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Evaluation of The Antimicrobial Potentials of Methanol Extract and Fractions of *Xylopia Aethiopica* Seed Pods against Selected Clinical Bacterial and Fungal Isolates

Alozie, M. F.*[1], Anwana, U. U.[2], and Henshaw, E. E.[2]

[1]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria*
[2]*Department of Biochemistry, Faculty of Biological Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria*

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Corresponding Author: **Mfonobong F. Alozie**
E-Mail: **mfonobongalozie@uniuyo.edu.ng**

ABSTRACT

Xylopia aethiopica is a medicinal plant widely used in traditional medicine as a spice and for the treatment of ailments such as diarrhoea. This study evaluated the antimicrobial activity of the methanol seed pod extract of X. aethiopica using the agar diffusion method. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) were determined using standard agar dilution techniques. Five clinical isolates—Salmonella typhi, Staphylococcus aureus, Escherichia coli, Candida albicans, and Trichophyton tonsurans—were obtained from the University of Uyo Health Center and used for the analysis. The crude extract and its fractions exhibited varying degrees of antimicrobial activity, with more pronounced effects against fungal isolates, even at low concentrations. The crude extract demonstrated broad-spectrum antifungal activity at concentrations ranging from 12.5 ± 0.02 to 100 ± 0.01 mg/mL, producing inhibition zones against all tested fungal organisms, while antibacterial activity was comparatively limited. Among the fractions, the ethyl acetate fraction exhibited both bacteriostatic and bactericidal effects against Staphylococcus aureus and Salmonella typhi, respectively, at 200 ± 0.01 mg/mL. The butanol fraction showed the strongest fungicidal activity, with an MFC of 3.125 ± 0.02 mg/mL. The MBC/MIC and MFC/MIC ratios ranged from 1 to 4, indicating true bactericidal and fungicidal effects. These findings suggest that X. aethiopica possesses promising antifungal potential, and further studies are recommended to investigate its therapeutic applications and safety profile in the management of fungal infections.

Keywords: *Xylopia aethiopica, antimicrobial sensitivity testing, fractions, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC), Minimum Bactericidal Index, Minimum Fungicidal Index.*

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Medicinal plants like *Xylopia aethiopica* offer promising natural alternatives in the fight against infectious diseases, especially amidst rising antimicrobial resistance. This study investigated the antimicrobial potential of methanol extracts from *X. aethiopica* seed pods and found significant antibacterial and antifungal activity, particularly against fungal pathogens at low concentrations. These findings highlight the plant's potential as a source for developing novel, plant-based antimicrobial agents to combat resistant infections and improve global health outcomes.

INTRODUCTION

Xylopia aethiopica (Figure 1.1) is an aromatic, evergreen member of the Annonaceae family that typically reaches a height of 20 meters. Its nomenclature reflects its physical and historical roots: the genus name is a Greek compression of *xylon pikron*, meaning "bitter wood," while the specific epithet *aethiopica* refers to its Ethiopian origins. Although named for Ethiopia, the tree is currently most prominent as a crop and native species throughout the lowland rainforests and moist fringe forests of the African savanna zones [1]. It is widely distributed across Tropical Africa, appearing frequently in coastal rainforests, riverine ecosystems, and even serving as a hardy pioneer species within arid savanna regions [2].

The morphology of *Xylopia aethiopica* is defined by a straight, tall stem that is occasionally supported by buttressed roots[1]. Its smooth, grey-brown bark transitions into branches that range from reddish-brown to blackish, characterized by a high density of conspicuous lenticels. The foliage consists of oblong-elliptic to ovate leaves, measuring 8.0–16.5 cm in length. During anthesis, the tree produces strongly scented, greenish-white to yellow flowers that occur either solitary or in small clusters of two to five [2]. The resulting fruits comprise numerous cylindrical, hairless carpels up to 6 cm long; these pods are often marked by diagonal ridges and shift in color from green to a deep reddish hue upon maturity [3].

MORPHOLOGY OF *Xylopia aethiopica*
Kingdom: Plantae (Plants)
Phylum: Spermatophyta (Seed plants)
Sub-phylum: Angiosperms (Flowering plants)
Class: Magnoliopsida (Dicotyledon)
Order: Magnoliales
Family: Annonaceae (Custard apple family)
Genus: *Xylopia*
Species: *Xylopia aethiopica*

Figure 1.1: Fruits of Xylopia aethiopica
Source: *Royal Botanic Garden Edinburgh (2025)*

Figure 1.2: Seeds of Xylopia aethiopica
Source: *Field data (2025)*

2. Pharmacological Uses of *Xylopia aethiopica*

The dried fruits of *X. aethiopica* (Grains of Selim) also known as Ethiopian pepper, is used as a spice and in herbal medicine. An infusion made from the bark or fruit of *X. aethiopica* has been useful in the treatment of bronchitis and dysenteric conditions, or as a mouthwash to treat toothaches. [4]

It has also been used as a medicine for biliousness, female infertility, hemorrhoids, uterine fibroids, malaria, cough, diabetes and febrile pains. The seeds (Figure 1.2) are known to be used traditionally to enhance postpartum placental expulsion. The bark, when steeped in palm wine is used to treat asthma, stomach aches, and rheumatism [4].

A study by Ezenobi *et al*. [5] shows that the fruit of the plant *Xylopia aethiopica* has been used in ethno-medicine in southern Nigeria for treating dysentery, cough, and bacterial infections. The reviewed studies confirm the therapeutic promise of *X. aethiopica.* However, significant gaps remain. There is limited uniformity in extraction methods and microbial assays, and few studies link phytochemical profiles directly with antimicrobial activity.

In a study by Larayetan [6], it shows that volatile oil from the fruit of *X. aethiopica* exhibited good antimicrobial activity against the tested organism and was found to show no cytotoxicity. This could be an indication of the safety of the fruit oil as a targeted drug for mammalian organisms. In addition to this, the volatile oil from the plant could be used as an excellent substitute to synthetic antiplasmodial and antimicrobial therapeutic drugs to battle malaria and infectious ailments from various bacterial strains.

This research work seeks to bridge these gaps by performing antimicrobial assessment of *X. aethiopica* seed pods using validated microbiological protocols.

3.0 METHODOLOGY

3.1 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations (MIC) of the methanol extracts and fractions of the seed pods of *Xylopia aethiopica* for the bacterial and fungal isolates were determined following the reference standard agar dilution technique (ADT) and assay plates incubated at 37 °C for 24 h.[7,8,9] Inoculated MHA plates without the extracts served as a negative control. MIC was taken as the least concentration of the extracts and fractions that inhibited growth.

3.2 Determination of Minimum Biocidal Concentration (MBC) and Mode of Activity

The Minimum Biocidal Concentrations (MBCs) of the methanol extracts and fractions of *Xylopia aethiopica* were determined after reincubating the plates from the overnight agar dilution test with no visible growth at 37 °C for 24 h.[8], [9] MBCs were taken as the least concentrations that did not show growth (kill the cultures). The mode of activity (MBC/MIC index) of the extracts and fractions was determined as either static or –cidal [10].

4.0 RESULTS

4.1 Antimicrobial Activity Results

Table 4.1.1 Antimicrobial sensitivity tests of methanol extract and fractions of Xylopia aethiopica (inhibition zone diameters (± 0.02 mm) on bacterial isolates

Extract/Fraction	Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
CE	SA	22	20	12	10
	EC	20	15	12	8
	ST	22	18	15	14
AF	SA	20	15	10	8
	EC	20	15	12	8
	ST	15	10	8	_
HF	SA	20	15	10	8
	EC	20	15	10	8
	ST	20	15	12	10
BF	SA	20	18	15	10
	EC	20	15	12	10
	ST	20	15	10	8
EF	SA	20	18	15	12
	EC	20	15	12	10
	ST	20	15	10	8
DF	SA	20	15	10	_
	EC	20	15	15	12
	ST	20	15	10	10

Key: SA- *Staphylococcus aureus,* ***EC-*** *Escherichia coli,* ***ST-*** *Salmonella typhi,* ***CAD-*** *Candida albicans,* ***TT-*** *Trichophyton tonsurans, CE- Crude extract, AF-Aqueous Fraction, BF- Butanol fraction, DF- Dichloromethane Fraction, EF- Ethylacetate Fraction, HF- Hexane Fraction.*

Table 4.1.2 Antimicrobial sensitivity tests of methanol extract and fractions of Xylopia aethiopica (inhibition zone diameters (± 0.02 mm) on fungal isolates

Extract/Fraction	Microorganisms	100 mg/ml	50 mg/ml	25mg/ml	12.5 mg/ml
CE	CAD	35	30	25	20
	TT	33	20	20	18
AF	CAD	20	15	15	12
	TT	25	20	15	12
HF	CAD	22	20	20	15
	TT	25	20	20	15
EF	CAD	30	20	15	15
	TT	25	20	20	15
BF	CAD	20	20	18	15
	TT	25	20	18	15
DF	CAD	25	18	18	15
	TT	20	20	15	12

Table 4.1.3 Antibacterial activities of drugs used as positive controls

CON TROL	MICRO ORGANISMS	100 mg/ml	50 mg/ml
GENTAMICIN	SA	50	30
	EC	50	30
	ST	35	25
CIPROFLAXIN	SA	30	20
	EC	25	20
	ST	35	25
KETOCONAZOLE	CAD	30	25
	TT	40	35
FLUCONAXOLE	CAD	30	25

Key: SA- *Staphylococcus aureus,* ***EC-*** *Escherichia coli,* ***ST-*** *Salmonella typhi,* ***CAD-*** *Candida albicans,* ***TT-*** *Trichophyton tonsurans*

4.2 Minimum Inhibitory Concentration Results

Table 4.2.1 Minimum inhibitory concentration values of fractions of Xylopia aethiopica against bacterial isolates

Fraction	Microorganisms	200 mg/ml	100 mg/ml	50 mg/ml
AF	EC	+	+	+
	SA	+	+	+
	ST	+	+	+
HF	EC	+	+	+
	SA	+	+	+
	ST	+	+	+
EF	EC	+	+	+
	SA	-	+	+
	ST	-	+	+
BF	EC	+	+	+
	SA	+	+	+
	ST	+	+	+
DF	EC	+	+	+
	SA	+	+	+
	ST	+	+	+

Key: (+) presence of bacterial growth, (-) absence of bacterial growth, SA- *Staphylococcus aureus,* ***(EC)*** *Escherichia coli,* ***(ST)*** *Salmonella typhi, AF-Aqueous Fraction, BF- Butanol fraction, DF- Dichloromethane Fraction, EF- Ethyl acetate Fraction, HF- Hexane Fraction*

Table 4.2.2 Minimum inhibitory concentration values of fractions of Xylopia aethiopica against fungal isolates

Fraction	Microorganisms	25 mg/ml	12.5 mg/ml	6.25mg/ml	3.125 mg/ml
AF	CAD	+	+	+	+
	TT	+	+	+	+
HF	CAD	-	-	-	+
	TT	-	-	-	+
EF	CAD	-	-	-	+
	TT	-	-	-	+
BF	CAD	-	-	-	-
	TT	-	-	-	-
DF	CAD	+	+	+	+
	TT	+	+	+	+

Key: (+) presence of fungal growth, (-) absence of fungal growth, CAD- *Candida albicans,* ***TT-*** *Trichophyton tonsurans, AF-Aqueous Fraction, BF- Butanol fraction, DF- Dichloromethane Fraction, EF- Ethylacetate Fraction, HF- Hexane Fraction*

4.3 MINIMUM BACTERICIDAL and FUNFICIDAL CONCENTRATION (MBC) RESULTS

4.3.1 Minimum Bactericidal Concentration (MBC) Values For Bacterialsolates

Fraction	Microorganisms	200 mg/ml	100 mg/ml	50 mg/ml
EF	EC	+	+	+
	ST	+	+	+
	SAL	-	+	+

Key: (+) presence of bacterial growth, (-) absence of bacterial growth, (ST) *Staphylococcus aureus,* ***(EC)*** *Escherichia coli,* ***(SAL)*** *Salmonella EF- ethyl acetate fraction*

Table 4.3.2 Minimum Fungicidal concentration results of fractions of Xylopia aethiopica on fungal isolates

Fraction	Microorganisms	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
AF	CAD	+	+	+	+
	TT	+	+	+	+
HF	CAD	-	+	+	+
	TT	-	+	+	+
EF	CAD	-	-	+	+
	TT	-	-	+	+
BF	CAD	-	-	-	-
	TT	-	-	-	-
DF	CAD	+	+	+	+
	TT	+	+	+	+

Key: (+) presence of fungal growth, (-) absence of fungal growth, (CAD) *Candida albicans,* ***(TT)*** *Trichophyton tonsurans, AF-Aqueous Fraction, BF- Butanol fraction, DF- Dichloromethane Fraction, EF- Ethylacetate Fraction, HF- Hexane Fraction.*

4.4 RESULTS FOR MINIMUM BACTERICIDAL INDEX AND MINIMUM FUNGICIDAL INDEX

4.4.1 MINIMUM BACTERICIDAL INDEX

Fraction	Microorganisms	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC RATIO (mg/ml)	INTERPRETATION
EF	SA	200	-	-	Bacteriostatic at 200mg/ml
	ST	200	200	1	Bactericidal

Key: SA- *Staphylococcus aureus,* ***ST-*** *Salmonella typhi, EF- Ethyl acetate Fraction*

The results for Minimum Bactericidal Index indicate that the only fraction with bacteriostatic and bactericidal activity is the ethyl acetate fraction (Table 4.4.1).

Table 4.4.2 Minimum Fungicidal Index

Fraction	Microorganisms	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC RATIO (mg/ml)	INTERPRETATION
HF	CAD	6.25	25	4	Fungicidal at 25 mg/ml
	TT	6.25	25	4	Fungicidal at 25 mg/ml
EF	CAD	6.25	12.5	2	Fungicidal at 12.5 mg/ml
	TT	6.25	12.5	2	Fungicidal
BF	CAD	3.125	3.125	1	Fungicidal at 3.125 mg/ml
	TT	3.125	3.125	1	Fungicidal

Key: CAD- Candida albicans, TT- Trichophyton tonsurans, BF- Butanol fraction, EF-Ethylacetate Fraction, HF- Hexane Fraction

DISCUSSION

The menace of antimicrobial resistance has led to the incessant drive to seek for new compounds of natural origin from plants, animals, soil etc. and with the mandate from the World Health Organization (WHO), the search is still ongoing.

Antibiotic susceptibility testing is the process of evaluating the sensitivity of bacteria to specific antimicrobial agents in order to determine which antibiotics are most effective for treating a particular infection.

The crude extract (methanol extract of *X. aethiopica* seed pods), had more activity against fungi (*Candida albicans* and *Trichophyton tonsurans*) than bacteria at all concentrations (12.5 ± 0.02 -100 ± 0.01 mg/ml). The effectiveness of the extract varies with the type of microorganism, indicating that the plant extract does not possess broad-spectrum antimicrobial activity but shows selectivity toward fungal organisms. This implies that the cell structure or physiology of the tested fungi makes them more vulnerable to the phytochemicals in *Xylopia aethiopica* than the tested bacterial species. The result suggests that the crude extract has promising potential for development as a natural antifungal remedy rather than as an antibacterial drug.

This implies that the crude methanol extract of *Xylopia aethiopica* seed pods possesses stronger antifungal activity than antibacterial activity. This indicates that the extract is more suitable for combating fungal infections and suggests the presence of phytochemicals with greater efficacy against fungi. Therefore, the plant has greater potential for development as an antifungal agent, warranting further antifungal-focused research.

For the aqueous fraction, there was some antibacterial activity against *Salmonella typhi* and *Escherichia coli,* though slightly higher at 25mg/ml. There was no activity against *Salmonella typhi* at 12.5 ± 0.01 mg/ml (the least concentration). There was also higher activity for *Candida albicans* at 25.0 mg/ml and 12.5 mg/ml respectively. Higher activity for *Trichophyton tonsurans* at all concentrations ranging from 12.5 -100mg/ml was observed. The dichloromethane fraction had no activity against *Staphylococcus aureus* at the least concentration of 12.5mg/ml.

Summarily, all the fractions had better antifungal activity than antibacterial activity as evidenced by the Inhibition Zone Diameters. Also, the Inhibition Zone Diameters were concentration dependent *i.e.* the greater the concentration the larger the Inhibition Zone Diameter. This corroborates the work of previous researches on antimicrobial activity being concentration dependent. This observation is in conformity with Okigbo *et al*.[12] and Jagtap *et al*. [13]. The increase in inhibitory effects of the extracts as the concentrations increased may be attributed to the ethanol extracting most of the active ingredients of the plants.[11]

MINIMUM INHIBITORY CONCENTRATION

Ethyl acetate fraction had no Minimum Inhibitory values against *Staphylococcus aureus* and *Salmonella typhi* at 200.0 mg/ml, indicating its lack of activity against Staphylococcal infections.

MIC AGAINST FUNGAL ISOLATES

Aqueous and Dichloromethane fractions had no Minimum inhibitory Concentration values starting from 3.125 – 25.0 mg/ml against the fungal isolates, indicating a lack of antifungal property by both fractions of the methanol extract of *X. aethiopica* seed pods.

Hexane and Ethyl acetate fractions had MIC values at 6.25 mg/ml for the fungal isolates- *Candida albicans* and *Trichophyton tonsurans,* whereas Butanol fraction had MIC values at 3.125 mg/ml, indicating their probable use topically for the treatment of fungal infections.

MINIMUM BACTERICIDAL CONCENTRATION

Ethyl acetate fraction had MBC value at 200.0 mg/ml for *Salmonella typhi* but no MBC values at 50.0 – 200.0 mg/ml for *Escherichia coli* and *Staphylococcus aureus*. Hexane fraction had MBC at 25mg/ml for the fungal isolates- *Candida albicans* and *Trichophyton tonsurans,* whereas Ethyl acetate fraction had MBC at 12.5 mg/ml and the best fraction was Butanol fraction with MBC values at 3.125 mg/ml for the fungal isolates, indicating where further research can be carried out. Activity varies depending on the type of organism and the solvent fraction used.

Conclusion: The methanol extract and fractions of *Xylopia aethiopica* seed pods exhibit significant antimicrobial activity, with a stronger and more consistent effect against fungal pathogens than bacterial strains. The findings highlight the plant's potential as a source of antifungal agents, particularly the butanol and ethyl acetate fractions. The findings imply that the extract exhibits selective antimicrobial activity. The ethyl acetate fraction is weakly bactericidal against *Salmonella typhi* but ineffective against *E. coli* and *S. aureus*. In contrast, all fractions showed antifungal activity, with the butanol fraction demonstrating the highest potency (lowest MBC). This suggests that the extract is more effective as an antifungal agent and that the butanol fraction contains the most active antifungal constituents represents the best candidate for Isolation of active compounds, Further purification and Development of potential antifungal agents thus making it suitable for further research and drug development.

These results support traditional use of *X. aethiopica* and suggest the need for further studies to validate its efficacy and safety for clinical application, especially in the treatment of fungal infections.

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Ethical Permission

No ethical permission was required for the study as it did not involve human or animal subjects.

Authors Contribution

The research work was carried out in collaboration between Authors. Author HEE carried out the laboratory investigations and wrote the first draft of the manuscript, MFA designed and supervised the work, while MFA and AUU managed the literature search and analyses of the study. All authors read and approved the final manuscript for submission.

Conflicts of Interests

Authors declare no conflict of interest

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