DRUG DISCOVERY

16(37), 2022

Phytochemical and antimicrobial studies of *xylopia aethiopica* stem bark extracts

Babalola SA[1*], Gabriel AF[2]

To Cite:
Babalola SA, Gabriel AF. Phytochemical and antimicrobial studies of *xylopia aethiopica* stem bark extracts. *Drug Discovery*, 2022, 16(37), 68-75

Author Affiliation:
[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]Department of Chemistry, University of Abuja, Gwagwalada-Abuja, FCT, Nigeria.

[*]**Corresponding author**:
Babalola Sodeeq Adewale, Department of Pharmaceutical and Medicinal Chemistry
Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Email: wale.babalola91@gmail.com, Tel: +2347058199796
ORCID: 0000-0003-0580-0595

Peer-Review History
Received: 03 February 2022
Reviewed & Revised: 10/February/2022 to 18/April/2022
Accepted: 20 April 2022
Published: 23 April 2022

Peer-review
External peer-review was done through double-blind method.

ABSTRACT

The preliminary screening of phytochemical constituents of extracts of *Xylopia aethiopica* was evaluated. The n-hexane extract revealed the presence of terpenoids, alkaloids, and steroids, while the presence of alkaloids, terpenoids, saponins, steroids, and phenolics was indicated in ethyl acetate extract, and the methanol extract contained tannins, flavonoids, phenolics, terpenoids, saponins, alkaloids, steroids. The medicinal property of the extracts was investigated using *in-vitro* antimicrobial assays. The hexane extract indicates potent activity against Pseudomonas aeruginosa with MIC of 100mg/ml and ZI of 20 mm. Methanol and ethyl acetate extracts had inhibitory activities against *Candida albican* with MIC of 100mg/ml while the standard antibiotics have no inhibitory activities against *Candida albican* suggesting that these extracts contain certain phytochemical that are active against *Candida albican*. Saponins which are constituents of methanol and ethyl acetate extracts from the phytochemical screening are known to be antifungal, especially against *Candida albican*. This suggests that the presence of saponins in both ethyl acetate and methanol extracts may be responsible for the inhibitory activities against *Candida albican.*

Keywords: *Xylopia aethiopica* stem bark, Antimicrobial, Phytochemical, *Pseudomonas aeruginosa*, *Candida albican*

1. INTRODUCTION

The World Health Organization (WHO) recognizes traditional medicine, plant medicine is of particular importance as a possible substitute for the healthcare delivery system for a large portion of the world's population. Infectious diseases are the number one cause of death worldwide which accounted for almost one-half of all deaths in tropical countries especially sub-Sahara Africans [1]. The rises are attributed to a high occurrence of respiratory tract infections and HIV/AIDS. Another issue is antibiotic resistance in nosocomial and community-acquired infections [1]. These worrying health trends demand prompt infectious disease solutions from the medical and public health communities, as well as updated treatment and prevention approaches like immunization, improved monitoring, and the creation of novel medicines. One of these treatments is the discovery of new antimicrobials. [2]. It's possible that man has almost eaten or chewed all kinds of herbs in order to relieve or

DISCOVERY SCIENTIFIC SOCIETY

cure illness. He identified the usefulness of different plants against a variety of ailments through trials and errors, which were the first crude extracts trials. Various ethnic cultures have vast amounts of information on the medicinal properties of various plants and animals, which can be used to develop new pharmaceuticals [3].

Xylopia aethiopica is an evergreen, aromatic tree with a height approximately 20m high, and a width of 100cm, with unbending boles. It is often found in moist fringe forests and low-land rain forests of savanna zones of Africa. Various parts of the plant have been employed traditionally in different therapeutic formulations [4].

The fruits of the *Xylopia aethiopica* tree are used for various therapeutic purposes such as antitussive, sedative, laxative, and analgesic. The fruit is a common ingredient in some parts of Nigeria. Its spice smoke is used in the treatment of asthma in Liberia [5]. The fruit extract is also used in the treatment of analgesia and chronic inflammatory diseases, headache, neuralgia (pains in the nerves), and colic pain in Ghana [6].

It is also employed in inducing placental discharge post-partum due to its abortifacient [6]. The fruit is often subsumed in formulation for endemic and external uses, owing to its analgesic property for any part of the body associated with pain and in the treatment of acute inflammation. The fruit decoction is useful as of diuretic, anti-inflammatory, and also airway inflammation.
The infusion of the stem bark extract in palm wine dosage rate of one or two glasses per day is useful in the treatment of airway inflammation, inflammation of the intestine, and chronic inflammatory diseases and as medicine for bulimia (eating disorder) in Congo [7]. The plant is also used in the treatment of cancer and gastrointestinal ulceration conditions via its various traditional therapeutic preparations in Nigeria [8].

2. EXPERIMENTAL METHODS

A fresh sample of *Xylopia aethiopica* stem bark was collected from Iyara town, Ijumu local government, Kogi state. The plant material was authenticated in the herbarium of the University. The stem bark of *Xylopia aethiopica* was then air-dried and pulverized into powdery form in the Chemistry department laboratory, University of Abuja. The powdered stem bark was then weighed.

Extraction of Xylopia aethiopica stem bark with n-hexane, ethyl acetate, and methanol

The extraction method followed that of Gabriel *et al.*, 2016 [9]. 69.73g powdered sample was successively extracted with solvents of varying polarities which includes n-hexane, ethyl-acetate, and methanol using soxhlet extractor in the order of their increasing polarities.

n-Hexane extract

250 ml of n-hexane was used to extract 69.73 g of the powdered stem bark using soxhlet. The sample was extracted at 65 ^{o}C temperature of the heating mantle. The sample was continuously extracted under reflux until the yellow color of a solvent-extract system in the glass thimble turned colorless. A yellow-colored n-hexane extract was obtained and the extract was poured into a 100ml flask. n-Hexane extract of the sample was evaporated on a thermostat water bath to afford gummy yellow extract.

Ethyl acetate extract

150 ml of ethyl acetate was used to extract 69.73 g of the powdered stem bark using soxhlet. The sample was extracted at 78 ^{o}C temperature of the heating mantle. The sample was continuously extracted under reflux until the green color of a solvent-extract system in the glass thimble turned colorless. A green-colored ethyl acetate extract was obtained and the extract was transferred into a 100ml flask. Ethyl acetate extract of the sample was evaporated on a thermostat water bath to afford a sticky green extract.

Methanol extract

150 ml of methanol was used to extract 69.73 g of the powdered stem bark using soxhlet. The sample was extracted at 68 ^{o}C temperature of the heating mantle. The sample was continuously extracted under reflux until the brown color of a solvent-extract system in the glass thimble turned colorless. A brown-colored methanol extract was obtained and the extract was transferred into a 100ml flask. Methanol extract of the sample was evaporated on a thermostat water bath to afford slurry brown extract.

Phytochemical screening of the extracts

The qualitative phytochemical screening procedure followed those described by Barathidasan *et al.* [10], Jennifer Adline *et al.* [11], and P. Brindha *et al.* [12].

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

Antimicrobial analysis of the extracts

Bacteriological techniques

The bacteria logical techniques followed were those described by Cheesbrough *et al.* [12], Burdon and Williams *et al.* [14], Brooks *et al.* [15].

The standard bacterial strains *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa,* and a fungus *Candida albican* were obtained from the Microbiology laboratory of the University of Abuja Teaching Hospital, Gwagwalada, Abuja.

The strains were: gram-positive: *Staphylococcus aureus, Candida albican*. And gram negative: *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*.

The bacteria and fungus strains were activated and cloned three successive times in CLED agar and nutrient agar respectively, and they were stored in nutrient slants at 4 ^{o}C, subsequent activation and test were done on nutrient agar medium.

Control

Cotrimoxazole (septrin), Gentamycin, and Tetracycline were used at concentrations ranging from 40mg/ml to 100mg/ml as positive control while the solvents were used as the negative control.

3. RESULTS

Table 1 shows the physical characteristic, masses of extracts of powdered *Xylopia aethiopica* stem bark obtained, and their percentage yield from 69.73 g powdered sample. The percentage yield was calculated using the formula below.

Phytochemical screening of t n-hexane, ethyl acetate, and methanol extracts of *X. aethiopica* stem bark was carried out and the result is as shown in Table 2.

Table 3 shows the Zones of Inhibition (mm) and the Minimum Inhibitory Concentration (MIC) of n-hexane, ethyl acetate, and methanol extracts.

$$\% \text{ Yield} = \frac{\text{Weight of crude extract}}{\text{Weight of the sample}} \text{ X } 100\%$$

Figure 1: Percentage yield formula

Table 1: Physical characteristics, masses, and percentage yield crude extract yield and their percentage yield of the extracts.

Powdered Sample	Extraction Solvent	Physical Characteristic	Mass of Crude extract (g)	% yield of Crude extract
Stem bark of *Xylopia aethiopica*	n-Hexane	Yellow color	2.14	3.07
	Ethyl acetate	Green color	0.94	1.35
	Methanol	Brown color	4.29	6.15

ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

Table 2: Phytochemical screening results of stem bark extracts of *X. aethiopica*.

Phytochemical	n-Hexane extract	Ethyl acetate extract	Methanol extract
Saponns	-ve	+ve	+ve
Tannins	-ve	-ve	+ve
Alkaloids	+ve	+ve	+ve
Flavonoids	-ve	-ve	+ve
Terpenoids	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Phenolics	-ve	+ve	+ve

Keywords: +ve = Present, -ve = Absent

Table 3: Result for antimicrobial tests on n-hexane, ethyl acetate, and methanol extracts.

	Zones of inhibition (ZI)					
CONC.(mg/ml) / Micro-organism	100mg/ml	200mg/ml	300mg/ml	400mg/ml	500mg/mg	600mg/ml
	METHANOL EXTRACT					
Pseudomonas aeruginosa	2 mm	2 mm	3 mm	3 mm	3 mm	4 mm
Escherichia coli	2 mm	2 mm	2 mm	2 mm	3 mm	3 mm
Candida albican	3 mm	4 mm	4 mm	3 mm	5 mm	6 mm
Klebssiella pneumonia	2 mm	2 mm	2 mm	2 mm	3 mm	3 mm
Staphylococcus aureus	------	-------	-------	------	-------	-------

ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

	ETHYL ACETATE EXTRACT					
Pseudomonas aeruginosa	------	------	2 mm	4 mm	6 mm	6 mm
Escherichia coli	2 mm	2 mm	4 mm	3 mm	4 mm	7 mm
Candida albican	2 mm	2 mm	2 mm	2 mm	2 mm	2 mm
Klebsiella pneumonia	------	------	------	------	-------	------
Staphylococcus aureus	-----	------	------	------	-------	------
	n-HEXANE					
Pseudomonas aeruginosa	20 mm	25 mm	25 mm	28 mm	28 mm	31 mm
Escherichia coli	-------	-------	-------	-------	------	-------
Candida albican	-------	-------	-------	------	------	------
Klebsiella pneumonia	-------	2 mm	2 mm	3 mm	3 mm	4 mm
Staphylococcus aureus	-------	-------	-------	------	------	------

Table 4: Positive Control (known antibiotics)

Micro-organism	Tetracycline 40 mg/ml	Gentanycin 20mg/ml	Cotrimoxazole 100 mg/ml
Pseudomona aeruginosa	30 mm	27 mm	15 mm
Escherichia coli	30 mm	25 mm	18 mm
Klebsiella			

ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

Pneumonia	30 mm	25 mm	18 mm
Staphylococcus aureus	10mm	14mm	---------
Candida albican	--------	--------	---------

Table 5: Negative control (Ethyl acetate)

Micro-organism	Solvent (Ethyl acetate)
Pseudomonas aeruginosa	------
Escherichia coli	------
Klebsiella pneumonia	------
Staphylococcus aureus	------
Candida albican	------

4. DISCUSSION

Phytochemical Screening

The result of preliminary screening of phytochemical constituents of n-hexane extract indicates the presence of alkaloids, terpenoids, and steroids in the extract. Ethyl acetate extract showed the presence of saponins, alkaloids, terpenoids, steroids, and phenolics while in methanol extract the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, and phenolics were all observed according to the result in Table 2 above. Preliminary studies showed that *X. aethiopica* stem bark contains pharmaceutical constituents such as saponins which are known to be antifungal, especially against *Candida albican*, it is also known to lower cholesterol levels in the body. Saponins have been found to increase the effectiveness of vaccines [16].

Alkaloids and terpenoids which are bioactive have been known to be bactericidal, pesticidal, and fungicidal [17]. Alkaloids are also used in medicine because of their quick action on specific areas of the central nervous system, the effectiveness of alkaloids on humans has led to the development of powerful painkiller vaccines. They are the active components of many relaxants, tranquilizers, sedatives, stimulants, and anesthetics [18]. Some terpenoids are useful flavoring agents. Steroids are mostly secondary metabolites that are capable of producing definite physiological actions on the body [17].

Tannins in plants are known to be astringents, which help in wound healing and the treatment of hemorrhoids, tonsillitis, pharyngitis, and skin eruptions [19]. They are used in treating intestinal disorders such as diarrhea and dysentery and are known to show curative activity against several pathogens [20]. Tannins are also used as antidotes for metallic, alkaloidal, and glycosidic poisons [18].

Antimicrobial Analysis

Table 3 showed the antimicrobial activity of n-hexane, ethyl acetate, and methanol extracts of *X. aethiopica* stem bark at different concentrations.

Methanol extract has inhibitory activity against *Pseudomonas aeruginosa, Escherichia coli, Candida albican,* and *Klebsiella pneumonia* at all concentrations (100 mg/ml – 600 mg/ml). The extract had no inhibitory effect against *Staphylococcus aureus* at all concentrations. The highest zone of inhibition was observed with *Candida albican*.

Ethyl acetate extract has inhibitory activity against *Pseudomonas aeruginosa, Escherichia coli,* and *Candida albican.* The ethyl acetate extract has no inhibitory effect against *Klebsiella pneumonia* and *Staphylococcus aureus* at all concentrations (100 mg/ml – 600 mg/ml) and only indicated activity against *Pseudomonas aeruginosa* at concentration 300 mg/ml to 600 mg/ml. The highest zone of inhibition was observed against *Klebsiella pneumonia* at 600 mg/ml.

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

The n-Hexane extract had an inhibitory effect against *Pseudomonas aeruginosa* at all concentrations (100 mg/ml – 600 mg/ml) and only had an inhibitory effect against *Klebsiella pneumonia* at 200 mg/ml to 600 mg/ml. The extract had no inhibitory effect against *Escherichia coli, Candida albican,* and *Staphylococcus aureus* at all concentrations (100 mg/ml – 600 mg/ml). the n-Hexane extract has very high zones of inhibition against *Pseudomonas aeruginosa* at all concentrations making it the most potent extract of all the three extracts against *Pseudomonas aeruginosa.* The three extracts had no observable inhibitory effect against *Staphylococcus aureus*.

The Minimum Inhibitory Concentration (MIC) of the extracts observed for *Pseudomonas aeruginosa* was 100 mg/ml of methanol extract, 300 mg/ml for ethyl acetate extract, and 100 mg/ml of n-hexane extract. MIC of methanol and ethyl acetate extracts was 100 mg/ml for *Escherichia coli* and *Candida albican,* and the MIC for *Klebsiella pneumonia* was 100 mg/ml of methanol and n-hexane extracts.

Methanol and ethyl acetate extracts have an inhibitory effect against *Candida albican* while the standard antibiotics had no inhibitory effect against *Candida albican* suggesting that these extracts contain certain phytochemicals that are active against *Candida albican*. Saponins which are constituents of methanol and ethyl acetate extracts from the phytochemical screening result in Table 2 above are known to be antifungal, especially against *Candida albican*. This suggests that the inhibitory effect of methanol and ethyl acetate extracts may be due to the presence of saponins in the two extracts.

The antimicrobial results of the crude extracts indicate that these extracts are sources of useful potential medicines that will help in the treatments of diseases related to the inhibited pathogens. Generally, an increase in activity with an increase in concentration was observed from the results.

Control

The antibiotics used as the positive control showed an inhibitory effect against all the microbes except for *Candida albican* which showed resistance to all the antibiotics. The highest zone of inhibition was observed against *Klebsiella pneumonia.* Cotrimoxazole (septrin) had no effect against *Staphylococcus aureus* suggesting that septrin has no effect against gram-positive strains (*Staphylococcus aureus* and *Candida albican*). This indicates that septrin has a selective spectrum of action.

Methanol, ethyl acetate, and n-hexane solvents were used as the negative control. The three solvents did not show any observable inhibition against the micro-organisms.

5. CONCLUSION

Phytochemical are essential regimens for infectious diseases caused by bacteria and fungi. More attentions are currently toward phytochemical for their effectiveness in the treatment of infectious diseases and simultaneous alleviation of many of the adverse events caused by conventional antimicrobials drugs. This research demonstrated that *Xylopia aethiopica* stem bark contains bioactive compounds that can be used in the treatment of diseases that are related to inhibited microbes.

Acknowledgment

The authors acknowledge the contribution of Prof (Mrs) Osanaiye for the supply of sample materials from Iyara.

Funding

We acknowledge Dr. Olayiwola Bello of the University of Ilorin for moral and financial support.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for experimentation. The ethical guidelines for microbial studies are followed in the study.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

REFERENCES

1. Pinner, R., Teutsch, S., Simonsen, L., Klug, L., Graber, J., Clarke, M. and Berkelman, R. (1996). Trends in infectious diseases mortality in the United States. Journal of America Medical Association 275, 189-193.
2. Fauci, A. (1998). New and re-emerging diseases: The importance of biomedical research. Emerging Infectious Diseases 4, 3.
3. Pandey, R.C. (1998). Prospecting for potentially new pharmaceuticals from natural products. Medical Research Review 18, 33-346.
4. Donning, G.H., Agyare, C. and Ennison, B. (2004). Antimicrobial activity of some medicinal plants from Ghana. Fitoterpia 75: 65-67.
5. Dan Acquaye, Marianna Smith, Wudeneh Letchamo, and Jim Simon "*Xylopia*"
6. Eric Woode *et al* (2013): Analgesic effects of an ethanol extract of the fruits of *Xylopia aethiopica* and Xylopic acid in murine models of pain.
7. Tona *et al.* (1999); Anti-amoebic and phytochemical screening of some comgotese medicinal plants. J. Ethnopharmacology., 61(1): 57-65.
8. Burkill, H, M. (1999). The useful plants of West Africa. 4th edition. Macmillan Press. 1:34-36.
9. Akin-Osanaiye B.C., Gabriel A.F., Omoniyi A.O., Ezeani S.C. (2016). Scientific Approach on the Antimicrobial Potentials of Bioactive Phytochemicals of Trema Orientalis Leaves and Stalk. European Academic Research - Vol. III, Issue 12. ISSN 2286-4822
10. Barathidasan R *et al*. (2013) "Quantitative, qualitative phytochemical analysis and in vitro antibacterial activity of *Bauhinia tomentosa L.* J. Nat. Prod. Plant Resour. 3(2): 31-36. ISBN: 2231-3184 CODEN (USA): JNPP137.
11. Jennifer Adline and Anchana Devi. (2014). "A study on phytochemical screening and antibacterial activity of *Moringa oleifera*.: International Journal of Research in Applied, Natural and Social Sciences. Vol. 2, Issue 5,169-176
12. P. Brindha *et al*. (1982) "Bull Medico-Ethnobotanical Res. 3: 84-97.
13. Cheesbrough, M., (1984). Culture Media in Medical laboratory mammal for tropical countries. Tropical Health Technology and Butterworth-Heineman Cambridge. Vol 3. 60(69): 407-428.
14. Burdon, K.L., and Williams. (1968). Microscopes and microscopic methods in microbiology. 6th ed. Macmillan company London pp: 74-76.
15. Brooks, G. F., J. S. Butel, and S. A. Morse, (2001). Antimicrobial chemotherapy in medical microbiology. 22nd ed. Appleton and large. USA, 170:222-223.
16. Usman H and Osuji J.C. (2007). Phytochemicals and in-vitro Antimicrobial assay of the leaf extract of Newbouldia leavis. African Journal of Traditional Complementary and Alternative Medicine 4(4):476-480.
17. Akin-Osanaiye C.B and Ahmad Rukayyah. (2014). Phytochemical analysis, antimicrobial screening, and anti-oxidant activity of the seed of *Trema Orientalis.*
18. Kurtikar K.F, Basu B.D. (1980). India medicinal plants 2nd Ed. Vol. 1 P.264.
19. Dharmananda, S. (2003). Gallnuts and the uses of Tannins in Chinese Medicine. In: Proceedings of Institute of Traditional Medicine, Portland, Oregon

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS