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# Phytochemistry and Antibacterial Activity of *Platycerium coronarium* (Tanduk Rasa Fern) Leaf Extracts

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**Abstract:** Medicinal plants have been utilized for centuries and continue to be a global resource for healthcare. They offer a potentially safer and more cost-effective alternative to conventional pharmaceuticals, which can pose health risks and financial burdens, especially in low-income communities. This study investigated the antimicrobial properties of *Platycerium coronarium* leaf extracts against *Staphylococcus aureus* and *Escherichia coli*. The extraction process involved maceration with methanol, ethanol, and chloroform solvents. Each extract was independently tested for antibacterial activity using the agar well diffusion method. Qualitative phytochemical analysis was conducted, revealing the presence of saponins, flavonoids, alkaloids, and glycosides in the methanol and ethanol extracts. Minimum Inhibitory Concentration (MIC) values were determined for the methanol extract against *S. aureus*. Results indicate that the methanol extract exhibited the highest antimicrobial activity (8.5mm) against *S. aureus*, followed by the ethanol extract (7mm), while the chloroform extract showed no antimicrobial effect. None of the extracts showed antimicrobial activity against *E. coli*. These findings suggest that *Platycerium coronarium* leaf extracts hold promise as natural antimicrobial agents. Further research, including in vivo studies, is required to evaluate their efficacy, safety, and mechanism of action against Gram-positive bacterial infections. Such investigations could contribute to the development of affordable alternatives to combat antibiotic resistance and alleviate medication costs in underserved communities.

**Keywords:** Medicinal Plants, Antimicrobial Properties, *Staphylococcus aureus*, *Escherichia coli*, MIC

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## 1. Introduction

Throughout history, medicinal plants have served as essential resources in healthcare, offering a perceived safer and more cost-effective alternative to conventional pharmaceuticals. The World Health Organization (WHO) [1] acknowledges the significance of traditional medicines in global healthcare, reporting that nearly 80% of the world's population relies on these remedies, particularly phytomedicines, to address fundamental healthcare needs. This reliance transcends borders, covering both developing nations and more affluent countries where plant-based treatments constitute over a quarter of authorized therapies [2].

Ancient medicinal systems recognized the therapeutic

potential of ferns, with references dating back to texts such as the Sushruta and Charaka Samhitas in Ayurveda (circa 100 AD) [3]. These ancient texts prescribed the use of certain ferns, and similar endorsements can be found in the Unani system of medicine [4]. Traditional Chinese medicine also embraced ferns as valuable remedies [5]. Across Africa, traditional medicine plays a significant role in healthcare, and pharmaceuticals with countries like Ghana, Nigeria, Ethiopia, South Africa, and Tanzania relying heavily on plant-based treatments [1]. According to Alebie *et al.*, (2017) [7] over 8,000 plant species are used in traditional medicine, in Nigeria and about 80% of the population use traditional medicine for their primary healthcare needs.

However, allopathic medicine remains the predominant treatment modality in developed nations, not without its drawbacks [8]. Synthetic compounds in allopathic drugs,

often characterized by complex chemical structures, can have adverse health effects when used extensively or in high doses [9]. Furthermore, overuse of these drugs has led to the emergence of antibiotic-resistant bacteria, a global health concern [10]. In contrast, medicinal plants, with their historical use in traditional medicine, are generally regarded as safe when used appropriately [11]. They tend to produce fewer side effects and are often more financially accessible than their allopathic counterparts [12].

In Uganda, where allopathic medicine is the primary healthcare system, the average cost of prescribed antibiotics per patient is approximately \$0.5 (1,800/- UGX), exceeding the per capita medication budget of \$0.3. This cost discrepancy often results in the inappropriate use of antibiotics in rural communities, where patients may opt for under-dosing due to financial constraints [12]. This problem significantly impacts patient health and healthcare costs, contributing to the unavailability of antibiotics and undermining the effectiveness of treatment [12].

The unavailability of antibiotics also erodes trust in the healthcare system, forcing prescribers to limit their choices based on medication availability rather than clinical appropriateness [13, 14]. A more affordable, accessible, and effective alternative is urgently required. Medicinal plants present a promising avenue for the development of safe, cost-effective alternatives.

Evaluating the antimicrobial properties of *Platynerium coronarium* leaf extracts against specific bacterial strains such as *Staphylococcus aureus* and *Escherichia coli* has the potential to discover valuable and cost-effective local sources of medication. However, our current understanding of the phytochemistry and antimicrobial effects of these leaf extracts remains limited, specifically their interactions with bacteria. There is lack of research comparing the antimicrobial efficacy of *Platynerium coronarium* crude extracts with standard pharmaceutical drugs. This highlights the importance of conducting an assessment. This can provide valuable insights into the use of *Platynerium*

*coronarium* leaf extracts as potential substitutes for conventional antibiotics. It can also help address healthcare challenges by making treatments more accessible and cost-effective.

Hence, this study was focused on evaluating the antimicrobial activity of *Platynerium coronarium* leaf extracts against selected bacterial organisms, specifically, one gram-positive bacterium (*Staphylococcus aureus*) and one gram-negative bacterium (*Escherichia coli*).

## 2. Materials and Methods

### 2.1. Study Area

The research was conducted within Kyambogo University, situated in the eastern part of Kampala, the capital city of Uganda (Figure 1). Kyambogo University is located in the Kyambogo suburb of Kampala and is bounded by the neighborhoods of Banda, Ntinda, Nakawa, and Mbuya. The geographical coordinates of Kyambogo University are approximately 0.3433°N latitude and 32.6039°E longitude. The climate in Kampala is tropical, characterized by temperatures ranging from 21°C to 29°C year-round. The region experiences a rainy season from March to May and from October to November, with a dry season occurring from December to February and from June to August. Moderate rainfall is distributed throughout the year, creating favorable conditions for the growth of *Platynerium coronarium*. The adjacent areas of Banda, Ntinda, Nakawa, and Mbuya feature a blend of residential and commercial properties, with some pockets of natural vegetation.

Kyambogo University was selected as the study site due to the abundant presence of freely growing *Platynerium coronarium* on various tree branches, facilitating the collection of plant materials for the study. The research laboratory within the Department of Biological Sciences at Kyambogo University served as the primary facility for conducting experiments and subsequent analysis.

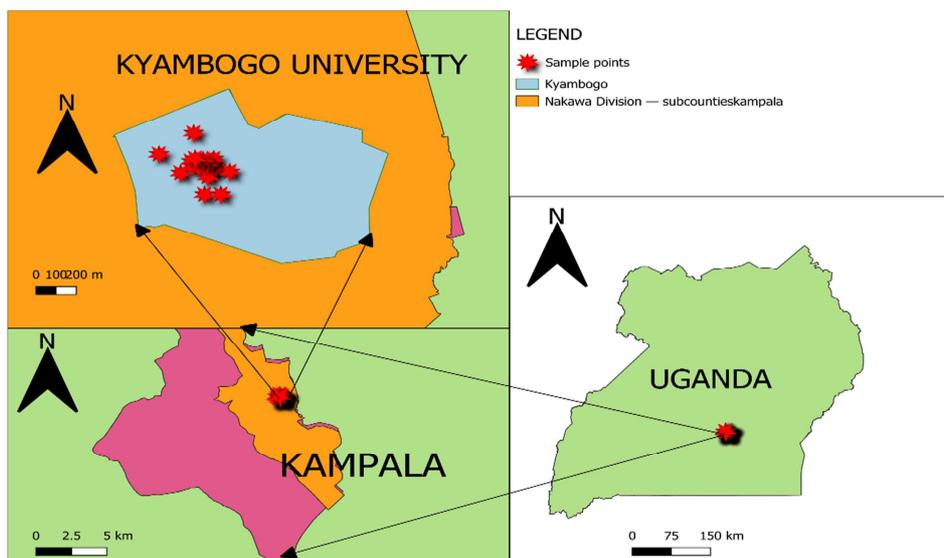


Figure 1. The Map and Location of Kyambogo University on The Map of Uganda.

## 2.2. Research Design

The study followed an experimental research design where laboratory experimental investigations to determine the bioactivity of *Platycerium coronarium* leaf extracts against *S. aureus* and *E. coli* and also phytochemical analyses were carried out. The antimicrobial activity of the plant extracts was evaluated using the agar well diffusion method against standard strains of bacteria *Staphylococcus aureus* and *Escherichia coli*. Tetracycline was used as a positive control at a concentration of 10 mg/ml to compare the efficacy of the plant extracts against the standard strains, while the negative control was Dimethyl Sulphoxide (DMSO).

### 2.2.1. Determination of the Antimicrobial Activity of *P. coronarium*

#### (i). Plant Material Collection and Processing

Fresh leaves of the *Platycerium coronarium* plant were harvested from the tree branches around Kyambogo University Main Campus. Fresh mature leaves were thoroughly cleaned 3 times with running tap water. The collected material was then air dried for 20 days (away from direct sunlight to avoid deterioration) as shown in Figure 2 and ground into a fine powder using a mechanical blender (Saachi NL-BL-4361; Batch No: 102010; Made in China).



Figure 2. Fresh Leaves (A) and Dried Chopped Leaves (B).

#### (ii). Chemicals and Standards

The pure *Staphylococcus aureus* stock culture, ATCC 452300, and *Escherichia coli* stock culture, ATCC 2592, were obtained from the Microbiology Research Laboratory, at the Biological Science Department, Kyambogo University. During the extraction process three solvents were used and they include; ethanol, methanol, and chloroform. For qualitative phytochemical analysis, the following reagents were used: Iodine crystals, Potassium iodide, Copper (II) sulphate, Ferric chloride, Acetone, Copper acetate, Sodium hydroxide, Sulphuric acid, Hydrochloric acid, and Dimethyl Sulphoxide. Those chemicals were purchased from the laboratory suppliers, and they were of analytical grade.

#### (iii). Extraction Process

The powdered leaves were then sieved to ensure uniformity. The leaf extracts were prepared by maceration

protocols outlined by Banu & Cathrine, (2015) with slight modifications. Twenty (20) grams of the powdered leaves were soaked separately in 200ml of methanol, ethanol and 7.39g of powdered leaves in 70ml chloroform solvents for 72 hours as shown in figure 3. The mixtures were shaken continuously, filtered using Whatman filter paper number one, and the filtrates were concentrated to near dryness in a hot air oven at 40°C for four days. The concentrates were preserved in a vial, weighed, labeled appropriately and stored in the refrigerator at 4°C until needed for analysis.



Figure 1. The Processes of Maceration (A); and Filtering (B).

#### (iv). Media Preparation

Mueller Hinton Agar (MHA) was prepared by dissolving 38g of MHA into a liter of distilled water as per the manufacturer's instructions. It was mixed thoroughly, using the laboratory magnetic stirrer (UK type) for complete dissolution, and then autoclaved using a stainless-steel autoclave (Indian model) at 121°C for 15 minutes for sterilization. The media was then allowed to cool under the Laminar flow hood (LAF, Stainless steel, Indian made) to about 55°C. The sterile MHA was then dispensed in sterile plastic Petri dishes (20ml) and left in the sterilized biosafety cabinet (LAF) until the media solidified.

#### (v). Inoculum Preparation

The *Staphylococcus aureus* inoculum and *Escherichia coli* inoculum were prepared by direct colony method as described by Manilal *et al.*, (2020) and Siddiqui, (2021). The *S. Aureus* and *E. coli* standards were sub cultured on different MHA plates and incubated at 37°C for 24 hours. Discrete colonies were aseptically picked directly from the plate with a sterile wire loop and suspended in 0.85% saline. The suspension was adjusted to a concentration of 10<sup>8</sup> colony-forming units (CFU) per millilitre, by comparison with 0.5 McFarland standard. The *S. aureus* and *E. coli* suspensions were then stored in the refrigerator at 4°C until required for analysis.

#### (vi). Antimicrobial Efficacy

The antimicrobial efficacy of *Platycerium coronarium* was determined by Agar well diffusion assay as described by [16]. Sterile MHA was dispensed onto Petri plates to a depth of 4mm under aseptic conditions, and left to dry. Agar plates were inoculated with 100µl of *S. aureus* and *E. coli*

suspensions containing  $10^8$ cfu/ml using a sterile P200 micropipette and spread over the entire sterile agar surface using a swabbing stick by rotating the agar plate for uniform distribution of the inoculum. Five wells each of 5mm diameter for each bacterium were bored in agar plates using a sterile 5mm cork borer. The extracts (ethanol, chloroform and methanol) of concentration 50mg/ml each and standard tetracycline (10mg/ml) were prepared using Dimethyl Sulphoxide (DMSO) as the solvent. The standard and the test extracts (100 $\mu$ l) were added in the wells using sterile micropipettes and incubated at 37°C for 24hours. Tetracycline and DMSO were used for positive and negative tests respectively. The zone of inhibition of each extract and the standard was measured using a ruler scale. The assays were conducted in duplicates so as to validate the findings statistically.

### 2.2.2. The Minimum Inhibitory Concentration (MIC)

The MIC of *Platyserium coronarium* leaf extracts was determined using Broth dilution method based on procedures described by [17]. The MIC was determined by serial diluting the extracts (methanol, and ethanol) independently to required concentrations (32, 16, 8, 4, 2, 1 and 0.5mg/ml). Equal volumes of 1ml extracts (methanol and ethanol) at the concentration of 64mg/ml was added to 1ml of nutrient broth and serially diluted to obtain exact concentrations of 32, 16, 8, 4, 2, 1 and 0.5mg/ml in seven (7) different test tubes as shown in figure 7 below. The positive control was setup with only plant extract and broth without the inoculum, while the negative control was only the inoculum and broth. Chloroform extracts did not show any antimicrobial efficacy, thus there was no need to carry out their MIC.

To each test tube, 100 $\mu$ l of  $1 \times 10^8$ cfu/ml of *Staphylococcus aureus* strain was inoculated with an equal volume of Mueller Hinton Broth (MHB) Medium. The test tubes were incubated at 37°C for 24 hours. The lowest concentration of the extract that produced no visible bacterial growth (no turbidity) in the 24 hours when compared with the control test tubes was considered the MIC. The MIC was determined for ethanol, and methanol extracts. The same procedures were not done for *Escherichia coli* because *Platyserium coronarium* leaf extracts did not show antimicrobial efficacy on *E. coli*.



A



B

**Figure 2.** Serial dilution of the leaf extracts. A for ethanol extracts and B for Ethanol extracts.

### 2.2.3. Phytochemical Analysis of *Platyserium coronarium* Extracts

The phytochemicals (secondary metabolites) present in the methanol, and ethanol extracts of *Platyserium coronarium* were identified using the standard procedures as described by [18].

#### (i). Alkaloids

2g of powdered leaf extract was mixed with 1% Sulphuric acid and allowed to stand for 2 hours and filtered. To the filtrate, five drops of Wagner's reagent (made by dissolving 2g of iodine and 3g of potassium iodide are dissolved in a small amount of distilled water and then made up to 100ml of water) was added. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

#### (ii). Saponins

In test tubes, 2mg of crude extract were suspended in 20 ml of distilled water and boiled for 5 minutes. Then, 10 ml of the filtrate and 5 ml of distilled water were mixed well to develop the froth. The development of froth confirmed the presence of saponins.

#### (iii). Flavonoids

To 2ml of aqueous crude extract in the test, tube will be added 5ml of dilute ammonia solution, followed by 2ml of concentrated Sulphuric acid. A yellow coloration in the solution on standing indicated the presence of Flavonoids.

#### (iv). Glycosides

To 2mg of dried plant extract were hydrolyzed with 5 ml concentrated hydrochloric acid in a water bath for 2 hours and filtered using Whatman filter papers. In a test tube, 2 ml of filtered hydrolysate was taken and into it added 3 ml of chloroform and mixed well. A chloroform layer was separated and to it 10% ammonia solution was added. A pink color formation indicated the presence of glycosides.

#### (v). Phenolic Compounds

To 2ml of Iron (III) chloride solution, 2ml of the extract were added. Formation of a deep bluish-green solution confirmed the presence of phenol.

#### (vi). Proteins

In a test tube, 2mg of extract will be dissolved in 5ml of

distilled water and filtered through Whatman no: 1 filter paper. To 2 ml of filtrate, 2ml of dilute Sodium hydroxide followed by 3drops of Copper (II) sulphate were added. A pink coloration indicated the presence of proteins.

#### (vii). Diterpenes

2mg of extract was dissolved in water and treated with 4 drops of copper acetate solution. The formation of bright green color indicated the presence of diterpenes.

#### (viii). Phytosterols/Triterpenes

2mg of the extract was treated with chloroform and filtered. The filtrate was then treated with 3 drops of Concentrated Sulphuric acid, mixed carefully and allowed to stand. Appearance of golden yellow color indicated the presence of triterpenes.

#### (ix). Tannins

In a test tube, 2ml of extract was added followed by 2 drops of 5% aqueous ferric chloride solution. A bluish black color which disappears on addition of a few ml of Sulphuric acid indicated the presence of tannins.

#### (x). Resins

In a test tube, 2ml of the extract were mixed with acetone followed by small amount of water and shaken. The appearance of turbid solution indicated the presence of resins.

### 2.3. Ethical Considerations

No human subjects were involved in the study; therefore, no ethical issues are concerned with the study. For the laboratory investigations, standard bacterial strains whose sensitivity to antibacterial agents are known were used.

### 2.4. Data Analysis

The data was analyzed using Graph pad prism version 9.5.1 (733) and presented in form of graphs, and tables. For statistical analysis; the statistical analysis was performed at a 95% confidence interval to test for significant differences between the activities of the solvents by performing one ANOVA.

## 3. Results

### 3.1. Antimicrobial Efficacy of *P. coronarium* Leaf Extracts Against *Staphylococcus Aureus*

Ethanol and methanolic leaf extracts of *Platycerium coronarium* showed activity against *Staphylococcus aureus* as represented by the zones of inhibition in figure 8. Chloroform extracts of *Platycerium coronarium* leaf extracts never showed any activity against *Staphylococcus aureus* as observed in figure 5.

The ethanol extracts exhibited the highest activity with a mean zone of 8.5mm against *S. aureus*; while methanol extracts followed with a mean zone of inhibition of 07mm against *S. aureus*.

Chloroform extracts never showed activity against

*Staphylococcus aureus*, probably due to the smaller amount of the powdered extract used initially (7.395g), for the maceration process, thus no active compounds were extracted in the solvent.



**Figure 5.** Agar plate inoculated with *S. aureus* after 24hour period, with it's wells treated as follows +ve; Tetracycline; M; methanol extract; E; ethanol extract and C; chloroform.

### 3.2. Antimicrobial Efficacy Against *Escherichia coli*

The extracts from the three solvents (ethanol, methanol and chloroform) never showed antimicrobial activity against *E. coli* as represented by no zones of inhibition in figure 6. *Escherichia coli* being a gram-negative bacterium, it has an additional outer membrane composed of lipopolysaccharides. This cell wall structure could have affected the susceptibility of *E. coli* to the active compounds that were present in the plant extracts.



**Figure 3.** Agar plate inoculated with *E. coli* after 24hour period, with it's wells treated as follows +ve; Tetracycline; M; methanol extract; E; ethanol extract and C; chloroform.

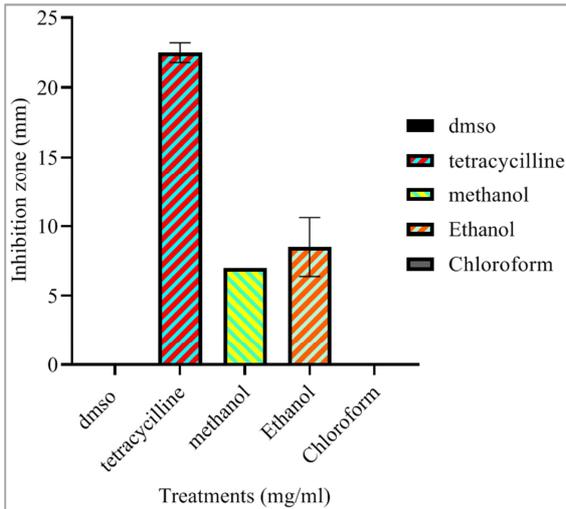


Figure 7. Mean zone of inhibition for methanol and ethanol extracts; positive and negative controls on *Staphylococcus aureus*.

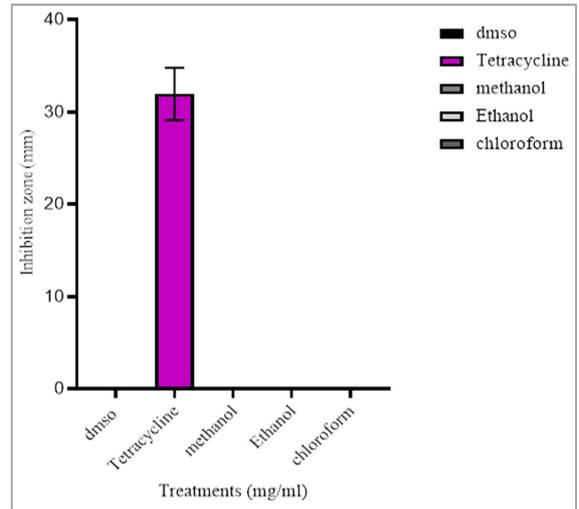


Figure 8. Mean zone of inhibition for methanol and ethanol extracts; positive and negative controls on *Escherichia coli*.

Table 1. Mean zones of inhibition of *Staphylococcus aureus*, after the various treatments.

Treatments	Inhibition zone (mm)
DMSO	0.00 ± 0.00
Tetracycline	22.50 ± 0.00
Methanol	7.00 ± 0.00
Ethanol	8.50 ± 0.00
Chloroform	0.00 ± 0.00

Table 2. Tukey's Multiple Comparisons ANOVA test comparing Ethanolic and Methanolic leaf extracts of *Platycerium coronarium* on *Staphylococcus aureus*.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
DMSO vs. Tetracycline	-22.50	-26.51 to -18.49	Yes	<0.0001
DMSO vs. Methanol	-7.000	-11.01 to -2.988	Yes	0.0049
DMSO vs. Ethanol	-8.500	-12.51 to -4.488	Yes	0.0020
DMSO vs. Chloroform	0.000	-4.012 to 4.012	No	>0.9999
Tetracycline vs. methanol	15.50	11.49 to 19.51	Yes	0.0001
Tetracycline vs. Ethanol	14.00	9.988 to 18.01	Yes	0.0002
Tetracycline vs. Chloroform	22.50	18.49 to 26.51	Yes	<0.0001
Methanol vs. Ethanol	-1.500	-5.512 to 2.512	No	0.6026
Methanol vs. Chloroform	7.000	2.988 to 11.01	Yes	0.0049
Ethanol vs. Chloroform	8.500	4.488 to 12.51	Yes	0.0020

Table 3. One-way ANOVA comparing the various treatments above.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	677.4	4	169.4	F (4, 5) = 169.4	P<0.0001
Residual (within columns)	5.000	5	1.000		
Total	682.4	9			

Table 4. Mean zones of inhibition of *Escherichia coli*, after the various treatments.

Treatment	Inhibition zone
DMSO	0.00 ± 0.00
Tetracycline	32.00 ± 0.00
Methanol	0.00 ± 0.00
Ethanol	0.00 ± 0.00
Chloroform	0.00 ± 0.00

Table 5. Tukey's Multiple Comparisons ANOVA test comparing Ethanolic and Methanolic leaf extracts of *Platycerium coronarium* on *Escherichia coli*.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
DMSO vs. Tetracycline	-32.00	-37.07 to -26.93	Yes	<0.0001
DMSO vs. methanol	0.000	-5.074 to 5.074	No	>0.9999
DMSO vs. Ethanol	0.000	-5.074 to 5.074	No	>0.9999

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
DMSO vs. chloroform	0.000	-5.074 to 5.074	No	>0.9999
Tetracycline vs. methanol	32.00	26.93 to 37.07	Yes	<0.0001
Tetracycline vs. Ethanol	32.00	26.93 to 37.07	Yes	<0.0001
Tetracycline vs. chloroform	32.00	26.93 to 37.07	Yes	<0.0001
Methanol vs. Ethanol	0.000	-5.074 to 5.074	No	>0.9999
Methanol vs. chloroform	0.000	-5.074 to 5.074	No	>0.9999
Ethanol vs. chloroform	0.000	-5.074 to 5.074	No	>0.9999

### 3.3. Minimum Inhibitory Concentration of *Platycerium coronarium* leaf Extracts

The MICs of ethanol and methanol extracts of *P. coronarium* leaf extracts were determined and compared for the test bacterial isolates. As shown in table 6 below, the methanolic extracts showed greater activity than the ethanolic extracts, as they inhibited the growth of *Staphylococcus aureus* at a lower concentration of 16 mg/ml as compared to the ethanolic extracts, which did so at a slightly higher concentration of 32 mg/ml. However, all the extracts never showed antibacterial activity against *E. coli*.

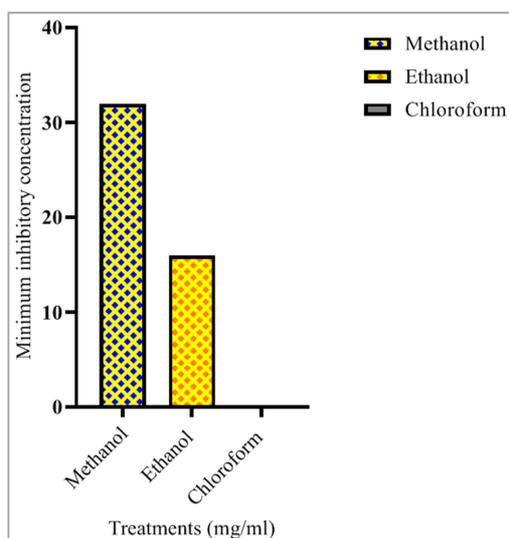


Figure 4. MICs of *P. coronarium* leaf extracts in methanol solvents and ethanol solvents against *S. aureus*.

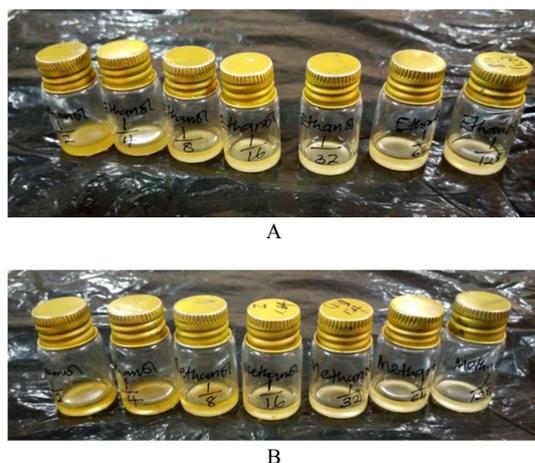


Figure 5. MICs of leaf extracts of *Platycerium coronarium*. (A) Ethanol Extracts; (B) Methanol Extracts.

Table 6. MIC of *Platycerium coronarium* leaf extracts against *Staphylococcus aureus* in the two solvents.

Solvent	MIC (mg/ml)
Methanol	16
Ethanol	32
Chloroform	0

### 3.4. Phytochemical Analysis

Six (6) phytochemicals, including phenolics, tannins, saponins, flavonoids, glycosides, and alkaloids, were detected in the methanol leaf extracts of *P. coronarium*. Phytochemical tests on proteins, resins, diterpenes, and phytosterols for the methanol extracts similarly came out negative.

The ethanol extracts, on the other hand, tested positive for a number of phytochemicals, including tannins, phenolics, alkaloids, saponins, flavonoids, glycosides, and phytosterols.

The different phytochemicals that were tested for are listed in table 7 below; the (+) sign denotes the presence of phytochemicals, while the (-) sign denotes their absence, for each solvent that was examined.

The presence or absence of specific phytochemicals was correlated with the polarity of the extracting solvent. Polar solvents (methanol and ethanol) extracted active compounds but non-polar solvents (chloroform) did not extract significant active compounds as this was evidenced by no zones of inhibition for *S. aureus*. Methanol is highly polar, followed by ethanol which is slightly polar and lastly chloroform, which is completely non-polar. The polarity arises due to the OH groups, with the oxygen atoms having high electronegativity, thus forming partial positive and partial negative charges in solution that aid in bonding with other polar compounds.

Table 7. Phytochemicals present in the two solvents Methanol and Ethanol.

Phytochemical	Solvent	
	Methanol	Ethanol
Saponins	+	+
Flavonoids	+	+
Phenolics	+	+
Tannins	+	+
Proteins	-	+
Diterpenes	-	+
Alkaloids	+	+
Glycosides	+	+
Resins	-	+
Phytosterols	-	+

(+); Present (-); Absent

## 4. Discussion

### 4.1. Antimicrobial Activity of *Platycerium coronarium* Leaf Extracts Against *Staphylococcus Aureus*

The ethanolic and methanolic leaf extracts of *Platycerium coronarium* leaves that were used during this study showed activity as presented in various tables and graphs used during results interpretation, against *Staphylococcus aureus* but the effect was not seen on *Escherichia coli*. This might be due to the fact that *E. coli* can change their genetic constitution, at a rapid rate. *E. coli* like other gram-negative bacteria show resistance because of their cell walls, by altering it slightly so the antibiotics cannot attach, or produce enzymes that break down the antibiotics to harmless substance [19]. The results agreed with findings by [20], who also found out that the ethanol extracts of neem plant (*Azadirachta indica*) had antibacterial activity against *S. aureus* but no effect on *Escherichia coli*. This was also consistent with the findings of [21], which showed a similar report of antibacterial activity against gram positive *S. aureus* ATCC 2592 using a different species of *Platycerium* (*Platycerium elephantiasis*) but no effect on *E. coli*. These results were in disagreement with the findings by [19], who showed that methanol and ethanol plant extracts of *Maesa lanceolata* had high antimicrobial activity against *Escherichia coli* but no notable effect against *S. aureus*. Li *et al.* (2019) [22], showed different findings from the above results when he assessed the antibacterial activity and mechanism of a laccase-catalyzed chitosan-gallic acid derivative against *Escherichia coli* and *Staphylococcus aureus*, there was no notable effect against *Staphylococcus aureus* but higher antimicrobial activity against *E. coli*.

This study also showed that in comparison to ethanol, the methanol extract showed greater activity against *Staphylococcus aureus*. This was in line with the findings of [23], who found that *S. aureus* was more vulnerable to methanol extracts than to ethanol or chloroform extracts when he evaluated different solvents for phytochemical constituents of *Severinia buxifolia*. The leaf extracts had antibacterial effect on *S. aureus*. This was linked to the fact that plants build up secondary metabolites in various regions of their bodies. These metabolites are biologically active and have therapeutic characteristics, which essentially defend the plants against microbial attacks. These results were consistent with Pang *et al.* (2021) [24], who reported that plants produce different phytochemicals during cell metabolism such as flavonoids, tannins, steroids and flavonoids that are biologically active. These findings matched with those of Salmerón-Manzano *et al.* (2020) [26], who suggested that alkaloids, flavonoids, and saponins are responsible for many medicinal plants' strongest antibacterial effects. It was confirmed that these compounds were found in the methanol and ethanol leaf extracts of *Platycerium coronarium*. According to Wang *et al.* (2020) [24], such phytochemicals damage membranes and precipitate proteins from cell walls, some prevent the formation of microtubules and cell proteins inside the cells, both of which are crucial for

maintaining the stability of the cytoskeleton, this results in the death of bacterial cells.

Chloroform leaf extracts of *Platycerium coronarium* did not exhibit any antibacterial activity against *Staphylococcus aureus*. On the other hand, the ethanol and methanol solvent extracts showed antibacterial activity against this bacterium. These results were consistent with the findings by El Houda Lezoul *et al.* (2020) [28], when he tried to extract bioactive compounds from *Passiflora caerulea*, *Physalis peruviana* and *Solanum muricatum* plants, he found out that the use of certain solvents could lead to small extraction yield of secondary metabolites and sometimes no yield at all of these bioactive compounds due to the polarity properties of the solvents used during extraction process. The lack of antibacterial activity of chloroform leaf extracts on *Staphylococcus aureus* could be attributed to the fact that the active compounds responsible for the antibacterial effect are not readily soluble in chloroform. Chloroform is a non-polar solvent; this could have been the reason for absence of antibacterial activity in its extracts since most of the secondary metabolites were polar.

None of the solvent extracts of the leaves of *Platycerium coronarium*, including ethanol and methanol, exhibited antibacterial activity against *Escherichia coli*. These findings suggest that the antibacterial properties of *Platycerium coronarium* leaf extracts are influenced by the solvent used for extraction, as well as the specific bacterial strain being tested. The absence of antibacterial activity against *Escherichia coli* in all solvent extracts, including ethanol and methanol, suggests that *Platycerium coronarium* leaf extracts may not contain compounds that are effective against this particular bacterium. It is possible that the bioactive compounds in the plant extract have a specific target or mode of action that is not effective against *Escherichia coli*. *Escherichia coli* being a gram-negative bacterium, it has an additional outer membrane composed of lipopolysaccharides. This cell wall structure could have affected the susceptibility of *E. coli* to the active compounds that were present in the plant extracts.

Similar results have been reported in the study by El Houda Lezoul *et al.* (2020) [28], who revealed that for a gram positive *S. aureus* there was a maximum zone of inhibition displayed by *Citrus reticulata* peel extract (28mm), while gram negative *E. coli*, *S. typhi*, and *P. aeruginosa*, were found to be resistant to the peel extracts.

### 4.2. Minimum Inhibitory Concentration of *Platycerium coronarium* Against *Staphylococcus Aureus*

Both the methanolic and ethanolic extracts were active, thus inhibiting *S. aureus* growth at low doses. This shows that leaves of *Platycerium coronarium* plants contain significant levels of bioactive components including as saponins, alkaloids, steroids, and tannins, which are required in low amounts to inhibit antimicrobial growth. These phytochemicals were also reported by Salmerón-Manzano [26], to produce precipitation of the bacterial cell wall, membrane disruption that leaks the cell contents, and

inhibition of microtubule production, which forms the bacterium cytoskeleton and so causes bacterial death. These results are in line with Sowndhararajan [26], whose works on *Callistemon lanceolatus*, showed minimum inhibitory effect against the methicillin-resistant *S. aureus* (MRSA), *Staphylococcus epidermidis*, and *S. aureus*, with the ethyl acetate extract that indicated a lower MIC of 10mg/ml. However, *E. coli* was not tested for, because all the extracts never antibacterial activity against this bacterium. The increased activity of the *Platyserium coronarium* leaf methanolic extract against *S. aureus*, as evidenced by the lower MIC value, could be attributed to methanol being a more polar solvent than ethanol. Because of its stronger polarity, methanol can extract a wider range of bioactive chemicals from plant material, such as saponins, alkaloids, steroids, and tannins.

### 4.3. Phytochemical Analysis

The results of the phytochemical analysis revealed the presence of six different phytochemicals in the methanol leaf extract of *P. coronarium*. These included phenolics, tannins, saponins, flavonoids, glycosides, and alkaloids. This was in line with V [7], who suggested that phytochemicals accumulate in different concentrations in the different plant parts.

The phytochemicals in *P. coronarium* play various roles where some are antimicrobial and others are not hence making *Platyserium coronarium* a valuable plant. The non-antimicrobial properties of *Platyserium coronarium* include: Phytosterols, which are cholesterol modulators, they reduce cholesterol absorption and increase bile acids synthesis [30]. Resins are used as varnishes and cement, preparation of zinc oxide plasters and some resin sugars are used for coating tablets, whereas the glycosides are important phytohormones that regulate growth, development and responses to environmental stresses and they include abscisic acid, gibberellins and auxins [31]. The antimicrobial phytochemicals of *Platyserium coronarium* include; Saponins, these have pharmaceutical properties such as anti-inflammatory, antifungal, antibacterial, anti-parasitic and antiviral activities [18]. The flavonoids have antifungal activities, nutraceutical, pharmaceutical and antioxidants, antimutagenic and anticarcinogenic properties [32].

## 5. Conclusion

The ethanolic and methanolic leaf extracts of *Platyserium coronarium* exhibited significant antibacterial activity against *Staphylococcus aureus* and by their ability to inhibit bacterial growth at low concentrations. However, no notable effect was observed against *Escherichia coli*. This suggests that the leaf extracts of *Platyserium coronarium* have selective antimicrobial properties and may be more effective against Gram-positive bacteria like *Staphylococcus aureus*. The phytochemical analysis revealed the presence of several bioactive compounds in the methanol and ethanol leaf extracts of *Platyserium coronarium*. These findings

demonstrate the diverse phytochemical profile of *Platyserium coronarium* leaves and provide valuable information into the bioactive compounds responsible for its antimicrobial activity.

## 6. Recommendations

With findings of this study, it is suggested that more research and practical studies be conducted to increase the understanding and application of *Platyserium coronarium* as an antibacterial agent. This comprises isolating and fractionating certain bioactive molecules responsible for antimicrobial activity for inclusion in pharmaceutical products targeting Gram-positive bacteria, specifically *Staphylococcus aureus*. Investigating the full in vivo potential and toxicity of *Platyserium coronarium* extracts at higher concentrations is important in controlling *S. aureus* infections and determining the minimal bactericidal concentration (MBC).

## Study Limitations

The study was conducted during the semester so the author faced challenges of time management since he had to attend lectures while conducting research at the same time. The author also faced challenges of financial support since he was an undergraduate student with no job and the only source of income was family. These two must affected the outcomes.

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## Conflicts of Interests

The authors declare no conflicts of interest.

## References

- [1] World Health Organization (WHO), "WHO Traditional Medicine Strategy 2014-2023," *World Heal. Organ.*, pp. 1–76, 2013, doi: 2013.

- [2] T. Mohammed, B. Erko, and M. Giday, "Evaluation of antimalarial activity of leaves of *Acokanthera schimperi* and *Croton macrostachyus* against *Plasmodium berghei* in Swiss albino mice," *BMC Complement. Altern. Med.*, vol. 14, no. 1, pp. 1–10, 2014, doi: 10.1186/1472-6882-14-314.
- [3] X. ravi Baskaran, A. varuvel Geo Vigila, S. Zhou Zhang, S. Xiu Feng, and W. bo Liao, "A review of the use of pteridophytes for treating human ailments," *J. Zhejiang Univ. Sci. B*, vol. 19, no. 2, Feb. 2018, doi: 10.1631/JZUS.B1600344.
- [4] S. Bouazzi *et al.*, "Cytotoxic and antiviral activities of the essential oils from Tunisian Fern, *Osmunda regalis*," *South African J. Bot.*, vol. 118, pp. 52–57, 2018, doi: 10.1016/j.sajb.2018.06.015.
- [5] T. Dutta, U. Anand, S. C. Saha, A. B. Mane, and A. Dey, "Advancing urban ethnopharmacology: a modern concept of sustainability, conservation and cross-cultural adaptations of medicinal plant lore in the urban environment," vol. 9, pp. 1–20, 2021, doi: 10.1093/conphys/coab073. Introduction.
- [6] G. Alebie, B. Urga, and A. Worku, "Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: Trends and perspectives," *Malar. J.*, vol. 16, no. 1, pp. 1–13, 2017, doi: 10.1186/s12936-017-1953-2.
- [7] J. A. Ampofo, A. Andoh, W. Tetteh, and M. Bello, "Microbiological Profile of Some Ghanaian Herbal Preparations—Safety Issues and Implications for the Health Professions," *Open J. Med. Microbiol.*, vol. 02, no. 03, pp. 121–130, 2012, doi: 10.4236/ojmm.2012.23018.
- [8] T. Dvirna, V. Minarchenko, and I. Tymchenko, "A review of the medicinal Lycopodiophyta of Ukraine," *Scr. Sci. Pharm.*, vol. 6, no. 1, p. 7, 2019, doi: 10.14748/ssp.v6i1.6206.
- [9] B. Narayan Biswal, S. Narayan Das, B. Kumar Das, and R. Rath, "Alteration of cellular metabolism in cancer cells and its therapeutic," *J. oral Maxillofac. Pathol.*, vol. 21, no. 3, pp. 244–51, 2017, doi: 10.4103/jomfp.JOMFP.
- [10] V. Silva *et al.*, "Antimicrobial Resistance and Molecular Epidemiology of *Staphylococcus aureus* from Hunters and Hunting Dogs," *Pathogens*, vol. 11, no. 5, pp. 1–14, 2022, doi: 10.3390/pathogens11050548.
- [11] J. E. Ssenku *et al.*, "Medicinal plant use, conservation, and the associated traditional knowledge in rural communities in Eastern Uganda," *Trop. Med. Health*, vol. 50, no. 1, pp. 1–10, 2022, doi: 10.1186/s41182-022-00428-1.
- [12] N. Kamere *et al.*, "Scoping Review of National Antimicrobial Stewardship Activities in Eight African Countries and Adaptable Recommendations," *Antibiotics*, vol. 11, no. 9, pp. 1–17, 2022, doi: 10.3390/antibiotics11091149.
- [13] A. W. Garedow and G. T. Tesfaye, "Evaluation of Antibiotics Use and its Predictors at Pediatrics Ward of Jimma Medical Center: Hospital Based Prospective Cross-sectional Study," *Infect. Drug Resist.*, vol. 15, pp. 5365–5375, 2022, doi: 10.2147/IDR.S381999.
- [14] H. Nantongo, R. Kiguba, V. Batwala, and J. Mukonzo, "An Evaluation of Antibiotic Prescription Rationality at Lower Primary Healthcare Facilities in Three Districts of South-Western Uganda," *J. Multidiscip. Healthc.*, vol. 15, no. September, pp. 2249–2259, 2022, doi: 10.2147/JMDH.S384297.
- [15] M. Siddiqui, "Phytochemical Analysis of Some Medicinal Plants," *Liaquat Med. Res. J.*, vol. 3, no. 8, pp. 1–5, 2021, doi: 10.38106/lmrj.2021.36.
- [16] L. Damian and S. Pațachia, "Method for Testing the Antimicrobial Character of the Materials and Their Fitting to the Scope," *Bull. Transilv. Univ. Brașov*, vol. 7, no. 56, pp. 37–44, 2014, [Online]. Available: [http://webbut.unitbv.ro/BU2013/2014/Series\\_I/BULETIN\\_I/Damian N.pdf](http://webbut.unitbv.ro/BU2013/2014/Series_I/BULETIN_I/Damian N.pdf)
- [17] M. Balouiri, M. Sadiki, and S. K. Ibsouda, "Methods for in vitro evaluating antimicrobial activity: A review," *J. Pharm. Anal.*, vol. 6, no. 2, pp. 71–79, 2016, doi: 10.1016/j.jppha.2015.11.005.
- [18] A. F. Okiti and O. T. Osuntokun, "Antimicrobial, Phytochemical Analysis and Molecular Docking (In-silico Approach) of *Tithonia diversifolia* (Hemsl.) A. Gray and *Jatropha gossypifolia* L on Selected Clinical and Multi-Drug Resistant Isolates," *J. Adv. Microbiol.*, vol. 20, no. 6, pp. 1–18, 2020, doi: 10.9734/jamb/2020/v20i630248.
- [19] I. L. Elisha, F. S. Botha, L. J. McGaw, and J. N. Eloff, "The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts," *BMC Complement. Altern. Med.*, vol. 17, no. 1, p. 133, 2017, doi: 10.1186/s12906-017-1645-z.
- [20] N. Peetambari, H. Region, and S. Gojendro, "A comparative study on antibacterial activity of integumentary extract of selected freshwater fish Species and Neem extracts against gram-positive and Gram-Negative bacteria," *J. Entomol. Zool. Stud.*, vol. 7, no. 2, pp. 1352–1355, 2019.
- [21] A. Chandra Iwansyah, *From Local Wisdom: Preliminary Antibacterial Activity of "Tanduk Rusa" Fern (*Platyserium Coronarium*) Studies on water-soluble extract of *Labisia pumila var. alata* of Selected Geographic Origins View project*, no. March. 2017. [Online]. Available: <https://www.researchgate.net/publication/314209551>
- [22] K. Li, G. Guan, J. Zhu, H. Wu, and Q. Sun, "Antibacterial activity and mechanism of a laccase-catalyzed chitosan-gallic acid derivative against *Escherichia coli* and *Staphylococcus aureus*," *Food Control*, vol. 96, pp. 234–243, Feb. 2019, doi: 10.1016/J.FOODCONT.2018.09.021.
- [23] D. H. Truong, D. H. Nguyen, N. T. A. Ta, A. V. Bui, T. H. Do, and H. C. Nguyen, "Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *severinia buxifolia*," *J. Food Qual.*, vol. 2019, 2019, doi: 10.1155/2019/8178294.
- [24] Z. Pang *et al.*, "Linking Plant Secondary Metabolites and Plant Microbiomes: A Review," *Front. Plant Sci.*, vol. 12, no. March, 2021, doi: 10.3389/fpls.2021.621276.
- [25] E. Salmerón-Manzano, J. A. Garrido-Cardenas, and F. Manzano-Agugliaro, "Worldwide research trends on medicinal plants," *Int. J. Environ. Res. Public Health*, vol. 17, no. 10, 2020, doi: 10.3390/ijerph17103376.
- [26] G. Cappelli and F. Mariani, "A systematic review on the antimicrobial properties of mediterranean wild edible plants: We still know too little about them, but what we do know makes persistent investigation worthwhile," *Foods*, vol. 10, no. 9, 2021, doi: 10.3390/foods10092217.

- [27] Y. N. Wang, L. H. Meng, and B. G. Wang, "Progress in Research on Bioactive Secondary Metabolites from Deep-Sea Derived Microorganisms," *Mar. Drugs*, vol. 18, no. 12, 2020, doi: 10.3390/md18120614.
- [28] N. El Houda Lezoul, M. Belkadi, F. Habibi, and F. Guillén, "Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants," *Molecules*, vol. 25, no. 20, 2020, doi: 10.3390/molecules25204672.
- [29] K. Sowndhararajan, "A review of the chemical composition and biological activities of *Callistemon lanceolatus* (Sm.) Sweet," *J. Appl. Pharm. Sci.*, vol. 11, no. 12, pp. 65–73, 2021, doi: 10.7324/japs.2021.1101204.
- [30] J. Tan, U. H. Engelhardt, Z. Lin, N. Kaiser, and B. Maiwald, "Flavonoids, phenolic acids, alkaloids and theanine in different types of authentic Chinese white tea samples," *J. Food Compos. Anal.*, vol. 57, pp. 8–15, 2017, doi: 10.1016/j.jfca.2016.12.011.
- [31] M. Wink, "Current Understanding of Modes of Action of Multicomponent Bioactive Phytochemicals: Potential for Nutraceuticals and Antimicrobials," <https://doi.org/10.1146/annurev-food-052720-100326>, vol. 13, pp. 337–359, Mar. 2022, doi: 10.1146/ANNUREV-FOOD-052720-100326.
- [32] B. Xavier-ravi, G. vigila Antony-varuvel, P. Thangaraj, M. R. Doualathabad, and K. Rajan, "Antioxidant, anti-inflammatory activities and HPLC quantification of flavonoids in *Pteris tripartita* Sw. a critically endangered medicinal fern from India," *Biocatal. Agric. Biotechnol.*, vol. 21, no. August, p. 101304, 2019, doi: 10.1016/j.bcab.2019.101304.