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Harnessing *Blighia sapida* as a Source of Novel Antimicrobials: Activity of Crude and Fractionated Extracts Against MDR Pathogens

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Abstract

The rapid proliferation of multidrug-resistant pathogens has elevated antimicrobial resistance to a global crisis, necessitating the search for alternative antimicrobial sources. This study evaluated the antibacterial activity of crude and fractionated extracts of *Blighia sapida* against selected multidrug-resistant (MDR) clinical isolates. The crude extract was prepared by maceration and subsequently fractionated using solvents of varying polarity. Antibacterial activity was assessed through standard microbiological assays, and phytochemical constituents were profiled using high-performance liquid chromatography (HPLC). The crude extract exhibited broad-spectrum antibacterial activity, with inhibition zones increasing in a concentration-dependent manner. *Escherichia coli* was the most susceptible (20.0 mm at 100 mg/mL), followed by *Klebsiella pneumoniae* (17.0 mm), with minimum inhibitory concentrations (MICs) ranging from 25–50 mg/mL. Fractionation produced varied activities: the n-hexane fraction demonstrated minimal inhibition (≤10 mm), the ethanolic fraction showed moderate activity against *Enterococcus faecalis*, *Acinetobacter baumannii*, and *K. pneumoniae* (5–10 mm), while the aqueous fraction was the most active, particularly against *A. baumannii* (11.0 mm) and *E. coli* (10.0 mm). Fraction MICs were higher (50–>100 mg/mL), indicating reduced potency compared to the crude extract. HPLC analysis identified kaempferol, gallic acid, chlorogenic acid, myricetin, quercetin, and caffeine as major constituents. These findings demonstrate that *B. sapida* possesses promising antimicrobial phytochemicals and highlight its potential as a valuable plant-based source for combating MDR bacteria.

Keywords: *Blighia sapida*; multidrug-resistant bacteria; fractionation; HPLC; phytochemicals.

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health crisis that threatens to reverse decades of medical progress. Driven by the misuse and overuse of antibiotics in human medicine, agriculture, and livestock, AMR increasingly undermines conventional therapies, resulting in treatment failures, higher morbidity and mortality, and substantial strain on healthcare systems worldwide (Awolope et al., 2020; Salam et al., 2023; Ahmed et al., 2024; Osei et al., 2024). The rapid evolution of resistance has outpaced the development of new antibiotics, largely due to high research costs and limited commercial incentives, prompting many pharmaceutical companies to scale back antimicrobial discovery efforts (Ahmed et al., 2024; Brüssow et al., 2024).

This urgent need for safe and cost-effective alternatives has renewed interest in natural products, particularly medicinal plants, which have been used for centuries to manage infectious diseases and represent a rich reservoir of bioactive metabolites (Sorokina & Steinbeck, 2020; Najmi et al., 2022). Phytochemicals exert diverse antimicrobial mechanisms, including disruption of microbial membranes, inhibition of enzymatic activity, interference with biofilm formation, and modulation of virulence factors (Zou et al., 2021; Roy et al., 2022). Their structural diversity often confers broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, and even drug-resistant strains (Bouyahya et al., 2022). Ethnobotanical knowledge provides a valuable framework for identifying plants with promising antimicrobial potential and guiding scientific evaluation of their bioactive constituents.

Blighia sapida (Sapindaceae), commonly known as ackee, is native to West Africa and has spread to the Caribbean, South America, and other tropical regions (Wray et al., 2020). This evergreen tree reaches up to ten meters in height, bearing compound leaves and pear-shaped fruits that split when ripe to reveal three black seeds surrounded by cream-colored arils (Wireko-Manu et al., 2024). In Jamaica, the arils are a key ingredient in the national dish, ackee and saltfish, underscoring the plant's cultural and economic significance (Kozlova et al., 2021).

Beyond its culinary value, *B. sapida* has a long history of medicinal use across West Africa and the Caribbean. Leaves, bark, seeds, and arils are employed to

manage fever, inflammation, gastrointestinal disorders, parasitic infections, respiratory ailments, and anemia (Stephen-Amzat, 2023; Origbemisoye and Ifesan, 2024). Leaf decoctions are used to control fever, inflammation, and respiratory conditions, and are applied topically to wounds due to their antimicrobial and anti-inflammatory properties (Alatise and Adedokun, 2020; Odunayo, 2022; Ibrahim et al., 2023). Bark decoctions or poultices are applied to gastrointestinal and skin disorders, with tannins and saponins contributing to astringent and antimicrobial effects (Ojo et al., 2018; Adekola et al., 2022; Effiong and Jacob, 2022). Seeds, though containing toxic hypoglycins, are occasionally used against parasites after detoxification, while the nutrient-rich arils are employed to manage anemia, malnutrition, and general weakness (Oladiji et al., 2013; Kibiti and Afolayan, 2015; Adarkwa-Yiadom, 2018). Despite its ethnomedicinal significance, systematic studies of *B. sapida*'s antimicrobial activity are limited, highlighting the need for scientific validation (Ibrahim et al., 2023).

Although traditionally used to treat infections and inflammation, scientific evidence for *Blighia sapida*'s efficacy against multidrug-resistant (MDR) bacteria is limited. Evaluating crude and fractionated extracts from different plant parts may reveal bioactive compounds with therapeutic potential, bridging traditional knowledge and modern medicine. Its wide availability, cultural acceptance, and chemically diverse phytoconstituents make *B. sapida* a promising source of novel, affordable, plant-based antimicrobials. This study therefore aimed to assess the antibacterial activity of crude and partially purified extracts of *B. sapida* against selected MDR bacterial isolates.

METHODS

Plant Material and Preparation of *Blighia sapida* Extracts

Fresh leaves of akee apple (*Blighia sapida* König; family Sapindaceae) were collected from the Akungba-Akoko area. The plant material was identified and authenticated at the Herbarium and Taxonomic Unit, Department of Plant Science and Biotechnology, Adekunle Ajasin University, where a voucher specimen (PSBH 727) was deposited. The leaves were rinsed thoroughly with clean water, air-dried at room temperature for three weeks, and milled into a fine powder using an electric blender. Extraction was carried out by maceration: 600 g of the powdered material was soaked in 4 L of 70% ethanol for 96 hours with intermittent agitation (Oluyele and Oladunmoye, 2017). The mixture was filtered three times through muslin cloth, and the combined filtrate was concentrated by air-drying. The resulting crude extract was stored in a sterile, airtight container until use.

Fractionation of the Crude Extract

The crude ethanol extract of *Blighia sapida* was sequentially fractionated using n-hexane, ethanol, and water following a previous protocol, with minor modification (Oluyele, 2025). For the non-polar fraction, the crude extract was dissolved in 100 mL of n-hexane in a 500 mL separatory funnel, shaken vigorously, and allowed to stand for 15 minutes to achieve phase separation. The n-hexane layer was collected into a clean flask, and the extraction was repeated three times until the solvent layer became nearly colorless. The combined n-hexane extracts were concentrated to dryness under reduced pressure using a rotary evaporator. The resulting n-hexane fraction was stored at –20 °C until further use. The residual aqueous layer was re-concentrated under reduced pressure to remove traces of n-hexane. This layer was then subjected to the same extraction procedure using ethanol to obtain the intermediate-polarity fraction. Finally, the remaining aqueous residue was extracted with distilled water following the same protocol to yield the polar (aqueous) fraction. All fractions were weighed, stored in sterile amber vials, and kept at –20 °C until further analysis.

Test Organisms and Inoculum Standardization

Five multidrug resistant (MDR) bacterial isolates, *Enteroccocus faecalis, Staphylococcus aureus, Escherichia coli, Acenectobacter baumanni, Klebisella pneumonia w*ere used for this study. These organisms were obtained and collected aseptically from the Microbiology laboratory, in Adekunle Ajasin University, Akungba Akoko. Isolates were maintained on nutrient agar slants at 4 °C and subcultured on fresh nutrient agar prior to testing. Bacterial suspensions were prepared in sterile saline and adjusted to match a 0.5 McFarland standard. For susceptibility testing, suspensions were diluted 1:100 in sterile saline to yield a working inoculum of approximately 1×10^6 CFU/mL (Oluyele *et al*., 2023).

Antimicrobial susceptibility test

The antimicrobial potential of the extracts were evaluated using the agar well diffusion method (Oluyele and Akinyeke, 2025). A 1 ml aliquot of each standardized test organism suspension was spread evenly on sterilized Mueller-Hinton agar plates. After drying, uniform wells (6mm in diameter) were made in the plates, and 50 µL of the extract (100 mg/mL in 5% dimethyl sulfoxide) was added to the wells, with Ciprofloxacin/Augmentin as a control. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined using tube-dilution and plating methods (Oluyele and Oladunmoye, 2017). For MIC, various concentrations of the extract (ranging from 100 to 3.125 mg/ml) were prepared, followed by adding 0.5 ml of the

test inoculum into each tube. Negative and positive control tubes were also prepared. The tubes were incubated at 37°C for 24 hours, and the MIC was determined as the lowest concentration with no visible turbidity. To determine the MBC, samples from MIC tubes and other non-turbid tubes were subcultured onto fresh Mueller-Hinton agar, incubated at 37°C, and the MBC was identified as the concentration with no visible growth.

High-Performance Liquid Chromatography (HPLC) Profiling

Phytochemical profiling of the ethanolic extract was performed using an Agilent 1200 RP-HPLC system equipped with a Hypersil BDS C18 column (250 mm × 4.0 mm i.d.). Two grams of the extract were dissolved in 20 mL of acetonitrile–methanol (1:1 v/v), sonicated for 30 min, filtered, and made up to 25 mL. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) at a flow rate of 0.6 mL/min. The injection volume was 20 µL, and detection was carried out at 280 nm. Identification of compounds was achieved by comparing retention times and UV spectra with authenticated reference standards (Oluyele, 2025).

RESULTS

Antibacterial Activity of the Crude Extract of *B. sapida*

The crude extract exhibited broad-spectrum antibacterial activity against all tested MDR pathogens, with inhibition zones increasing in a concentration-dependent manner (Table 1). *Escherichia coli* showed the greatest susceptibility (20.0 mm at 100 mg/ml), followed by *Klebsiella pneumoniae* (17.0 mm) and *Staphylococcus aureus* (15.0 mm), while *Acinetobacter baumannii* demonstrated weak activity and no inhibition at lower concentrations. MIC values further confirmed higher potency of the crude extract, ranging from 25–50 mg/ml across organisms (Table 5).

Table 1. Zone of inhibition of *B. sapida* crude extract against MDR Pathogens.

Test Organisms	Zone of inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Enterococcus faecalis	13.0 ± 0.17	12.0 ± 0.17	9.0 ± 0.33	6.0 ± 0.33
Staphylococcus aureus	15.0 ± 0.17	13.0 ± 0.17	12.0 ± 0.17	8.0 ± 0.33
Escherichia coli	20.0 ± 0.33	17.0 ± 0.17	15.0 ± 0.17	11 ± 0.17
Acinectobacter baumannii	12.0 ± 0.17	10.0 ± 0.17	0.0	0.0
Klebsiella pneumoniae	17.0 ± 0.00	15.0 ± 0.33	13.0 ± 0.17	10.0 ± 0.17

Antibacterial Activity of *B. sapida* Fractions

Fractionation of the crude extract produced notable variations in activity (Tables 2, 3 and 4). The n-hexane fraction displayed the lowest antibacterial efficiency, with inhibition zones typically ≤10 mm and no activity at lower concentrations for most organisms. The ethanolic fraction showed moderate activity, particularly against *E. faecalis*, *A. baumannii*, and *K. pneumoniae*, but with smaller zones (5–10 mm). The aqueous fraction exhibited the highest activity among the fractions, especially against *A. baumannii* (11.0 mm) and *E. coli* (10.0 mm). MIC values for the fractions (Table 5) were generally higher (50 – >100 mg/ml), indicating reduced potency compared to the crude extract.

Table 2. Zone of inhibition of *B. sapida* N-Hexane fraction against MDR pathogens.

Test Organisms	Zone of inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Enterococcus faecalis	10.0 ± 0.00	8.0 ± 0.33	0.0	0.0
Staphylococcus aureus	5.0 ± 0.17	4.0 ± 0.17	2.0 ± 0.17	0.0
Escherichia coli	10.0 ± 0.17	7.0 ± 0.17	0.0	0.0
Acinectobacter baumannii	11.0 ± 0.17	8.0 ± 0.17	4.0 ± 0.17	0.0
Klebsiella pneumoniae	8.0 ± 0.17	6.0 ± 0.33	0.0	0.0

Table 3. Zone of inhibition of *B. sapida* Ethanolic fraction against MDR pathogens.

Test Organisms	Zone of inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Enterococcus faecalis	10.0 ± 0.33	7.0 ± 0.17	5.0 ± 0.00	0.0
Staphylococcus aureus	8.0 ± 0.33	6.0 ± 0.33	0.0	0.0
Escherichia coli	7.0 ± 0.17	5.0 ± 0.33	0.0	0.0
Acinectobacter baumannii	9.0 ± 0.17	7.0 ± 0.00	6.0 ± 0.00	0.0
Klebsiella pneumoniae	9.0 ± 0.33	8.0 ± 0.17	6.0 ± 0.00	0.0

Table 4. Zone of inhibition of *B. sapida* Aqueous fraction against MDR pathogens.

Test Organisms	Zone of inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Enterococcus faecalis	7.0 ± 0.00	5.0 ± 0.17	0.0	0.0
Staphylococcus aureus	10.0 ± 0.17	8.0 ± 0.33	5.0 ± 0.33	0.0
Escherichia coli	10.0 ± 0.00	7.0 ± 0.17	4.0 ± 0.33	0.0
Acinectobacter baumannii	11.0 ± 0.00	9.0 ± 0.33	8.0 ± 0.33	0.0
Klebsiella pneumoniae	8.0 ± 0.17	5.0 ± 0.17	0.0	0.0

Table 5. Minimum Inhibitory Concentration (MIC) of *B. sapida* Extracts against MDR Pathogens.

Test Organisms	MIC (mg/ml)			
	Crude extract	n-Hexane fraction	Ethanolic fraction	Aqueous fraction
Enteroccocus faecalis	50	100	100	>100
Staphylococcus aureus	50	>100	100	100
Escherichia coli	25	100	>100	100
Acinectobacter baumannii	50	100	>100	50
Klebsiella pneumoniae	50	100	50	100

HPLC Profiling of Phytoconstituents in *B. sapida*

HPLC analysis identified diverse bioactive compounds (Table 6; Figure 1), including phenolic acids (gallic acid, caffeic acid, ferulic acid, chlorogenic acid), flavonoids (myricetin, kaempferol, quercetin, catechin), alkaloids (nicotine, quinine), pyrogallol, curcumin, and caffeine. Kaempferol exhibited the highest peak area, followed by nicotine and curcumin, suggesting these compounds may contribute significantly to the antimicrobial activity.

Table 6. HPLC Identified Compounds in *B. sapida* Crude Extract.

Component	Retention Time (min)	Area	Height
Gallic Acid	1.266	1091.9290	32.172
Nicotine	2.750	2650.7045	25.578
Caffeic Acid	4.450	880.2335	14.235
Cinnamic Acid	6.483	207.6050	9.258
Pyrogallol	7.333	81.9950	5.385
Myricetin	8.150	82.3060	5.048
Kaempferol	10.234	7853.2795	147.374
Curcumin	12.166	2222.5360	47.738
Catechin	13.700	652.5060	17.225
Quinine	14.816	175.3440	0.439
Caffeine	15.716	164.7435	6.464
Ferulic Acid	16.250	103.2780	5.271
Quercetin	17.233	119.0285	6.397
Chlorogenic Acid	17.616	581.3260	8.544

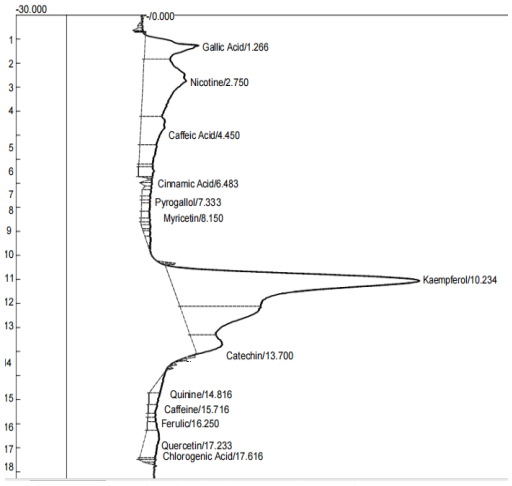

Figure 1. Chromatograph of *Blighia sapida* Crude Extract.

DISCUSSION

Amid escalating antimicrobial resistance, medicinal plants remain a promising source of bioactive compounds. In this study, the crude extract of *Blighia sapida* exhibited superior antibacterial activity against both Gram-positive and Gram-negative multidrug-resistant (MDR) bacteria, likely due to the combined action of its constituent phytochemicals. HPLC profiling revealed a mixture of potent antimicrobial agents, including gallic acid, quercetin, kaempferol, chlorogenic acid, catechin, nicotine, and curcumin—all previously reported for antimicrobial mecahnisms (Jan *et al*., 2022; Sun and Shahrajabian, 2023; Nguyen *et al*., 2024). The strong susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* suggests that phenolic acids in the extract

may interact effectively with the lipopolysaccharide layer of Gram-negative bacteria, enhancing permeability and facilitating compound entry. This finding aligns with previous reports indicating that crude extracts of medicinal plants often exhibit greater antibacterial activity than isolated constituents (Oluyele, 2025; Oluyele et al., 2025)

In contrast, the antibacterial activity of the solvent fractions declined substantially, reflecting the loss of synergistic interactions among phytochemicals during partitioning. Each solvent selectively extracts compounds of specific polarity, yielding a narrower chemical profile and reduced potency. The n-hexane fraction, rich in non-polar constituents, showed minimal activity, whereas the ethanolic fraction retained some flavonoids and phenolic acids but at lower concentrations, resulting in moderate inhibition. Notably, the aqueous fraction performed better than other fractions against *Acinetobacter baumannii*, likely due to the presence of polar antimicrobial compounds capable of penetrating its highly impermeable outer membrane.

MIC results further confirmed the superior activity of the crude extract, with values as low as 25 mg/mL for *E. coli*. Gram-positive bacteria, particularly *Staphylococcus aureus* and *Enterococcus faecalis*, also showed strong susceptibility, indicating that key phytochemicals target the peptidoglycan layer or intracellular processes. Higher MIC values for the fractions (>100 mg/mL in several cases) underscore the importance of phytochemical synergy. The lower susceptibility of *A. baumannii* can be attributed to its robust membrane, efflux pumps, and biofilm formation (Verma et al., 2021; Mohamed et al., 2023), although the aqueous fraction still achieved notable inhibition.

The presence of kaempferol, curcumin, nicotine, and gallic acid supports the observed antibacterial activity. Kaempferol and gallic acid are known to inhibit DNA gyrase, disrupt membranes, and suppress efflux pumps (Tian et al., 2021; Jan et al., 2022; Luo et al., 2025). Curcumin acts broadly against bacteria by inhibiting quorum sensing, virulence expression, and biofilm formation. (Zheng et al. 2020; Hussain et al., 2022), while chlorogenic acid enhances membrane permeability and interferes with protein synthesis (Lou et al., 2011; Nguyen et al., 2024). The cumulative and interactive effects of these compounds likely underpin the crude extract's superior potency.

CONCLUSION

The crude extract of *Blighia sapida* demonstrates strong, broad-spectrum antibacterial activity against multidrug-resistant strains, outperforming all solvent fractions. HPLC profiling revealed abundant phenolics, flavonoids, alkaloids, and curcuminoids—including kaempferol, gallic acid, caffeic acid, quercetin, chlorogenic acid, catechin, nicotine, and curcumin—whose synergistic interactions likely drive this efficacy. These findings highlight the therapeutic potential of *B. sapida* and warrant further studies on isolation of active compounds, mechanistic and computational analyses, nanoformulation for improved bioavailability, broader screening against MDR strains, and in vivo evaluation for safety and efficacy.

Ethics Approval and Consent to Participate: not applicable

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: Olumide Oluyele: Conceptualization, supervision, data analysis, manuscript writing, and corresponding author. Precious Temidayo Bimboye: data curation and methodology

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