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Phytochemical Composition and Antimicrobial Activity of Combined Stem, Leaf and Root Extracts of *Anogeissus leiocarpus* Against Selected Pathogenic Microorganisms

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Abstract

Antimicrobial resistance limits the efficacy of conventional antibiotics, prompting interest in medicinal plants as potential sources of antimicrobial agents. This study evaluated the phytochemical composition and antimicrobial activity of combined stem bark, leaf, and root extracts of *Anogeissus leiocarpus* collected from Girei Local Government Area, Adamawa State, Nigeria. The combined plant material was extracted separately with ethanol and distilled water using cold maceration. The resulting ethanolic (EECPP) and aqueous (AECPP) extracts underwent qualitative and quantitative phytochemical screening. Antimicrobial activity was assessed against *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus niger* using agar well diffusion and minimum inhibitory concentration (MIC) determination. Alkaloids, flavonoids, phenolics, tannins, saponins, phytosteroids, and anthraquinones were detected in both extracts, whereas glycosides, coumarins, and triterpenoids were not detected. AECPP contained higher concentrations of flavonoids (4.53 mg/g) and tannins (0.381 mg/g), while EECPP contained higher concentrations of alkaloids (39.88 mg/g) and total phenolics (0.98 mg/g). Saponin and steroid levels were similar between extracts. Across all four microorganisms, EECPP consistently produced larger inhibition zones and lower MICs than AECPP. The largest inhibition zone was observed against *C. albicans* at an extract concentration of 400 mg/mL (26.77 mm); this organism also exhibited the lowest MIC (3.125 mg/mL). These findings indicate that ethanol was more effective than water in extracting antimicrobial

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constituents from the combined plant parts. The results provide preliminary experimental support for the traditional use of *A. leiocarpus* against infectious diseases and highlight its potential as a source of plant-derived antimicrobial agents.

Keywords: *Anogeissus leiocarpus*; Phytochemical Composition; Antimicrobial Activity; Minimum Inhibitory Concentration; Solvent Extraction

INTRODUCTION

The growing global burden of antimicrobial resistance (AMR) continues to weaken the effectiveness of traditional antibiotics, contributing to extended illness, increased death and rising healthcare expenditures (World Health Organization, 2021; Murray *et al*., 2022). Slowing rates of new antibiotic discovery have increased interest in plant-derived antimicrobial agents as complementary or alternative therapeutic options, while multidrug-resistant pathogens like Staphylococcus aureus and Salmonella species have become more challenging to treat (Ekor, 2014; Dadgostar, 2019).

African birch, or *Anogeissus leiocarpus* (DC.) Guill. and Perr, is a member of the *Combretaceae* family and is found throughout the savanna of West and East Africa, including Ethiopia, Sudan, Nigeria, and Ghana (Ouédraogo *et al*., 2020). Phytochemical research has revealed flavonoids, tannins, alkaloids, and other bioactive components thought to underpin the plant's antibacterial, antifungal, and antioxidant qualities. The plant has long been used to treat parasitic infections, diabetes, malaria, cough, and other illnesses (Adekunle *et al*., 2024a; Issa *et al*., 2021).

The use of all three plant parts as a single pooled extract has received relatively little attention, despite the possibility that pooling may integrate a wider range of complementary bioactive constituents. In contrast, the antimicrobial potential of individual parts of *A. leiocarpus* (stem bark, leaf, or root) has been documented separately in the literature. In order to assess the antimicrobial activity of both extracts against two clinically relevant bacteria (Staphylococcus aureus and Salmonella typhi) and two fungal pathogens (Candida albicans and Aspergillus niger), the current study set out to ascertain the qualitative and quantitative phytochemical composition of aqueous and ethanolic extracts of a combined stem, leaf, and root preparation of A. leiocarpus.

MATERIALS AND METHODS

Plant Material

Samples of *A. leiocarpus* stem bark, leaves, and roots were taken from the Girei Local Government Area in Adamawa State, Nigeria, and verified by a taxonomist at the Department of Botany, Modibbo Adama University, Yola. Before being used, each plant part was cleaned, allowed to air dry for two weeks at room temperature, then processed into a fine powder using a mechanical grinder and kept apart in airtight containers.

Extraction Procedure

To create 100 g of a homogenous mixture, equal quantities (33.3 g each) of the powdered stem, leaf, and root were mixed. The combined powder was separately macerated for 72 hours at room temperature with sporadic stirring in 500 milliliters of ethanol and 500 milliliters of distilled water. The ethanolic (EECPP) and aqueous (AECPP) crude extracts were obtained by filtering each mixture through muslin cloth and Whatman No. 1 filter paper. The filtrates were then concentrated by solvent evaporation at room temperature and stored in airtight containers until analysis (Tiwari *et al.*, 2018; Okoro *et al.*, 2022).

Phytochemical Screening

Both extracts were subjected to qualitative screening for alkaloids, flavonoids, tannins, saponins, phytosterols/triterpenoids, phenolics, glycosides, anthraquinones, and coumarins using standard color-reaction tests (Mayer's test, alkaline reagent test, Braymer's test, frothing test, Salkowski's test, ferric chloride test, Baljet's test, ammonium hydroxide test, and the sodium hydroxide test, respectively).

Alkaloids, flavonoids, tannins, saponins, phenolics, and steroids were measured using colorimetric analysis after each extract had been properly derivatized. Harborne (1998) was used to assess absorbance at the wavelength specific to each assay: 565 nm for alkaloids, 510 nm for flavonoids using quercetin as standard, 720 nm for tannins, 544 nm for saponins using diosgenin as standard, 750 nm for phenolics, and 780 nm for steroids.

Test Microorganisms

Staphylococcus aureus and Salmonella typhi were utilized as bacterial test organisms while Candida albicans and Aspergillus niger were used as fungal test organisms. Gram staining, spore staining, conventional biochemical assays (indole, methyl red, Voges-

Proskauer, citrate utilization, urease, and hydrogen sulfide generation), and lactophenol cotton blue staining for fungal isolates were used to identify the isolates.

Antimicrobial Evaluation

To achieve working concentrations of 100, 200, 300, and 400 mg/mL, stock solutions of each extract were serially diluted (CLSI, 2023). The agar well diffusion method was used to measure the antibacterial and antifungal activities. Standardized microbial suspensions were seeded onto nutrient agar (for bacteria) or potato dextrose agar (for fungi), wells measuring about 10 mm in diameter were bored into the agar, and the extract concentrations were added to the wells. Positive controls for the bacterial and fungal tests were ciprofloxacin and fluconazole, respectively. The widths of the inhibitory zones were determined in millimeters following a 24-hour incubation period at 37 °C (Balouiri *et al.*, 2020).

The lowest concentration exhibiting no discernible growth following a 24-hour incubation period at 37 °C was used to calculate the minimum inhibitory concentration (MIC) of each extract against each organism using broth dilution. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), which are the lowest concentrations causing no growth following subculture, were found by subculturing MIC tubes onto fresh agar.

Statistical Analysis

Every experiment was conducted in triplicate, and the mean ± standard deviation is used to express the results. One-way analysis of variance (ANOVA) was used to analyze the data, and $p < 0.05$ was considered statistically significant..

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

Table 1 presents the qualitative phytochemical profile of the aqueous (AECPP) and ethanolic (EECPP) extracts of the combined plant parts.

Table 1: Qualitative phytochemical constituents of AECPP and EECPP

Constituent	AECPP	EECPP
Alkaloid	+	+
Glycoside	–	–

Constituent	AECPP	EECPP
Flavonoid	+	+
Phenolic compound	+	+
Tannins	+	+
Saponins	+	+
Phytosteroids	+	+
Anthraquinone	+	+
Coumarins	–	–
Triterpenoids	–	–

Key: + = present/detected; – = absent/not detected.

Alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosteroids, and anthraquinones were present in both extracts, which had the same qualitative profile. Glycosides, coumarins, and triterpenoids were not found in either extract. This agreement shows that A. leiocarpus's predominant secondary metabolites are significantly soluble in polar (water) and moderately polar (ethanol) solvent systems. The inhibitory activity seen against S. typhi and *S. aureus* in this study is plausibly explained by flavonoids, tannins, and phenolic compounds, which have been shown to disrupt microbial cell membranes, inactivate enzymes, and interfere with nucleic acid synthesis. These findings are consistent with those of Salihu *et al.* (2025) and Nauzo *et al.* (2024) for other *A. leiocarpus* extracts. According to Okafor *et al.* (2026), the co-occurrence of alkaloids strengthens the extracts' antibacterial efficacy, while saponins are known to break microbial membranes due to their surface-active characteristics. Given the richness of the other ingredient classes found, the absence of glycosides, coumarins, and triterpenoids is unlikely to affect the bioactivity of the extracts..

Quantitative Phytochemical Composition

Table 2: Quantitative phytochemical composition of AECPP and EECPP

Constituent	AECPP (mg/g)	EECPP (mg/g)
Alkaloid content	11.41	39.88
Flavonoid content	4.53	0.49
Phenolic content	0.401	0.98
Tannin content	0.381	0.145
Saponin content	0.135	0.117
Steroid content	0.056	0.051

Since ethanol is more effective at rupturing plant cell membranes and dissolving nitrogenous alkaloid ring structures, the alkaloid content in EECPP (39.88 mg/g) was nearly 3.5 times higher than that of AECPP (11.41 mg/g). On the other hand, AECPP had about ten times more flavonoids (4.53 mg/g) than EECPP (0.49 mg/g); EECPP had a higher total phenolic content (0.98 mg/g) than AECPP (0.401 mg/g); and AECPP had a higher tannin content (0.381 mg/g) than EECPP (0.145 mg/g), indicating the tannins' strong water solubility resulting from their abundant hydroxyl groups. The two extracts' saponin and steroid concentrations were largely similar, indicating that the solvent polarity in this plant matrix had little effect on these constituent classes.

Overall, the data show a distinct solvent-dependent extraction pattern: water favored tannins and, surprisingly, flavonoids, whereas ethanol favored the recovery of alkaloids and phenolics. The antimicrobial activity described here has a chemical foundation thanks to the hydroxyl and carboxyl functional groups found in phenolic compounds, tannins, and flavonoids, which are known for their roles in both antimicrobial action and metal-ion binding (Nnaji *et al.*, 2022).

Identification of Test Organisms

Table 3: Biochemical characteristics of isolated test organisms

Isolate	Gram/Stain	Citrate	Methyl Red	Oxidase	Urease	Indole
Staphylococcus aureus	+ve rod	–	–	+	–	–
Salmonella typhi	–ve	+	+	–	–	–
Candida albicans	+ (white)	–	–	–	–	–
Aspergillus niger	– (black spores)	–	–	–	–	–

The test organisms were identified by biochemical and microscopic characterization as Staphylococcus aureus, Salmonella typhi, Candida albicans, and Aspergillus niger. These organisms are commonly linked to bacterial and fungal infections in humans and have been widely employed in previous antimicrobial screening of *A. leiocarpus* extracts (Adekunle *et al.*, 2024b; Fatima *et al.*, 2025).

Minimum Inhibitory Concentration

Table 4: MIC values of aqueous and ethanolic extracts (mg/mL)

Organism	Aqueous Extract	Ethanolic Extract
Staphylococcus aureus	50.00	6.25
Salmonella typhi	50.00	12.50
Candida albicans	25.00	3.125
Aspergillus niger	25.00	12.50

For all four organisms, the ethanolic extract consistently produced lower MIC values than the aqueous extract, indicating higher potency. With a minimum inhibitory concentration (MIC) of 3.125 mg/mL for the ethanolic extract, Candida albicans was the most vulnerable organism. This result is similar to what Odumosu *et al.* (2019) reported for methanolic *A. leiocarpus* stem extracts against Candida species. The sub-milligram to low milligram range described by Salihu *et al.* (2025) and Nauzo *et al.* (2024) for comparable *A. leiocarpus* preparations is generally compatible with the MIC of 6.25 mg/mL achieved against *S. aureus*.

Antimicrobial Activity (Zones of Inhibition)

Table 5: Zones of inhibition (mm) produced by aqueous and ethanolic extracts at 400 mg/mL

Organism	Aqueous Extract (mm)	Ethanolic Extract (mm)
Staphylococcus aureus	15.47 ± 1.33	24.56 ± 0.67
Salmonella typhi	14.17 ± 1.02	24.12 ± 0.23
Candida albicans	18.16 ± 0.55	26.77 ± 0.88
Aspergillus niger	14.00 ± 0.22	21.22 ± 2.35

All four test organisms were inhibited in a concentration-dependent manner by both extracts, although the ethanolic extract consistently outperformed the aqueous extract at all tested concentrations. While the matching aqueous-extract zones ranged from 14.00 to 18.16 mm, the ethanolic extract produced its biggest inhibition zone against Candida albicans (26.77 mm) at the maximal concentration (400 mg/mL), followed by S. aureus (24.56 mm), S. typhi (24.12 mm), and A. niger (21.22 mm). Because of its tough, spore-forming cell wall, Aspergillus niger was consistently the least susceptible organism to both extracts.

A large amount of research on *A. leiocarpus* is consistent with the ethanolic extract's improved performance. While Salihu *et al.* (2025) reported a zone of 25.0 ± 1.41 mm against the same organism using root bark extract, Nauzo *et al.* (2024) reported inhibitory zones of up to 26.24 mm against S. aureus using methanolic stem bark extracts, which nearly matched the 24.56 mm recorded here. The strong antifungal activity against Candida albicans is consistent with previous findings that *A. leiocarpus* extracts have broad-spectrum antifungal efficacy (Koudokpon *et al.*, 2020; Odumosu *et al.*, 2019). Ethanol's superior extraction of tannins, flavonoids, alkaloids, and phenolic compounds—all of which obstruct the production of microbial cell walls, membrane permeability, protein synthesis,

and enzymatic activity—is responsible for its increased effectiveness (Mann *et al.*, 2008; Okafor *et al.*, 2026).

CONCLUSION

Alkaloids, flavonoids, tannins, phenolics, saponins, phytosteroids, and anthraquinones are among the many bioactive phytochemicals found in *Anogeissus leiocarpus* stem, leaf, and root extracts. The concentration of these compounds varies according on the extraction solvent utilized. Staphylococcus aureus, Salmonella typhi, Candida albicans, and Aspergillus niger were all susceptible to concentration-dependent antibiotic activity from both the aqueous and ethanolic extracts, with the ethanolic extract continuously generating lower MIC values and broader inhibition zones. With a minimum inhibitory concentration (MIC) of 3.125 mg/mL for the ethanolic extract, Candida albicans was the most vulnerable organism overall. These findings validate the traditional therapeutic use of *A. leiocarpus* and support its potential as a source of natural antimicrobial agents, warranting further isolation and characterisation of the specific bioactive compounds responsible for this activity, as well as in vivo safety and efficacy evaluation prior to pharmaceutical development.

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