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PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACTS OF *Polyalthia longifolia* AND *Annona senegalensis* PLANTS

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Abstract

This study investigates the phytochemical profile and antimicrobial potential of methanolic extracts of *Polyalthia longifolia* and *Annona senegalensis*, medicinal plants extensively used in African traditional medicine. The extracts underwent preliminary phytochemical screening to detect the presence of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids, steroids, glycosides, and anthraquinones using standard protocols. The antimicrobial activity of the extracts was assessed against *Escherichia coli, Salmonella typhi, Bacillus subtilis,* and *Candida albicans* using the agar well diffusion method. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, tannins, saponins, steroids, and terpenoids in

Introduction

The reliance on medicinal plants for the treatment and management of various human diseases dates back to antiquity and remains significant in contemporary healthcare systems, especially in developing countries. It is estimated that over 80% of the world's population depends on traditional medicine, predominantly based on plant-derived substances, for primary healthcare (World Health Organization, 2002). The global search for novel

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different plant parts. Extracts of P. longifolia, particularly stem bark and root, exhibited broad-spectrum antimicrobial effects with zones of inhibition ranging from 21.0–27.0 mm, in some cases exceeding the efficacy of standard antibiotics. A. senegalensis root extract showed moderate antibacterial effects but was most active against C. albicans. These findings substantiate the traditional use of these plants and indicate the potential of their phytoconstituents for the development of novel antimicrobial agents

Keywords: Phytochemicals, antimicrobial, *Polyalthia longifolia, Annona senegalensis*, traditional medicine

Antimicrobial agents has reignited interest in medicinal plants as potential sources of bioactive compounds, particularly in the face of rising antibiotic resistance (Newman and Cragg, 2020).

Plants synthesize a wide array of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and phenolic compounds, many of which exhibit potent pharmacological activities (Cowan, 1999). These phytochemicals have been shown to possess antimicrobial, anti-inflammatory, antioxidant, and anticancer properties, making plants a valuable reservoir for drug discovery and development (Fabricant and Farnsworth, 2001).

Polyalthia longifolia (Family: *Annonaceae*) which is commonly known as an Indian mast tree is a versatile plant known for its use in environmental beautification and herbal medicine. It has been shown to possess a variety of medicinal properties in indigenous systems of medicine. Almost all parts of the plant are used in traditional medicine to treat various human diseases. The stem bark of *P. longifolia* is used to treat, among other ailments; inflammation, digestive disorders, gonorrhea, mouth ulcers, menorrhagia and leucorrhoea (Yao *et al.,* 2019; Bisht *et al.,* 2024). Extract of leaf and bark are used as teas in the treatment of fever, diabetes and skin diseases (Yao *et al.,* 2019). Ethno-pharmacological reports suggest that *P. longifolia* leaf depresses heart muscles, lowers blood pressure, and stimulates respiration (Firdous *et al.,* 2022). Different solvent extracts of various parts of *P. longifolia* (e.g. leaves, root bark, stem bark, green berries, flowers, etc.) have been reported to demonstrate antimicrobial properties (Bisht *et al.,* 2024). Anticancer potential of *P. longifolia* tested in various cell lines with the mechanism of apoptosis induction has also been

reported (Soundararajan *et al.,* 2023). Bioactive principles such as diterpenes, alkaloids, steroids, and lactones have been isolated from different parts of the plant and reported to possess anti-inflammatory, antibacterial, antifungal, anti-tumor, antioxidant activities (Manjula *et al.,* 2010).

Annona senegalensis is a traditional medicinal plant commonly used in traditional medicine for the treatment of various diseases. It also belongs to a family of Annonaceae and commonly called Wild Custard Apple and Wild Soursop, etc (Babalola *et al.,* 2021; Okhale *et al.,* 2016). It is a shrub or small tree 2-6 m tall but may reach 11m under favorable conditions. It is widely spread throughout tropics and sub-tropics. It is commonly used for therapeutic fighting of major diseases including dysentery, diabetes mellitus, heart stroke, epilepsy, parasite and worm infestations, constipation, hemorrhage, dysuria, fever, ulcer, and cancers. The plant has also been reported for several biological activities including anti-oxidant, antidiarrheal, antimicrobial, antiparasitic, anticonvulsant, antitrypanosomal, antimalarial, anti-inflammatory, anti-snake venom, and antinociceptive.

Given the profound medicinal potentials of these two plants, and the pressing need for new antimicrobial agents, this study focused on the phytochemical screening and evaluation of antimicrobial activities of the methanolic extracts of the stem bark and roots of *Polyalthia*

Materials and Methods

Sample Collection, Preparation and Extraction

Fresh samples of leaves, stem bark, and roots of *Polyalthia longifolia* and *Annona senegalensis* were collected from Mubi North Local Government Area of Adamawa State, Nigeria, in December, 2024. The plants samples were authenticated by a botanist from the Botany Department of Modibbo Adama University, Yola, and voucher specimens were assigned for future reference.

Collected plant samples were washed with distilled water, air-dried under shade at ambient temperature (25–28°C) for 14 days, and pulverized using a mechanical grinder. Exactly 100 g of each powdered sample was subjected to Soxhlet extraction with 500 mL of methanol (analytical grade; Sigma-Aldrich) for 6 hours. Extracts were filtered and concentrated using a rotary evaporator (Büchi Rotavapor R-300) at 40°C. and kept for further use.

Phytochemical Screening

The methanolic extracts were subjected to standard qualitative phytochemical screening to detect the presence of major classes of secondary metabolites based on established methods:

- **Alkaloids**: Detected using Dragendorff's and Mayer's reagents. A positive test was indicated by the formation of a precipitate.
- **Flavonoids**: Confirmed by the lead acetate and alkaline reagent tests, indicated by yellow coloration or turbidity.
- **Tannins**: Identified using ferric chloride solution, with a blue-black or greenish coloration indicating presence.
- **Saponins**: Detected via frothing test by vigorous shaking and observation of stable foam.
- **Glycosides**: Keller-Kiliani test was used, characterized by the formation of a reddish-brown ring at the interface.
- **Steroids and Terpenoids**: Detected through Salkowski's test, indicated by red or green color development.
- **Phenolic compounds**: Ferric chloride test was employed, showing blue or green color for positive samples.

Antimicrobial Activity Assay

Microbial Strains

Clinical isolates of Bacillus subtilis (Gram-positive bacterium), Salmonella typhi (Gram-negative bacterium), Escherichia coli (Gram-negative bacterium), and Candida albicans (fungus) were obtained from the Microbiology Department, Adamawa State University Mubi.

Preparation of Inoculum

Bacterial strains were cultured on nutrient agar, while Candida albicans was cultured on Sabouraud dextrose agar. Fresh colonies were suspended in sterile saline and adjusted to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

Agar Well Diffusion Method

The antimicrobial activity was evaluated by the agar well diffusion method: Mueller-Hinton agar plates (for bacteria) and Sabouraud dextrose agar plates (for fungi) were uniformly seeded with the microbial inocula. Wells of 6 mm diameter were bored into the agar using a sterile cork borer. 100 µL of the plant extract solutions at concentrations of 50, 75, and 100 mg/mL were introduced into respective wells. Positive controls used were Gentamycin (10 µg/mL) for bacterial isolates and Fluconazole (10 µg/mL) for *Candida albicans*. Plates were incubated at 37°C for 24 hours (bacteria) and 48 hours (fungi). Zones of inhibition (diameter in mm) were measured with a calibrated ruler.

Results and Discussion

The present study provides strong evidence supporting the traditional use of *Polyalthia longifolia* and *Annona senegalensis* as effective agents for managing infectious diseases. The phytochemical screening and antimicrobial activity assessments of their methanolic extracts revealed a diverse array of bioactive compounds and potent activity against selected pathogenic microorganisms, reinforcing their ethnopharmacological relevance.

Phytochemical Screening of Polyalthia longifolia

The phytochemical analysis of methanolic extracts of *P. longifolia* revealed a diverse array of secondary metabolites across the plant's leaves, stem bark, and root.

Table 1: Phytochemical Screening of the Methanolic Extracts of *P. longifolia* Plant

Test	PLL (Leaves)	PLS (Stem Bark)	PLR (Root)
Alkaloid	+	+	+
Anthraquinones	-	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Phenols	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+

Key: + = Present, - = Absent

All tested parts were found to contain alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids, while anthraquinones were selectively detected in the stem bark and root extracts. The broad distribution of these bioactive compounds suggests a well-balanced phytochemical profile with significant pharmacological potential.

The universal presence of alkaloids, flavonoids, and phenols in all plant parts aligns with previous findings and indicates a strong potential for antioxidant and antimicrobial activities. These phytochemicals are known to play vital roles in protecting plant tissues and have been widely reported for their therapeutic relevance. Alkaloids, for instance, exhibit a wide range of bioactivities including antimicrobial, analgesic, and anticancer effects (Tungmunnithum *et al.,* 2018), while flavonoids and phenolics are potent antioxidants capable of neutralizing free radicals and mitigating oxidative stress (Srinivasan *et al.,* 2014; Alara *et al.,* 2021).

Saponins and glycosides, also found across all plant parts, are reputed for their surface-active properties and capacity to disrupt microbial membranes, contributing to their antimicrobial and anti-inflammatory effects (Rasouli *et al.,* 2021). The presence of steroids and terpenoids—both of which were consistently detected—further supports the plant's wide-ranging use in traditional medicine. These compounds have been linked to anti-inflammatory, hepatoprotective, and immune-modulatory effects (Kurek, 2019).

The selective occurrence of anthraquinones in the stem bark and root, but not in the leaves, is particularly notable. This suggests a tissue-specific biosynthesis and storage of certain compounds, which could inform future bioactivity-guided fractionation efforts. Anthraquinones are well-documented for their antimicrobial, laxative, and cytotoxic properties (Lin *et al.,* 2021), and their absence in the leaves may indicate limited therapeutic overlap between plant parts.

These results are consistent with the findings of Agbo *et al.,* (2015) and Rajakaruna et *al.,* (2002), who also reported the phytochemical abundance of *Polyalthia longifolia* in ethnomedicinal contexts. Earlier foundational work by Raghunathan and Mitra (1982) similarly recorded the presence of major phytochemical groups in *Polyalthia* species, reinforcing the present study's observations. Furthermore, the antimicrobial and antioxidant potential associated with the detected flavonoids and

alkaloids is well-supported by broader literature (Cowan, 1999; Othman *et al.,* 2019).

Overall, the findings from this study confirm that *Polyalthia longifolia* is a rich source of therapeutically relevant phytochemicals, affirming its value for future drug discovery and the formulation of standardized herbal medicines.

Phytochemical Screening of Annona senegalensis

The phytochemical analysis of the methanolic extracts of *A. senegalensis* revealed the presence of a rich diversity of bioactive secondary metabolites across the leaves, stem bark, and root (Table 2).

Table 2: Phytochemical screening of the Methanolic Extracts of *A. senegalensis*

Test	ASL (Leaves)	ASS (Stem Bark)	ASR (Root)
Alkaloid	+	+	+
Anthraquinones	+	+	+
Flavonoids	+	+	+
Glycosides	+	-	+
Phenols	+	+	+
Saponins	+	+	+
Steroids	+	-	-
Tannins	+	+	+
Terpenoids	+	-	-

Key: + = Present, - = Absent

All parts tested positive for alkaloids, anthraquinones, flavonoids, phenols, saponins, and tannins. However, glycosides were absent in the stem bark, while steroids and terpenoids were not detected in both the stem bark and root. The broad distribution of phytoconstituents in the leaves compared to other plant parts suggests it may serve as the most chemically diverse component of the species.

The consistent presence of alkaloids, flavonoids, and phenols in all plant parts indicates a strong potential for antioxidant, antimicrobial, and anti-inflammatory activity. Alkaloids, known for their bactericidal and antiparasitic actions, are among the most pharmacologically active classes of natural products (Tungmunnithum *et al.,* 2018). Flavonoids and phenols, on the other hand, play a critical role in

scavenging free radicals and modulating enzymatic activity involved in microbial survival (Othman *et al.,* 2019; Alara *et al.,* 2021). Their ubiquitous presence in *A. senegalensis* aligns with traditional claims of the plant's therapeutic potential against infections and inflammation.

Saponins, detected in all parts, are known for their membrane-permeabilizing properties and are believed to enhance the absorption of other phytochemicals, suggesting a synergistic role in the plant's overall bioactivity (Rasouli *et al.,* 2021). Tannins, also present across all plant parts, are polyphenolic compounds widely reported for their antimicrobial, antioxidant, and astringent effects, and are often associated with the inhibition of bacterial adhesion and biofilm formation (Serrano *et al.,* 2009).

The absence of steroids and terpenoids in the stem bark and root suggests a selective biosynthetic pathway or differential accumulation, which could inform targeted extraction depending on the desired bioactivity. Notably, anthraquinones were consistently detected in all parts, indicating a possible role in the plant's antimicrobial or laxative effects, as supported by literature (Lin *et al.,* 2021).

These findings are consistent with earlier studies by Akinmoladun *et al. (*2021) and Atawodi *et al.* (2014), which reported similar phytochemical profiles in *A. senegalensis* extracts. The absence of glycosides in the stem bark but their presence in the root and leaf reflects tissue-specific variation, a phenomenon common in medicinal plants due to environmental or physiological factors.

Altogether, the phytochemical diversity exhibited by *A. senegalensis* reinforces its role in ethnomedicine and its potential as a source of bioactive compounds for pharmaceutical applications.

Antimicrobial Activity of Polyalthia longifolia

The methanolic extracts of *P. longifolia*, particularly those derived from the stem bark and root, demonstrated significant antibacterial activity against most tested microorganisms —*Salmonella typhi*, *Bacillus subtilis*, and *Escherichia coli* but insignificant against *Candidate albida* (Table 3).

Table 3: The Antimicrobial Activity of Methanolic Stem Bark Extract of *P. longifolia*

Test Organism	Concentration (mg/mL)	Zone of Inhibition (mm)	Standard Drug	Standard ZI (mm)
Salmonella typhi	100	24.0	Gentamycin	20.0
	75	27.0		
	50	23.0		
Bacillus subtilis	100	24.0	Gentamycin	20.0
	75	23.0		
	50	25.0		
Escherichia coli	100	21.0	Fluconazole	20.0
	75	23.0		
	50	25.0		
Candida albida	100	NA	Fluconazole	20.0
	75	NA		
	50	NA		

Key: NA = No Activity

The observed zones of inhibition were consistently high and, in several instances, surpassed those of conventional antibiotics. Notably, the root extract at 75 mg/mL produced a zone of inhibition measuring 27.0 mm against *S. typhi*, which exceeded the 20.0 mm zone produced by the standard antibiotic gentamycin. This suggests a strong antibacterial effect that warrants further investigation for potential pharmaceutical development.

These findings are consistent with previous studies highlighting the broad-spectrum antimicrobial potential of *P. longifolia*. El-Mahmood *et al.,* (2010) reported similar inhibitory effects of the plant's extracts against both Gram-positive and Gram-negative bacteria, attributing this to the rich presence of phytochemicals such as alkaloids, flavonoids, tannins, and terpenoids. Alkaloids, in particular, are known to disrupt microbial DNA and protein synthesis, while flavonoids and tannins have been shown to compromise cell membrane integrity and inhibit bacterial adhesion (Cowan, 1999; Rasouli *et al.,* 2021).

The higher potency of the stem bark and root extracts compared to the leaves may be explained by the differential distribution and concentration of bioactive constituents. Studies have shown that the root and bark tissues of many medicinal

plants often accumulate higher levels of secondary metabolites, especially those involved in defense mechanisms (Tungmunnithum *et al.,* 2018). The presence of anthraquinones—detected in the stem bark and root but absent in the leaves—may further enhance antimicrobial efficacy, as these compounds possess proven bacteriostatic and bactericidal activities (Lin *et al.,* 2021).

The effectiveness of *P. longifolia* extracts against *E. coli* and *B. subtilis* also indicates a non-specific antimicrobial mechanism, capable of acting on both Gram-negative and Gram-positive cell structures. Such broad-spectrum activity is particularly relevant in the context of rising antibiotic resistance, as it opens the door for the development of plant-based alternatives or adjuvants to conventional antibiotics.

In conclusion, the substantial antimicrobial activity demonstrated by *P. longifolia* methanolic extracts supports its traditional use in treating infectious diseases. It also underscores the need for further bioassay-guided fractionation and toxicological assessment to isolate and characterize the specific constituents responsible for this activity.

Antimicrobial Activity of Annona senegalensis

The methanolic root extract of *Annona senegalensis* exhibited moderate antimicrobial activity across the tested organisms, with the most pronounced effect observed against the fungal strain *Candida albicans* (Table 4).

Table 4: The Antimicrobial Activity of Methanolic Root Extract of *A. senegalensis*

Test Organism	Concentration (mg/mL)	Zone of Inhibition (mm)	Standard Drug	Standard ZI (mm)
Salmonella typhi	100	19.5	Gentamycin	20.0
	75	16.5		
	50	15.5		
Bacillus subtilis	100	15.5	Gentamycin	20.0
	75	14.0		
	50	11.5		
Escherichia coli	100	17.0	Fluconazole	20.0
	75	18.0		
	50	14.0		
Candida albida	100	21.0	Fluconazole	20.0
	75	19.0		
	50	19.5		

At a concentration of 100 mg/mL, the extract produced a 21.0 mm zone of inhibition, slightly exceeded the inhibition zone obtained by the standard antifungal drug fluconazole (20.0 mm). This finding reinforces the plant's folkloric use in the treatment of fungal infections, particularly candidiasis.

The antibacterial activity of the extract was comparatively lower. Zones of inhibition against *Bacillus subtilis* and *Salmonella typhi* were 15.5 mm and 19.5 mm, respectively, at the highest test concentration. While these values are modest in comparison to those obtained from *P. longifolia* extracts in this study, they remain within the range reported in previous literature. For instance, Adesegun *et al.,* (2008) and Atawodi *et al.,* (2014) confirmed the antimicrobial potential of *A. senegalensis*, attributing the effects to the presence of alkaloids, flavonoids, and tannins in the roots and bark.

Interestingly, the extract demonstrated a greater inhibitory effect against *C. albicans* than against the bacterial strains tested, suggesting that its bioactive compounds may exhibit stronger affinity or specificity for fungal cellular structures. Phytochemicals such as flavonoids and phenols have been reported to exert antifungal actions through mechanisms like disruption of ergosterol synthesis, oxidative stress induction, and interference with fungal cell wall biosynthesis (Rasouli *et al.,* 2021). The dominance of these compounds in the phytochemical profile of *A. senegalensis* may explain its superior antifungal efficacy.

This observation supports traditional medicinal practices where *A. senegalensis* is commonly employed to manage fungal skin infections, oral thrush, and vaginal candidiasis. Ibrahim *et al.* (2014) further emphasized the relevance of *A. senegalensis* in ethnomedicine, noting its effectiveness in treating dermatological and urogenital infections in rural communities.

In comparison to *Polyalthia longifolia,* which exhibited broader antibacterial potency, *Annona senegalensis* appears more specialized in antifungal activity. This complementary bioactivity pattern suggests the potential for synergistic use in polyherbal formulations aimed at managing mixed microbial infections.

Conclusion

This study revealed that methanolic extracts of *Polyalthia longifolia* and *Annona senegalensis* are rich in diverse secondary metabolites, including alkaloids,

flavonoids, phenols, tannins, saponins, glycosides, steroids, terpenoids, and anthraquinones. The extracts demonstrated remarkable antimicrobial activities, with *P. longifolia* stem bark and root showing broad-spectrum antibacterial effects (zones of inhibition 21.0–27.0 mm), in some cases exceeding standard antibiotics, while *A. senegalensis* root extract exhibited moderate antibacterial activity but superior antifungal potency against *Candida albicans* (21.0 mm), slightly surpassing fluconazole. These findings validate the ethnomedicinal use of both plants and highlight their complementary antimicrobial potential.

Further studies are recommended to isolate and characterize the bioactive constituents, evaluate their safety profiles, and elucidate their mechanisms of action. Standardization of extracts and development of polyherbal formulations may enhance their efficacy, while testing against antibiotic-resistant pathogens and conducting clinical trials will be critical steps toward their potential integration into modern antimicrobial therapy.

Conflict of interest: The authors declare no competing interest.

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