GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Phytochemical and *In vitro* Antimicrobial Activities of the Fruit Extracts of *Xylopia aethiopica* [Dun] A. Rich. (Annonaceae)

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GSC Biological and Pharmaceutical Sciences, 2023, 22(03), 082–087

Publication history: Received on 29 April 2022; revised on 05 March 2023; accepted on 08 March 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.22.3.0172

Abstract

Aim: The fruit of the plant *Xylopia aethiopica* has been used in ethno-medicine in southern Nigeria for treating dysentery, cough and bacterial infections. This present study investigates the phytochemical and antimicrobial activities of the different extracts of *X aethiopica* fruit.

Method: The powdered dried fruits of *Xylopia aethiopica* was extracted with 95% ethanol and further fractionated into *n*-hexane and ethyl acetate fractions. The clinically isolated strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* were obtained from their stock cultures and characterized using biochemical tests and then standardized with 0.5 McFarland. The antimicrobial activities of the different extracts were investigated using agar diffusion method.

Results: The *n*-hexane extract was most effective against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* with a minimum inhibitory concentration (MIC) of 6.25, 50 and 50 mg/ml respectively while the ethyl acetate extract was active against *Pseudomonas aeruginosa* and *Candida albicans* with MIC of 6.25 and 12.5 mg/ml respectively. The crude ethanol extract showed most activity against *Pseudomonas aeruginosa* with MIC of 3.125 mg/ml. The phytochemical studies on the crude extract revealed the presence of triterpenoids /steroids, carbohydrates, cardenolides and saponins.

Conclusion: The antimicrobial activities of the plant justify its usage in traditional healthcare practices especially in infections involving *Pseudomonas aeruginosa* which showed the highest sensitivity.

Keywords: *Xylopia aethiopica*; Antimicrobial study; MIC; Phytochemical

1. Introduction

While antibiotics remain the main basis for treating microbial infections, the microorganisms responsible for community or hospital-acquired infections are continually mutating and developing resistance to the best and most effective antibiotics [1,2] . The resultant burden of microbial drug resistance includes loss of finance, prolonged hospitalization due to treatment failures, increased risk of chronic diseases (cancer and diabetes etc.), decreased productivity and increased mortality rate [3]. The effect of multidrug- resistance by microorganisms and the emergence of new infectious disease agents are worsened by the low number of promising compounds in the product development pipeline. There is therefore urgent need for new and active antimicrobial compounds with significant activity against antibiotic resistance or as complementing antibiotics against the disease [4–6]. Plant derived compounds because of their chemical diversity continues to play an important role both in treatment and prevention of infections [7,8]. These

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phytochemicals are synthesized in the secondary metabolism of the plant and includes tannins, alkaloids, terpenoids, phenolic compounds and glycosides.

The plant *Xylopia aethiopica* (Annonaceae) is a medicinal plant widely distributed in most tropical countries particularly in West Africa [9]. It is known by different names such as 'Uda' (Igbo –Southern Nigeria), Negro pepper, Ethiopian pepper and West African pepper. In nutrition it is used as a spice and blood replenishment in women after child delivery. While in ethno-medicine, it is widely used for colic, toothache, rheumatism and cough. The fruit and stem bark are used in the treatment of bronchitis, dysentery, boils and sores. Previous studies using essential oil from different parts of the plant showed different degrees of antimicrobial activities [10]. Previous investigation on the fruit extract in combination with different antibiotics showed different levels of activity against bacteria ranging from indifference, antagonism to synergism [11]. Hence in the present investigation we report on the phytochemical and antimicrobial properties of the different fruit extracts of *Xylopia aethiopica*.

2. Material and methods

Fresh fruits of *Xylopia aethiopica* were bought from a market in Choba, Rivers State, Nigeria and the identity of the fruits was authenticated by the taxonomical section of the Plant Science and Botany department, University of Port Harcourt, Nigeria. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, University of Port Harcourt, Nigeria under a voucher number UPH/C/131.

The Fresh fruits were air dried and then powdered using a Corona hand mill. The powder (350 g) was macerated with 95% ethanol (2 L) for 72 h with intermittent shaking at room temperature (25^{o}C). The sample was then filtered and concentrated *in vacuo* using a rotary evaporator, to achieve a reduced volume. The sample was dried over a water bath set at 39^{o}C to obtain a crude semisolid extract 56.40 g. This was kept in a freezer in an air-tight container.

The crude ethanolic extract (50.00 g) obtained above was suspended in 100 mL of sterile pure water and partitioned sequentially with *n*-hexane (500 ml× 5) and ethyl acetate (500 ml × 5) using a separating funnel to give *n*-hexane fraction (9.32 g) and ethyl acetate fraction.

2.1. Antimicrobial Studies

Pure clinical slants of four organisms namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* were obtained from Divic medical Laboratory, Rumosi, Rivers State. The identity of the organisms were first ascertained by subculturing into a fresh nutrient media and were maintained on nutrient slants and preserved at 4 - 5^{o}C before screening for antimicrobial activity.

2.2. Purification of test Organisms

The identities of the organisms were confirmed by subculturing unto fresh nutrient media.

A sterile wire loop was used to collect a little of each stock and the organism was streaked on sterile nutrient agar plates. Subsequent culturing into their selective media for the different organism was carried out in following manner. The organism, *Staphylococcus aureus* was sub- cultured into a mannitol salt agar, *Escherichia coli* into MacConkey agar, *Pseudomonas aeruginosa* into Cetrimide and *Candida albicans* into a Sabouraud dextrose agar and incubated at 37^{o}C for 24 h. All culture media were prepared according to standard methods.

2.3. Biochemical tests

The physical properties (color, smell and texture) of the microbial growth were noted. Also, conventional biochemical tests (catalase, oxidase, coagulase, Indole, citrate and Nitrate tests) and gram stain were employed to identify, confirm and differentiate between the organisms.

2.4. Standardization of Microbial Isolates

Each colony of organism was transferred using a sterilized wire loop into a 4 ml of peptone water. The inoculum suspension was incubated at 35^{o}C until the turbidity (microbial population) of 0.5 McFarland standard was achieved which is approximately 1.0 × 10^{8} cfu/ml.

2.5. Preparation of drug stock solution

Stock solutions of ciprofloxacin (antibacterial), fluconazole and nystatin (antifungal agents) were prepared on each occasion by weighing and subsequent dissolution in a calculated volume of dimethyl sulfoxide (DMSO) to get the desired concentration. Aseptic conditions were maintained in each case. DMSO was also used as a negative control.

2.6. Sensitivity testing of the plant extracts and standard antibiotics on the clinical isolates

Susceptibility of the microbial clinical strains to plant extract and the standard antibiotics were assayed using agar diffusion method. The plant extracts were reconstituted in DMSO to obtain an initial starting concentration of 100 mg/ml and then diluted further to 10 mg/ml. A 0.1 ml volume of the standard culture of organism was seeded into sterile Mueller Hinton agar and Sabouraud dextrose for bacteria and fungi respectively. These were mixed uniformly and made to stand at room temperature for 3 h and then poured into a sterile petri dish which was then allowed to solidify. Using a sterile cork borer of 6 mm diameter wells were made on the seeded plates and these were filled separately with plant extract and antibiotics of known concentration. The plates were incubated at 35^{o}C for 24 h and the zones of inhibition measured.

2.7. Determination of the minimum inhibitory concentration (MIC) of the plant extracts

A two-fold serial dilution using dimethyl sulfoxide (DMSO) as the solvent of dilution was done on the plant extract (100 mg/ml). Lower concentrations were obtained by transferring 2 ml of plant extract solution into 2 ml of DMSO to give 50, 25, 12.5, 6.25 and 3.125 mg/ml. The method used for antimicrobial determination (MIC) was the agar diffusion method. The Mueller Hinton agar (MHA) plates (for bacteria) and Sabouraud dextrose agar plate (for fungi) were prepared by pouring 20 ml of molten media (already seeded with 0.1 ml of standardized inoculum) into sterile plates. The plates were allowed to solidify for 5 minutes. Using a sterile cork borer of 6 mm diameter wells were made on the seeded plates and these were filled separately with 50 µl of plant extract, positive control (ciprofloxacin for bacteria and fluconazole for fungi) and DMSO as the negative/solvent control. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 24 h at 37^{o}C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the tested compounds. The zones of inhibition measured in mm.

2.8. Phytochemical screening of the plant extracts

Standard phytochemical screening procedures [12] was carried out on the crude extract of the plant.

3. Results

3.1. The biochemical tests result

The bacterial samples were identified and classified morphologically and biochemically into their species using biochemical and Gram-stain tests (Table 1). The biochemical tests are used to confirm the purity and identity of the isolated organisms. The microorganisms chosen for the study were common causative agents responsible for some common bacterial and fungal Infections.

Table 1 Identification of bacterial species using biochemical tests

Name of Test	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Catalase	+	+	+
Indole	+	-	-
Oxidase	-	-	+
Nitrate	-	+	-
Coagulase	-	-	-
Gram - stain	-	+	-
Shape of organism	Rod	Cocci	Rods

Key: + = Positive, - = not detected

3.2. Antimicrobial Sensitivity of *Xylopia aethiopica* fruit extracts

The sensitivity pattern of the bacterial and fungal isolates to the different fruit extracts (100 mg/ml) were compared with that of Ciprofloxacin (1 mg/ml) which is a standard antibiotic and Nystatin, which is a standard antifungal agent (Table 2). The sensitivity tests showed that the standard drug ciprofloxacin (1mg/ml) was very sensitive to both Gram-positive and negative bacteria and this accounts for its use as a broad-spectrum antibiotic. The crude ethanol extract, ethyl acetate and n-hexane fractions at 100 mg respectively showed antimicrobial activity against the entire microorganism including the fungus *Candida albicans*. This is evidenced by the zone of inhibition diameter observed with the different fractions of *Xylopia aethiopica*.

Table 2 The sensitivity pattern of *Xylopia aethiopica* against test bacterial isolates

Bacterial and fungi Isolates	**Zones of Inhibition (mm)**				
	***Xylopia aethiopica* (100 mg/ml)**				
	***n*-Hexane fraction**	**Ethyl acetate fraction**	**Ethanol (crude) fraction**	**Ciprofloxacin (1mg/ml)**	**Nystatin (25mg/ml)**
Staphylococcus aureus	12 ± 0.71	11 ± 1.40	11 ± 0.56	18.5 ± 0.02	-
Escherichia coli	9 ± 0.84	11 ± 0.71	12 ± 1.00	25 ± 1.00	-
Pseudomonas aeruginosa	11 ± 1.20	12 ± 1.58	11 ± 0.89	>30 ± 0.00	-
Candida albicans	9 ± 0.00	8 ± 1.00	10.5 ± 0.00	-	14 ± 0.30

3.3. The Minimum Inhibitory Concentrations (MIC) of the *Xylopia aethiopica* fruit extracts

The minimum inhibitory concentrations (MIC) in mg/mL of the fruit extracts against the microorganisms are shown in Table 3. The MIC refers to the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism. The crude ethanol extract has more antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* while it has least activity against *E. coli*. The ethyl acetate fraction has more activity against *Pseudomonas aeruginosa* and the fungus *Candida albicans*. The MIC result of 100 mg/ml of the different extracts against *E. coli* shows poor activity this organism.

Table 3 The minimum inhibitory concentrations (MIC) of the *Xylopia aethiopica* fruit extracts

Bacterial and fungi Isolates	**Minimum Inhibitory concentration (mg/mL)**		
	***Xylopia aethiopica* fruit extracts**		
	***n*-Hexane fraction**	**Ethyl acetate fraction**	**Ethanol (crude) fraction**
Staphylococcus aureus	50	-	50
Escherichia coli	100	100	100
Pseudomonas aeruginosa	6.25	6.25	3.125
Candida albicans	50	12.5	-

3.4. Phytochemical Screening of *Xylopia aethiopica* fruit extracts

The phytochemical screening result of the *Xylopia aethiopica* fruit extracts is shown in Table 4. The fruit extracts show a rich presence of steroids, tannins, glycosides and reducing sugar.

Table 4 Phytochemical screening of *Xylopia aethiopica* fruit extracts

Phytochemical test	*n*-Hexane fraction	Ethyl acetate fraction	Ethanol (crude) fraction
Alkaloids	±	±	±
Steroids/Triterpenoids	+	+	+
Saponins	±	±	+
Tannins	-	-	-
Flavonoids	-	-	-
Glycosides	+	+	+
Reducing sugar	+	+	+
Anthraquinones	-	-	-

Key: ± = Trace, + = present, - = absent

4. Discussion

The search for novel antimicrobial compounds of ethno-medicinal origin is of significant importance due to the problems of microbial resistance, high cost of medicine and side effects encountered using some of the existing antibiotics. The use of traditional medicine for healthcare is highly practiced among the rural dwellers, hence it is important to justify the use of some of these herbs as medicine [13].The antimicrobial activities of the plant *Xylopia aethiopica* (Annonaceae) fruit extract was investigated using three human bacterial isolates (*Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*) and a fungus *Candida albicans.* The antimicrobial effects expressed may be due to the presence of bioactive phytochemicals or secondary metabolite such as tannins, steroids, glycosides and saponins [14]. The presence of these phytochemicals in *Xylopia aethiopica* were also confirmed in the findings of John-Dewole *et al*., [15]. Their research work also confirmed the antibacterial activity of *Xylopia aethiopica* fruit extract against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* These secondary metabolites from plants has been a source of chemical diversity and lead compounds useful in drug development [16]. The antifungal activity of the ethanol extract of *Xylopia aethiopica* fruit was also reported by Kanife *et al*., [17].

5. Conclusion

Based on the results emanating from this study, the fruit extract of *Xylopia aethiopica* has antimicrobial activity due to the presence of phytochemical compounds which can be isolated and characterized as source of lead compound.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the Laboratory staff of Pharmaceutical microbiology, Faculty of Pharmaceutical Sciences and Plant Science and Botany Department of University of Port Harcourt, Rivers State, Nigeria.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper

References

[1] Davies J and, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010, 74(3):417–33.

[2] Blair JM, Webber MA, Baylay AJ, Ogbolu DO, J PL. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015, 13(1):42–51.

[3] Chandy SJ, Naik GS, Balaji V, Jeyaseelan V, Thomas K, Lundborg CS. High cost burden and health consequences of antibiotic resistance: the price to pay. J Infect Dev Ctries. 2014, 8(9):1096–102.

[4] Alsheikh HM Al, Sultan I, Kumar V, Rather IA, Al-sheikh H, Jan AT, et al. Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. Antibiotics. 2020, 9(8):1–23.

[5] Giamarellou H. "Multidrug-resistant Gram-negative bacteria: how to treat and for how long." Int J Antimicrob Agents. 2010, 36:S50–4.

[6] Murphy Cowan M. Plant Products as Antimicrobial agents. Clin Microbiol Rev. 1999, 12(4):564–82.

[7] Manandhar S, Luitel S, Dahal R. In Vitro Antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med. 2019, 2019.

[8] Chinaka CN, Omotoso AE, Obinwugo EC. *In vitro* antibacterial effects of different solvent extracts of the leaves of Nicotiana tabacum Linn (Solanaceae) on clinical isolates from otitis media patients. Eur J Adv Res Biol Life Sci. 2018, 6(1).

[9] Abarikwu SO, Ogunlaja A, Otuechere CA, Gideon O. Effect of ethanolic extract from seeds or pods of Xylopia aethiopica (dunal) a. rich (annonaceae) on the testicular function of adult male rats. Indian J Clin Biochem. 2017, 32(4):420–8.

[10] Fleischer T, Mensah MLK, Mensah AY, Komlaga G, Gbedema SY, Skaltsa H. Antimicrobial Activity Of Essential Oils Of Xylopia aethiopica. African J Tradit Complement Altern Med. 2008, 5(4):391–3.

[11] Ilusanya OAF, Odunbaku OA, Adesetan TO, Amosun OT. Antimicrobial activity of fruit extracts of Xylopia aethiopica and its combination with antibiotics against clinical bacterial pathogens. J Biol Agric Healthc. 2012, 2(9):1–9.

[12] Sofowora AO. Medicinal plants and traditional medicine in Africa. 2nd Editio. Ibadan, Nigeria: University of Ife Press., 1993. 320 p.

[13] Ginovyan M, Petrosyan M and, Trchounian A. Screening of some plant materials used in Armenian traditional medicine for their antimicrobial activity. BMC Complement Altern Med [Internet]. 2017, (1750):1–9. Available from: http://dx.doi.org/10.1186/s12906-017-1573-y

[14] Mandal SM, Roy A, Ghosh AK, Hazra TK, Basak A. Challenges and future prospects of antibiotic therapy : from peptides to phages utilization. Front Pharmacol. 2014, 5(May):1–12.

[15] John-Dewole O., Agunbiade S., Alao O, Arojojoye OA. Phytochemical and antimicrobial studies of extract of the fruit of Xylopia aethiopica for medicinal importance. J Biotechnol Pharm Res. 2012, 3(6):118–22.

[16] Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, et al. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. J Ethno- Pharmacol. 2005, 97:421–7.

[17] Kanife UC, Doherty F, Nwakanma NM, Adamu GO. Antifungal Activity of Xylopia aethiopica on Some Clinical Organisms in Nigeria. Hamdard Med. 2012, 55(1):14–7.