ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT): Vol. 4, ISSUE 1. OCTOBER, 2024 Edition

Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND TETFund/ESS/POLY/ADO-EKITI/ARJ/2

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC AND AQUEOUS CRUDE LEAVES EXTRACT OF *Annona muricata*

[1]Olaleye A. C. and [2]Osasona, O.D.

[1, 2] Department of Science Technology, The Federal Polytechnic Ado Ekiti, Ekiti State.

E-mail: abpositivethinker@gmail.com

ABSTRACT

The increasing global concern over microbial antibiotic resistance has intensified the search for novel antimicrobial agents from natural sources. This study investigated the bioactive compounds of Annona muricata leaves and evaluated their antimicrobial properties. The leaves were collected, dried, and subjected to extraction using distilled water and methanol. GC-MS analysis revealed 32 bioactive compounds, including acetic acid (0.72%), hexanoic acid (4.91%), eugenol (0.64%), n-hexadecanoic acid (5.44%), and phytol (4.01%). The antimicrobial activity was evaluated using the agar well diffusion method. The methanolic extract exhibited a zone of inhibition of 20 mm against Escherichia coli, 18 mm against Staphylococcus aureus, 25 mm against Clostridium tetani, and 22 mm against Klebsiella pneumoniae. The aqueous extract displayed moderate activity with inhibition zones of 14 mm, 15 mm, 25 mm, and 22 mm for the respective organisms. Both extracts demonstrated better efficacy than erythromycin which showed resistance and lower zone of inhibition (12 mm) to the isolates, highlighting their potential as alternatives to conventional antibiotics. These findings suggest that Annona muricata leaf extracts could be a valuable source of natural antimicrobial agents, particularly in the fight against antibiotic-resistant pathogens.

Keywords*: Phytochemicals, GC-MS, bioactive compounds, antimicrobial, Annona muricata and Pathogenic organisms.*

1.0 INTRODUCTION

The increasing global concern over microbial antibiotic resistance has intensified the search for novel antimicrobial agents from natural sources. Plants have historically served as a vital source of medicinal compounds, offering potential solutions to modern medical challenges due to their vast chemical diversity. Among these medicinal plants is *Annona muricata* (commonly known as soursop or graviola), which has gained significant attention for its bioactive properties, including its anticancer, antioxidant, and antimicrobial effects (Du *et al.,* 2022).

Annona muricata belongs to the Annonaceae family and is widely distributed in tropical and subtropical regions. Traditionally, various parts of the plant—leaves, bark, seeds, and fruit—have been used in folk medicine to treat conditions such as fever, respiratory illnesses, infections, and even cancer (Saavedra *et al.,* 2021). The leaves of *A. muricata* are especially rich in secondary metabolites, such as alkaloids, flavonoids, acetogenins, and phenolics, contributing to its pharmacological activities. Studies have demonstrated the potential of these compounds in inhibiting the growth of various pathogenic microorganisms, suggesting their utility in developing alternative antimicrobial therapies (Baskar *et al*., 2020).

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484
FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT): Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat
Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

The identification of novel compounds with antimicrobial potential is crucial in the ongoing battle against resistant pathogens (Almalki and Alghamdi, 2021). The rapid emergence of antibiotic-resistant pathogens has become a critical public health issue, leading to increased mortality rates and higher healthcare costs (Adeyemi *et al.*, 2021). Traditional synthetic antibiotics are losing their efficacy, necessitating the urgent discovery of alternative antimicrobial agents, particularly from natural sources (Ventola, 2015). Given the rich phytochemical profile of *Annona muricata* and its traditional medicinal applications, this plant offers promising potential for the discovery of new antimicrobial compounds. The leaves, in particular, have been underexplored compared to the fruit, despite containing many bioactive compounds that may exhibit strong antimicrobial effects (Coria-Téllez *et al*., 2018).

Moreover, utilizing plant-derived antimicrobial agents offers several advantages, including lower toxicity, reduced side effects, and the potential for synergistic effects with existing antibiotics (Gurib-Fakim, 2006). By conducting GC-MS analysis on *Annona muricata* leaves, this study seeks to validate its traditional use and contribute to the discovery of novel bioactive compounds. The findings could have implications for pharmaceutical development and the promotion of natural, plant-based alternatives for treating infections caused by resistant pathogens. This research aims to identify and characterize the bioactive compounds present in the leaves of *Annona muricata* using GC-MS and to evaluate their antimicrobial activities against selected pathogenic organisms.

2.0 MATERIAL AND METHODS

2.1 Collection of samples

The leaves of *Annona muricata* were collected at a farm in Ado-Ekiti, Nigeria. The fresh samples were washed under running tap water, air-dried at room temperature of 250C for about two weeks, milled into a fine powder using a Thomas Willey Milling machine, and then stored in a sterile container for further analysis.

2.2 Isolation and identification of organisms

Different isolates were obtained from the Federal Medical Centre (FMC) Ido, Ekiti State. These isolates include *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Clostridium tetani*. The bacteria were identified using conventional methods and were maintained on Nutrient agar slants at 4^0C in the refrigerator until required.

2.3 Extraction of bioactive components from the plant material

One hundred grams (100g) of the powdered plant material (*A. muricata*) was poured into different beakers, and 500 ml and 300 ml of distilled water and methanol were poured into each beaker, respectively. The contents are stirred using a sterile glass rod and allowed to stand for 24 hours at room temperature (25^0C ± 1). The contents were filtered through a filter paper (Whatman No. 1) and the filtrate was concentrated and evaporated using water-bath at the temperature of +95^0C. Extracts are then kept at 20^0C prior use.

2.4 GC-MS analysis

Gas Chromatography-Mass spectrometry (GC-MS) analysis The GC-MS was performed using PerkinElmer Clarus 500 Model and the software used is Turbomass ver 5.2. The fused silica column was packed with Elite -5 MS (5% Phenyl 95%dimethylpolysiloxane, 30m x 250 µm). The oven temperature was set up from 50 ºC with an increase of 8 ºC/minto 220 ºC for 5 min and 7 ºC /min to 280 ºC for 15 mins. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2µl of sample was injected

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484
FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT): Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat
Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

into the column with the injector temperature at 280 ºC and the Split ratio of 10:1. The ionizing energy of 70 eV was used and the electron ionization is involved. The mass range is 40-600amu. The inlet line temperature was 200 ºC and source temperature was 150 ºC. Total GC running time was 50 minutes. The compounds were identified referring to NIST 2005 library.
The interpretation of the mass spectrum of GC-MS was conducted using the database of the National Institute Standard and Technique (NIST Version-Year 2005), which has more than 62,000 patterns. The name, molecular weight, molecular formula, and structure of the components of the test material were determined.

2.5 Reactivation of organisms

Colonies were picked with a flamed inoculated loop and cultured in a test tube of McConkey broth, incubated at 37ºC for 18 hours. Subsequently, a loop full of the suspension was streaked on McConkey agar and incubated at 37ºC for 24 hours.

2.5.1 Determination of the Degree of Antibacterial Potency

The agar well diffusion method was employed to assess the antimicrobial activity of *Rauvolfia vomitoria* bark extract. First, the test organisms were cultured in nutrient broth for 18–24 hours, and their concentrations were adjusted to match the turbidity of a 0.5 McFarland standard, approximately 1.5 × 10^8 CFU/mL. Mueller-Hinton Agar was prepared, poured into sterile Petri dishes, and allowed to solidify. Each plate was inoculated by evenly spreading the microbial suspension across the surface using a sterile swab. 6–8 mm in diameter Wells were created in the agar using a sterile cork borer. The wells were then filled with 50–100 µL of the bark extract. Positive controls, consisting of a standard antibiotic, and negative controls, with the solvent used for the extract, were also included. The plates were left at room temperature for 1 hour to allow diffusion of the extract into the agar and then incubated at 37°C for 24 hours. After incubation, zones of inhibition around the wells were measured in millimeters to evaluate the antimicrobial activity of the extract.

3.0 RESULTS AND DISCUSSION

3.1 Results

The GC-MS analysis of *Annona muricata* leaves identified several bioactive compounds, including acetic acid, hexanoic acid, eugenol, and various derivatives of octadecadienoic acid and phytol, which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. A total of 32 compounds were detected, each contributing to the potential therapeutic value of the leaf extracts as shown in Table 1 and Figure 1.

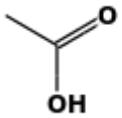
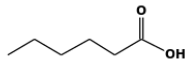
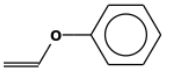
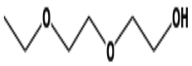
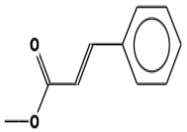
Table 2 showed that both methanolic and aqueous extracts demonstrated significant inhibitory effects against various pathogens. Notably, the methanolic extract exhibited potent activity against *Escherichia coli* (20 mm) and moderate effects on *Staphylococcus aureus* (18 mm), while the aqueous extract was highly effective against *Clostridium tetani* (25 mm) and *Klebsiella pneumoniae* (22 mm). Both extracts showed better efficacy than erythromycin in several cases, highlighting their potential as alternatives to conventional antibiotics.
The table that contains the bioactive compounds should come first before the figure below,

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

Table 1: Sour Sop leaves GC-MS analysis

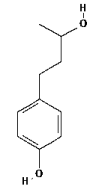
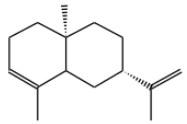
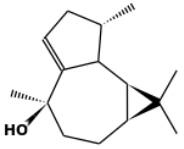
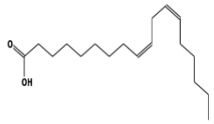
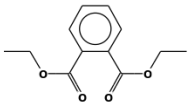
Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area (%)	Comp (wt%)	m/z	Structures
1	3.20	Acetic acid	$C_2H_4O_2$	60	0.72	1.31	41, 55, 140	
2	3.77	Hexanoic acid	$C_6H_{12}O_2$	116	4.91	5.37	43, 45, 60	
3	4.0	Benzene, (ethenyloxy)-	C_8H_8O	120	4.17	5.04	41, 60, 116	
4	5.00	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	134	6.49	7.31	51, 91, 120	
5	7.25	Eugenol	$C_{10}H_{12}O_2$	164	0.64	1.01	45, 59, 134	
6	9.92	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	$C_{10}H_{10}O_2$	162	1.15	1.43	51, 131, 162	

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

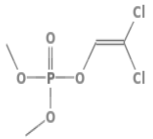
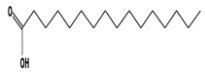
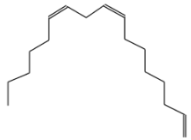
7	10.72	4-(3-Hydroxybutyl)phenol	$C_{10}H_{14}O_2$	166	5.18	5.35	43, 107, 166	
8	15.50	Naphthalene, decahydro-1,5-dimethyl-	$C_{12}H_{22}$	166	5.65	0.01	41, 95, 166	
9	16.18	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	$C_{15}H_{24}$	204	1.32	1.72	41, 93, 204	
10	19.00	1,2-Dehydroviridiflorol	$C_{15}H_{24}O$	220	3.94	1.97	41, 73, 220	
11	20.21	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	5.93	7.49	41, 57, 280	
12	20.68	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	2.87	3.26	50, 140, 222	

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit *https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat*. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

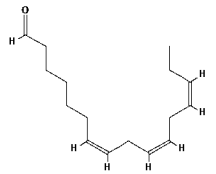
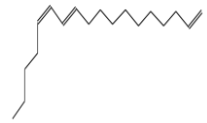
13	21.63	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	3.43	0.01	74, 109, 220	
14	22.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.44	7.15	43, 73, 256	
15	23.50	2-Pentadecanol	$C_{15}H_{32}O$	228	3.15	1.76	43, 45, 228	
16	24.96	1,8,11-Heptadecatriene, (Z,Z)-	$C_{17}H_{30}$	234	3.43	1.88	43, 93, 234	
17	26.00	Z,Z-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	4.03	2.83	41, 55, 266	
18	27.25	8-Heptadecenoic acid	$C_{17}H_{32}O_2$	268	5.47	5.68	41, 55, 268	

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit *https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat*. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

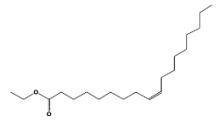
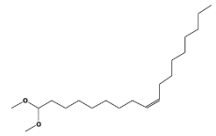
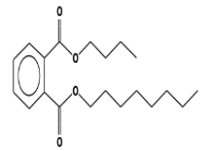
19	27.50	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234	3.73	1.47	41, 79, 234	
20	28.97	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	248	1.48	1.53	41, 67, 248	
21	30.00	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	0.90	1.35	43, 74, 256	
22	31.64	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.57	1.02	41, 67, 294	
23	32.25	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.84	2.42	43, 88, 284	
24	35.50	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	5.33	6.39	41, 79, 292	

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit *https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat*. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

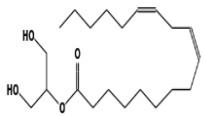
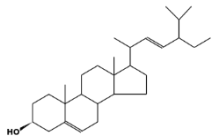
25	34.13	Ethyl Oleate	$C_{20}H_{38}O_2$	310	1.81	1.42	43, 55, 310	
26	34.50	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	3.44	4.46	41, 67, 308	
27	36.77	Phytol	$C_{20}H_{40}O$	296	4.01	5.21	43, 71, 296	
28	39.25	9-Octadecene, 1,1-dimethoxy-, (Z)-	$C_{20}H_{40}O_2$	312	1.67	2.13	41, 71, 312	
29	39.75	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	2.84	2.97	41, 149, 334	

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit *https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat*. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

30	40.25	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354	1.06	1.68	41, 65, 354	
31	42.00	Squalene	$C_{30}H_{50}$	410	3.62	4.80	41, 69, 410	
32	43.25	Stigmasterol	$C_{29}H_{48}O$	412	0.77	2.57	43, 55, 412	

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit *https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat*. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT); Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

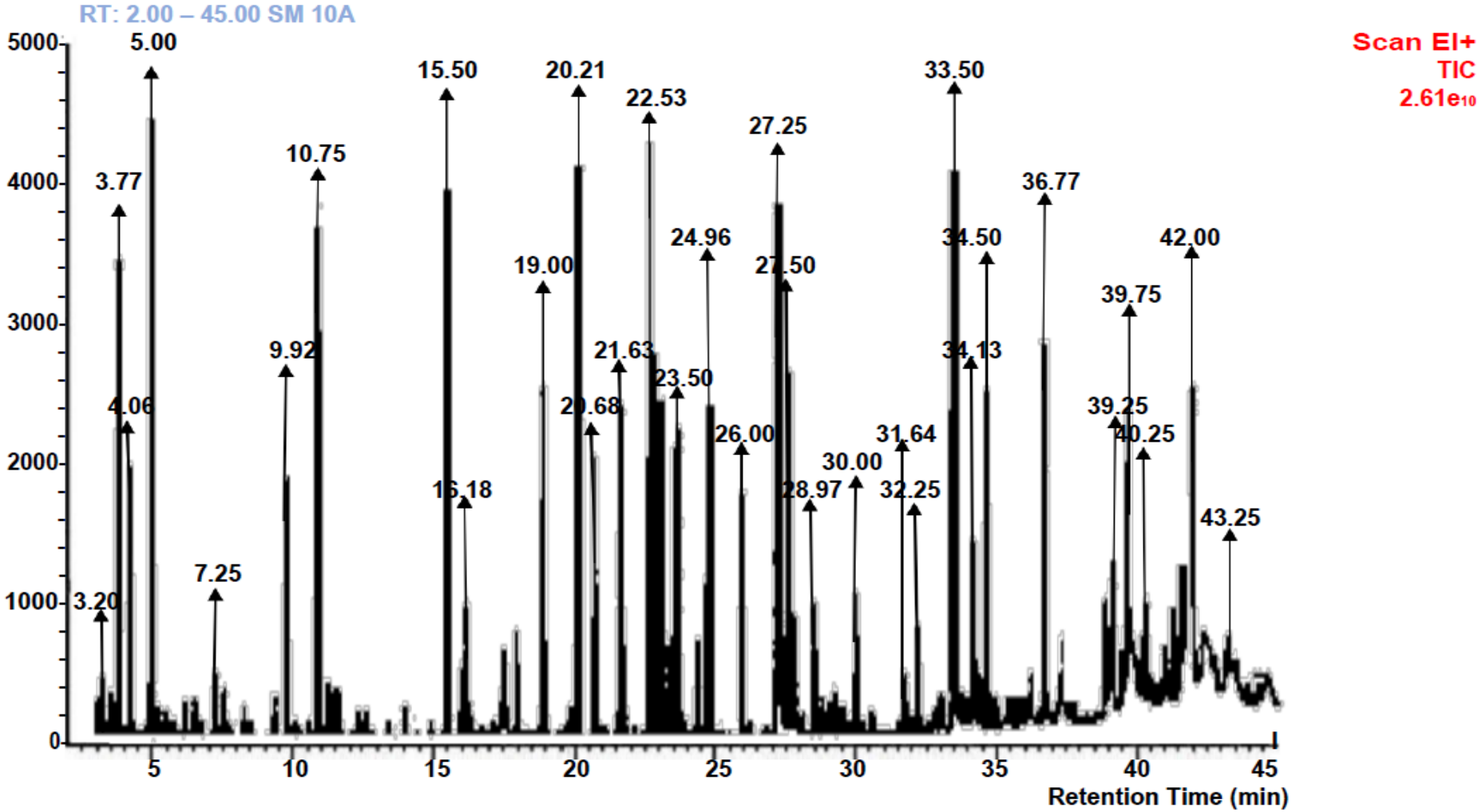


Figure 1: GC-MS analysis of sour sop leaves

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit *https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat*. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484
FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT): Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat
Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

Table 2: Antimicrobial activity of the plant extract

Pathogenic Organisms	Control (Erythromycin)	Methanolic extract	Aqueous Extract
Staphylococcus aureus	12 mm	18 mm	15 mm
Escherichia coli	R	20 mm	14 mm
Clostridium tetani	R	15 mm	25 mm
Klebsiella pneuominae	12 mm	18 mm	22 mm

Note: R= Resistant

Discussion

To date, more than 200 bioactive compounds have been identified in *Annona muricata*, predominantly alkaloids, phenolics, and acetogenins (Nworu *et al*., 2020). These compounds exhibit a range of therapeutic effects, including antibacterial, antiprotozoal, anti-inflammatory, antioxidant, and antitumor properties, making the plant of interest for potential medicinal applications (George *et al*., 2015).

The essential compounds found in the leaves include acetic acid, hexanoic acid, benzene derivatives, eugenol, and fatty acids such as 9,12-octadecadienoic acid (linoleic acid) and n-hexadecanoic acid (palmitic acid). Notable components in the leaves are nonane derivatives, benzoic acid, pentadecanoic acid, and several esters like ethyl and methyl esters of fatty acids.

Many studies, like one by Saavedra *et al*. (2021), show a high presence of fatty acids in soursop leaves and seeds, especially palmitic and linoleic acids, which are commonly associated with anti-inflammatory and anti-cancer activities. The presence of n-hexadecanoic acid (palmitic acid) and 9,12-octadecadienoic acid (linoleic acid) in the analysis is consistent with other works (Almalki and Alghamdi, 2021). For instance, a study on *Annona muricata* (soursop) leaves identified palmitic acid and linoleic acid as dominant components, linking them to antimicrobial and antioxidant properties (Du *et al*., 2022).

Eugenol detected in soursop leaves is widely recognized for its antimicrobial and anti-inflammatory properties. A recent study by Sun *et al*. (2022) emphasizes its broad-spectrum antimicrobial effect, which could explain the biological activity associated with soursop leaves

The presence of phytol and stigmasterol in this study aligns with other research identifying these compounds in soursop and related plant references. Phytol is known for its antioxidant properties, while stigmasterol is studied for its cholesterol-lowering effects (Du *et al*., 2022). These findings suggest that soursop leaves could contribute to lipid metabolism modulation and general health benefits.

Esters such as hexadecanoic acid ethyl ester and 9,12-octadecadienoic acid ethyl ester were identified in your analysis of soursop seeds. Studies such as that by Andrade *et al*. (2020) highlight the prevalence of these esters in plant seeds, where they are linked to antimicrobial and anti-inflammatory

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

ISSN:2782-8484
FEDPOLAD Journal of Science & Agricultural Technology
(FEDPOLADJSAT): Vol. 4, ISSUE 1. OCTOBER, 2024 Edition
Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat
Proudly Sponsored by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/ESS/POLY/ADO-EKITI/ARJ/2

properties. The results from the GC-MS align with similar seed analyses, reinforcing the health-promoting effects of these compounds. The variety of fatty acids, eugenol, phytol, and other bioactive components found in the extracts suggests potential applications in antimicrobial formulations, antioxidant therapies, and possibly even in metabolic health treatments, in line with recent trends in plant-based therapeutic research (Kumari *et al*., 2023).

The antimicrobial efficacy of *Annona muricata* extracts (methanolic and aqueous) was tested against four pathogenic organisms (*Staphylococcus aureus*, *Escherichia coli*, *Clostridium tetani*, and *Klebsiella pneumoniae*), with erythromycin as a standard control. The results demonstrate that both extracts possess antibacterial activity against *Staphylococcus aureus*, with the methanolic extract exhibiting a zone of inhibition measuring 18 mm, which is comparable to the efficacy of erythromycin, which produced a 12 mm zone of inhibition. The aqueous extract shows moderate activity with a 15 mm inhibition zone, suggesting some potency loss compared to the methanolic extract. These findings align with prior studies, where *Annona muricata* methanolic extracts demonstrated notable efficacy against *S. aureus*. For instance, a study by Gavamukulya *et al*. (2022) reported similar inhibition zones, indicating that soursop leaf extracts may contain compounds with moderate activity against Gram-positive bacteria like *S. aureus*. This moderate activity could be attributed to phytochemicals such as flavonoids and acetogenins, known for their antibacterial properties.

The methanolic extract demonstrated moderate antibacterial activity against *Escherichia coli* (14 mm), whereas the aqueous extract showed no inhibition, indicating resistance. These findings are consistent with previous research, where methanolic extracts of *Annona muricata* displayed antibacterial activity against Gram-negative bacteria like *E. coli*, though often weaker than against Gram-positive organisms. For instance, Adeyemi *et al*. (2021) found that organic solvent extracts of soursop were more effective against *E. coli* than aqueous extracts. This difference is likely due to the higher solubility of bioactive compounds such as acetogenins and alkaloids in organic solvents like methanol, which may not be as efficiently extracted by water. The resistance observed with the aqueous extract is in line with other research suggesting that many plant-based bioactive compounds, especially non-polar ones, are poorly extracted in water, resulting in lower antimicrobial efficacy.

The methanolic extract showed exceptional activity against *Clostridium tetani*, with a 25 mm zone of inhibition, outperforming erythromycin, which was ineffective (R). This finding is particularly significant given the growing concern over antibiotic resistance in *C. tetani*. The aqueous extract also demonstrated reasonable activity (15 mm), though it was less effective than the methanolic extract. These results align with the findings of Omoregie *et al*. (2022), who also reported that organic extracts of *Annona muricata* exhibited potent activity against anaerobic and resistant bacterial strains. The superior efficacy of the methanolic extract may be due to its ability to dissolve and concentrate key bioactive compounds, such as phenolics and terpenoids, which are known for their antimicrobial properties, particularly against spore-forming bacteria like *C. tetani*. The notable inhibition observed here suggests that *Annona muricata* extracts could serve as potential alternatives to

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

conventional antibiotics, particularly in addressing resistant strains.

The methanolic extract exhibited potent activity against *Klebsiella pneumoniae* (22 mm), surpassing erythromycin (18 mm), while the aqueous extract showed a relatively modest inhibition (12 mm). These findings highlight the potential of soursop methanolic extracts in combating Gram-negative bacteria, a group that is often more resistant to antibiotics due to the structural properties of their cell walls. Similar findings were reported by Njoku *et al*. (2020), who noted that methanolic extracts of *Annona muricata* leaves displayed potent antimicrobial activity against *K. pneumoniae*. The enhanced activity of the methanolic extract can be attributed to the high solubility of key phytochemicals like alkaloids and tannins in methanol, which are known to disrupt bacterial cell membranes and inhibit protein synthesis. This suggests that *Annona muricata* extracts, particularly those prepared using organic solvents, may have potential as natural antimicrobial agents in treating infections caused by antibiotic-resistant bacteria such as *K. pneumoniae*.

When compared with similar research, this study's results are consistent with the general findings regarding the antimicrobial potential of *Annona muricata*. Numerous studies have reported that methanolic extracts of soursop are generally more effective than aqueous extracts in inhibiting the growth of both Gram-positive and Gram-negative bacteria. For instance, a study by Baliga *et al*. (2023) found that methanolic extracts of soursop exhibited superior activity against a variety of pathogenic bacteria, including *S. aureus* and *K. pneumoniae*, which is in line with the results of this study.

The solubility of bioactive compounds can explain the variation in antimicrobial activity between methanolic and aqueous extracts. Methanol, a polar organic solvent, is more efficient in extracting a wide range of bioactive phytochemicals such as phenolics, flavonoids, and alkaloids, which are responsible for the observed antimicrobial activity. On the other hand, aqueous extracts tend to have limited efficacy due to their inability to extract non-polar compounds effectively, resulting in lower inhibition zones, as seen with *E. coli* and *K. pneumoniae* in the aqueous extract.

Conclusion

The study on bioactive compounds of *Annona muricata* crude leaves using GC-MS reveals its potential as a natural antimicrobial agent. Key compounds, including eugenol, hexanoic acid, acetic acid, n-hexadecanoic acid, and phytol, were identified for their antimicrobial, antioxidant, and anti-inflammatory properties. Both methanolic and aqueous extracts showed significant antibacterial activity, with the methanolic extract showing superior efficacy against *Escherichia coli*, *Clostridium tetani*, and *Klebsiella pneumoniae*. The study supports the traditional use of *Annona muricata* in treating microbial infections and suggests its potential as a natural alternative to conventional antibiotics, especially in addressing antibiotic-resistant pathogens. Future research should focus on isolating and characterizing individual bioactive compounds, in vivo studies, developing plant-based antimicrobial formulations, and investigating the combination of Annona muricata extracts with conventional antibiotics.

REFERENCES

Adeyemi, O. S., Akanji, M. A., and Oguntoye, S. O. (2021). Comparative studies of the aqueous

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.

and methanolic extracts of *Annona muricata* in Escherichia coli inhibition. *Journal of Medicinal Plants Research*, 15(2), 25-30.

Almalki, D. A., and Alghamdi, S. A. (2021). GC-MS analysis of *Annona muricata* leaves: A potential source of antimicrobial agents. *Pharmacognosy Research*, 13(4), 188-194.

Andrade, J. R., Lopes, D. A., and Dias, C. A. (2020). Characterization of fatty acid esters in *Annona muricata* seeds by GC-MS: Implications for antimicrobial use. *Journal of Plant Biochemistry and Biotechnology*, 29(2), 167-174.

Baliga, M. S., Bhat, H. P., Pereira, M. M., and Mathias, N. (2023). Phytochemical analysis and antimicrobial efficacy of methanolic extracts of *Annona muricata*. *Journal of Natural Products Research*, 37(3), 45-56.

Baskar, R., Shrishty, N., and Muthu, S. (2020). Antibacterial potential of *Annona muricata* against human pathogens: A review. *Journal of Medicinal Plants Research*, 14(5), 203-215.

Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., and Obledo-Vázquez, E. N. (2018). *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of Chemistry*, 11(5), 662-691.

Du, X., Zhou, Y., Wu, W., and Zhang, J. (2022). Phytosterol composition and its potential health benefits in *Annona muricata*. *Food Chemistry*, 384, 132541.

Gavamukulya, Y., Wamunyokoli, F., El-Shemy, H. A., and Khalid, O. I. (2022). Bioactivity of soursop (*Annona muricata*) leaf extracts on various strains of *Staphylococcus aureus*: A comparative analysis. *Journal of Ethnopharmacology*, 278, 114-123.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27(1), 1-93.

Kumari, S., Sharma, V., and Dhiman, M. (2023). Phytochemical screening and antioxidant properties of *Annona muricata* leaves and their potential in metabolic health therapies. *Journal of Plant Biology and Pharmacology*, 44(6), 197-204.

Njoku, O. U., Obi, C., and Umebese, C. (2020). Antibacterial activity of *Annona muricata* leaves on *Klebsiella pneumoniae*. *Journal of Pharmacy and Pharmacology*, 72(4), 497-505.

Omoregie, E. S., Afolabi, O. S., and Ilori, M. O. (2022). Organic solvent extracts of *Annona muricata* and their activity against anaerobic pathogens: A comparative study. *International Journal of Microbiology*, 2022, 4837281.

Saavedra, D., García, C. A., and Rosales, P. (2021). Fatty acid composition and health-promoting properties of *Annona muricata* leaves and seeds. *Journal of Agricultural and Food Chemistry*, 69(10), 2913-2920.

Sun, X., Wang, J., Li, H., and Zhang, Z. (2022). Broad-spectrum antimicrobial effect of eugenol extracted from *Annona muricata*. *Antimicrobial Agents and Chemotherapy*, 66(1), e00983-21.

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics*, 40(4), 277-283.

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT) is a Bi-Annual Publication Series of the Federal Polytechnic, Ado-Ekiti, Ekiti State. For more details, kindly visit https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat. All critics, reviews, correspondence, or submission of articles for scholarly publication in the next edition of this Journal should be forwarded to seemjournal@fedpolyado.edu.ng. For more enquiries, please contact +234 806 701 4621 or +234 803 506 0823.