

Antimicrobial activities and phytochemical properties of *Annona muricata* leaf

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Abstract: *Annona muricata* is a well-known economic and traditional plant of Nigeria. The study investigated the properties of constituents and antimicrobial activities of extracts of the leaf of *A. muricata*. The extracts were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* by the agar well diffusion method at concentration of 20 mg/mL. The methanol (AM2) and ethyl acetate (AM4) extracts were characterized using UV and IR spectroscopy. The morphological structure of the *A. muricata* leaf was observed at a magnification of 20,000X using SEM and then subjected to EDX analysis. The results of the phytochemical screening revealed the presence of phenolic compounds, flavonoids, tannins, alkaloids, saponins and cardiac glycosides in the extracts. Anthraquinone was found absent. The ethyl acetate extract was found highly active against gram positive bacteria, *S. aureus* (ZI of 42 mm; AI = 1.31) and ampicillin resistant gram negative *P. aeruginosa* (ZI of 34 mm; AI = 1.13). The IR spectra of AM2 and AM4 extracts of *A. muricata* showed peaks at a frequency of 3377 cm⁻¹ to 3440 cm⁻¹ indicating the presence of a phenolic OH stretch. This supports the phenolics detected chemically. The UV spectrum of methanol extract showed peaks that are typical of flavonoids and phenolics. The SEM revealed the sizes of the leaf particles as inhomogeneous. EDX results showed high oxygen concentration

of 73.5 % and carbon concentration of 26.5 %. The findings proved the anti-infective potential of *Annona muricata* leaf and established physico-chemical markers for the active extracts.

Keywords: *Annona muricata*, phytochemical, antimicrobial, infections

Introduction

Annona muricata belongs to the family of Annonaceae. It is reputed for its edible fruit that is commonly called soursop [1]. The fruit has slight acidic taste when ripe. *Annona muricata* is used in traditional medicine in many regions. The fresh leaves when crushed are applied on skin eruption for quick healing. A poultice of young *A. muricata* leaf is applied on the skin to alleviate rheumatism and other skins like eczema. When applied during the healing of wounds it results in less or no skin scars [2]. The decoction can also be used as wet compress on swollen feet and other inflammations. The juice of the fruits is taken orally as herb remedy of arthritis, haematuria and liver ailments [3]. The leaf tea is used for catarrh in the Peruvian Andes. The seed extracts are used to kill external parasites, head lice, and worms [4, 5]. In the Peruvian Amazon, the bark, root and leaves are used for the treatment of diabetes as well as sedative and antispasmodic drugs. The leaf or bark tea or combination of both is used as a sedative and heart tonic by the indigenes of Guyana. In the Brazilian Amazon, a leaf tea is used for liver problems and the oil of the leaves and unripe fruit is mixed with olive oil and used externally for neuralgia, rheumatism, and arthritic pain [3].

It was reported [6] that *A. muricata* crude extract samples exhibited different level of cytotoxicity toward breast cancer cell lines. The selected B1 AMCE reduced the tumor's size and weight, showed anti-metastatic features, and induced apoptosis in vitro

and in vivo of the 4 T1 cells. Furthermore, it decreased the level of nitric oxide and malondialdehyde in tumor while also increased the level of white blood cell, T-cell, and natural killer cell population. In another work, *A. muricata* was investigated and reported [7] that a chloroform/methanol (1:1) leaf extract showed a 67 % inhibition of *Plasmodium falciparum* F32 *in vitro* at 20 µg/mL.

The present study evaluated the antimicrobial potential, phytochemical constituents and the physico-chemical characteristics of the leaf extracts of *A. muricata*.

Materials and Methods

The leaf of *Annona muricata* was collected from Ota, Nigeria. The plant was identified at the herbarium in the Biological Department, Covenant University, Ota, Nigeria. The plant leaf was air-dried for two weeks then crushed and stored in a clean container at room temperature for further analysis.

Extraction: The leaf was defatted then extracted with methanol on cold maceration. Extract was strained and filtered over anhydrous sodium sulphate and then evaporated in *vacuo* at 45° C. The crude extract was partitioned into chloroform, ethyl acetate and aqueous layers successively using a separating funnel. The concentrated fractions were dried in desiccator and kept for further analysis.

Phytochemical screening and analyses: The dried sample of *A. muricata* (AM) leaf was extracted with 50:50 methanol-water mixture and the leaf extract were tested for tannins,

phenolics, anthraquinones, flavonoids, alkaloids, saponins and cardiac glycosides according to the modified standard procedures [8]. The UV-Visible spectra were obtained in MeOH on a Genesys 10 UV spectrophotometer while FTIR measurement was run on Perkin-Elmer 2 spectrometer. Scanning electron microscope was run at a magnification of 20 μm to observe clear image of the plant leaf particles in colloid well dispersed with uniform size. The elemental concentration of the plant species was determined by EDX.

Antimicrobial test assay: Antimicrobial activity was tested against the following clinical strains of micro-organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. The modified standard procedures [9, 10] were adopted for the assay.

Antibacterial activity assay: Antibacterial activity of the extracts was determined by well diffusion method on nutrient agar medium. Nutrient agar medium is poured in to the petri-plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. A standard cork borer of 9 mm diameter was used to bore a well and equal dose (20 mg/mL) of each of the test samples was transferred into the well with needle syringe. Gentamicin is taken as positive control. The plates were incubated at 37 $^{\circ}\text{C}$ for 24 hrs. Then antibacterial activity

was determined by measuring the diameter of zone of inhibition and Activity Index was calculated.

Antifungal activity assay: Antifungal activity of the extracts was determined by well diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. And 0.2 ml of the test sample was introduced into the well at a concentration of 20 mg/mL. The plates were incubated at room temperature of 25 $^{\circ}\text{C}$ for 48 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibition and Activity Index was calculated.

Results and Discussion

Phytochemical Screening

The results of the phytochemical screening (Table 1) revealed the presence of phenolic compounds, flavonoids, tannins, alkaloids, saponins and cardiac glycosides as secondary metabolites in the leaf extract of *Annona muricata*. However, Anthraquinone was found absent in the leaf sample. The previous works [3, 11] agree with the findings of this study. Various reports have indicated tannins, flavonoids and phenolics as free-radical scavengers possessing anti-oxidative properties, antimicrobial properties as well as strong anti-cancer properties [12-14].

Table1. Classes of secondary metabolites in *Annona muricata* leaf

Constituent	Intensity
Phenols	+++
Flavonoids	++
Tannins	+++
Alkaloids	+
Cardiac glycosides	++
Saponins	+
Saponin glycosides	+
Anthraquinones	-

Keys: (+++) Intense, (++) Moderate, (+) Mild, (-) Absent.

UV-Visible spectral analysis

The UV-Vis spectrum of methanol extract showed peaks at 280, 325 and 664 nm which are typical of flavonoids and phenolics. The peak at 325 nm

disappeared in the spectrum of ethyl acetate extract which suggests that the metabolite in that region is likely more polar and hydroxylated.

TEST SETUP
GENESYS 10S v1.200 2L7J355002

Scanning 15:11 24Mar16
Test Name MAM
Measurement Mode Absorbance
Start Wavelength 190nm
Stop Wavelength 800nm
Sample Positioner 1-Cell Platform
Scan Speed Fast
ID# (0=OFF) Off
Auto Print Off

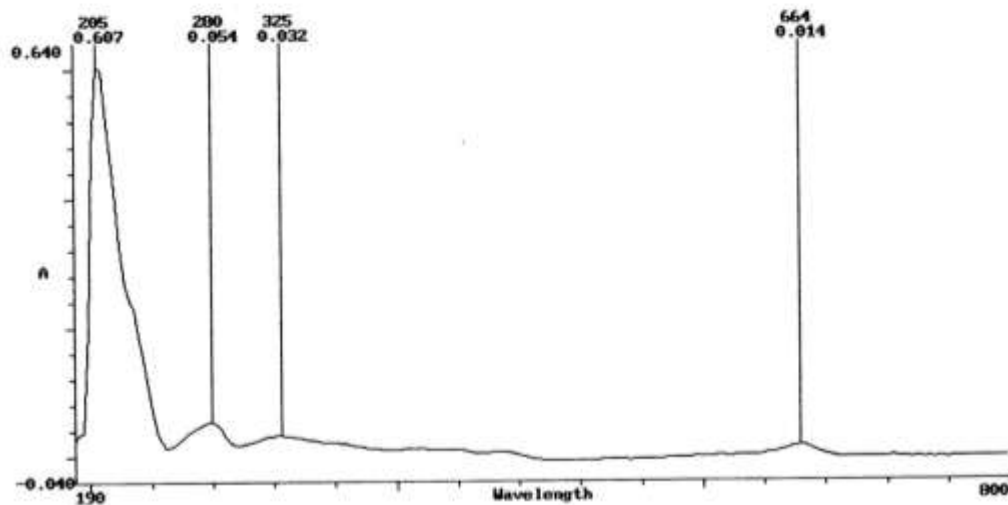


Figure 1. UV spectrum of the methanol extract of *Annona muricata*

3.3. Fourier transform infra-red spectrum: FTIR measurement was

carried out for the identification of functional groups (Table 2). The IR

spectra of the ethyl acetate extract and the methanol extract of *A. muricata* showed peaks at a frequency of 3377 cm^{-1} to 3500 cm^{-1} which indicated a broad hydroxyl group due to hydrogen bonding molecules. The broad OH strongly overlapped the $\text{C}=\text{C}-\text{H}_{\text{str}}$ ($3100\text{--}3000\text{ cm}^{-1}$) in MeOH extract spectrum (Figure 2) but little hump showed in EtoAC extract spectrum (Figure 3) due to reduction in hydrogen bonding effect. Also the presence of peaks at frequency of 1612 cm^{-1} – 1450 cm^{-1} by the extracts indicated the presence of an aromatic ring $\text{C}=\text{C}=\text{C}_{\text{str}}$. The two functional groups pool together suggest the possibility of the presence of a phenolic compound. The data support the presence of phenolics detected

chemically. Phenolic compounds are known to possess good antioxidant property [15]. In the study carried out by Muthu and Durairaj [5], they reported that the hydroalcoholic extract of *Annona muricata* leaves had total antioxidant capacity (TAC) of IC_{50} value of $44.2474\text{ }\mu\text{g/mL}$. Radical scavenging potentials against superoxide, nitric oxide, hydroxyl, and hydrogen peroxide were found to be effective with IC_{50} values $59.05 + 0.103$, $70.12 + 0.023$, $134.21 + 0.063$ and $43.4 + 0.102\text{ }\mu\text{g/mL}$ respectively. The findings suggest *Annona muricata* leaves to be good source of antioxidants and radical scavenging agent suitable for prevention of human diseases caused by oxidative stress.

Table 2. IR spectral values of *Annona muricata* extracts

Sample	ν (in solid state, cm^{-1})
AM2 (MeOH Extract)	3400 (OH_{str} Broad, phenol), $\text{C}=\text{C}-\text{H}_{\text{str}}$ (strongly obscured by the broad OH overlapped), 2924, 2854 ($\text{C}-\text{H}_{\text{str}}$ aliphatic), 1738 ($\text{C}=\text{O}_{\text{str}}$), 1612, 1516, 1447 ($\text{C}-\text{C}=\text{C}_{\text{str}}$)
AM4 (EtOAC fraction)	3400 (OH_{str} Broad, phenol), 2923, 2854 ($\text{C}-\text{H}_{\text{str}}$ aliphatic), 1731 ($\text{C}=\text{O}_{\text{str}}$), 1608, 1514, 1450 ($\text{C}-\text{C}=\text{C}_{\text{str}}$ aromatic ring)

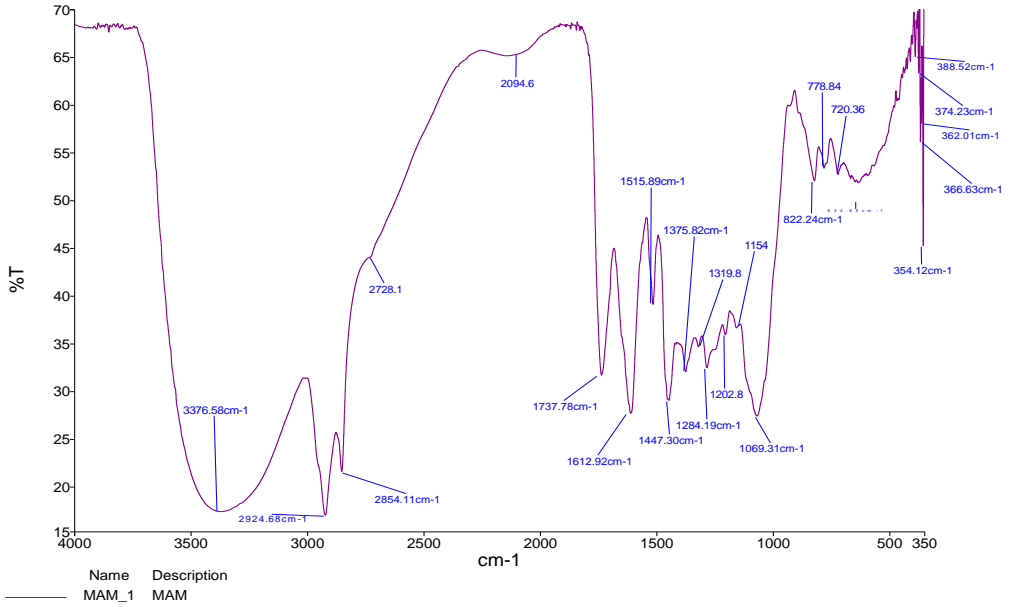


Figure 2. IR spectrum of the methanol extract of *Annona muricata* (MAM)

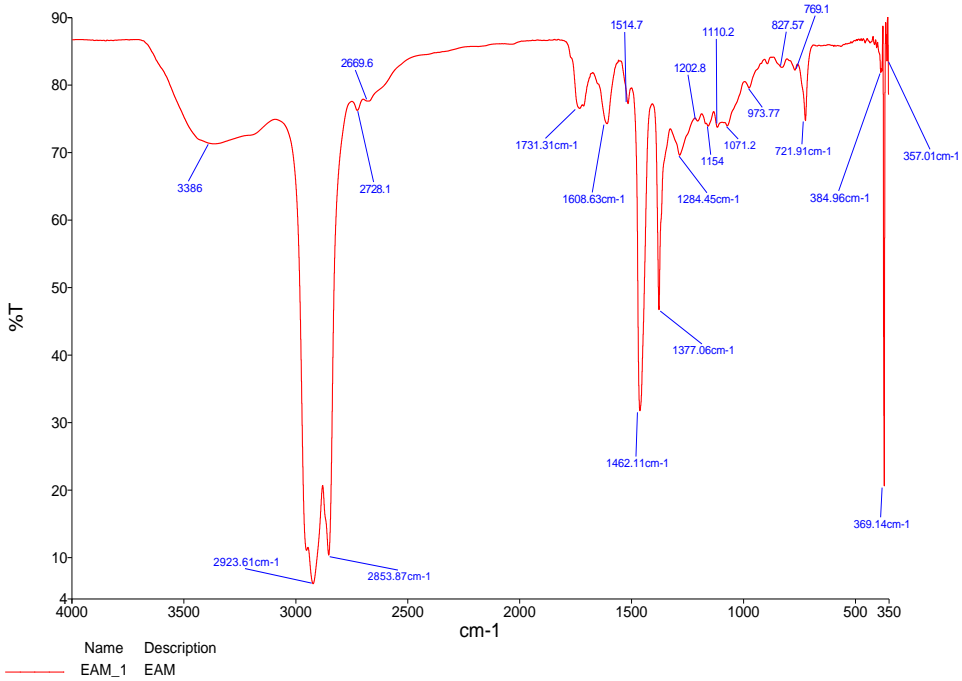


Figure 3. IR spectrum of the ethyl acetate extract of *Annona muricata* (EAM)

Scanning Electron Microscope

The scanning electron microscope (SEM) image at the magnification of 20,000X showing the sample's surface

topography is displayed in Figure 4. It reveals the texture as coarse and the leaf particles showed inhomogeneous sizes.

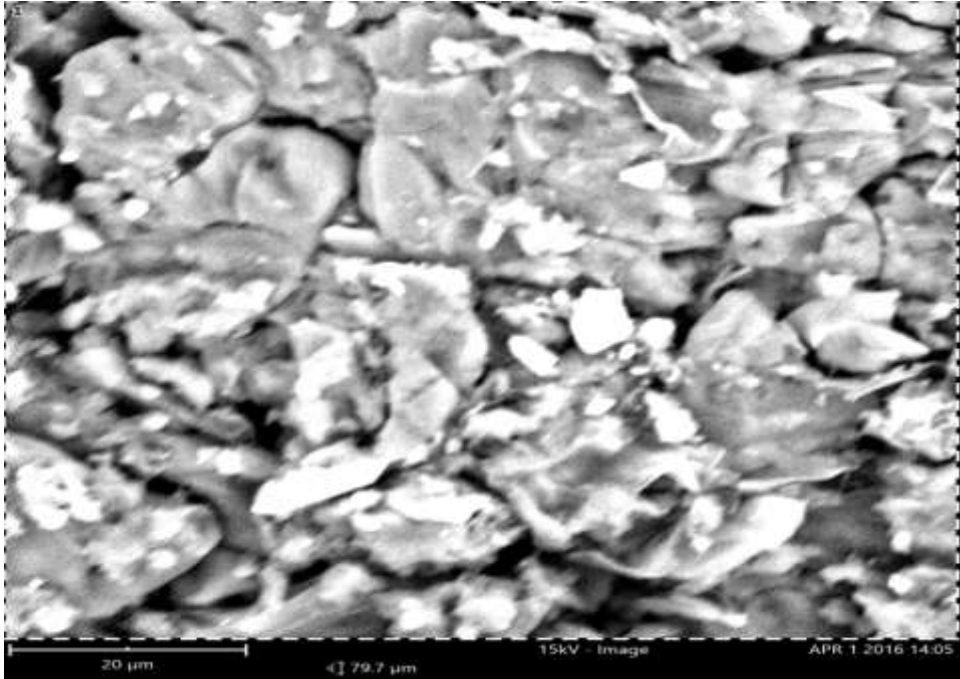


Figure 4. Scanning electron microscopy image of *Annona muricata*

Energy Dispersive X-Ray

The EDX analysis (Figure 5 and Table 3) gives information on the composition of the elements in the sample. EDX

results showed high oxygen concentration of 73.5 % and carbon concentration of 26.5 %.

Table 3. EDX result of *Annona muricata*

Element Number	Element Symbol	Element Name	Confidence	Concentration	Error
6	C	Carbon	100.0	26.5	1.4
8	O	Oxygen	100.0	73.5	2.1

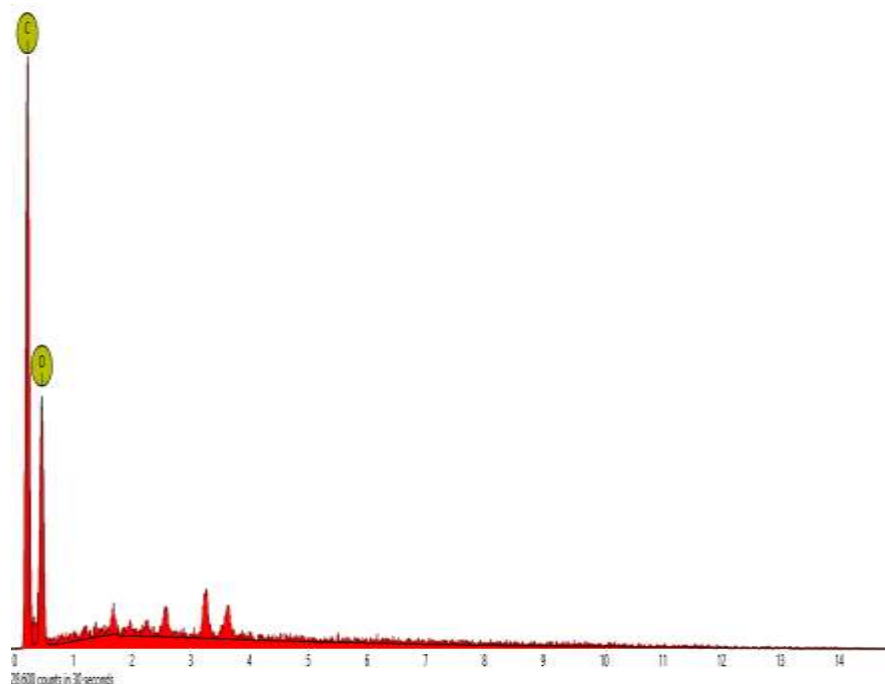


Figure 5. EDX spectrum of elemental concentration of *Annona muricata* leaf

Antimicrobial activities

The antimicrobial activities of *Annona muricata* leaf extracts were displayed in Table 4. The antimicrobial results of the test extracts at a potency of 20 mg/mL were compared with the activity of the standard, Gentamicin (20 µg/mL). The results revealed that the extracts exhibited high to moderate antimicrobial activities with the exception of aqueous extract (AM₅). Ethyl acetate extract (AM₄) was found highly active against gram positive bacteria, *S. aureus* (ZI of 42 mm; AI = 1.31) and ampicillin resistant gram negative *P. aeruginosa* (ZI of 34 mm; AI = 1.13). Also, AM₄ exhibited moderate activities against *E. coli* (ZI of 22 mm; AI = 0.73) and *C. albicans* (ZI of 28 mm; AI = 0.89). The hexane extract of *Annona muricata*

(AM₁) had the highest zone of inhibition (42 mm) and activity index (1.40) against *E. coli* followed by the MeOH extract (ZI = 38 mm; AI = 1.27). The aqueous fraction generally exhibited low activity against the test pathogens, the activity indexes range from 0.73 to 0.63 as compared with reference drug. Solomon-Wisdom *et al.* (2014) reported that the methanol extract of the plant leaf showed maximum antibacterial activity than aqueous extract. In this work, among the different solvent extracts studied ethyl acetate and hexane showed highest antimicrobial property. The study supports the ethnomedicinal use of *Annona muricata* for the treatment of infections.

Table 4. Antimicrobial results of extracts of *A. muricata* leaf.

Organism	Zone of inhibition (mm)					
	AM ₁	AM ₂	AM ₃	AM ₄	AM ₅	Gentamicin
<i>Escherichia coli</i>	42	38	20	22	22	30
A.I	1.40	1.27	0.67	0.73	0.73	
<i>Staphylococcus aureus</i>	28	30	24	42	22	32
A.I	0.88	0.94	0.75	1.31	0.69	
<i>Pseudomonas aeruginosa</i>	22	24	24	34	22	30
A.I	0.73	0.80	0.80	1.13	0.73	
<i>Candida albicans</i>	24	22	22	28	20	32
A.I	0.75	0.69	0.69	0.89	0.63	

Assay run @ 20 mg/mL; AI = activity Index = ZI of test sample/ ZI of standard.; ZI = zone of inhibition

Conclusion

In this study, *Annona muricata* leaf has been found rich in phenolic compounds, flavonoids, tannins, alkaloids, saponins and cardiac glycosides as secondary metabolites. However, Anthraquinone was found absent in the leaf sample. The antimicrobial work demonstrated that among the different solvent extracts studied ethyl acetate and hexane showed highest antimicrobial property. The results suggest that purification of methanol extract which was the crude extract enhances the potency of its fractions. Therefore, from the activity

indexes, it proves that the ethyl acetate and hexane fractions of *A. muricata* possess good anti-infective property. The findings revealed the anti-infective potential of *Annona muricata* leaf and established physico-chemical markers (UV-Vis, IR, SEM, EDX Analyses) for the active extracts which could be valuable in the bioassay guided fractionation and standardization of the vegetable drug. The good antimicrobial property demonstrated by *Annona muricata* leaf would provide valuable use to the health management if further processed.

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