



COMPARATIVE STUDY OF ANTIMICROBIAL SUSCEPTIBILITY AND PHYTOCONSTITUENT ANALYSIS OF *NEWBOULDIA LAEVIS* ROOT AND STEM BARKS

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ABSTRACT

Newbouldia laevis is widely used in traditional medicine for managing microbial infections. This study evaluated the phytochemical components and antimicrobial properties of its stem and root bark extracts. Stem and root barks were collected from Agbani, Enugu State, authenticated, dried, pulverized, and extracted using methanol, ethyl acetate, and n-hexane. Qualitative phytochemical screening was conducted using standard methods. Thin Layer Chromatography (TLC) was performed with the three solvent systems. Antimicrobial activity was assessed against *Escherichia coli*, *Staphylococcus aureus*, *Penicillium marneffeii*, and *Aspergillus flavus* using the broth dilution method to determine Minimum Inhibitory Concentrations (MICs). Methanol extracts of both stem and root bark exhibited the highest phytochemical diversity, containing alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, and glycosides. Resins were absent in all extracts. TLC revealed 1–3 spots with retention factor (R_f) values ranging from 0.33 to 0.95 across all solvent systems. The methanol stem extract demonstrated MICs of 6.25 mg/ml against *A. flavus*, 12.5 mg/ml against *P. marneffeii*, and 25 mg/ml against *S. aureus*. MICs for the root extract were slightly higher, showing 25–50 mg/ml against bacteria and fungi, with *E. coli* being the least susceptible (MIC: 50 mg/ml). The findings reveal that methanol extracts of the stem and root bark of *N. laevis* are rich in bioactive compounds with strong antimicrobial activity, particularly against *A. flavus* and *P. marneffeii*. In the face of rising multidrug-resistant pathogens, the potent bioactivity of *N. laevis* supports its therapeutic relevance and positions it as a promising candidate for development into novel antimicrobial agents to address global resistance threats.

KEYWORDS: Antimicrobial activity, *Newbouldia laevis*, methanol extract, multidrug resistance, phytoconstituents, thin layer chromatography

INTRODUCTION

The growing prevalence of "superbugs" or multidrug-resistant (MDR) bacteria, particularly in hospital settings, poses a significant global threat to humans, animals, and the environment (Ugwoke *et al.*, 2024). Although antimicrobials are widely used, the overuse and misuse of antibiotics have led to the emergence of resistant bacterial strains, thereby compromising the effectiveness of treatment, especially in critically ill patients (Zhen *et al.*, 2019). While synthetic antimicrobial agents provide some level of protection, they are often limited by toxicity, adverse side effects, and difficulty in developing drugs suitable for both human and veterinary use (Ekwealor *et al.*, 2024). These conventional agents may harm mammalian cells along with microbial pathogens, leading to toxicity or undesirable interactions (De Lucca *et al.*, 1999).

Consequently, there is an urgent need to explore alternative sources of antimicrobial agents. Recent studies have shown that a substantial proportion of modern antimicrobials are derived from natural products (Vanreppelen *et al.*, 2023; Ekwealor *et al.*, 2024). In this context, plant-based compounds with antimicrobial properties present a promising avenue for combating antimicrobial resistance. For centuries, plants have been used in traditional medicine to treat a wide range of disorders. The natural products derived from medicinal plants have inspired the development of numerous therapeutic agents (Ugwoke *et al.*, 2024). The use of medicinal plants is a widespread and ancient practice across all cultures, particularly in African societies. This tradition continues to thrive in many developing countries, prompting researchers to intensify efforts to discover and develop effective plant-based medicines (Adesanya *et al.*, 1994).

Newbouldia laevis, commonly referred to as the boundary tree (Ugwoke *et al.*, 2024), is a medium-sized angiosperm belonging to the Bignoniaceae family. It is known as *Aduruku* in Hausa, *Ogirisi* in Igbo, and *Akoko* in Yoruba (Akerele *et al.*, 2011). Typically, it grows to a height of 7–15 meters and often forms clumps of gnarled branches. Native to Africa, it thrives in environments ranging from Guinea savannas to dense forests, particularly on moist, well-drained soils. The plant is found in secondary forests extending from Senegal to Cameroon, Gabon, the Democratic Republic of Congo, and Angola (Udeozo *et al.*, 2014).

Ethnobotanical evidence highlights the extensive traditional use of *N. laevis* across West Africa, especially in Nigeria and Ghana. In Nigeria, the stem bark is traditionally chewed and swallowed to relieve stomach pain, diarrhea, and toothache (Ushie *et al.*, 2021). It has also been employed in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, piles, and as a vermifuge for roundworms (Rashed, 2021). Various parts of the plant, such as the leaves, stem, root, and fruit, are used for wound dressing and to manage chest pain, earache, sore feet, epilepsy, and convulsions in children (Ugwu *et al.*, 2019). Its applications extend to both infectious and non-infectious diseases, including malaria, sexually transmitted infections, dental caries, peptic ulcers, hemorrhoids, constipation, skin and eye problems (Rashed, 2021).

In women's health, the plant is traditionally used to treat pelvic pain, infertility, and to support childbirth (Ugwoke *et al.*, 2024). In both Nigeria and Ghana, the cooked stem bark is a common remedy for breast tumors (Bothon *et al.*, 2014). Furthermore, *N. laevis* is used in managing respiratory and systemic conditions such as pneumonia, cold, fever, and cough. Its efficacy is reportedly

enhanced when combined with local clay (lay) and red pepper (Akande *et al.*, 2020). These diverse uses underscore the plant's therapeutic potential and justify continued scientific investigation into its pharmacologically active constituents.

Phytochemical analyses have identified the presence of alkaloids and phenylpropanoids in the roots (Akerele *et al.*, 2011), as well as flavonoids and tannins in the leaves (Chukwuma & Nwachukwu, 2016). However, earlier studies on the leaves and bark of *N. laevis* collected from Congo reported the absence of flavonoids, saponins, quinones, terpenes, and steroids (Okonkwo *et al.*, 2023). These discrepancies may result from environmental factors, genetic variation, plant maturity, extraction methods, degradation of bioactive compounds, seasonal influences, and limitations in analytical techniques (Hamrick & Godt, 1996; Tungmunthum *et al.*, 2018; ElGamal *et al.*, 2023).

The rising human population and expansion of industrial activities have increased the reliance on *Newbouldia laevis* for medicinal applications. This trend emphasizes the need for comprehensive research into its therapeutic properties. Accordingly, the present study focused on the comparative evaluation of the phytochemical constituents and antimicrobial activities of the stem and root barks of *Newbouldia laevis* (Bignoniaceae) collected from Agbani, Enugu State, with the objective of exploring their potential for treating bacterial and fungal infections.

MATERIALS AND METHODS

Collection, identification, and preparation of the plant materials

The stem and root barks of the plant were collected from Agbani, Enugu State, in April 2023. Prof. Ezech of the Department of

Applied Biological and Biotechnology, Enugu State University of Science and Technology, Enugu State, Nigeria, identified the plant specimen. The samples were chopped into smaller sizes, washed, and dried at room temperature for 14 days before being pulverized with a grinder. They were stored in airtight containers until needed for analysis.

Phytochemical screening

Qualitative analysis of the phytochemical component of the stem and root extracts was carried out based on the methods described by Dubale *et al.* (2023).

Qualitative Phytochemical Analysis

The confirmatory qualitative phytochemical screening of the leaf extract was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, steroids, terpenoids, glycosides, and resins) present in the extract following standard protocols as described in the literature (Dubale *et al.*, 2023).

Test for Tannins

200 mg of the stem and root extracts was boiled with 10 ml of distilled water, and 0.1% Ferric chloride was added to the mixture, which was then observed for blue-black coloration, indicating the presence of tannins.

Test for Saponins

1 g of the powdered dried sample was separately boiled with 10 ml of distilled water in a water bath for 10 min. The mixture was filtered while hot and allowed to cool.

2.5 ml of the filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 min (frothing indicated the presence of saponin in the filtrate). (Ajayi *et al.*, 2011)

Test for flavonoids:

1 g of the powdered dried leaf sample was boiled with 10 ml of distilled water for 5 min and filtered while hot. A few drops of sodium hydroxide (20%) solution were added to 1 ml of the cooled filtrate. The development of a

yellow colour that turned colourless upon acid addition suggested the presence of flavonoids.

Test for Alkaloids:

The plant extract was dissolved in 100 ml of water, filtered, and heated in steam with 2 ml of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

Test for Steroids

1 ml of the crude extract was combined with 10 ml of chloroform and 10 ml of sulfuric acid. The formation of the bilayer (red top layer and greenish bottom layer) revealed the presence of steroids

Test for terpenoids (Salkowski test):

Five ml of each extract was mixed with 2 ml of chloroform, and 3 ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for Glycosides

1 ml of distilled water and solid sodium hydroxide (NaOH) were added to 0.5 ml of the crude extracts of the leaves. The formation of a yellowish colour indicated the presence of glycosides.

Test for Resins

The presence of resins in the plant extracts was determined using the alcohol precipitation method. A small quantity (approximately 0.5 g) of each extract was dissolved in 5 mL of 95% ethanol and shaken thoroughly. The solution was then poured into 20 ml of distilled water in a beaker. Formation of a precipitate indicated the presence of resins in the extract (Harborne, 1998). This procedure was repeated for all solvent extracts of both the stem and root bark of *Newbouldia laevis*.

Thin Layer Chromatography (TLC)

TLC was performed to separate the phytoconstituents of the various extracts. Pre-coated silica gel plates (GF254) were used as the stationary phase. The plant extracts were spotted using capillary tubes 1 cm above the base of the plate. The mobile phases used were: n-hexane: ethyl acetate (3:1 and 3:2 v/v) and n-hexane: ethyl acetate: methanol (2:2:1 v/v/v). The developed plates were air-dried, visualized under UV light (254 nm and 365 nm), and then sprayed with vanillin-sulphuric acid reagent and heated at 110 °C to enhance spot detection. The retention factor (R_f) values of the resolved spots were calculated using the formula:

$$R_f = \frac{\text{Distance traveled by substance}}{\text{Distance traveled by the solvent front}}$$

Preparation of Test Organisms

Clinical strains of *Escherichia coli*, *Staphylococcus aureus*, *Penicillium marneffei*, and *Aspergillus flavus* were obtained from ESUT Teaching Hospital at Parklane, Enugu State. They were preserved as stock cultures at 4 °C in the Department of Molecular Biology, National Arbovirus and Vector Research Centre, Enugu. Characterization of the bacterial and fungal isolates was carried out for confirmation. The organisms were maintained on Nutrient Agar and Sabouraud Dextrose Agar slants, respectively, and subcultured prior to use.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the methanol extracts of *N. laevis* stem and root barks was determined using the broth dilution method as described by

Cheesbrough (2006). Serial dilutions of the extracts were prepared to obtain final concentrations ranging from 200 mg/mL to 6.25 mg/mL. Each concentration was inoculated with standardized microbial

suspensions (equivalent to 0.5 McFarland standard) and incubated at 37 °C for bacteria and 28 °C for fungi for 24–48 hrs. The MIC was recorded as the lowest concentration of extract that showed no visible growth.

Results and Discussion

Table 1: Phytochemical screening of N-hexane, ethyl acetate, and methanol extract of *Newbouldia laevis* stem and root bark

Phytoconstituents	Stem barks			Root barks		
	N-hexane	Ethylacetate	Methanol	N-hexane	Ethylacetate	Methanol
Saponin	+	+	--	+	+	+
Glycoside	+	--	+	--	--	+
Alkaloids	+	--	+	+	+	+
Tannin	+	+	+	+	--	+
Flavonoids	--	--	+	--	+	+
Steroids	+	--	+	+	+	+
Resin	--	--	--	--	--	--
Terpenoid	--	--	+	+	+	+

Key: + = positive, --- = negative

Table 2: Thin layer chromatographic characteristics of *Newbouldia laevis* stem and root bark extracts of N-hexane, ethyl acetate, and methanol

Sample Extracts	Stem Bark		Root Bark	
	Number of spots	R _f Value	Number of spots	R _f Value
N-hexane/ethylacetate (3:1)	3	0.4, 0.6 & 0.7	2	0.65 & 0.83
N-hexane/ethylacetate (3:2)	2	0.63 & 0.77	2	0.33 & 0.75
N-hexane/ethylacetate/methanol (2:2:1)	10.95	2	2	0.84 & 0.93

Table 3: Extract concentration (mg/ml) and minimum inhibitory concentration (MIC) of *Newbouldia laevis* stem bark methanol extracts

Conc of methanol extracts (mg/ml)	Test organisms mic			
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. marneffeii</i>	<i>A. flavus</i>
200	0.86	0.29	0.20	0.00
100	0.62	0.14	0.09	0.00
50	0.38	0.04	0.00	0.00
25	0.12	0.00	0.00	0.00
12.5	0.008	0.00	0.00	0.00
6.5	0.00	0.00	0.00	0.00

Table 4: Minimum extract concentration (mg/ml) and minimum inhibitory concentration (mic) of *Newbouldia laevis* root bark methanol extracts

Conc of methanol extracts (mg/ml)	Test organisms MIC			
	<i>E. coli</i>	<i>S.aureus</i>	<i>P. marneffeii</i>	<i>A. flavus</i>
200	1.26	0.89	0.46	0.00
100	0.82	0.44	0.19	0.00
50	0.58	0.15	0.08	0.00
25	0.32	0.02	0.00	0.00
12.5	0.00	0.00	0.00	0.00
6.5	0.00	0.00	0.00	0.00

The phytochemical analysis on the stem and root barks of *Newbouldia laevis* (as shown in Table 1) using n-hexane, ethyl acetate, and methanol as solvents for extraction revealed that saponin, alkaloids, tannin, and steroids were present in the n-hexane extract of both stem and root barks of *Newbouldia laevis*. All the analysed phytoconstituents were present in the methanol extract of both samples, except resin and saponin, which were absent in the stem bark. Few of the analysed secondary metabolites were present in the ethyl acetate extract, only saponin and tannin in the stem bark. In contrast, saponin, alkaloids, flavonoids, steroids, and terpenoids were detected in the root bark. The root bark contained more of the analysed secondary metabolites (such as saponin, alkaloids, steroids, and terpenoids) than the stem bark.

All the secondary metabolites present proved the efficacy of the plant stem bark and root bark in herbal medicine. The presence of alkaloids in the stem and root bark extracts of *Newbouldia laevis* suggests potential pharmacological effects, as alkaloids are known to exhibit diverse biological activities, including local anesthetic and stimulant properties, similar to compounds like cocaine, caffeine, and nicotine. Saponins have been used in the treatment of a number of cardiovascular disorders (Ushie *et al.*, 2021). They also facilitate and ease the process of digestion (Udeozo *et al.*, 2014). The presence of tannins in all the extracts depicted the antibacterial and antifungal effects of the stem and root barks of *Newbouldia laevis* (Funagawa *et al.*, 2004). The presence of flavonoids in the samples indicated that the plant is a good source of antioxidants, which mop up free radicals in the system and help the immune system to function properly. The concentration of tannin in the sample proved the authentic ability to act as an antidote to poisoning by an alkaloid and its ability to heal fresh injury.

Tannin is used as a parasite expeller from the body due to its antiseptic properties (Udeozo *et al.*, 2015). Thin layer chromatography (TLC) of the stem and root bark extracts (Table 2) revealed three components in the stem bark and two in the root bark, with R_f values of 0.40, 0.60, and 0.70 for the stem, and 0.65 and 0.83 for the root, when N-hexane/ethyl acetate (3:1) was used as the solvent system. Using N-hexane/ethyl acetate in a 3:2 Ratio, two spots were observed in each extract, with R_f values of 0.63 and 0.77 for the stem bark, and 0.33 and 0.75 for the root bark. The N-hexane/ethylacetate/methanol extract in the ratio of 2:2:1 showed one and two components in the stem & root bark with R_f values of 0.95 and 0.84 and 0.93, respectively. The TLC results confirmed the presence of some components in the extracts and their high purity.

The results of the susceptibility testing of the methanol extracts of *Newbouldia laevis* stem and root barks (Tables 3 and 4) showed that at concentrations below 12.5 mg/ml, the extracts could not inhibit the growth of *S. aureus*, *P. marnerifei*, *A. flavus*, and *E. coli*, except for the stem bark, which showed some activity against *E. coli*. None of the extracts affected *A. flavus*, but exhibited increasing activity against the other tested organisms as the concentration increased.

The results showed more activity with the root bark extract. The results also showed that the extract has a more antibacterial effect than an antifungal effect. This aligns with the findings of Ugwu *et al.*, (2019), who reported a broad-spectrum activity of the root extract of *Newbouldia laevis* against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria, with a higher MIC of 50 mg/ml for both organisms. In contrast, our study recorded a lower MIC of 25 mg/ml for the same bacterial isolates. This observation agrees with the report by Akande *et al.*,

(2020), who also recorded an MIC of 25 mg/ml against *E. coli* and *S. aureus*. The results of this study suggest that *N. laevis* possesses broad-spectrum antibacterial activity, which is consistent with the findings of earlier studies by Ogbe *et al.*, (2020) and Muhammad *et al.*, (2023)

CONCLUSION AND RECOMMENDATION

The phytochemical screening of the stem and root bark extracts of *Newbouldia laevis* confirmed the presence of several bioactive constituents, supporting the traditional use of this plant in herbal medicine. Thin Layer Chromatography (TLC) analysis indicated a high level of purity in the crude fractions; however, the specific fractions analyzed were not clearly identified, and further clarification is needed.

The extracts demonstrated notable antimicrobial activity against *S. aureus*, *P. marneffei*, and *E. coli*, with the root bark extract exhibiting stronger activity. However, neither the stem nor root extracts showed inhibitory effects against *A. flavus*. Given the promising antimicrobial properties observed, especially in the root extract, the traditional medicinal use of *N. laevis* stem and root bark in local communities should be encouraged. However, further research is essential to isolate and characterize the specific active compounds, determine their structures, assess their toxicological profiles, and establish safe and effective dosage guidelines for clinical use.

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