Antimicrobial Susceptibility Test of Mangosteen Fruit Ethanol Extract (*Garcinia mangostana Linn)* Against *Escherichia coli* Bacteria

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Abstract

This study was conducted to determine the Antimicrobial Susceptibility Test (AST) of the Mangosteen fruit ethanol extract (*Garcinia mangostana Linn)* against Escherichia coli (E. coli) bacteria. The study used an experimental research design and made different concentrations of Mangosteen fruit ethanol extract, with concentrations consisting of: Tube 1, 100% (no distilled water), Tube 2, 75% (250 uL [0.25 mL] of distilled water), Tube 3, 50% (500 uL [0.5 mL] of distilled water), and Tube 4, 25% (750uL [0.75 mL] of distilled water). The positive control contains the antibiotic (Gentamicin), and negative control which is made of distilled water.

The researchers used a two-way analysis of variance (ANOVA) as statistical analysis. The computed P-value in treatments is <0.0001 that indicates that there is a significant difference in the zone of inhibition of E. coli against various concentrations of Mangosteen Fruit Ethanol Extract. While the computed P-value in trials is 1.0000 that indicates that there is no significant difference in the zone of inhibition of E. coli against various concentrations of Mangosteen Fruit Ethanol Extract. It is therefore concluded that the different concentrations of Mangosteen Fruit Ethanol Extract has no effect in inhibiting the growth of E. coli. Meanwhile when it comes to the effectiveness of concentrations (100%,75%,50%,25% of Mangosteen Fruit Ethanol Extract) in the inhibitory zone for antimicrobial E. coli, the result shows that *it is ≤ 12;* hence all of the concentration falls under Resistant category on the Kirby-Bauer Method

Keywords: Mangosteen fruit ethanol extraction; Gentamicin; Kirby-Bauer disk diffusion test; Escherichia coli; Zone of inhibition; Antimicrobial susceptibility testing.

1. Introduction

. Mangosteen juice has grown in popularity as a result of the high antioxidant content of Mangosteen fruit, which is regarded to provide health benefits and it contains secondary metabolites such as prenylated compounds and polyphenols. It has recently been noted that Mangosteen contains a plentiful source of a class of polyphenols known as xanthones. From ancient times, the fruit known as Mangosteen, or Garcinia mangostana, has also been utilized as an antibacterial agent. Although the pericarp of the Mangosteen has

been shown to have antibacterial properties, its impact on cariogenic organisms has not been studied. In order to better grasp the therapeutic potential of the Mangosteen pericarp's antibacterial impact on cariogenic bacteria, the current study set out to better comprehend this effect. (Janardhanan, S., Mahendra, J., Girija, A. S., Mahendra, L., & Priyadharsini, V. 2017)

In modern day, the southern United States' channel catfish (Ictalurus punctatus) industry suffers significantly from bacterial infections caused by the gram-negative bacterium *Edwardsiella ictaluri*, and columnaris disease, caused by the rod-shaped, gram-negative bacterium *Flavobacterium columnare*. Catfish farmers typically rely on commercial antibiotics and efficacious alternatives to the currently used antibiotics and chemicals that will tremendously help the catfish aquaculture industry. They studied ethyl acetate and methanol extracts of Mangosteen *Garcinia mangostana* fruit pericarp using bioassay-guided fractionation as part of our ongoing efforts in the hunt for such new chemicals. Any species of bacteria or fungus that may be important for the patient's therapy and whose treatment susceptibility may not be known is subjected to susceptibility testing. (Meepagala, K. M., & Schrader, K. K. 2018). Modern healthcare is under danger from infections brought on by germs resistant to antibiotics. Estimating their prevalence, side effects, and associated mortality is difficult. Due to the creation and spread of antibiotic resistance among bacterial infections, antibiotics frequently lose their effectiveness over time. Among the most common Gram-positive and Gram-negative species, such as *Staphylococcus aureus, Enterococcus spp., Pseudomonas aeruginosa*, and Acinetobacter spp., strains resistant to numerous antibiotic classes have arisen. Together with *Neisseria gonorrhoeae, Enterobacteriaceae*. (Prestinaci, F., Pezzotti, P., & Pantosti, A. 2015) Meanwhile, E. coli is becoming more resistant, as reported from all around the world. Both wealthy and developing nations are becoming more concerned about E. coli. Treatment of infections is made more difficult by an increase in bacterial resistance to antibiotics. In general, instances with severe symptoms are treated in up to 95% of cases without doing a bacteriological study. (MU Rasheed · 2014)

This has made it necessary to conduct a study and establish an environmentally safe, biodegradable, affordable and readily available Antimicrobial Susceptibility Test for one of the most resistant bacteria, reported around the world. Hence, we, the researchers, aim to determine the efficacy of Mangosteen Fruit Ethanol Extract *(Garcinia mangostana Linn)* extract as an organic antimicrobial susceptibility against *Escherichia coli* bacteria.

2. Methodology

2.1. Research design

The researchers used an experimental research design in evaluating the extract of Mangosteen fruit as an antimicrobial agent against *Escherichia coli*. To determine the susceptibility of the bacteria the Antimicrobial Susceptibility Testing is applied which is also known as Kirby-Bauer Diffusion Test. There will also be a positive control which consists of Mueller-Hinton Agar + antibiotic (gentamicin), and for the negative control Mueller-Hinton Agar + distilled water.

2.2. Sample concentration

The study uses a micro broth dilution to recognize the organic alternatives that can be used in eliminating pathogenic bacteria using Mangosteen *(Garcinia mangostana Linn)* fruit ethanol extract. In making the broth dilution, the Mangosteen fruit was dried in the oven before being blended and squeezed on a clean cloth to extract all of the fruit's extract. Each treatment's concentration is shown in the table.

Table 1. Mangosteen Fruit Ethanol Extract concentrations for micro broth dilution.

	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5
Label (%)	100%	75%	50%	25%	Bacterial suspension of E. Coli
Mangosteen Fruit (Garcinia Mangostana Linn) Extract (uL)	1000 uL (1 mL)	750 uL (0.75 mL)	500 uL (0.5 mL)	250 uL (0.25 mL)	None
Saline (uL)	None	250 uL (0.25 mL)	500 uL (0.5 mL)	750 uL (0.75 mL)	8 mL

After making the broth dilution, 20 pieces of Whatman paper number 1 is used and a hole is created using a sterile puncher, which is then placed in each tube with the Mangosteen fruit and saline solution. The filter paper is soaked for 24 hours (1 day) then it is dry pat.

Prepare the subculture of the E. coli and get an inoculum. A McFarland Standard (0.5) was used as a comparator to the bacterial suspension E. coli. Prepare the Mueller-Hinton Agar (MHA). Streak the bacteria coming from the bacterial suspension to the MHA. Then put the different concentration of the disc of the Mangosteen fruit ethanol extract to its specified treatments. For the plate of positive control, the MHA and gentamicin is used and for the plate of negative control, it is composed of MHA and distilled water.

Table 2. Number and composition of each plate

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Label	Contents	MHA Plates
Trial 1	Mangosteen Fruit Ethanol Extract, consisting of different concentrations (25%, 50%, 75%, 100%)	2 plates
Trial 2		2 plates
Trial 3		2 plates
Positive Control	Gentamicin	1 plate
Negative Control	Distilled water	1 plate
Total Number of plates		8 plates

2.3. Research instrument

*Garcinia mangostana Linn (*Mangosteen) fruit ethanol extract is the main focus of the study. This extract will be used as an antimicrobial agent against *Escherichia coli* Bacteria.

2.4. Locale of the study

The experimentation process will be carried out at PHINMA Saint Jude College laboratory under the Department of Medical Laboratory Science at Dimasalang corner Don Quijote Streets, Sampaloc, Manila, Philippines.

2.5. Ethical considerations

The researchers sent a letter of request signed by Ms. Snowie Balansag, Program Head of Medical Laboratory Science, and Mr. Jejomar Quiros, Dean of Allied Health at PHINMA Saint Jude College. The

letters were addressed to the Bureau of Plants and Industry for the identification and certification of the Mangosteen Fruit and to Ms. Sarah Forto, laboratory facilitator, for permission to use the PHINMA Saint Jude College laboratory and equipment for carrying out the experimentation

The researchers ensure that personal protective equipment was worn throughout the experimentation process. All sources such as literature and articles that were included in the related literature for this study was noted with proper citation. The research study was carried out in the laboratory of PHINMA Saint Jude College, at Dimasalang corner Don Quijote Streets, Sampaloc, Manila, Philippines applying the safety protocols in the laboratory. After the experiment, the researchers clean the equipment used and sanitize the working place before leaving the laboratory.

2.6. Research procedure

2.6.1. Research tools and materials

The tools that were used are autoclave, stirring glass, blender, petri dish, automatic pipette (1000µL), Erlenmeyer, measuring glass, beaker glass, incubator, analytical balance, tube rack, cotton swab, and spatula.

The ingredients are Mangosteen fruit, Gentamicin, distilled water and Mueller-Hinton Agar (MHA). The test bacteria that will be used is *Escherichia coli* obtained from a laboratory in Marikina.

2.6.2. Preparation of Mangosteen fruit ethanol extract

The Mangosteen fruit was placed inside the oven to dry the fruit before it was blended. A blender will be used to collect the extract from the Mangosteen fruit, by slicing it first and placing the fruit in the blender. After the extraction of the fruit, this will be filtered using a clean cloth to remove the pulps leaving only the extract, 2 mL of Mangosteen must be collected.

2.6.3. Preparation of bacterial suspension

An aliquot of 0.5 mL of a 0.048 mol/liter $BaCl_2$ (1.175% wt/vol $BaCl_2 \cdot 2H_2O$) to 99.5 mL of 0.18 mol/liter H_2SO_4 (1% vol/vol) is added with constant stirring for maintaining the suspension.

The 5 mL barium sulfate suspension is transferred into screw-cap tubes that are used in standardizing bacterial suspensions. The tightly sealed tubes were stored in the dark at room temperature.

2.6.4. Preparation of Mueller-Hinton agar

A 17.1 gram Mueller-Hinton Agar is added and mixed into a 450 mL of water. It is heated with frequent agitation in a hot plate at a high temperature and boiled for 1 minute for the components to dissolve. As the components dissolve, it is then autoclave at 121°C for 15 minutes, then the mixture is then poured in the plastic petri dish and allowed to solidify at room temperature and stored in the refrigerator.

2.7. Antimicrobial susceptibility test

A bacterial suspension is made where the bacterial isolates came from the MacConkey Agar (MAC). An MHA is prepared at a temperature of 35° C, which is still a liquid, then a 12 mL of the MHA is poured into a sterile petri dish, left to cool and solidify. Bacterial suspension *Escherichia coli* is spread with a cotton swab into the MHA and the different concentrations of Mangosteen fruit ethanol extract which are 25%, 50%, 75%, and 100% are planted into each media made, then incubated at 37°C for 24 hours.

Processing also includes the making of positive control that consist of MHA + antibiotic (gentamicin) and a negative control which is the MHA + distilled water. After incubation, the researchers observed the presence of the formed inhibition zone (mm) in the agar.

2.8. Data analysis

The 30th edition of CLSI's Performance Standards for Antimicrobial Susceptibility Testing was used by the researchers for the preparation of antibiotics in the experiment. Gentamicin will be used for the positive control and as for the negative control distilled water is the choice. The positive and negative controls are used as a comparator to determine the effectiveness of using Mangosteen fruit (*Garcinia mangostana Linn)* ethanol extract in different concentrations against *Escherichia coli* in terms of bacterial growth and its zone of inhibition. Category for interpretation of the results varies with the zone on inhibition of the fruit ethanol extract. Results of antimicrobial susceptibility testing shows if the bacteria is resistant (cannot be treated by fruit extract), intermediate (may be treated with drug, adjustment of concentration or dosage is require), susceptible (can be treated by fruit extract)

The ANOVA will be used to compare all the treatment with positive and negative control

3. Results and discussion

Table 1. Result of antibacterial activity test of Mangosteen (*Garcinia mangostana Linn)* fruit ethanol extract on *Escherichia coli* bacterial growth.

Treatment	Concentration	Trial 1	Trial 2	Trial 3	Average (mm)
1	100%	6 mm	6 mm	6 mm	6 mm
2	75%	6 mm	6 mm	6 mm	6 mm
3	50%	6 mm	6 mm	6 mm	6 mm
4	25%	6 mm	6 mm	6 mm	6 mm
Positive Control	Gentamicin	19 mm	19 mm	19 mm	19 mm
Negative Control	Distilled water	6 mm	6 mm	6 mm	6 mm

Based on the table 1 above it can be seen that all concentrations of ethanol extract of the Mangosteen (Garcinia mangostana Linn) cannot inhibit the growth of *Escherichia coli* bacteria by diffusion method on MHA that are planted for 24 hours

Table 2. This table displays the statistical analysis of antibacterial activities of experimental groups in various concentrations (100%, 75%, 50%, and 25%), positive control, and negative control using two-way analysis of variance.

Source of variance	% of Total variation	P value	Significant
Treatment	100.00	<0.0001	Yes
Trial	0.00	1.0000	No

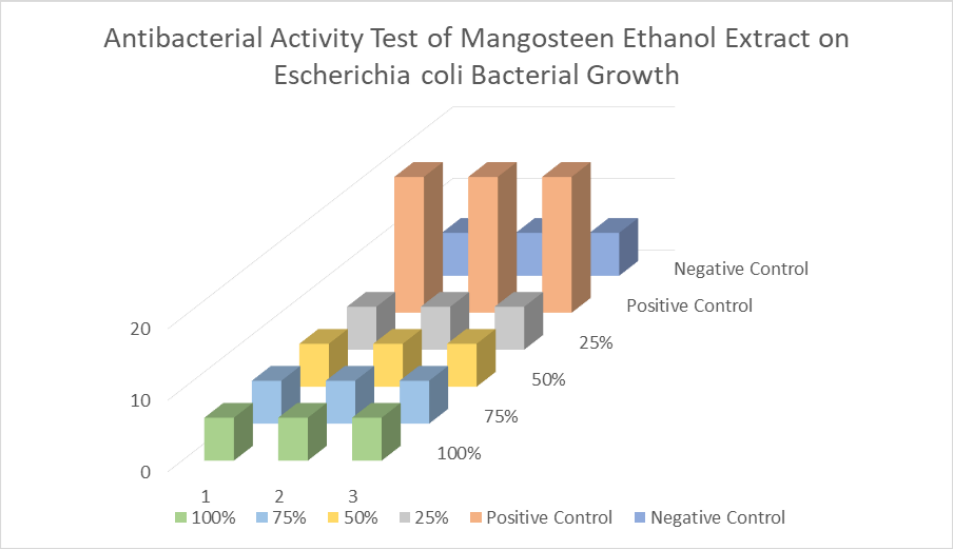
Table 2.1. P-value summary

Source of variance	P-value Summary	Significant
Treatment	SIGNIFICANT	Yes
Trial	NS	No

Table 2.2. Degree of freedom, sum of squares, and mean of square summary

Source of variance	DF	Sum of Squares	Mean Square
Treatment	5	420	85
Trial	2	0.00	0.00

Figure 1. Visual presentation of each treatments



The antibacterial activities of Mangosteen Fruit Ethanol Extract in various concentrations of 25%, 50%, 75%, and 100%. Gentamicin is used for positive control and distilled water for negative control. The Experiment has three trials with a mean of 6mm. The average for the positive control is 19mm. The computed P-value in ANOVA in treatments is 0.0001, indicating that there is a significant difference in different concentrations of Mangosteen (Garcinia mangostana Linn) fruit ethanol extract. The P-value in ANOVA in trials is greater than the confidence level of 0.05, indicating that there is no significant difference in the zone of inhibition of Escherichia coli against various concentrations of Mangosteen (Garcinia mangostana Linn) fruit ethanol extract.

As a result, there is no significant difference in the zone of inhibition between the antimicrobial components of extracted Mangosteen Fruit Ethanol Extract and Escherichia coli.

4. Conclusion

The computed P-value in ANOVA in treatments is <0.0001 which indicates that there is a significant difference in the zone of inhibition of *Escherichia coli* against various concentrations of Mangosteen (*Garcinia mangostana Linn)* fruit ethanol extract.

While the computed P-value in ANOVA in trials is 1.0000 which indicates that there is no significant

difference in the zone of inhibition of *Escherichia coli* against various concentrations of Mangosteen (*Garcinia mangostana Linn)* fruit ethanol extract.

Based on the data and results gathered, it can be therefore concluded that the different concentrations of Mangosteen (*Garcinia mangostana Linn)* fruit ethanol extract has no effect in inhibiting the growth of *Escherichia coli.* Meanwhile when it comes to the effectiveness of concentrations (100%, 75%, 50%, 25% of Mangosteen fruit ethanol extract*)* in the inhibitory zone for antimicrobial *Escherichia coli,* the result shows that *it is* $\leq$ *12; hence* all of the concentration falls under resistant category on the Kirby-Bauer Method.

Therefore, the null hypothesis is rejected based on the inhibited zoned concentrations that fall under resistance.

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