Differences for *Pseudomonas aeruginosa* among treatments. This would mean that each treatment is compared with the rest of the treatments in this study.

The obtained p-value of 1.00 suggests that there is no statistically significant difference between Treatment A and Treatments B, C, D, and E at the 0.05 alpha level. The results obtained indicate that there is no significant difference between the variables, thereby supporting the null hypothesis. The results indicate that there was no significant difference observed in the inhibitory zone among Treatment A, B, C, D, and E.

The calculated p-value of the comparison between Treatment A and Treatments F, G, and H is 0.000, which is below the predetermined alpha level of 0.05. The rejection of the null hypothesis is based on the significant difference observed. The findings suggest that Ciprofloxacin exhibits greater inhibitory zone compared to *Melastoma malabathricum* extract at a concentration of 7.5ml extract dissolved in 15 ml water solvent. In terms of the inhibitory zone, the positive control of amikacin at 30 micrograms (6 mm) outperforms the *Melastoma malabathricum* extract at 7.5ml extract diluted in 15 ml water solvent and the Negative Control (no treatment).

The calculated p-value for the comparison between Treatment B and Treatments C, D, and E is 1.00, which exceeds the predetermined alpha level of 0.05. This finding suggests that there is no statistically significant distinction, and therefore, the null hypothesis is upheld. Hence, the inhibition zone among Treatments B, C, D, and E exhibits no significant difference.

The calculated p-value of the comparison between Treatment B and Treatments F, G, and H is 0.000, which is below the predetermined alpha level of 0.05. This finding suggests a notable distinction, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, it has been observed that Ciprofloxacin outperforms *Melastoma malabathricum* extract when dissolved in a 15 ml water solvent at a concentration of 15 ml. In comparison to *Melastoma malabathricum* extract dissolved in a water solvent of 15 ml, the Positive Control Amikacin 30 ug (6 mm) exhibits superiority. The superiority of *Melastoma malabathricum* extract dissolved in 15 ml of water over Negative Control (no treatment) is evident.

The obtained p-value for the comparison between Treatment C and Treatment D as well as Treatment E is 1.00, exceeding the predetermined alpha level of 0.05. This finding suggests the absence of a noteworthy difference and supports the null hypothesis. Therefore, the inhibitory zone does not exhibit a significant difference among Treatment C, Treatment D, and Treatment E.

The calculated p-value of 0.000 for Treatment C in comparison to Treatment F, G, and H is below the alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, it has been observed that Ciprofloxacin outperforms *Melastoma malabathricum* extract when 30 ml of the extract is dissolved in 15 ml of water solvent. The inhibitory zone analysis revealed that Amikacin at 30 micrograms (6 mm) exhibited greater efficacy as the positive control compared to *Melastoma malabathricum* extract at 30 ml extract diluted in 15 ml water solvent. Furthermore. The superiority of *Melastoma malabathricum* extract diluted

in 15 ml of water over the negative control (no treatment) has been demonstrated.

The p-value calculated for the comparison between Treatment D and Treatment E is 1.00, exceeding the predetermined alpha level of 0.05. This finding suggests that there is no statistically significant difference, thereby providing support for the null hypothesis. Hence, the inhibitory zone does not exhibit a noteworthy distinction between Treatment D and Treatment E.

The calculated p-value of the comparison between Treatment D and Treatments F, G, and H is 0.000, which is below the predetermined alpha level of 0.05. This finding suggests the presence of a noteworthy disparity, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, it has been observed that Ciprofloxacin exhibits greater efficacy compared to *Melastoma malabathricum* extract when the latter is dissolved in a water solvent at a concentration of 45 ml extract per 15 ml solvent. The inhibitory zone analysis revealed that 30 micrograms of amikacin exhibited greater efficacy as a positive control compared to 45 milliliters of *Melastoma malabathricum* extract dissolved in 15 milliliters of water solvent. The superiority of the *Melastoma malabathricum* extract at 45 ml extract diluted in 15 ml water solvent over the negative control (no treatment) can be inferred.

The calculated p-value of Treatment E compared to Treatment F, G, and H is 0.000, indicating statistical significance at an alpha level of 0.05. This finding suggests the presence of a noteworthy disparity, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, ciprofloxacin exhibits superiority over 100% pure *Melastoma malabathricum* extract as a positive control. In terms of the inhibitory zone, it has been observed that amikacin at 30 ug (6 mm) exhibits better positive control than *Melastoma malabathricum* extract at 100%. The superiority of 100% *Melastoma malabathricum* extract over the negative control (no treatment) has been observed.

In comparing Treatment F to Treatment G, the obtained p-value of 0.000 is below the predetermined alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. The inhibitory zone analysis indicates that Ciprofloxacin outperforms the Positive Control Amikacin 30 ug (6 mm).

The computed p-value for Treatment F versus Treatment H is statistically significant at the alpha level of 0.05, with a value of 0.000. The rejection of the null hypothesis is based on the observed significant difference. Therefore, Ciprofloxacin is a more favorable option compared to the Negative Control (absence of treatment).

The p-value calculated for the comparison between Treatment G and Treatment H is 0.000, indicating statistical significance at the alpha level of 0.05. The rejection of the null hypothesis is based on the significant difference observed. The superiority of Positive Control Amikacin 30 ug (6 mm) over Negative Control (no treatment) has been established.

Table 6: Test for Significant Difference for *Staphylococcus aureus* Among Treatments.

Compared Group		p-value	Significance	Ho Decision
A	B	0.604	Not Significant	Accept
	C	0.004	Significant	Reject
	D	0.061	Not Significant	Accept
	E	0.000	Significant	Reject
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
B	C	0.015	Significant	Reject
	D	0.165	Not Significant	Accept
	E	0.000	Significant	Reject
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
C	D	0.263	Not Significant	Accept
	E	0.008	Significant	Reject
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
D	E	0.000	Significant	Reject
	F	0.000	Significant	Reject
	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
E	F	0.000	Significant	Reject

	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
F	G	0.000	Significant	Reject
	H	0.000	Significant	Reject
G	H	0.000	Significant	Reject
Legend: Treatment A= Approx 20ml; 7.5ml extract dissolved in 15 ml water solvent | Treatment B= Approx 20ml; 15 ml extract dissolved in 15 ml water solvent | Treatment C= Approx 20ml; 30 ml extract dissolved in 15 ml water solvent | Treatment D= Approx 20ml; 45 ml extract dissolved in 15 ml water solvent | Treatment E= Melastoma malabathricum Extract E (10 mm), F= 10 mm Ciprofloxacin | Treatment G= Positive Control Oxacillin 1 ug (6 mm) | Treatment H= Negative Control Sample- free disc (10 mm)				
**Significant at .05 alpha level*				

The preceding table shows the Test for Significant Differences for Staphylococcus aureus Among Treatments. This observation suggests that each treatment in the present study is being evaluated in comparison to the other treatments.

The calculated p-value for Treatment A compared to Treatment B is 0.604, exceeding the alpha level of .05. This finding would suggest that there is no statistically significant difference and support the null hypothesis. Hence, the inhibitory zone does not exhibit a noteworthy contrast between Treatment A and Treatment B.

The obtained p-value of .004 for the comparison between Treatment A and Treatment C is statistically significant at the alpha level of .05. This finding suggests the presence of a noteworthy disparity, leading to the rejection of the null hypothesis. The findings indicate that *Melastoma malabathricum* extract dissolved in a 30 ml extract to 15 ml water solvent ratio exhibits greater inhibitory zone compared to *Melastoma malabathricum* extract dissolved in a 7.5 ml extract to 15 ml water solvent ratio.

The p-value obtained for the comparison between Treatment A and Treatment D was 0.061, exceeding the predetermined alpha level of 0.05. This finding suggests that there is no statistically significant difference, thereby supporting the null hypothesis. Hence, the inhibitory zone does not exhibit a noteworthy distinction between Treatment A and Treatment D.

Comparing Treatment A to Treatment E, F, and G the calculated p-value is 0.000, which is less than the alpha level of 0.05. This finding suggests the presence of a notable discrepancy, leading to the rejection of the null hypothesis. In relation to the inhibitory zone, it has been observed that the effectiveness of 100% *Melastoma malabathricum* extract surpasses that of a diluted extract consisting of 7.5ml extract and 15ml water solvent. In relation to the inhibitory zone, it has been found that Ciprofloxacin outperforms *Melastoma malabathricum* extract when dissolved in a 15 ml water solvent at a concentration of 7.5ml. In terms of the inhibitory zone, the Oxacillin 1 ug (6 mm) positive control outperforms the *Melastoma malabathricum* extract 7.5 ml extract diluted in 15 ml water solvent.

The computed p-value for Treatment A compared to Treatment H is less than the predetermined alpha level of 0.05, with a value of 0.000. The rejection of the null hypothesis is based on the significant difference observed. The superiority of *Melastoma malabathricum* extract over Negative Control (no treatment) was observed when 7.5ml of the extract was diluted in 15 ml of water solvent.

In the comparison between Treatment B and Treatment C, the obtained p-value of 0.015 is below the predetermined alpha level of 0.05. The rejection of the null hypothesis suggests the presence of a notable difference. The findings indicate that *Melastoma malabathricum* extract at a concentration of 30 ml extract dissolved in 15 ml exhibits greater zone of inhibition compared to *Melastoma malabathricum* extract at a concentration of 15 ml extract dissolved in 15 ml.

The p-value obtained for the comparison between Treatment B and Treatment D was 0.165, exceeding the predetermined alpha level of .05. This finding suggests that there is no statistically significant difference, which lends support to the null hypothesis. Hence, the inhibitory zone does not exhibit a noteworthy contrast between Treatment B and Treatment D.

The calculated p-value of 0.000 for Treatment B in comparison to Treatment E, F, G, and H is below the alpha level of 0.05. This finding suggests the presence of a noteworthy disparity, leading to the rejection of the null hypothesis. The superior zone of inhibition was observed in 100% *Melastoma malabathricum* extract as compared to *Melastoma malabathricum* extract dissolved in 15 ml. In terms of the inhibitory zone, it has been observed that Ciprofloxacin outperforms *Melastoma malabathricum* extract dissolved in 15 ml of water. The inhibitory zone of Positive Control Oxacillin 1 ug (6 mm) is superior to *Melastoma malabathricum* extract at 15 ml extract dissolved in 15 ml water solvent. In conclusion, the 15 ml *Melastoma malabathricum* extract diluted in 15 ml water solvent exhibits superiority over the Negative Control (absence of treatment).

The calculated p-value for the comparison between Treatment C and Treatment D is not statistically significant, as it is greater than the predetermined alpha

level of 0.05. This finding suggests that there is no statistically significant difference, thereby providing support for the null hypothesis. Hence, the inhibitory zone does not exhibit a noteworthy distinction between Treatment C and Treatment D.

The obtained p-value of 0.008 for the comparison between Treatment C and Treatment E is below the predetermined alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. In relation to the inhibitory zone, it can be concluded that the efficacy of 100% *Melastoma malabathricum* extract surpasses that of *Melastoma malabathricum* extract dissolved in 15 ml of water.

The obtained p-value of 0.000 for the comparison between Treatment C and Treatments F, G, and H is below the predetermined alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, it has been found that Ciprofloxacin outperforms *Melastoma malabathricum* extract when 30 ml of the extract is dissolved in a 15 ml water solvent. In terms of the inhibitory zone, it has been determined that Positive Control Oxacillin 1 ug (6 mm) outperforms *Melastoma malabathricum* extract at 30 ml extract dissolved in 15 ml water solvent. The superiority of *Melastoma malabathricum* extract over Negative Control (no treatment) can be inferred, specifically when 30 ml extract is dissolved in 15 ml water solvent.

The calculated p-value for the comparison of Treatment D with Treatments E, F, G, and H is 0.000, which is below the alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. The findings indicate that the 100% *Melastoma malabathricum* extract exhibits greater zone of inhibition compared to the *Melastoma malabathricum* extract dissolved in a 45 ml extract and 15 ml water solvent. In terms of the inhibitory zone, it has been observed that Ciprofloxacin outperforms *Melastoma malabathricum* extract when dissolved in a 15 ml water solvent at a concentration of 45 ml extract. This finding is noteworthy. The comparative analysis of the zone of inhibition between the positive control and *Melastoma malabathricum* extract diluted in water revealed that 1 mg of oxacillin exhibits greater efficacy than 45 ml of the aforementioned extract. The superiority of *Melastoma malabathricum* extract mixed in 15 ml of solvent water over Negative Control (no treatment) has been demonstrated.

The calculated p-value of Treatment E in comparison to Treatment F, G, and H is 0.000, indicating statistical significance at an alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, the effectiveness of Ciprofloxacin surpasses that of 100% *Melastoma malabathricum* extract. The inhibitory zone of Positive Control Oxacillin 1 ug (6 mm) is superior to that of 100% *Melastoma malabathricum* extract. In conclusion, the inhibition zone of 100% *Melastoma malabathricum* extract is superior to that of the Negative Control (no treatment).

In comparing Treatment F to Treatment G and H, the resulting p-value of 0.000 is below the predetermined alpha level of 0.05. This finding suggests the presence of a notable disparity, leading to the rejection of the null hypothesis. In terms of the inhibitory zone, it can be concluded that Ciprofloxacin outperforms the Positive Control Oxacillin 1 ug (6 mm) and Negative Control.

The obtained p-value of 0.000 for the comparison between Treatment G and Treatment H is lower than the predetermined alpha level of 0.05. This finding suggests the presence of a noteworthy disparity, leading to the rejection of the null hypothesis. The superiority of Positive Control Oxacillin 1 ug (6 mm) over Negative Control Oxacillin 1 ug (6 mm) (no treatment) has been demonstrated.

Table 7: Test for Significant Difference Between *Pseudomonas aeruginosa* and *Staphylococcus aureus* per Treatment.

Pseudomonas aeruginosa	***Staphylococcus aureus***	**p-value**	**Significance**	**Ho Decision**
A	A	0.066	Not Significant	Accept
B	B	0.021	Significant	Reject
C	C	0.000	Significant	Reject
D	D	0.001	Significant	Reject
E	E	0.001	Significant	Reject
F	F	0.000	Significant	Reject
G	G	0.000	Significant	Reject
Legend: *Treatment A= Approx 20ml; 7.5ml extract dissolved in 15 ml water solvent | Treatment B= Approx 20ml; 15 ml extract dissolved in 15 ml water solvent | Treatment C= Approx 20ml; 30 ml extract dissolved in 15 ml water solvent | Treatment D= Approx 20ml; 45 ml extract dissolved in 15 ml water solvent | Treatment E= Melastoma malabathricum Extract E (10 mm), F= 10 mm Ciprofloxacin | Treatment G = Negative Control Sample- free disc (10 mm)*				
****Significant at .05 alpha level***				

The table above shows the Test for Significant Difference Between *Pseudomonas aeruginosa* and *Staphylococcus aureus* per Treatment.

The computed p-value for the comparison of *Pseudomonas aeruginosa* and *Staphylococcus aureus* using Treatment A, which is *Melastoma malabathricum* extract at 7.5ml dissolved in 15 ml water, is 0.066. This value is greater than the alpha level of .05. This finding suggests that there is no significant distinction, which provides support for the null hypothesis. The statistical analysis indicates that despite a variation in the zone of inhibition, the difference is not considered significant.

The computed p-value for the comparison of *Pseudomonas aeruginosa* and *Staphylococcus aureus* using Treatment B, which involves the use of *Melastoma malabathricum* extract dissolved in 15 ml of water, is 0.021. This value is lower than the specified alpha level of 0.05. The rejection of the null hypothesis is based on the significant difference observed. The efficacy of *Melastoma malabathricum* extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was compared in terms of zone of inhibition. Results showed that a 15 ml dilution of the extract in water was more effective against *Staphylococcus aureus* than *Pseudomonas aeruginosa.*

The study found that Treatment C, consisting of *Melastoma malabathricum* extract at 30 ml dissolved in 15 ml water solvent, resulted in a computed p-value of 0.000 when comparing the effectiveness against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This value is below the alpha level of 0.05. This finding suggests the presence of a notable discrepancy, leading to the rejection of the null hypothesis. The study reveals that *Melastoma malabathricum* extract diluted in water at a concentration of 30 ml/15 ml exhibits a greater zone of inhibition against *Staphylococcus aureus* compared to *Pseudomonas aeruginosa*.

The paper reveals that Treatment D, which involves the use of *Melastoma malabathricum* extract at 45 ml dissolved in 15 ml water, resulted in a computed p-value of 0.001 for the comparison between *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This value is lower than the predetermined alpha level of 0.05. This finding suggests the presence of a notable distinction, leading to the rejection of the null hypothesis. Thus, the study reveals that *Melastoma malabathricum* extract, when diluted in 15 ml water at a concentration of 45 ml, exhibits a higher degree of effectiveness against *Staphylococcus aureus* as compared to *Pseudomonas aeruginosa*, as evidenced by the zone of inhibition.

The computed p-value of 0.001 for the comparison between *Pseudomonas aeruginosa and Staphylococcus aureus* using *Melastoma malabathricum* Extract E (10 mm) is below the established alpha level of 0.05. This finding suggests a noteworthy disparity, leading to the rejection of the null hypothesis. In this dissertation, the effectiveness of 100% *Melastoma malabathricum* Extract (10 mm) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* is analyzed based on the zone of inhibition. The results indicate that the extract is significantly more effective against *Staphylococcus aureus* compared to *Pseudomonas aeruginosa*.

The paper reveals that Malatungaw fruit extracts were found to be more effective against Gram-positive bacteria Staphylococcus aureus as compared to Gram-negative bacteria E. coli, as per the Minimum Inhibitory Concentration (MIC) observed in disc diffusion experiments. Certain classes of flavonoid compounds exhibited greater efficacy in inhibiting the growth of Gram-positive bacteria in comparison to Gram-negative bacteria. This could be attribute to the concept of Gram-negative bacteria possess an outer membrane and a distinct periplasmic area, which is absent in Gram-positive bacteria. The role of lipopolysaccharide molecules located on the hydrophilic surface of the outer membrane of Gram-negative bacteria in impeding the entry of various antimicrobial agents may be a contributing factor to their resistance against antibacterial drugs. Basically, the lack of outer membrane and cell wall components in gram-positive bacteria renders them vulnerable to the effects of antibacterial drugs, which can readily disrupt their cell wall and cytoplasmic membrane, leading to cytoplasmic leakage (Gilmore & Denyer, 2023).

The p-value computed for the comparison between *Pseudomonas aeruginosa* and *Staphylococcus aureus* under Treatment F, with the positive control Ciprofloxacin, is less than the alpha level of 0.05, specifically 0.000. This finding suggests the presence of a notable discrepancy, leading to the rejection of the null hypothesis. Hence, Ciprofloxacin exhibits a higher level of effectiveness against *Pseudomonas aeruginosa* as compared to *Staphylococcus aureus*, as evidenced by the zone of inhibition.

Sharma et al. (2017) state that ciprofloxacin is a second-generation fluoroquinolone that exhibits a wide range of activity against both Gram-positive and Gram-negative bacteria. Second-generation quinolones exhibit efficacy against Pseudomonas species, specific Gram-positive bacteria like Staphylococcus aureus, and certain atypical pathogens, in contrast to their first-generation counterparts. Thai, Salisbury, and Zito (2021) reported that the inhibition of bacterial DNA topoisomerase and DNA-gyrase halts DNA replication. Ciprofloxacin, a fluoroquinolone, is the most effective treatment for gram-negative bacteria.

CONCLUSIONS

As a standard drug, ciprofloxacin demonstrated greater consistency in creating an inhibitory zone against *Staphylococcus aureus* than *Pseudomonas aeruginosa*. Malatungaw ethanolic fruit extract exhibits potential

antibacterial activity at concentrations tested against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Thus, this plant species could serve as a future resource for the development of alternative medications. The extract of Malatungaw is more effective against *Staphylococcus aureus* than *Pseudomonas aeruginosa*. The increasing concentration of Malatungaw ethanolic fruit extract had no effect on the *Pseudomonas aeruginosa* inhibition zone. Extract E, a 100% ethanolic Malatungaw fruit extract, is the most effective concentration of Malatungaw against *Staphylococcus aureus*. Positive control of oxacillin and amikacin works better than all concentrations of Malatungaw fruit ethanolic extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively.

Recommendation

The following recommendations are made in light of the conclusions:

1. Use different solvents or concentrations to obtain crude extracts from Malatungaw fruits to determine which solvent elicits the most antibacterial action against bacterial strains.
2. Undergo additional investigations, such as toxicity testing, chemical analysis, and drug formulation, on the crude extract from Malatungaw fruit.
3. Investigate further Malatungaw plant parts, particularly its flowers, and roots.
4. To study the antibacterial activity of the plant extract on various bacterial strains, further solvent extraction techniques and fractionates must be employed.
5. Perform antibacterial susceptibility testing in other pathogenic organisms such as a hospital- and community-acquired multidrug resistant organisms using the crude extract of Malatungaw fruits.

REFERENCES

1. Bayas, Q. E. B., Salvador, S. A. S. J., Ragragio, E. M., & Obico, J. J. A. Taxonomic survey of nickel hyperaccumulating plants in a Mining Site on Luzon Island, Philippines. *Philippine Journal of Systematic Biology*, 2018; *12*(1). https://doi.org/10.26757/pjsb.2018a12001
3. Elmido, C., Balangcod, K., & Balangcod, T. Inhibition of *Pseudomonas aeruginosa* by the extracts of indigenous plants from the Cordillera Region, Philippines. *Indian Journal of Traditional Knowledge*, 2018; *18*(1): 41-46.
4. Evans, W. C., Evans, D., & Trease, G. E. Trease and Evans pharmacognosy. Edinburgh: Saunders/Elsevier, 2009.
5. Farhadi, F., Khameneh, B., Iranshahi, M., & Iranshahy, M. *Antibacterial activity of flavonoids and their structure-activity relationship: An update review. Phytotherapy Research*, 2018. doi: 10.1002/ptr.6208.
6. Ferdous, N., Rahman, M., & Alamgir, A. N. M. Secondary metabolites and bioactivities of *Melastoma malabathricum* (L.) Smith: an anti-diarrheal plant of Bangladesh. *Chem J*, 2018; 1(3): 256-261.
7. Frieri, M., Kumar, K., & Boutin, A. Antibiotic resistance. Journal of Infection and Public Health, 2017; 10(4): 369–378. doi:10.1016/j.jiph.2016.08.007
8. Gilmore, B. F., & Denyer, S. P. (Eds.). *Hugo and Russell's Pharmaceutical Microbiology*. Wiley, 2023.
9. Górniak, I., Bartoszewski, R. & Króliczewski, J. *Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev.*, 2019; *18:* 241–272. doi: https://doi.org/10.1007/s11101-018-9591-z.
10. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *American Society for Microbiology*, 2016; 1-23. Retrieved from https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf
11. Hussein, R., & El-Anssary, A. (2019). *Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. Herbal Medicine.*
12. Khan, S., Imran, M., & Pindari N. Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant Candida spp. *Bioinformation*, 2017; *13*(6): 67–72. doi:10.6026/97320630013067
13. MacDougall, C., & Gallagher, J. C. *Antibiotics Simplified*. Jones & Bartlett Learning, 2022.
14. Mayers D.L., Lerner S.A., & Ouelette, M. *Antimicrobial Drug Resistance: Clinical and Epidemiological Aspects*. (2nd ed.). Springer Dordrecht Heidelberg; London, 2009; 2: 681–1347.
15. Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnology advances*, 2019; *37*(1): 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013
16. Pulhin, F. B., Torres, A. M., Pampolina, N. M., Lasco, R. D., & Alducente, A. M. (2020). Vegetation Analysis of Sanctuary and Forest Areas of Kalahan Forest Reserve Nueva Vizcaya and Pangasinan, Philippines. *Philippine Journal of Science*. https://philjournalsci.dost.gov.ph/.
17. Reduan, F. H., Shaari, R. M., Sayuti, N. S., Mustapha, N. M., Abu Bakar, M. Z., Sithambaram, S., & Hamzah, H. Acute and subacute dermal toxicity of Ethanolic extract of Melastoma malabathricum leaves in Sprague-Dawley rats. Toxicological Research, 2020; 36(3): 203–210. https://doi.org/10.1007/s43188-019-00013-5.
18. Suba, M., Arriola, A., & Alejandro, G. A checklist and conservation status of the medicinal plants of Mount Arayat National Park, Pampanga, Philippines. *Biodiversitas*, 2019; *20*(4): 1034-1041. doi: 10.13057/biodiv/d200414.

19. Sharma, D., Patel, R. P., Zaidi, S. T. R., Sarker, M. M. R., Lean, Q. Y., & Ming, L. C. Interplay of the Quality of Ciprofloxacin and Antibiotic Resistance in Developing Countries. Frontiers in Pharmacology, 2017; 8: doi:10.3389/fphar. 2017.00546.
20. Thai, T., Salisbury, B., & Zito, P. (2021). Ciprofloxacin. Retrieved from StatPearls: https://www.ncbi.nlm.nih.gov/books/NBK535454/.
21. Widowati, R., Handayani, S., & Al Fikr, A. R. Phytochemical Screening and Antibacterial Activities of Senggani (Melastoma malabathricum L.) Ethanolic Extract Leaves. *Jurnal Ilmu Pertanian Indonesia (JIPI)*, April 4, 2022; *27*(2). 10.18343/jipi.26.4.562
22. Wong, K., Ali, D., & Boey, P. Chemical constituents and antibacterial activity of *Melastoma malabathricum* L., *Natural Product Research*, 2012; 26:7, 609-618. DOI: 10.1080/14786419.2010.538395