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Phytochemical and in-vitro antibacterial activity of crude extracts of Xylopia aethiopica fruits (Dunal) A Rich (Annonaceae)

M. K. Bello*[1] and L. Lajide[2]

[1]Department of Pharmacology &Therapeutics, University of Ilorin, Ilorin, Kwara State, Nigeria
[2]Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria

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ABSTRACT: Phytochemical screening of the aqueous and methanolic extracts of the fruit of *Xylopia aethiopica* (Annonaceae) confirmed the presence of saponin ,cardiac glycosides, tannin and volatile oil. Spectrophotometric analysis of the fruit of *X.aethiopica* for trace metals, phosphorus and sulphur showed the plant contained Mn (0.037±0.002mg/100g), Zn (1.020 ± 0.001mg/100g), ,Cu (0.274±0.004mg/100g), Ni (1.099 ±0.001mg/100g), Fe (0.690 ±0.02mg/100g), P (30.62±0.002mg/100g) and S (100.50±0.51mg/100g). Antibacterial activity of the crude aqueous, methanolic and petroleum-ether extracts were evaluated in-vitro against pathogenic bacteria (*Staphylococcus aureus, Esherichia coli and Pseudomonas aeruginosa)* using Agar diffusion method.

The aqueous and Petroleum-ether extract showed significant growth inhibitory effects on *Staphylococcus aureus and Esherichia coli;* with petroleum-ether extract being more active.
Esherichia coli was more susceptible to petroleum-ether extract with zone of inhibition diameter of 20mm, than Staphylococcus aureus 12mm.*Pseudomonas aeruginosa* was however resistant to all the plant extracts.

The minimum inhibitory concentration (MIC) of methanolic crude extract of *Xylopia aethiopica* fruits on *Staphylococcus aureus* and *Esherichia coli* were 12.50 and 6.25mg/ml respectively.

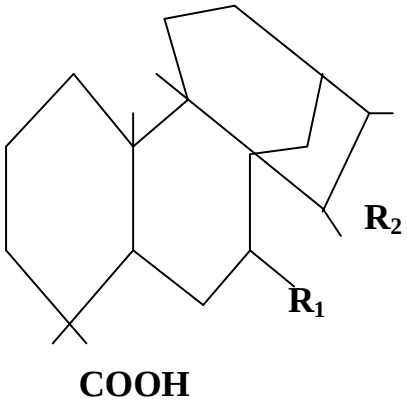
Keywords: *Xylopia aethoipica*, Agar diffusion method, Antibacterial activity, Spectrophotometric analysis.

Introduction

Xylopia aethiopica (Dunal) A Rich (Annonaceae) known as Eeru in Yoruba land, Nigeria, is a deciduous tree found in lowland, rainforest, costal brackish and fringing forest of Savannah zones. (Burkil 1971; Keay 1969). The deciduous tree popularly known as African pepper has reddish fruit containing 4-9 seeds, and used ethno-botanically as medicinal plants in the rural communities of Africa for the treatment of Broncho-pneumonia, dysentery, cancer and ulcer Sofowora. (1982), Lajide et al (1995).

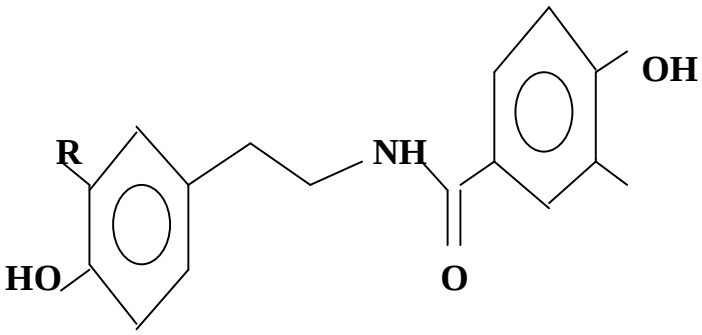
The active compounds isolated from hexane extract of the fruits of *X .aethiopica* with insect antifeediant are : 6-ent kaurene diterpenes, phenolic amides and lignamides, (Fig 1 and 2.) Lajide *et al*. (1992, 1995).

*To whom correspondence should be addressed.
Tel. No: 08035858083. E-mail address: mkaybello@yahoo.com



R_1 R_2
1= H, OAC
2 =H, H

Figure 1 Kaurene diterpenes form *Xylopia aethiopica*. Termite antifeedant



R_1
1= OCH_3
2= OH

Figure 2: Phenolic amides from *X. aethiopica*

Plate 1:Dried fruits of *Xylopia aethiopica*

Most rural communities in Nigeria also utilize the dried fruits of *X. aethiopica* in their herbal preparation (Plate 1). The efficacy of the plant, as used by the traditional medicine practitioners, has no scientific justification, because oral account of their use and efficacy were passed from generation to generation. Bubayaro (1986)

Ekong (1986) was of the view that mere isolation and elucidation of chemical structures of plant extracts may not be too significant, until appropriate bioassays are carried out to establish the **biological activity exhibited by the plant extracts**. Thus, the study investigated and reports on the phytochemical and antimicrobial activity of crude extracts of *Xylopia aethiopica.*

Material and Methods

Collection and Treatment of plant Material

Dried *Xylopia aethiopica* fruits was bought at different selling points, from traditional herbs seller at new market Baboko, Ilorin, Kwara State, Nigeria. The fruits of the plants were identified by Professor L. Lajide of Federal University of Technology, Akure, Ondo State and further authenticated by Mr. Jide Adeoye of Botany Department University of Ilorin, Kwara State. The fruits were air-dried at $32^oC \pm 2^oC$ for two weeks on a clean pavement. The dried bulk samples of the fruits were pulverized using a wooden pestle and mortar and sieved through a wire mesh to obtain powdered sample with particle size of $2mm^2$ .The powdered sample were mixed and quartered to obtain a representative sample used for the experimental work.

Plant Extraction

Fifty grams of powdered fruits of *Xylopia aethiopica* was weighed into the thimble of the Soxhlet continuously extracted using the soxhlet method with redistilled aqueous, methanol and petroleum-ether. The extracts were concentrated by gentle evaporation on a water bath.

Phytochemical Screening.

Qualitative and quantitative phytochemical screening of the extract of *Xylopia aethopica* was carried by methods described by Trease and Evans (1989) and Sofowora (1982)

Spectrophotometric Analysis:

Trace metal analysis was carried-out by method described by Howtz (1980) using Atomic absorption spectrophotometer model A 200. Spectrophotometric determination of phosphorus and sulphur were carried-out by vanadomolybdate and turbidometric methods respectively, using Camspect Digital Spectrophotmetric. Howtz (1980).Calibration curves for phosphorus and sulphur were prepared and reading converted to mg/100g

Screening of Bacterial Isolate

The test organisms used to screen for the antimicrobial activity of the extracts of *X.aethiopica* were pure clinical isolates of *Staphylococcus aureus, Esherichia coli, and Pseudomonas aeruginosa*. The test organisms were isolated and identified by Mr.Afolabi A. A. of the Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Kwara State.

Dilution of Extracts and Antibiotic

Fifty milligrams of the petroleum-ether extract was dissolved in 2ml of sterile Tween 20.While 50mg of the aqueous and methanolic extracts of *X.aethiopica* were dissolved in 2ml of sterile water to give a concentration of 25mg/ml

250mg of Ampicillin trihydrate and Tetracycline hydrochloride were dissolved in 10ml of sterile distilled water to give a final concentration of 25mg/ml. The dissolved portion of petroleum ether extract was kept at thermostated temperature of 40^{o}C in a water-bath. The extracts and the antibiotic solution were diluted to give 25mg/ml, 12.2mg/ml, 6.25mg/ml, 3.13mg/ml and 1.56mg/ml in a nutrient broth (Biotec), for the determination of Minimum Inhibitory concentration (MIC).

Determination of Antibacterial Activity of the crude Extracts

Agar diffusion and tube dilution methods described WHO (1991) and Oyeleke et al (2008) were used for this study. A loopful of the test organism was inoculated on nutrient broth and incubated for 24h. 0.2ml from the 24h broth culture of the test organism was dispensed in 20ml of sterile nutrient broth (1:100 dilution) and incubated for 3-5 h to standardize the culture to 10^{6} cfu/ml. Sterile swab sticks (sterilin) soaked in the inocula. was used to inoculate the surface of the prepared nutrient agar and labelled appropriately.

A Sterile cork borer (5mm) was used to make holes on each of the inoculated plates. These holes were filled with different concentrations of the extracts and incubated at 37^{o}C for 24 h.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the bioactive plant extracts was determined by tube dilution technique.WHO(1991).

Results

Table 1: Phytochemicals present in crude extract of *Xylopia aethiopica*

Secondary Metabolites	*X. aethiopica*
Saponin	**positive**
Cardiac glycoside	**positive**
Alkaloids	**positive**
Tannin	**negative**
Volatile Oil	**positive**
Balsam	**positive**

Table 2: Trace metal content of *Xylopia aethiopica.*

Elements	**Concentration mg/100g**
Mn	**0.37 ±0.002**
Zn	**1.20±0.001**
Cu	**0.27±0.004**
Co	**Nd**
Cd	**Nd**
Ni	**1.1±0.001**
Fe	**0.65±0.001**
Pb	**0.07±0.001**

*The values represent Mean ± SD (N =3)
Nd = Not Detectable

Table 3: Phosphorus and Sulphur content of *Xylopia aethiopica*

Element	**Concentration mg/100g**
Phosphorus	**30.62±0.02**
Sulphur	**100.50±0.51**

The values represent Mean ±SD (N=3)

Table 4: Antimicrobial activity of *Xylopia aethiopica* extract

PATHOGENS	ZONE DIAMETER (mm) OF GROWTH INHIBITION				
	Aqueous	Methanol	Pet.Ether	Ampicilline Control	Tetracycline Control
Staphylococcus aureus	10	0	12	22	26
Pseudomonas aeruginosa	0	0	0	0	0
Escherichia coli	0	0	20	26	27

Table 5 :The Minimum Inhibitory Concentrations of *Xylopia aethiopica on S.aureus and E.coli*

STAPHYLOCOCCUS AUREUS			***ESCHERICHIA COLI***		
Conc. in mg/ml	**Growth**	**MIC in mg/ml**	**Conc. in mg/ml**	**Growth**	**MIC mg/ml**
25.00	**-ve**	**-**	**25.00**	**-ve**	**-**
12.50	**-ve**	**12.50**	**12.50**	**-ve**	**-**
6.25	**+ve**	**-**	**6.25**	**-ve**	**6.25**
3.13	**+ve**	**-**	**3.13**	**+ve**	**-**
1.56	**+ve**	**-**	**1.56**	**+ve**	**-**

Key-: -ve : No growth,+ve : Positive growth

Discussion

The results of the phytochemical screening of *Xylopia aethiopica* (Table 1) showed the presence of saponin, cardiac glycoside, alkaloids ,tannin, volatile oil and basalm. Secondary metabolites from plants have some bioactivity and potential medicinal values. Lajide *et al* (1995) and Lajide et al (1992) isolated some bioactive compounds namely kaurene diterpenes, phenolic amides and lignamides from *Xylopia aethiopica*.. These compounds have bioactivity as anti-feedant on subterranean termites, *Reticulitermes speratus.*

Trace elements are required in small quantity in diet to serve various purposes in human physiology and metabolism. Tables 2 and 3 showed the trace metal, Phosphorus and Sulphur contents of the fruits *of Xylopia aethiopica*. These elements in small quantity serve as co-factor in the synthesis and metabolism of body enzymes, haemoglobin, vitamin B_{12} and thyroxin;while their deficiencies could lead to diseases and death. Kar and Kar (2002) observed that inorganic content of medicinal plants containing mineral elements have contributory roles in enhancing their medicinal properties. The concentration of the phytochemicals in the fruit *X. aethiopica* showed Suiphur > Zinc> Iron > Manganese >Copper.

The high concentration of sulphur (100.50±0.51mg/100g) in the fruit of *Xylopia aethiopica* could be responsible for its antimicrobial/medicinal properties. Sulphur containing compounds are known for their antimicrobial effects. The clinical effectiveness of sulphur containing antibiotics e.g. sulphanilamides, sulphathizole and sulphadiazines in control of bacterial infection is based on their interference with synthesis of folic acid, by utilization of para-amino-benzoic acid (PABA) necessary for the synthesis of trihydrifolic acid (Groth1974 and Stenlake 1979).

Table 4 gives the inhibitory effect of the fruit extract of *X. aethiopica* at 15mg/ml. *Staphylococcus aureus and Esherichoia. coil* were sensitive to petroleum-ether extract of *X.aethiopica*, with zones diameter of inhibition of 12mm and 20mm, compared to that of the antibiotic controls. *Pseudomonas aeruginosa* was resistant to all the plant extract.

Kaurene diterpernes, Phenolic amides and lignamides isolated from *Xylopia . aethiopica,* by Lajide et al (1995) with bioactivity as anti-feedant on subterranean termites, *Reticulitermes speratus* may also be responsible for the observed antimicrobial activity of this extract against *Staphylococcus aureus* and *Esherichia coli.* ,Lajide et al (!992),Lajide *et al* (1993

The minimum inhibitory concentration (MIC) of the plant extract on the pathogens is shown in table 5. The MIC of *X.aethiopica* on *Staphylococcus aureus* was 12.50mg/ml; while that of *Escherichia coli* was 6.25mg/ml. The minimum Bactericidal concentration (MBC) of petroleum ether extract of *X.aethiopica* on the pathogens also ranged from 12.50mg/ml to 25.00mg/ml

Phytochemical analysis and in-vitro antimicrobial assay of crude extracts of *X.aethiopica* showed that the medicinal plant has bioactivity and pharmacological effects on clinical isolates of *Staphylococcus aureus and Esherichia coli* .This justify the ethnobotanical and pharmcological uses of this plant in herbal medicine.

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