Antibacterial Activity of Acacia (*Samanea Saman*) Bark Extract Against *Escherichia Coli*

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Abstract. The World Health Organisation's Global Antimicrobial Surveillance System (GLASS) reported widespread antibiotic resistance among 500,000 people suspected of having bacterial infections, underscoring the need to identify a potent drug that can mitigate the health and economic havoc this crisis may cause. Tapping into phytochemicals has been a prospective solution for this global health concern. A sample plant, *Samanea saman*, known as Acacia in the Philippines, has a medicinal reputation for treating diarrhoea, tuberculosis, and stomach cancer, among other conditions, documented in various countries. Previous studies investigated its antibacterial activity. However, these studies were limited to fractionation rather than concentration, and the antibacterial assay used in susceptibility testing. The study used a true-experimental quantitative design to investigate the antibacterial properties of *S. saman* bark extract against *Escherichia coli*. Phytochemical screening of the extract revealed the presence of essential oil, steroids, coumarins, anthrones, phenols, tannins, and flavonoids. Antibacterial susceptibility testing revealed mean zones of inhibition of 13.17 mm, 13.73 mm, 11.58 mm, and 9.92 mm for 25%, 50%, 75%, and 100% concentrations of *S. saman* extract, respectively, on E. coli. Significant differences in the mean zones of inhibition were observed across concentrations and the positive control, Gentamicin, indicating that lower concentrations corresponded to wider zones of inhibition. This study proposes mechanisms by which *S. saman* bark extract can yield greater antibacterial susceptibility.

Keywords: Acacia; Phytochemicals; Antibacterial activity.

INTRODUCTION

The Global Antimicrobial Surveillance System of the World Health Organisation reported the widespread occurrence of antibiotic resistance among 500,000 people suspected of having bacterial infections across 22 countries. The World Health Organisation [1] reported that *Escherichia coli* is among the most commonly antibiotic-resistant bacteria. Due to misuse of antibiotics and the novel resistance mechanisms of pathogenic bacteria to the available antibiotics at present creating health and economic havoc like prolonged hospital stays therefore, higher medical costs and increased mortality for this manner, an urgent call for a change in the way professionals prescribe medicines, as well as for the public who use it, and the search for a potent drug that will mitigate the growing public health concern as posited [2]. In Metro Manila, Philippines, blood

culture confirmed bacterial bloodstream infection in 77 (5.9%) of 1,315 hospitalised patients with clinical suspicion of infection. Among those with confirmed infections, approximately 15.6% died, and one of the pathogens identified was *E.coli* as reported [3]. Phytochemicals like alkaloids, flavonoids, terpenoids, saponins, phytosterols, tannins, glycosides and many more produced mainly by plants possesses biological activity which are of great importance in pharmaceutical industry stated by [4] and has been the prospective solution to many health concerns for these substances can play a wide array of specific purposes in human health like anti-inflammatory, anti-allergic, antioxidants, antibacterial, antifungal, antispasmodic, chemo preventive, and hepato-protective are some examples in reference [5]. With this, a plant named *Samanea saman*, locally known as Acacia in the Philippines, has a documented medicinal history of treating diarrhoea, tuberculosis, and stomach cancer, among others, in various countries.

E. coli causes life-threatening bacterial infections that can be found in pumps and water wells, which are primary water sources for locals across Nueva Ecija, especially in far-flung areas. These bacterial infections are difficult to treat with available antibiotics, and the cost of treating them is high. As a result, locals can now opt for readily available alternative medicines, as the region is endowed with Acacia trees. Ultimately, this investigation paved the way for the possible use of Acacia bark extract to help alleviate the high medication costs faced by locals suffering from E. coli-related infections.

This study investigated the antibacterial activity of Acacia (*S. saman*) bark against E. coli. Specifically, it seeks to answer the following questions:

1) What are the phytochemical constituents of Acacia (*S. saman*) bark?

2) What is the zone of Inhibition of Acacia (*S. saman*) bark extract on *Escherichia coli* at different concentrations (25%, 50%, 75%, and 100%), and Gentamicin?

3) Is there a significant difference between the zone of Inhibition of Acacia (*S. saman*) bark extract on E. coli at different concentrations, Gentamicin and ethanol on *E. coli*?

a) Acacia bark extract at different concentrations and fentamicin on *E.coli*;

b) Acacia bark extract at different concentrations and ethanol on *E.coli*; and

c) Gentamicin and ethanol on *E.coli*

4) Is there a significant relationship between different concentrations of Acacia (*S.saman*) bark extract and the Zone of Inhibition?

Statement of Hypothesis. There is no significant difference in the zone of inhibition of: 1) Acacia bark extract at different concentrations and Gentamicin; 2) Acacia bark extract at different concentrations and ethanol on *E. coli*; 3) Gentamicin and ethanol on *E. coli.*

METHODS

Research Design. This quantitative study used a true-experimental research design to determine whether a significant difference exists in the zone of inhibition between plant extracts at various concentrations and the commercial antibiotic disc against the test bacterium *Escherichia coli*, and to test the relationship between concentration and zone of inhibition. The zone of inhibition was interpreted according to the Clinical & Laboratory Standards Institute guidelines for drug susceptibility, yielding Susceptible (S), Intermediate (I), and Resistant (R) results.

Sample Collection and Preparation of the Plant Material. The plant material was identified and validated by a taxonomist from the Department of Biological Sciences in Central Luzon State University on September 25, 2022. The researchers sent the voucher specimen for examination before the analysis. They collected acacia bark in Laur, Nueva Ecija, on February 17, 2023. The plant material was collected, thoroughly washed with distilled water, cut into small pieces, and ground to a powder using a mortar and pestle [6]. Finally, the sample was air-dried for 3 days and stored in a tightly sealed vessel in a cool, dark place before experimentation, as described in the study [7].

Solvent. The sample was treated with 80% ethanol as a solvent. A review by Abubakar and Haque [8], the factors to consider in choosing solvents and their rationale were as follows:

a) Selectivity is the ability of a solvent to extract active constituents while leaving inert material.

b) A solvent should be Safe, nontoxic and nonflammable. Ethanol is self-preservative at concentrations above 20% and nontoxic at low con-

centrations. It is also a good solvent for polyphenol extraction and is safe for human consumption.

c) The cost of the solvent must be cheap. In this case, ethanol is relatively cheaper compared to other solvents.

d) When it comes to Reactivity, an appropriate solvent for extraction should not react with the extract.

e) As for the Recovery, the solvent to be used should be readily recovered and dissociated from the extract.

f) Viscosity must be low to facilitate penetration.

g) The boiling temperature must be low to avoid degradation of active constituents. Ethanol has a boiling temperature of 78.37 °C, which makes it still an ideal solvent. Lastly, according to [9], ethanol yielded a moderately good extract of 12.2%, compared with Dichloromethane (4.95%), Chloroform (7.2%), and Acetone (8.6%).

Extraction. The study utilised the maceration method of extraction. It started with soaking plant materials in a stoppered container with the chosen solvent, then letting it stand at room temperature for at least 3 days, with frequent, careful shaking. Consequently, maceration did not require heating to release the phytochemicals in the preliminary step; thus, it is the safest for obtaining phytochemicals, unlike other conventional and non-conventional methods that use heat at the very first step to obtain extracts. Significantly, the simplicity and cost-effectiveness of the equipment and apparatuses used, namely the rotary evaporator, which is used for gradual, gentle yet efficient removal of solvents from samples by evaporation as stated [10], and the microbiological incubator, which housed the Acacia bark extracts for 16-18 hours at a temperature of 35°C from the study [11].

Preparation of the Concentrations. Following Hermoso, the researchers prepared the concentration solutions by separately soaking 100 g of each powdered bark sample in a 500-ml Erlenmeyer flask. Pour 300 ml of ethanol using a graduated cylinder, and tightly seal the flask opening with a cork. The researchers will set the mixtures aside for 3 days. They will then reflux on a hot plate for 1 hour. Each mixture will be filtered using Whatman No. 1 filter paper and funnelled into another Erlenmeyer flask. Each resulting filtrate constitutes 100% extract. The same procedure will be performed for the positive control, Gentamicin.

Preparation of 25% Concentration of Solution. To prepare a 25% concentration, add 2.5 ml of the plant stock extract to a 50 ml Erlenmeyer flask, then dilute to 7.5 ml with distilled water. The graduated cylinder is used to measure the volume of solute (2.5 ml) and solvent (7.5 ml) to prepare a 10 ml solution.

Preparation of 50% Concentration of Solution. To prepare a 50% concentration, measure 5 ml of the plant stock extract, then dilute to 5 ml with distilled water in a 50 ml Erlenmeyer flask. The graduated cylinder is used measure the volume of solute (5 ml) and solvent (5 ml) to prepare a 10 ml solution.

Preparation of 75% Concentration of Solution. To prepare a 75% concentration, measure 7.5 ml of the plant stock extract, then dilute to 2.5 ml with distilled water in a 50 ml Erlenmeyer flask. The graduated cylinder is used to measure the volume of solute (7.5 ml) and solvent (2.5 ml) to prepare a 10 ml solution.

Preparation of 100% Concentration of Solution. To prepare a 100% concentration, measure out 10 mL of the plant extract from the plant stock. The graduated cylinder is used to measure the volume of solute (10 ml) and solvent to prepare a 10 ml solution.

Phytochemical Screening. Thin-layer chromatography was used to identify the phytochemicals in Acacia bark extract. It starts with coating glass plates with a layer of silica gel (SiO_2), then placing the plant material on the plate and inserting the plate into a jar containing the solvent and a filter paper. Once the solvent has risen to near the top of the plate, it is removed, dried, and finally visualised using UV light.

Antibacterial Susceptibility Testing. The present study used the agar well diffusion method for susceptibility testing and Gentamicin as the reference control. The researchers punched the agar plate containing the inoculated bacteria with a 6–8 mm cork borer to create wells, then introduced 20–100 µl of plant extract into each well; they prepared separate wells for different extract concentrations. The plate is then incubated depending on the bacteria. In relation to this study, the pathogenic E. coli was incubated for 24 hours at 37°C. The wells were inoculated with 25%, 50%, 75%, and 100% Acacia bark extract, as well as the control gentamicin, as described in

the review of methods for antimicrobial activity [12].

In the current investigation, the researchers used Mueller–Hinton agar at three concentrations and placed the control without modification. According to [13], Mueller–Hinton agar is the preferred medium for susceptibility testing of nonfastidious bacteria because it provides acceptable reproducibility, contains low levels of sulfonamide, trimethoprim, and tetracycline inhibitors, supports rapid growth of nonfastidious microorganisms, and is widely used in susceptibility testing. The researchers prepared the agar according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Table 1 shows the result of the qualitative phytochemical screening of Acacia (*Samanea saman*) bark extract.

Table 1 – Qualitative Phytochemical Screening of Acacia (*Samanea saman*) Bark Extract

Phytochemicals
Essential oil
Steroids
Coumarins
Anthrones
Phenols
Tannins
Flavonoids

The plant extract was spotted on a marked and labelled Thin Layer Chromatography plate (7x4 cm) and developed in an acetate-methanol (7:3) mixture in the chamber. The spots for secondary metabolites were visualised on Thin Layer Chromatography plates and exposed to UV light and a hot plate for the separation of the different metabolites.

Vanillin-sulfuric acid ($C_8H_8O_3$-H_2SO_4) was utilised because it is a typical reagent for Thin Layer Chromatography. The reagent determined the presence of Phenols, Sterols, Triterpenes, and Essential oils. At the same time, the researchers used a methanolic potassium hydroxide (CH_3KO_2) solution to test for anthraquinones, coumarins, and anthrones, and they visualised phenolic compounds and tannins using a potassium ferricyanide ($C_6N_6FeK_3$)–ferric chloride ($FeCl_3$) reagent. Lastly, Dragendorff's reagent was used to detect alkaloids and Antimony chloride was used to spot the presence of Flavonoids.

Table 2 shows the zones of Inhibition of the Acacia (*S. saman*) crude extract, Gentamicin, and Ethanol on the test organism *E. coli*. It was measured to the nearest millimetre using a Vernier calliper.

Table 2 – Zone of Inhibition of Acacia (S.saman) bark extract on *Escherichia coli* at 25%, 50%, 75%, 100%, Gentamicin and Ethanol

Treatments	Mean	Descriptions
25%	13.17 mm	Intermediate
50%	13.73 mm	Intermediate
75%	11.58 mm	Intermediate
100%	9.92 mm	Resistant
Gentamicin	33.41 mm	Susceptible
80% Ethanol	6 mm	Resistant

Notes: Gentamicin (10 µg); Resistant < 12 mm; Intermediate 13-14 mm; Susceptible > 15 mm.

For 25% concentration, the mean was 13.17 mm interpreted as Intermediate (I). Treatment 50% had a mean of 13.73 mm and read as Intermediate (I). Followed by treatment 75%, with a mean of 11.58 mm, interpreted as Intermediate (I). Consequently, the 100% concentration had a mean of 9.92 mm, interpreted as Resistant (R), whereas Gentamicin had a mean of 33.41 mm, interpreted as Susceptible (S). Lastly, ethanol had a mean of 6 mm, indicating it was Resistant (R).

Susceptible indicated that the microorganism had responded well to the antimicrobial agent or possessed appropriate treatment for the pathogen. Describing the antimicrobial agent as Intermediate/partially active or moderately susceptible to the microorganism increased the likelihood of therapeutic success by adjusting the dose/concentration or by adjusting the agent's concentration at the site of infection. Ultimately, resistance meant that the microorganism was not inhibited by the concentrations achievable with usual dosages of the agent, thus not an appropriate concentration for inhibiting the microorganism.

Table 3 – F-Test Results of Various Concentrations of *Samanea Saman* (Acacia) Bark Extract and Its Zone of Inhibition against *E.coli*

	25%	50%	75%	100%	Gentamicin	Ethanol
Mean	13.17 mm	13.73 mm	11.58 mm	9.92 mm	33.41 mm	6 mm
sd	1.150	0.351	0.351	0.366	0.173	0.000
F-value	951.669					
df	5,12					
p-value	<0.001					

Table 3 showed the results of various concentrations of *Samanea Saman* (Acacia) Bark Extract and Its Zone of Inhibition against *E.coli*. As can be seen, the mean of the zone of inhibition with the concentration of 25% Acacia crude extract was 13.17 mm, while the mean of 50% was 13.73 mm, the mean of 75% was 11.58 mm, and the mean of 100% was 9.92 mm, while the mean under positive control was 33.40 mm. Lastly, the mean of ethanol was 6 mm. The F-test showed that significant differences existed in the zones of inhibition with the concentrations 25%, 75%, and 100% for Gentamicin and ethanol, and there was no significant difference in the zones of inhibition between the concentrations 25% and 50%: F=(958.661), df=5 and 12.

Conversely, a p-value greater than or equal to 0.05 indicated that no significant difference existed, as in the 25% vs 50% concentration. The p-value was 0.233, which is greater than 0.05. Based on the mean zones of inhibition (13.17 mm at 25% concentration and 13.73 mm at 50% concentration), the measurements showed little difference between the two concentrations, indicating no statistically significant difference.

In summary, the zone of inhibition showed that the bacteria stopped growing in the clear area due to the diffusion of the antimicrobial drug, measured to the nearest millimetre; i.e., Gentamicin inhibited *Escherichia coli* at a weighted mean of 33. 41 mm obtained from three replicates.

Table 4 presents the multiple-comparison results for various Gentamicin concentrations.

Table 4 – Multiple Comparison Results on Various Concentrations of Acacia (*S. saman*) Bark Extract – Various concentrations of acacia bark extract and Gentamicin

	Treatments, %	Mean difference	P-value
Gentamicin	25	20.25000	<0.001
	50	19.69667	
	75	21.84667	
	100	23.50000	

As shown in the table, the mean difference between Gentamicin and 25% is 20.25000. In comparison, Gentamicin vs 50% had a mean difference of 19.69667, followed by Gentamicin vs 75% (21.84667) and Gentamicin vs 100% (23.50000), with a p-value of <0.001, indicating a significant difference between Gentamicin and the various concentrations. Gentamicin was better than all of the concentrations.

Supported by the study [14] demonstrating the effects of various concentrations (25%, 50%, 75%, 100% and 200%) of MCEO and Gentamicin on different bacterial strains, including E. coli. The results showed that Gentamicin was the most effective (27 mm) compared to concentrations: 25% (No response), 50% (18 mm), 75% (17.83 mm), 100% (18.16 mm), and 200% (19.96 mm).

Table 5 shows the multiple-comparison results for various concentrations (25%, 50%, 75%, and 100%) and ethanol.

Table 5 – Multiple Comparison Results on Various Concentrations of Acacia (S. saman) Bark Extract – Various concentrations of acacia bark extract and ethanol

	Treatments	Mean difference	P-value
80% Ethanol	25%	-7.17333	<.001
	50%	-7.72667	
	75%	5.57667	
	100%	3.92333	

Presented in the table, the mean difference of ethanol vs. 25% was -7.17333, while ethanol vs. 50% had a mean difference of -7.72667. Consequently, the mean difference for ethanol vs 75% was 5.57667, and the mean difference for ethanol vs 3.92333 had a p-value of <0.001, indicating a significant difference between ethanol and the

various concentrations. Treatments 25%, 50%, 75%, and 100% was better than ethanol.

Table 6 shows the multiple-comparison results for Gentamicin and ethanol.

Table 6 – Multiple Comparison Results on Various Concentrations of Acacia (S. saman) Bark Extract – Gentamicin and Ethanol

		Mean difference	P-value
Gentamicin	80% Ethanol	27.42333	<0.001

As shown in the table, the mean difference between Gentamicin and ethanol is 27.42333, with a p-value of <0.001, indicating a significant difference between the two. Gentamicin was better than ethanol.

A study [15] showed that Gentamicin was more effective than Ethanol and Ciprofloxacin against *Escherichia coli* ATCC 25922, with Gentamicin exhibiting an inhibition zone of 20 mm, whereas Ethanol and Ciprofloxacin showed no activity.

Table 7 showed the correlation between various Acacia extract concentrations and the Zone of Inhibition.

Table 7 – Pearson-r correlation between Acacia extract concentrations (25%, 50%, 75%, and 100%) and zone of inhibitions

		Concentration	Zone of Inhibition
Concentration	Pearson Correlation	1	-0.896
	Sig. (2-tailed)		0.104
	N	4	4
Zone of Inhibition	Pearson Correlation	-0.896	1
	Sig. (2-tailed)	0.104	
	N	4	4

The table shows a negative correlation between extract concentrations and zones of inhibition (r =- 0.896), with a p-value > 0.05, indicating that as concentration increases, the zone of inhibition decreases.

Measuring the zone of inhibition in an antimicrobial assay to determine drug potency is a qualitative method; therefore, it is highly prone to misinterpretation. Other indicators include the water solubility of the drug molecule from the investigation [16], the antibiotic concentration, the agar medium, the duration and temperature of the diffusion phase before the incubation, and the incubation temperature from the study [17].

The dose-relationship or concentration of the antibiotic to plant extracts was not the same; for that reason, positive control vs each concentration of extract resulted in a highly significant difference. Moreover, agar was prepared by adding water; non-polar compounds cannot diffuse, but an intermediate-polarity compound can have the highest antimicrobial activity. Most significantly, the main mechanism for testing the potency of plant extracts against certain pathogens was protein denaturation. Authors [18] reported that absolute ethyl alcohol exhibits lower bactericidal activity than alcohol–water mixtures, as water accelerates protein denaturation. In these mixtures, water acts as a catalyst by penetrating the bacterial cell wall more effectively, permeating the entire cell, and promoting protein coagulation in the vegetative cell membranes, ultimately leading to bacterial cell death. Water content slowed evaporation, increasing surface contact time with the bacterial cell, which is why 25% and 50% concentrations exhibited larger inhibition zones than 75%, 100%, and ethanol.

CONCLUSION

The study showed that Acacia bark extract contained essential oils, steroids, coumarins, anthrones, phenols, tannins, and flavonoids, and its antibacterial activity may be attributed to these compounds. The agar well diffusion assay showed that the bark extract exhibited inhibitory effects against Escherichia coli; however, the standard antibiotic, Gentamicin, showed greater inhibitory effects. Interestingly, the findings showed that the lower the concentration, the higher the inhibitory effect suggesting that ratio of solvent to the crude extract and the diffusion properties may affect the antibacterial activity. This highlights the importance of strengthening ethnopharmacology in Nueva Ecija to make use of what nature has endowed people with.

Substantially, in making this study, it is recommended to: 1) isolate active compounds; 2) use antibacterial assays that are more rigorous; 3) determine the phytotoxicity of the *S. saman* bark.

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