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Research Article

Antibacterial Potential of *Polyalthia longifolia* (Sonn. Thwaites) Seed and Pericarp Extracts Against *Salmonella typhi* and *Escherichia coli*

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Abstract

This study evaluated the antibacterial activity of ethanolic extracts of *Polyalthia longifolia* seeds and pericarp against *Salmonella typhi* and *Escherichia coli*. Antibacterial screening using agar diffusion revealed concentration-dependent effects, with maximum inhibition observed at 100% concentration. The seed extract exhibited slightly higher activity against *S. typhi* (13 mm) compared to the pericarp extract (12 mm), while the pericarp extract showed greater inhibition of *E. coli* (9 mm) than the seed extract (7 mm). Both extracts displayed reduced activity at lower concentrations, consistent with the dose-dependent nature of crude plant extracts and reflecting the influence of bioactive metabolite concentration on antibacterial potency. Further insights were obtained through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The seed extract inhibited *E. coli* at 50 mg/mL and demonstrated bactericidal effects against both *S. typhi* and *E. coli* at 100 mg/mL. In contrast, the pericarp extract required 100 mg/mL to inhibit both organisms and primarily exhibited bacteriostatic activity. These findings indicate that the seed extract possesses greater antibacterial potency than the pericarp, particularly against *E. coli*, aligning with previous studies highlighting the antimicrobial potential of P. longifolia seeds and their bioactive phytochemicals. Although ciprofloxacin produced larger inhibition zones (16–22 mm), the antibacterial activity of both extracts remains pharmacologically relevant. Collectively, these results demonstrate that *P. longifolia* seeds and pericarp have significant antibacterial properties and support their potential as a natural source of bioactive compounds for managing enteric bacterial infections.

Keywords

Polyalthia longifolia, Seeds Extract, Pericarp Extract, Antibacterial Activity, *Salmonella Typhi*, *Escherichia Coli*

1. Introduction

Antibiotic resistance has become one of the most serious threats to global public health, indeed reversing much of the progress made in treating infectious diseases. It renders previously effective treatments futile, with results in prolonged illness, high mortality, and increased health care costs [17]. In 2019, antimicrobial-resistant infections were associated with about 4.95 million deaths globally, while 1.27 million of these deaths were directly attributable to drug-resistant bacteria [15]. For the last several decades, the burden of bacterial resistance continued to grow, reaching 4.71 million associated deaths

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globally in 2021 [16]. Therefore, antimicrobial resistance is on the list of the top global health challenges, and according to an estimate of the World Health Organization, AMR may cause up to 10 million deaths a year by 2050 if no control measures are taken, which will outrank cancer as the leading mortality cause [25].

Among the bacterial pathogens of greatest concern are multidrug-resistant strains of *Salmonella typhi* and *Escherichia coli*. For instance, *Salmonella typhi*, causing typhoid fever, is estimated to infect about 10.9 million people worldwide and kills approximately 100,000 people each year [16]. Since 2016, the emergence and spread of extensively drug-resistant (XDR) *S. typhi*, mostly from Pakistan and its vicinity, have posed a severe challenge to treatment options [9]. Similarly, *E. coli*, a leading cause of urinary tract and gastrointestinal infections, has shown rising resistance to commonly used antibiotics [10]. In several areas, resistance levels against trimethoprim-sulfamethoxazole are greater than 20%. Moreover, extended-spectrum β-lactamase (EBLS)-producing strains are resistant to third-generation cephalosporins, and their infection is becoming more prevalent, making management difficult at both community and outpatient levels [11].

The increasing incidence of multidrug-resistant *S. typhi* and *E. coli* infections has resulted in frequent therapeutic failures, prolonged regimens of treatment, and increased morbidity and mortality especially in poor and middle-income countries where advanced antimicrobial therapies are not easily accessible [12]. Despite the growing urgency of this problem, the development of new antibiotics has slowed considerably, prompting renewed interest in alternative and complementary sources of antimicrobial agents.

Medicinal plants have a rich reservoir of bioactive compounds with antimicrobial activity. For generations, plants have been used as medicines to treat various infectious diseases around the world [19]. Plants synthesize diverse secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds that may act on the bacteria either through bactericidal or bacteriostatic mechanisms, thus inhibiting growth or interfering with vital cellular processes [7]. In vitro activity has been demonstrated recently against several pathogenic antibiotic-resistant bacterial strains for extracts from such plant species as *Ocimum sanctum, Curcuma longa*, and *Quercus alba* [26].

Polyalthia longifolia (Sonn.) Thwaites, commonly known as the Masquerade Tree, is an aromatic plant belonging to the family Annonaceae with a wide distribution around the tropical regions [21]. During the traditional medical practices that prevail through India, Sri Lanka, and most parts of Africa, including Nigeria, it is utilized as a source of treatment for fever, skin diseases, inflammation, and gastrointestinal infections [22]. Scientific studies have supported these ethnomedicinal uses, with ethanol and methanol extracts of the leaves, bark, and roots demonstrating antibacterial, antifungal, antioxidant, and anti-inflammatory activities [1]. Phytochemical screening of this plant has indicated the presence of clerodane diterpenoids, aporphine and azafluorene alkaloids, flavonoids, and compounds known to act on bacterial cell membranes and affect biofilm formation [14].

Despite these findings, the seeds and pericarp of *P. longifolia* have remained underexplored for their antibacterial potential. Nutritional studies indicate the seeds as being rich in potassium and other leading essential micronutrients [13]. Preliminary reports have described pericarp extracts as having antimicrobial potentials superior to those of the leaf extracts against some selected bacterial species [5]. Thus, the extracts from the seeds and pericarp have not been systematically assessed for their antibacterial activity against clinically relevant drug-resistant pathogens. The increasing resistance of *S. typhi* and *E. coli* to standard antibiotics supports the exploration of the underutilized parts of *P. longifolia* in searching for novel, affordable, and plant-based agents capable of contributing to the management of resistant bacterial infections.

2. Materials and Methods

2.1. Study Area

The study was an *invitro* experimental design to evaluate the antibacterial activity of the ethanolic extracts of seed and pericarp of *Polyalthia longifolia* against bacteria isolates gotten from archived clinical cultures maintained at the University of Benin Microbiology Laboratory. The antibacterial analysis was conducted in a controlled Microbiology laboratory in University of Benin, Edo State, Nigeria.

2.1.1. Plant Collection and Identification

Fresh plant materials, specifically the seed and pericarp of *Polyalthia longifolia*, were collected from different locations in the main campus of the University of Delta, Agbor, Delta State. The plant was authenticated and issued a voucher number (UBH-M346) by a taxonomist at the Department of Plant Biology and Biotechnology, University of Benin, Benin city.

2.1.2. Plant Preparation

The collected parts of the plants were rinsed thoroughly with clean water, air-dried at room temperature for approximately six weeks until constant weight was achieved, then pulverised using a clean electric blender. The powdered samples were stored separately in clean, airtight containers at room temperature before to extraction.

2.1.3. Extraction Method

The powdered seed and pericarp of *Polyalthia longifolia* were extracted separately using absolute ethanol. 50 g of the powdered seed and pericarp were soaked in 300 mL of absolute ethanol. The container was sealed and allowed to macerate for 72 hours at room temperature, with periodic shaking to

improve solvent penetration and phytochemical release. After maceration, the ethanol mixture was sieved and filtered using Whatman No. 1 filter paper to obtain the ethanolic extract. Following filtration, the filtrates were concentrated using a water bath at 60°C until a thick extract was obtained. This was then stored in sterile containers and kept at 4°C prior to use.

2.2. Test Microorganisms

Bacterial isolates used in the study are *Escherichia coli* and *Salmonella typhi*. These were obtained from archived clinical cultures maintained in the Microbiology Laboratory of the University of Benin. The isolates were stored at 4°C and sub-cultured onto Mueller-Hinton agar plates, followed by incubation at 37°C for 24 hours to revive and ensure purity.

2.3. Antibacterial Screening

The antibacterial activity of the seed and pericarp was evaluated using the agar well diffusion method, as described by Valgas *et al.* (2007). Serial concentrations of the extracts were prepared to evaluate their dose-dependent effects: 12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL, using sterile distilled water as the diluent. Sterile nutrient agar plates were prepared and seeded with the standardized bacterial suspension using sterile swab sticks. Wells (6 mm) were bored into the agar and filled with the respective extract concentrations. Ciprofloxacin (5 µg/disc) was used as the positive control. The plates were incubated at 37°C for 24 hours and the zones of inhibition were measured in millimeters.

2.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC was determined using the broth dilution method. Serial dilutions of the extracts (12.5–100 mg/mL) were prepared in nutrient broth. Each test tube contained 9 mL of nutrient broth, 1 mL of extract, and 0.1 mL of standardized bacterial suspension. After 24 hours of incubation at 37°C, turbidity was assessed. The lowest concentration showing no turbidity was recorded as the MIC [2]. Aliquots from clear MIC tubes were streaked onto sterile nutrient agar and incubated at 37°C for 24 hours. Plates with no growth indicated bactericidal concentrations. The MBC was recorded as the lowest concentration that completely killed the bacteria [24].

3. Results

The antibacterial activity of *Polyalthia longifolia* ethanolic seed and pericarp extracts was determined against *S. typhi* and *E. coli*. The mean zones of inhibition (mm) from four independent replicates (12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL) are shown in Table 1. At the highest concentration tested (100mg/mL), both extracts showed notable antibacterial activity. For *S. typhi*, the seed extract produced a zone of inhibition of 13 mm, while the pericarp extract measured 12 mm. At 50% concentration, the seed and pericarp extracts yielded inhibition zones of 6 mm and 8 mm, respectively. At 25mg/mL, only the pericarp extract retained slight activity (3 mm), with no inhibition detected at 12.5 mg/mL. Against *E. coli*, the pericarp extract demonstrated greater activity, with inhibition zones of 9 mm (100mg/mL) and 4 mm (50mg/mL), compared to 7 mm and 3 mm from the seed extract. Lower concentrations (25mg/mL and 12.5mg/mL) were inactive. In contrast, ciprofloxacin exhibited significantly higher inhibition zones-22 mm against *S. typhi* and 16 mm against *E. coli* confirming standard antibiotic potency.

Table 1. *Antibacterial Activity of Ethanolic Extracts of* Polyalthia longifolia *on the bacterial isolates.*

Organism	Extract	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	CPX (5 µg/disc)
S. typhi	Seed	13.00 ± 0.00	6.00 ± 0.00	NI	NI	22.00 ± 0.00
	Pericarp	12.00 ± 0.00	8.00 ± 0.00	3.00 ± 0.00	NI	
E. coli	Seed	7.00 ± 0.00	3.00 ± 0.00	NI	NI	16.00 ± 0.00
	Pericarp	9.00 ± 0.00	4.00 ± 0.00	NI	NI	

Key: CPX – Ciprofloxacin (positive control): NI = No Inhibition.

The MIC values shown in Table 2 revealed that the seed extract had stronger inhibitory effects against *E. coli* (50 mg/mL) compared to the pericarp (100 mg/mL). Both extracts showed equal potency against *S. typhi* (MIC 100 mg/mL).

Table 2. *Minimum Inhibitory Concentration (MIC) of Ethanolic Seed and Pericarp Extracts of* Polyalthia longifolia *Against Bacterial Isolates.*

Organism	Seed Extract (mg/mL)	Pericarp Extract (mg/mL)
S. typhi	100.00 ± 0.00	100.00 ± 0.00
E. coli	50.00 ± 0.00	100.00 ± 0.00

The MBC results shown in Table 3 demonstrated bactericidal activity of the seed extract against *S. typhi* at 100 mg/mL, while the pericarp extract was bacteriostatic; against *E. coli*, both extracts were bacteriostatic at their respective MICs.

Table 3. *Minimum Bactericidal Concentration (MBC) of Ethanolic Seed and Pericarp Extracts of* Polyalthia longifolia *Against Bacterial Isolates.*

Organism	Seed Extract (mg/mL)	Activity	Pericarp Extract (mg/mL)	Activity
S. typhi	100.00 ± 0.00	Bactericidal	100.00 ± 0.00	Bacteriostatic
E. coli	50.00 ± 0.00	Bacteriostatic	100.00 ± 0.00	Bacteriostatic

4. Discussion

The ethanolic seed and pericarp extracts showed concentration-dependent antibacterial activity, as indicated by the agar diffusion method, with the highest zone of inhibition at the highest concentration of 100mg/mL gradually decreasing upon dilution. Both extracts inhibited *Salmonella typhi* and *Escherichia coli* at 100mg/mL and 50mg/mL, with no or little activity at lower concentrations. This dose-response pattern is typical for crude plant extracts whose bioactive compounds occur in low abundance, providing an API with higher antimicrobial activity at higher concentrations [7, 23]. Other parts of *Polyalthia longifolia* have also been reported widely to possess concentration-dependent antibacterial activity when using ethanolic and other organic solvents [16]. The seed extract yielded a slightly higher inhibition zone against *S. typhi* than the pericarp extract, with the inhibition zones being 13 mm and 12 mm, respectively, at 100mg/mL concentrations, both lower than the positive control, ciprofloxacin, which had an inhibition zone of 22 mm. The present study corroborates previous studies indicating moderate enteric pathogen inhibitory activity in fractions derived from the seeds of *P. longifolia*, which could be ascribed to clerodane diterpenoids and other related secondary metabolites [20]. Clerodane and diterpenoids are generally lipophilic, allowing them to penetrate the outer membrane of Gram-negative bacteria.. They disorganize phospholipid structures, increasing membrane permeability. This leads to leakage of intracellular contents (ions, proteins, nucleotides), ultimately causing cell death. Studies have shown that diterpenoids can destabilize lipopolysaccharide (LPS) structures, weakening the protective barrier of Gram-negative organisms. [20], This is, however, slightly significant, having *S. typhi* in mind for its clinical importance and the rising drug-resistance strains globally [8].

The pericarp extract was found to possess greater diffusion-based activity against *E. coli* (9 mm at 100mg/mL concentration) than the seed extract (7 mm) while, ciprofloxacin is still vastly superior (16 mm). The lower activity against *E. coli* in comparison with *S. typhi* has also been reported in previous studies and might be generally attributable to structural and functional properties of Gram-negative bacteria, such as low membrane permeability and efficient efflux mechanisms, which reduce the accumulation of phytochemicals inside the cells [8]. There have been reports on comparable inhibition zones against *E. coli* for the ethanolic extract of *P. longifolia*, reinforcing the reproducibility of moderate antibacterial activity against this organism when using crude extracts [18].

The MIC values further quantified the bacteriostatic activity of the extracts. Both seed and pericarp extracts were only able to inhibit *S. typhi* at 100 mg/mL, which correlates with the moderate inhibition observed in the diffusion assay. On the contrary, the seed extract was more potent against *E. coli* at a lower concentration of 50 mg/mL than the pericarp extract at 100 mg/mL, although it had a smaller inhibition zone on agar. The disparity between diffusion and broth dilution results has been thoroughly documented and is attributed to the difference in solubility, diffusion rate, and interaction with the agar matrix; conversely, MIC assays measure intrinsic antimicrobial potency in liquid media [24].

The MBC values equaled the corresponding MIC values for both extracts against the test organisms. A value for the MBC/MIC ratio of ≤ 4 is considered indicative of bactericidal activity, based on established interpretive criteria [6, 23]. This indicates that, at sufficiently high concentrations, the extracts exert bactericidal activity instead of merely inhibiting the growth of bacteria. A series of reports on similar high-concentration bactericidal activities for *P. longifolia* seed extracts and other plant parts-diterpenoids and fatty acids, for example, have implicated mechanisms of actions such as those affecting the integrity of cellular membranes and metabolic interference [3, 4].

Although ciprofloxacin displayed more potent antibacterial activity in all the assays, the observed moderate, yet consistently present, activity of the seed and pericarp extracts is undeniably relevant. Crude plant extracts are widely regarded as preliminary sources of antimicrobial lead compounds, rather than direct therapeutic agents, justifying further fractionation, phytochemical characterization and synergistic studies [7]. Given the rising global burden of antibiotic resistance, the extracts have demonstrated bactericidal efficacy at extremely high effective doses. As a result, *Polyalthia longifolia* should be investigated further for new sources of antibacterial compounds.

5. Conclusion

This study revealed that *Polyalthia longifolia* seeds and pericarp had strong antibacterial properties, which are most likely attributable to bioactive phytochemical compounds. Both extracts inhibited *Salmonella typhi* and *Escherichia coli*, but the seed extract was more effective overall, as shown by a lower MIC against *E. coli*, slightly higher inhibition zones, and bactericidal MBC results. These findings provide validity to *P. longifolia*'s traditional use and highlight the seed extract as a possible candidate for future development as a plant-based antibacterial agent. To enhance therapeutic potential, future studies should incorporate in vivo testing, molecular mechanism elucidation, extraction optimization, and phytomedicine standardization.

6. Recommendations

The findings of this study demonstrate that extracts from *Polyalthia longifolia*, particularly the seed and pericarp fraction, possess significant antibacterial activity against clinically relevant Gram-negative pathogens such as *E. coli* and *S. typhi.* The comparatively lower minimum inhibitory concentration (MIC), higher zones of inhibition, and bactericidal minimum bactericidal concentration (MBC) observed for the extracts indicate its superior efficacy and reinforce its potential as a promising source of bioactive antimicrobial compounds. These results provide scientific support for the ethnomedicinal use of *P. longifolia* and justify further exploration of its pharmacological applications.

To advance the translational potential of these findings, it is recommended that *in vivo* studies be conducted to validate the safety, pharmacokinetics, and therapeutic efficacy of the extracts under physiological conditions. Such studies are essential for bridging the gap between *in vitro* antimicrobial activity and clinical applicability. Secondly, there is a need for comprehensive molecular investigations to elucidate the precise mechanisms of antibacterial action

Furthermore, phytochemical standardization and characterization of the active constituents are necessary to ensure reproducibility, quality control, and regulatory compliance. Whilst the present study establishes *P. longifolia* as a promising candidate for plant-based antibacterial development, systematic and multidisciplinary investigations are required to fully harness its therapeutic potential and facilitate its progression into clinically relevant phytomedicines.

Abbreviations

MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
LPS	Lipopolysaccharides

Author Contributions

Pass Chidiebere Chijindu: Conceptualization, Supervision, Writing – original draft

Jude Chukwuemeke Igborgbor: Writing – review & editing

Michael Obinna Nwayo: Data curation, Methodology

Conflicts of Interest

The authors declare no conflict of interests.

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