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Original Research Article

PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATION OF ETHANOL AND METHANOL LEAVE EXTRACTS OF *Bligha sapida* K.D. KOENIG FAMILY SAPINDACEAE.

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ABSTRACT

Blighia sapida, a perennial herbaceous plant native to Western Tropical Africa, is renowned for its edible fruit and traditional medicinal uses. This work aimed at investigating the phytoconstituents composition and microbial inhibitory properties of aqueous ethanol and aqueous methanol extracts of *Blighia sapida* leaves. The leaves were collected, authenticated and extracted using aqueous methanol and aqueous ethanol solutions. Antimicrobial assay was carried out on the extract using the agar diffusion method to assess its antibacterial and anti-fungal activity. The presence of several bioactive compounds, including alkaloids, flavonoids, saponins, cardiac glycosides, tannins, and reducing sugars, was confirmed through the phytochemical analysis. Antimicrobial testing against various microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger, Candida albicans*, and *Bacillus subtilis,* showed that the methanol extract of the leaves of *B. sapida* was effective in hindering the growth of *Staphylococcus aureus* and *Bacillus subtilis,* with a similar result to the ethanol extract at higher concentrations. Both extracts were ineffective against *Pseudomonas aeruginosa*, *Escherichia coli,* and the fungal strains used in this study. The *Blighia sapida* extracts showed no antifungal activity compared to Nystatin, which inhibited *Candida albicans*. Levofloxacin, a standard antibacterial agent, exhibited greater inhibition zones against the tested bacteria, revealing a higher antimicrobial activity than *Blighia sapida* extracts. However, unlike plant extracts, it showed no inhibition against *Bacillus subtilis. Blighia sapida* has potential as a source of natural antibacterial agents against specific bacteria, but further research is needed to isolate active components and understand their mechanisms.

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INTRODUCTION

Throughout history, plants have served as a vital reservoir of natural compounds that support human health. The World Health Organisation (WHO) recognizes medicinal plants as one of the most promising sources for the development of diverse therapeutic agents. A wealth of recent research highlights the significant potential of these plants, especially in traditional and complementary medicine, for treating a wide range of human ailments [1]. Microbial diseases continue to pose a significant health threat, particularly in tropical regions. These infections can result in severe, potentially life-threatening complications. Due to the high cost of modern, effective pharmaceuticals and other limiting factors, herbal remedies have emerged as a necessary and more affordable

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alternative for managing these infections [2]. Among the plants used in Western Tropical Africa for their antimicrobial properties is Blighia sapida, a member of the Sapindaceae family [3]. *Bligha sapida* is commonly known as Ackee in English; the fruit also bears local names in Nigeria: Isin in Yoruba, Okpu in Igbo, and Gwanja Kusa in Hausa languages [4]. The plant is extensively cultivated across tropical and subtropical areas for its edible fruit [5]. Upon ripening, the Ackee fruit naturally splits open, exposing a creamy coat attached to an elongated black seed. The meaty fruit can also be substituted for meat in soups, stews, and curries. Before the fruit can be removed from the tree, it must be given time to fully open since hypoglycin and its derivatives are highly hazardous and can be found in overripe or unripe arils and seeds. The peculiar non-proteinogenic amino acids, hypoglycins A and B, are the toxic substances present [6]. The fruit contains more hypoglycin A than hypoglycin B. Hypoglycin A has been employed as a glucose inhibitor. Hypoglycin A consumption results in the formation of a metabolite known as methylenecyclopropane acetyl CoA (MCPACoA), which inhibits several dehydrogenases necessary for gluconeogenesis [7].

Blighia sapida holds significant value in traditional African medicine. It is commonly employed for the treatment of conditions such as oedema, epilepsy, and yellow fever, as a cathartic and diuretic, in Côte d'Ivoire [8]. The extracted sap from its terminal bud is traditionally applied to the eyes to manage ophthalmia and conjunctivitis. Additionally, the plant has been reported to possess antioxidant properties [2]. This study was designed to analyze the phytoconstituents and assess the antimicrobial activity of ethanol and methanol leaf extracts of *Blighia sapida*.

MATERIALS AND METHODS

Plant collection

The leaves of *Blighia sapida* were harvested from the wild in Ijegun, a village situated within the northern suburban region of Alimosho Local Government Area in Lagos State (6°30'15.2"N 3°14'36.0"E), and authenticated at the Herbarium of the Botany Department, University of Lagos, Akoka, Lagos, Nigeria and voucher number LUH 100225 was assigned.

Extraction

About 0.98 kg each of dried leaves of *B. sapida* were extracted using methanol (90 %) and ethanol (50 %), respectively. The mixture was thoroughly stirred and allowed to rest at ambient temperature for 48 hours. It was filtered, and the filtrate was concentrated using a rotary evaporator. The solution was agitated and then set aside at room temperature. for 48 hours, filtered, and the filtrate was concentrated using a rotary evaporator under reduced pressure. This procedure was repeated multiple times to increase the overall yield. Extracts were stored as methanol extract and ethanol extract in an airtight container until further use.

Screening of Phytoconstituents

The extracts underwent phytochemical screening using established standard methodologies to identify the presence of bioactive compounds [9].

Tests for Alkaloids

a. Dragendorff test: Approximately 1 mL of the plant extract was placed into a test tube and mixed with 1 mL of Dragendorff's reagent, mixed gently. The formation of an orange-red precipitate confirmed the presence of alkaloids [10].
b. Mayer's test: To screen for alkaloids, 1 mL of the plant extract was placed in a test tube and combined with 1 mL of Mayer's reagent. The mixture was gently shaken, and the appearance of a whitish or cream-colored precipitate indicated a positive result for alkaloids [11].

Test for Flavonoid

Lead acetate test: To detect flavonoids, 1 mL of the plant extract was mixed with 1 mL of a 10% lead acetate solution. Appearance of a yellow precipitate confirms the presence of alkaloids [12].

Test for Saponins

Frothing test: To identify the presence of saponins, 1 mL of the plant extract was combined with 1 mL of distilled water and shaken vigorously. The appearance of persistent froth indicated a positive result for saponins [13].

Test for Cardiac Glycosides

a. Keller-Killiani test: A solution composed of 0.5 mL glacial acetic acid and 2–3 drops of ferric chloride was added to 2 mL of the plant extract. About one millilitre of concentrated sulfuric acid (H_2SO_4) was gently layered along the inner wall of the test tube. The formation of a deep blue coloration at the interface between the two liquids was indicative of cardiac glycosides [14].
b. Liebermann test: The alcoholic extract of the substance was evaporated until completely dry, then re-extracted using chloroform ($CHCl_3$). A few drops of acetic anhydride were added, followed by careful addition of concentrated sulfuric acid along the inner wall of the test tube. The emergence of a violet ring that transitioned to blue signalled the presence of sterol compounds in the sample.
c. Borntrager's test: About 1 g of the extract was mixed with 10 mL of dilute HCl and boiled for 10 minutes on a water bath. After filtering the mixture, the filtrate was extracted with CCl_4. An equal amount of ammonia solution was then added to the filtrate and shaken. The formation of a pink to red color indicates the presence of an anthraquinone moiety [11].

Test for Tannins

Ferric Chloride test: To detect tannins, a few drops of 10% $FeCl_3$ solution were introduced to 2 mL of the heated ethanol extract of *B. sapida* (EEBS) and methanol extract of *B. sapida* (MEBS). The formation of a greenish precipitate signified a positive result for the presence of tannins.

Test for Reducing Sugars

Fehling's Test: To assess the presence of reducing sugars, equal volumes of Fehling's solutions A and B were mixed and added to approximately 2 mL of MEBS and EEBS. The resulting mixture was heated for five (5) minutes. The appearance of a red or deep red precipitate confirmed the presence of reducing sugars [15].

Antimicrobial Screening

The antibacterial and antifungal activity of the EEBS and MEBS was determined using the Agar diffusion method [16].

Preparation of Test Organisms

The microbial panel employed in this study comprised both bacterial and fungal strains. The bacterial species included *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli,* while the fungal isolates consisted of *Aspergillus niger* and which were obtained from stock cultures at the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutical Technology and Pharmaceutics, College of Medicine, Idi-Araba, Lagos. These microorganisms were identified based on their colonial morphology.

Each test organism was cultured in 10 mL of broth (Mueller-Hinton Agar was used for the bacterial strains, while Sabouraud Dextrose Agar was used for the fungal strains) and incubated at 37 °C for 18 to 24 hours. After incubation, the microbial cultures were standardized to a concentration of 1×10^8 CFU/mL in normal saline, following the McFarland turbidity standard [17].

Antibacterial Assay

The sample bottles were cleaned and dried thoroughly. Mueller Hinton Agar was prepared by adding 30.4 g of agar into a flask containing 800 mL of water. The resulting mixture was heated in a water bath for 45 minutes at 100 °C to ensure complete dissolution. Subsequently, 25 mL of the dissolved agar was dispensed into each sample bottle, which was autoclaved for two hours to sterilize the agar. For the bacterial assay, 1 mL of the bacterial organisms was inoculated into labelled Petri dishes, and 25 mL of the agar was poured into each dish containing the test organisms. The mixture was thoroughly mixed and allowed to dry before boring holes using a sterile cork borer with a diameter of 1mm. Approximately 1 mL of the crude plant extract (EEBS and MEBS) at concentrations of 50 %v/v and 100 %v/v was inoculated into the bored holes of the agar. The petri dishes were incubated at 37 °C for 24 hours before final readings were taken. The procedure was carried out in triplicate.

After a 24-hour incubation period, each tube was assessed for turbidity. Tubes that remained clear were marked as negative, indicating the absence of microbial growth, while those that appeared turbid were recorded as positive, signifying the presence of growth. Control plates were also prepared for each test organism, containing levofloxacin at the different concentrations (6.25, 12.5, 25, and 50 µg/mL) in the bored holes. The inhibition zone was measured to the nearest millimetre for each sample [18].

Antifungal assay

The sample bottles were thoroughly washed and dried. Saboraud Dextrose Agar was prepared using 26 g of Saboraud dextrose dissolved in 100 mL of water. The resulting mixture was heated in a water bath for 15 minutes at 100 °C to ensure complete dissolution. About 25 mL of the dissolved agar was dispensed into each sample bottle, which was then sterilized in an autoclave for 2 hours.

About 1 mL of the fungal organisms was introduced into well-labelled sterile Petri dishes. 25 mL of agar was poured into each petri dish containing the test organisms and mixed thoroughly. The mixture was allowed to dry completely before boring holes using a 1 mm sterile cork bore. Then, 1 mL of 50 % v/v and 100 % v/v crude plant extract was inoculated into the bored holes in the agar. The plates were incubated for 24 hours at 37 °C, after which the final observations were recorded. Control plates were also prepared for each test organism, using Nystatin at concentrations of 150 IU, 75 IU, 37.5 IU, and 18.75 IU in the bored holes. The zones of inhibition were quantified and measured with precision to the nearest millimetre [19]. Each assay was performed in triplicate to ensure accuracy and reproducibility. After 24 hours, the tubes were inspected for signs of turbidity. Samples that remained clear were noted as negative, indicating no microbial growth, while those exhibiting turbidity were classified as positive, confirming microbial proliferation.

Statistical analysis

Statistical analysis was carried out using an Excel spreadsheet Independent T-test (two-tailed, heteroscedastic) for both the antibacterial and antifungal assays, using 0.05 significance values at a 95% confidence interval.

RESULTS

Screening of Phytochemical

The phytochemical screening of the ethanol and methanol extracts of *B. sapida* is presented in Table 1.

Antibacterial assay

The antibacterial assay of the standard (levofloxacin) and the extracts (ethanol and methanol) are presented as zones of inhibition in Tables 2 and 3, respectively. Tables 4 and 5

Table 1: Phytochemical Screening

S/N	Phytochemical tested	EEBS	MEBS
i.	Saponins	+	+
ii.	Flavonoids	+	+
iii.	Alkaloids	+	+
iv.	Cardiac Glycosides	+	+
v.	Tannins	+	+
vi.	Reducing sugars	+	+
vii.	Anthraquinone	-	-

EEBS: Ethanol Extract of *Bligha sapida;* MEBS: Methanol Extract of *Bligha sapida, +*: *Positive* means presence of phytochemical, −: *Negative* means absence of phytochemical

Table 2: Zone of Inhibition of Levofloxacin Standard against some Selected Bacteria

Levofloxacin (µg/mL)	50 (mm)	25 (mm)	12.5 (mm)	6.25 (mm)
Pseudomonas aeruginosa	21 ± 9.97	15.5 ± 0.50	-	-
Staphylococcus aureus	21 ± 9.97	17.5 ± 0.50	15.5 ± 0.50	12.5 ± 0.50
Escherichia coli	25.5 ± 0.50	25.5 ± 0.50	20.0 ± 0.00	24.5 ± 0.50
Bacillus subtilis	-	-	-	-

−: *No inhibition*

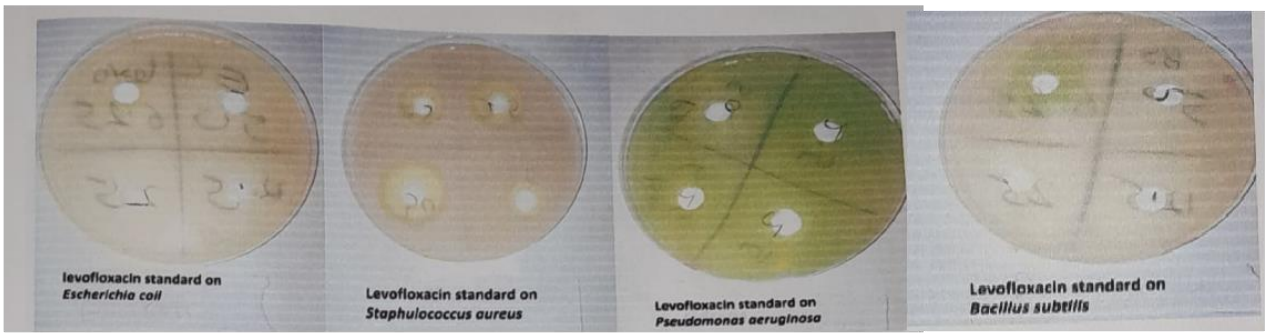

Figure 1: Levofloxacin standard against selected bacterial organisms

Table 3: Zone of Inhibition of the Extract against Bacteria

	MEBS (mm)		EEBS (mm)	
Crude Extract (%v/v)	**50**	**100**	**50**	**100**
Pseudomonas aeruginosa	-	-	-	-
Staphylococcus aureus	8.5 ± 0.50	9.0 ± 0.99	-	8.0 ± 0.99
Escherichia coli	-	-	-	-
Bacillus subtilis	10.0 ± 0.00	10.0 ± 0.00	-	9.5 ± 0.50

EEBS: Ethanol Extract of *Bligha sapida;* MEBS: Methanol Extract of *Bligha sapida;* − = *No inhibition*

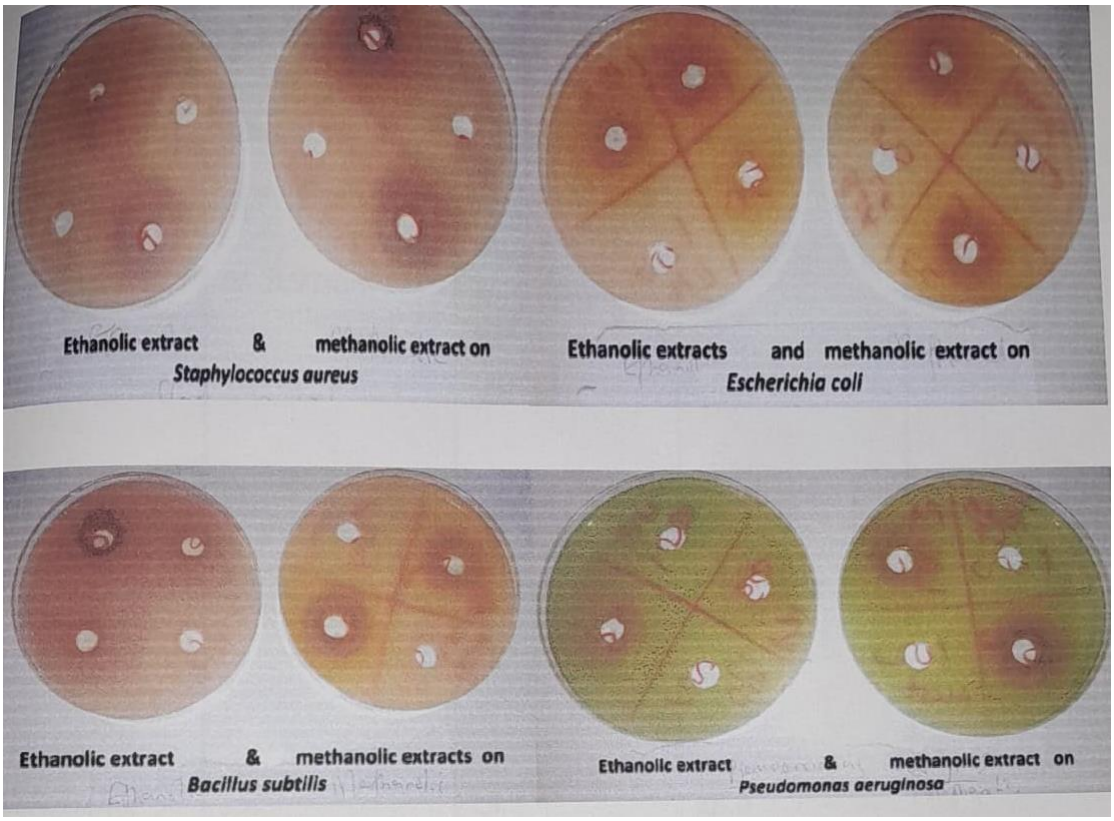

Figure 2: Methanol and ethanol extracts against selected bacterial organisms

Table 4: Zone of Inhibition of Nystatin against Fungi

Nystatin	150 IU (mm)	75 IU (mm)	37.5 IU	18.75 IU
Candida albicans	19 ± 0.99	13 ± 0.00	-	-
Aspergillus niger	-	-	-	-

− = No inhibition

Table 5: Zone of Inhibition of Crude Extract against Fungi

	MEBS		EEBS	
Extract concentration (%v/v)	**50**	**100**	**50**	**100**
Candida albicans	-	-	-	-
Aspergillus niger	-	-	-	-

EEBS: Ethanol Extract of *Bligha sapida;* MEBS: Methanol Extract of *Bligha sapida*, −: *No inhibition*

represent the antifungal assay of the standard (nystatin) and the extracts (ethanol and methanol), respectively. Figure 1 and 2 shows pictures of the petri dishes showing zones of inhibition for the standard and extract, respectively.

DISCUSSION

Analysis of *B. sapida* leaf extracts revealed a rich profile of bioactive constituents, including reducing sugars, alkaloids, flavonoids, cardiac glycosides, tannins, and saponins, present in both aqueous ethanol and methanol extractions (Table 1). This group of compounds are known to possess an extensive biological effect, such as antimicrobial, antioxidant, and therapeutic activities [20]. Their presence supports the potential of *B. sapida* as a promising source of natural agents for microbial control and health applications.

The crude extracts of *B. sapida* were evaluated using four (4) bacterial strains: *Pseudomonas aeruginosa, Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus. aureus*. Findings indicated that both methanol and ethanol extracts displayed variable antibacterial effects, with the methanol extract showing a broader spectrum of action (Table 3). It was particularly effective against *Staphylococcus aureus* and *Bacillus subtilis* at all tested concentrations, highlighting its strong activity against gram-positive bacteria. In contrast, its inhibitory effect on *E. coli* and *Pseudomonas aeruginosa* was less pronounced or inconsistent.

The ethanol extract of *B. sapida* displayed antibacterial effects only at elevated concentrations, suggesting its activity is dependent on dosage. Both methanol and ethanol extracts were ineffective against *E. coli* and *P. aeruginosa*, indicating resistance or lack of antimicrobial potency against these microbes. In contrast, the extracts showed selective efficacy, particularly against *B. subtilis* and *S. aureus*. However, standard antibiotic levofloxacin consistently produced higher inhibition zones across all bacterial strains, underscoring its superior antibacterial potency. Despite this, the plant extracts still demonstrated meaningful inhibitory effects against specific gram-positive bacteria.

Notably, *B. sapida* demonstrated effective inhibition against *Bacillus subtilis,* a bacterial strain that showed resistance to levofloxacin. This contrast underscores the potential of the plant's crude extracts to combat microorganisms that may elude conventional antibiotics. Such findings suggest that *B. sapida* could serve as a complementary or alternative treatment, particularly for infections caused by drug-resistant Gram-positive bacteria.

The methanol and ethanol extracts derived from *B. sapida* demonstrated no inhibitory effect on the C. *albicans* and *A. niger*, as signified by the absence of inhibition zones at all tested concentrations. This contrasts with earlier findings which reported that crude seed protein from *B. sapida* exhibited notable antifungal effects, comparable to the reference antifungal drug Ticonazole [21]. Meanwhile, Nystatin, used as a standard control, effectively inhibited Candida albicans but showed no activity against *Aspergillus niger,* with its potency increasing at higher concentrations. These observations suggest that while Nystatin selectively targets certain fungal species, the tested extracts of *B. sapida* lack broad-spectrum antifungal properties.

CONCLUSION

The research revealed that leaf extracts of *Blighia sapida* contain notable phytochemicals that demonstrate antibacterial activity largely against *Staphylococcus aureus* and *Bacillus subtilis*. However, these extracts showed no inhibitory effects on *E. coli*, *P. aeruginosa*, *C. albicans*, or *A. niger*. These findings indicate that while the plant may serve as a natural antibacterial option, its effectiveness is selective and limited to certain microbial strains. The study confirms the antibacterial potential of the extracts, though not across all tested pathogens.

AUTHORS' CONTRIBUTION

AAO was involved in research concept and design, data collection, assembly, analysis, interpretation, and writing the article; DRO and AAA were involved in data collection and assembly, while HBC was involved in critical revision of the article and final approval of the article.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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