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Antibacterial Activity of *Polyalthia longifolia* Leaf Extracts against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Polyalthia longifolia, commonly known as the masquerade plant is a tropical evergreen plant with cultural and medicinal importance in Nigeria. This study used the disc diffusion method to analyze the antibacterial activity of Polyalthia longifolia leaves extracts against Staphylococcus aureus and Escherichia coli. The leaves were dried grounded, and ethanolic and aqueous extracts were used for antibacterial screening against the test isolates. The test isolates were confirmed based on cell morphology, gram reaction, and biochemical tests. The leaf extracts were subjected to phytochemical screening for alkaloids, saponins, flavonoids, steroids, phytosterols, and tannins. Phytochemical analysis of the leaf extracts of Polyalthia longifolia revealed that the leaves of this plant contain alkaloids, anthraquinone, phenols acid, and saponins, while flavonoids and Steroids were absent. The results of the antibacterial screening for aqueous and ethanolic extracts of the plant indicated that ethanol and aqueous extracts of the plant exhibited antibacterial activities with Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations of 100mg/mL for both MIC and MBC.In conclusion, the leaves of the Polyalthia longifolia plant can be used as an alternative medicine for treating bacterial infections caused by Staphylococcus aureus and Escherichia coli.

Keywords: Antibacterial activity, phytochemical analyses, Polyalthia longifoliaextracts.

INTRODUCTION

Polyalthia longifolia is a plant with a rich history in traditional medicine across various cultures (Gupta *et al.*, 2022). It has been utilized for its diverse medicinal properties, including antibacterial effects. *Staphylococcus aureus* and *Escherichia coli* are two significant bacterial pathogens that cause many human infections, with increasing concerns about antibiotic resistance (Gupta *et al.*, 2022).

According to Ghosh *et al.* (2021), *Staphylococcus aureus* is a gram-positive bacterium that commonly colonizes human skin and nasal passages. Although it is usually not harmful, it can lead to various infections, ranging from mild skin infections to potentially fatal illnesses like endocarditis, pneumonia, and bloodstream infections. However, the gram-negative bacterium *Escherichia coli*is frequently discovered in human and animal intestines. A few strains can result in serious bloodstream infections, urinary tract infections, and severe gastrointestinal infections, even though most are benign (Ghosh*et al.*, 2021).

Understanding the potential antibacterial properties of the *Polyalthia longifolia* plant against these two bacterial pathogens is crucial for exploring alternative treatment options, particularly in the context of increasing antibiotic resistance. Natural plant-based compounds have gained attention due to their potential to provide effective antibacterial activity while minimizing the risk of resistance development (Yongabi and Njock, 2020).

Recently, interest in exploring natural sources for novel antibacterial compounds, given the limitations and challenges associated with conventional antibiotics. *Polyalthia longifolia* has shown promising antibacterial activity against some pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*, making it a potential candidate for further

investigation (Gupta *et al.*, 2022). However, despite these initial findings, further analysis is needed to comprehensively evaluate the antibacterial strength of *Polyalthia longifolia* against *Staphylococcus aureus* and *Escherichia coli*. Additionally, recent research has focused on identifying and characterizing the specific bioactive compounds responsible for these antibacterial properties.

MATERIALS AND METHOD

Collection of *Polyalthia longifolia* leaves and authentication.

The *Polyalthia longifolia* leaves were collected from the Kawo market in the Kaduna metropolis. A botanist from the Department of Biological Sciences at Kaduna State University, Kaduna authenticated the collected leaves.

Confirmation of the test organisms

The test isolates were verified based on cell appearance, gram response, and biochemical testing. The biochemical tests consist of the following: the coagulase and catalase tests for S. *aureus*, the Indole, Methyl red, Vougesproskeur, and citrate utilization for *E. coli* (Holt *et al.*, 2019).

Preparation of ethanol extract of *Polyalthia longifolia* leaves.

At room temperature, fifty (50) grams of *P. longifolia* powdered leaves were steeped in 250 mL of ethanol for a whole day. To prevent contamination and evaporation, the container was sealed firmly. Periodically, the components that had been soaked were stirred. Whitman filter paper (No. 1) was used on several filtration configurations to filter the soaked materials after 24 hours. Over a water bath, the filtrate was dried out by evaporation. The dried extract was then scraped from the bottom of the evaporating dish and was weighed to determine the percentage yield and stored in a sterile container (Cheesebrough, 2002).

Preparation of aqueous extract of *Polyalthia longifolia* leaves

Two hundred and fifty milliliters (250mL) of distilled water were macerated with fifty (50) grams of dried powdered *Polyalthia longifolia* leaves, and the mixture was left to stand for 24 hours with periodic stirring. Whitman filter paper (No. 1) was used on several filtration configurations to filter the soaked materials after 24 hours. Over a water bath, the filtrate was dried out by evaporation. The dried extract was then scraped from the bottom of the evaporating dish and was weighed to determine the percentage yield and stored in a sterile container (Cheesebrough, 2002).

Phytochemical screening

The leaf extracts obtained were subjected to phytochemical screening for alkaloids, saponins, flavonoids, steroids, phytosterols, and tannins, as described by Sharma *et al.* (2020).

Screening for Antibacterial activity

A suspension of the S. *aureus* and *E. coli* isolates were prepared in sterile distilled water and the turbidity of the suspension was adjusted to match the turbidity of a 0.5 McFarland standard. The surface of both organisms' Muller Hinton agar plates was swabbed with the S. *aureus* and the *E. coli* suspension using a sterile cotton swab. The inoculated plates were allowed to dry for a few minutes (CLSI, 2020).

Sterile filter paper discs were soaked in the prepared different concentrations of *Polyalthia longifolia* leaf extract for 30 seconds, and gently removed the excess liquid from the discs using sterile forcep. The soaked discs were dried in an oven and then placed on the surface of the inoculated agar plates using sterile forceps. The inoculated plates were incubated upside down in an incubator for 24 to 48 hours at 37°C (Ngamsurach and Praipipat, 2022).

Measurement of the zone of inhibition

Following incubation, the plates were checked to see any clear areas surrounding the discs. The plant extract's antibacterial activity was assessed by measuring the diameter of the zones in millimeters (mm). The results were recorded and compared to those of Ciprofloxacin used as control discs (Oyeleke and Manga, 2008).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was calculated with 200, 100, 50, 25, and 6.25 mg/mL extract concentrations. Using 2 mL syringes, two milliliters of Muller Hinton broth were added to test tubes 1 through 8. As a

positive control, tube 7 held two milliliters of the isolate-containing medium, while tube 8, which held two milliliters of Muller-Hinton broth with extract, was a negative control. Using 0.5 mL volumes of the working inoculums that had been previously adjusted to 0.5 cells/mL (0.5 McFarland Turbidity Standard), doubling dilutions were made from tubes 2 and then transferred into tubes 1-6. Two milliliters (2.0 mL) of broth and 0.5 mL of the working inoculums without the extracts were found in tube 7; 2.0 mL of broth and extract were found in tube 8; and 2 mL of broth, inoculum, and antibiotics were found in tube 9 which is the positive control. For a whole day, each test tube was incubated at 37^0C. The MIC was determined by taking concentrations at which the test organism does not appear to be growing (Sharma *et al.*, 2020).

Determination of Minimum Bactericidal Concentration (MBC)

A loop of broth from each set of test tubes used in the MIC determination was then inoculated on a sterile Muller Hinton agar if no visible growth was observed. The control was provided using Muller Hinton agar plates streaked with the test organisms. For a whole day, the plates were incubated at 370C. The Minimum Bactericidal Concentration (MBC) was the lowest concentration at which no discernible growth occurred (Ochei and Kolhatkar, 2007).

RESULTS

The phytochemical analysis indicates the presence of saponins, tannins, anthraquinones, terpenoids, alkaloids, and cardiac glycosides in theEthanol extract as shown in Table 1.

The plant showed antimicrobial activity against the tested microorganisms at different concentrations, according to the results of the antimicrobial screening for the ethanolic and aqueous extract of *Polyalthia longifolia*. The zone of inhibition was noted and is shown in Tables 2 and 3, respectively. The highest zones of inhibition were obtained at a concentration of 200mg/mL for the ethanolic and aqueous extract, which was the highest concentration used in this study. The diameter of inhibition zones for each sample was compared with standard antibiotic ciprofloxacin.

Table 1: Phytochemical constituents of the leaf extracts

Phytochemical	Ethanol extract	Aqueous extract
Saponins	+	+
Alkaloids	+	+
Steroids	-	-
Flavonoids	-	-
Anthraquinone	+	+
Phenols	+	+
Tannins	+	+
Terpenoid		-
Cardiac glycocide	+	-

Keys: + = present, and _is absent

Table 2: Antibacterial activity of the ethanolic leaf extract of *Polyalthia longifolia*

Organism	Concentrations/Zone of Inhibition (mm)				Antibiotic
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	CF
S. aureus	22	17	14	8	24
E. coli	18	14	10	NA	31

Key: NA=no activity, CF-ciprofloxacin

Table 3: Antibacterial activity of aqueous leaf extract of *Polyalthia longifolia* plant

Organism	Concentrations/Zone of Inhibition (mm)			
	200mg/ml	100mg/ml	50mg/ml	25mg/ml
S.aureus	8	5	0	
E. coli	6.5	0	0	0

The MIC and MBC of *Staphylococcus aureus* and *Escherichia coli* were presented in Tables 4 and 5, respectively, as shown below.

Table 4: Minimum inhibitory concentration of *Polyalthia longifolia* leaves extract against *Staphylococcus aureus*and *Escherichia coli*

	Concentrations of extract			
Organism	**200mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**
S.aureus(mm)	_	*	+	+
E. coli (mm)	_	*	+	+

Key: + = Presence of Organism (Turbid) - = Absent of Organism (Clear) * = MIC value

Table 5: Minimum bactericidal concentration of *Polyalthia longifolia* leaf extract against *Staphylococcus aureus* and *Escherichia coli*

	Concentration of extract (mg/m)			
Organism	**25mg/ml**	**50mg/ml**	**100mg/ml**	**200mg/ml**
S. aureus (mm)	+	+	*	_
E. coli (mm)	+	+	*	_

Key: + = Presence of Organism (Turbid) - = Absent of Organism (Clear) *=MBCvalue

DISCUSSION

Alkaloids, saponins, anthraquinone, phenols, tannins, terpenoids, and cardiac glucoside were found in leaf extracts from *Polyalthia longifolia* plants. However, flavonoids and steroids were not. Alkaloids, saponins, phenols, tannins, and anthraquinone are among the aqueous extract's phytochemical components; flavonoids, terpenoids, cardiac glucoside, and steroids are not. Saponins exhibit antioxidant, anti-cancer, anti-inflammatory, anti-hyperglycemia, anti-lipogenic, and anti-loss properties. Nebyu et al. (2023) claim that saponin is a plant's antibacterial agent. Phenolic compounds have been shown to have anti-inflammatory, anticarcinogenic, anti-oxidative, antimutagenic, and antidiabetic properties (Hamad and Al Mamari, 2021).

Compared to the aqueous extract, the ethanolic extract showed stronger antibacterial properties. This might be because ethanol extracts more phytoconstituents than water. After all, it is an organic solvent. This was further supported by Medini*et al.*'s (2014) earlier investigation. The results showed that the bacterial isolates' susceptibility to *Polyalthia longifolia* leaf extracts was similar to that of Ciprofloxacin (positive standard), suggesting that *Polyalthia longifolia* leaf extracts could be used as an additional or alternative antibacterial agent. The bacterial isolates chosen for this investigation were susceptible to *Polyalthia longifolia* leaf extracts in both aqueous and ethanolic forms at different doses, with MIC and MBC values of 100 mg/mL.

Babatunde et al. (2023) showed that crude ethanol extract from Polyalthia longifolia leaf was effective in inhibiting *E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. This suggests that the activities of the extracts were dose dependent. Since *E. coli* is a Gram-negative bacterium with lipopolysaccharides, which may have prevented the active components of the extracts from penetrating, the isolates' varying susceptibilities to different concentrations could be explained by differences in the composition of the bacteria's cell walls. *Staphylococcus aureus*, a Gram-positive bacterium, tends to allow diffusion of the active components.

Because of the permeability barriers provided by its outer membrane, which comprises lipopolysaccharides, *E. coli* is typically resistant to most antibiotics, which may also account for *S. aureus*'s increased sensitivity. This was also consistent with the findings of Yahia*et al.* (2020), who found that Gram-positive bacteria exhibited a larger zone of inhibition than Gram-negative bacteria. However, Abubakar *et al.* (2018) found that at 150 mg/ml concentration, *E. coli* exhibited greater susceptibility than *S. aureus*. However, all of the extract's actions were dose-dependent, which is consistent with studies by Sakha *et al.* (2018) that found that the extract's antibacterial activity increased at higher concentrations. Also, Uzama Danlami*et al.* (2011) reported that the leaf extract of *Polyalthia longifolia*showed the highest activity against *S.aureus* and *E.coli, and the* lowest activity was observed in *Bacillus subtilis.*

CONCLUSION

Phytochemical analyses of the leaf extract of *Polyalthia longifolia*revealed the presence of alkaloids, anthraquinone, phenols, terpenoid, cardiac glucoside, and saponins, while the aqueous extract revealed similar constituents excluding terpenoids and cardiac glucoside. The results of the antibacterial activity for aqueous and ethanol extract of *Polyalthia longifolia* leaf

exhibited antibacterial activity, and the extent of the activity was concentration-dependent. The plant was more effective on gram-positive S. *aureus* than *E. coli,* which is gram-negative. The leaves of the *Polyalthia longifolia* plant have antibacterial properties that make them useful for making medications that treat bacterial illnesses.

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