



PHYTOCHEMICAL PROFILING, GC-MS CHARACTERIZATION
OF ISOLATES AND CORRELATED ANTIOXIDANT POTENTIAL
FROM LEAVES EXTRACT OF *Annona muricata* LINN. GROWN IN
GOMBE

ISHAKA IBRAHIM HAMMA¹; ISMAIL MAIGARI ADAMU¹; &
IDRISS BAMIDELE MUSBA²U²

¹Department of Science Laboratory Technology, Gombe State Polytechnic, Bajoga. ²Department of
Chemical Sciences, North-Eastern University, Gombe State.

Corresponding Author: ishakaibrahim@gspb.edu.ng

DOI: <https://doi.org/10.70382/caijbhr.v10i3.006>

Abstracts

Annona muricata Linn. leaves have been used traditionally for the treatment of various diseases including cancer. Despite its widespread use and numerous reported beneficial uses, there is a few or no research on its antioxidant potential in the study area. The present study was conducted to evaluate the quantitative phytochemical constituents, antioxidant activity and FTIR and GC-MS characterization of the leaves extracts. The screening was carried out using standard methods and procedures. The antioxidant properties of methanol extracts were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP). The activity of the extract against the free radicals was concentration dependent. The result revealed a linear correlation between the extract and its polyphenolics contents. The reducing ability of the extract also occurred in a dose dependent manner. The study provides evidence that the leaves of *A. muricata* contain essential constituents which may serve as natural antioxidants. Serial exhaustive extraction of *A. muricata* dried leaves using n-hexane, ethyl acetate, and methanol (48 h each, orbital shaker, 150 rpm), followed by rotary evaporation, yielded an ethyl acetate fraction enriched in bioactive secondary metabolites. Open column chromatography (silica gel, hexane-EtOAc gradient) afforded Fraction 2 (pale yellow oil: τ -cadinol, methyl oleate), Fraction 3 (off-white solid: lupeol acetate, olean-12-en-3-yl acetate, urs12-en-3-yl acetate), and Fraction 6 (yellow semi-solid: 2,4-di-tert-butylphenol, ethyl linolenate). FTIR and GC-EI-MS (70 eV, NIST library >90% match) provided structural elucidation via diagnostic fragmentation pathways. τ -Cadinol ($[M]^+$ m/z 222) exhibited dehydration (m/z 204) $\rightarrow$ allylic cleavage (m/z 161, cadinane marker) $\rightarrow$ aromatization (m/z 134/119/105). Methyl oleate (m/z 296) showed McLafferty $-\text{CH}_3\text{OH}$ (m/z 264) $\rightarrow$ -allylic ladder (m/z 222/180, base m/z 55). Triterpene acetates (m/z 468) displayed conserved acetyl loss (m/z 425) $\rightarrow$ skeletal rearrangements (lupeol: m/z 393/357/189; oleanane: m/z 313/257; ursane: m/z 409/325), distinguishing skeletons. Phenol (m/z 206 $\rightarrow$ base m/z 191) and ethyl linolenate (m/z 306 $\rightarrow$ m/z 261/191/79/55) confirmed antioxidant-lipid synergy. Therefore, it could be concluded that the plant contains phytochemicals needed in the treatment and management of various ailments.

Keywords: *Annona muricata*, GC-MS fragmentation, Lupeol Acetate, Olean-12-en-3-yl Acetate, Urs-12en-3-yl Acetate, τ -cadinol.

Introduction

Cancer is a major public health problem that has a significant impact on both developed and developing countries. In 2018, around 18 million cancer cases emerged globally, it is estimated to increase to more than 23million new cases annually by 2030 (Bray *et al.*, 2018). In addition to causing serious harm to the patient's body, cancer also imposes a huge economic burden on patients. The GLOBOCAN 2020 estimate shows that the burden of cancer is rising and is projected to rise much faster in developing countries (Sung *et al.*, 2021). During the 20th century, the cancer death rate was found to increase, primarily due to abnormal lifestyles, such as excessive tobacco and alcohol use, physical and chemical carcinogens (Siegel *et al.*, 2020; Sanford *et al.*, 2020; Bhatia *et al.*, 2020), unhealthy diet, and biological carcinogens (Hartwig *et al.*, 2020; Khaltayev & Axelrod, 2020). Heavy metals from refuse dump, atmospheric deposition, livestock manure, irrigation with wastewater, pesticides, herbicides, phosphate-based fertilizers, and sewage sludge in the soil environment and agriculture (Rai *et al.*, 2019). In Africa, an estimated 1.1 million new cases and 711,429 deaths occurred due to neoplasms in 2020. By 2040, the burden of all neoplasms combined is forecasted to increase to 2.1 million new cases and 1.4 million deaths (Sharma *et al.*, 2022). The total number of new cases in Nigeria in 2020 was 124,815, of which 51,398 occurred in males with prostate cancer as the most common at 29.8% and 73,417 occurred in females with breast cancer being the commonest at 38.7%, followed by cervical cancer at 16.4% (The Global Cancer Observatory, 2021). Generally, in Africa, available funding is limited, lack of resources and other major health problems (Mbele *et al.*, 2017), hence, the need to explore more of our local plant species that are cost effective. *A. muricata* grown in gombe have shown promise in the traditional treatment of cancer, suggesting that they contain bioactive compounds with potent anticancer properties. However, the specific phytochemicals responsible for these effects, its cytotoxic and antioxidant activity in the study area remain largely unexplored.

Considering the high profile nature of the disease, its treatment has been a constant struggle with relatively less success, the disease is hard to treat and has a high chance of reversal after treatment. Therefore, to tackle the problems associated with current therapies in cancer treatment, there is still a search for anticancer agents with enhanced efficiency and minimal side effects (Ranjan *et al.*, 2019). Phytochemicals are naturally occurring chemicals derived from plants, and these biologically active compounds have shown promising results in anti-cancer effects and other human ailments with no or less adverse effects (Ding *et al.*, 2023, Anwar *et al.*, 2020). Plants act as useful anticancer agents, with more than 3000 plants evaluated for their role in fighting cancer by targeting apoptotic pathways (Mir *et al.*, 2022).

Soursop (*Annona muricata*) is a fruit-bearing plant that belongs to the kingdom Plantae, the division Angiospermae (Magnoliophyta), the class Magnolid, the order Magnoliales, the family Annonaceae family, and the genus Annona (Gavamukulya *et al.*, 2017). The tree grows at altitudes below 1200 m above sea level, at a relative humidity of 60%–80%, a temperature ranges of 25–28 °C, and with more than 1500 mm of annual rainfall (Gavamukulya *et al.*, 2017). *A. muricata* is an evergreen plant that

blooms and bears fruit almost throughout the year (Pinto *et al.*, 2018). *Annona muricata* known as guanabana, soursop, graviola, or Brazilian paw paw (Moghadamtousi *et al.*, 2015). This plant is distributed widely throughout Southeast Asia, South America, and the rainforests of Africa (Moghadamtousi *et al.*, 2015). *A. muricata* is commonly known as soursop because of the sweet and sour taste of its fruit. The major chemical compounds present in soursop are acetogenins, alkaloids, flavonoids, essential oils, vitamins, carotenoids, amides, and cyclopeptides (Coria-Téllez *et al.*, 2018). Additionally, the plant contains minerals such as K, Ca, Na, Cu, Fe, and Mg (Gyamfi *et al.*, 2011). *A. muricata* have been used in traditional medicine in Gombe to successfully manage various types of cancer disorders and different ailments, indicating the presence of bioactive compounds that could be harnessed for modern therapeutics. Therefore, it is important to screen and validate the use of this potential plant specie scientifically. The study could lead to the discovery of new anti-cancer agents with enhanced efficiency and minimal side effects.

Description of the Study Area

Gombe state is located in the Sudan savannah zone and it was created in October 1996 from the defunct Bauchi State (Discover Nigeria, 2011). Geographically, it is located in northeastern Nigeria, between latitudes 9°30' and 12°30'N and longitudes 8°45' and 11°45'E of the Greenwich Meridian. The State has an area of 20,265 km² and a population of around 3,588,219 people as projected from the 2006 National Population Census. Gombe State has two distinct climates: the dry season (November-March) and the rainy season (April-October).

Plant Collection and Identification

The leaves of *Annona muricata* was collected from the study area with the help of practitioners and the collected samples was taken to Herbarium unit in the Department of Plant Science, Faculty of Science, Gombe State University for identification and authentication by taxonomist.

Extraction and Fractionation Protocol

Air-dried *Annona muricata* leaves (250 g) underwent serial exhaustive maceration at room temperature: n-hexane (48 h, orbital shaker, 150 rpm), followed by ethyl acetate (48 h), then methanol (48 h), with sequential filtrations after each step. Ethyl acetate extracts were concentrated via rotary evaporation (rotavap, 40°C) to yield a viscous residue, which was subjected to open column chromatography (silica gel 60–200 mesh, hexane: EtOAc 100:0 → 0:100, isocratic steps) to afford Fraction 2 (sesquiterpene-lipid, non-polar eluate), Fraction 3 (triterpene acetates, midpolarity), and Fraction 6 (phenol-polyunsaturated fatty acid-PUFA, higher polarity) (Najmi *et al.*, 2022).

Analytical Methods for Spectral Characterization

Fourier-transform infrared (FTIR) spectra were recorded on a PerkinElmer spectrometer using KBr pellets (4000–400 cm⁻¹, 4 cm⁻¹ resolution, 32 scans). Gas chromatography-electron ionization mass spectrometry (GC-EI-MS) employed an Agilent 7890B GC coupled to a 5977B MSD (70 eV, 250°C transfer line, HP-5MS column: 30 m × 0.25 mm, 0.25 µm film; helium carrier 1 mL/min;

temperature program: 60°C (2 min), 10°C/min to 280°C (10 min)). Compound identification relied on retention indices (RI), NIST 2020 library matching (similarity >90%), and fragmentation pattern concordance with literature. Fragmentation mechanisms were deduced via established EI rules (α/β -cleavage, dehydrations, 1,2-shifts), validated against reference spectra. (Najmi *et al.*, 2022).

Quantitative Phytochemical Screening

Total phenolic content (TPC): According to Folin-Ciocalteu method, 100 μ l of FolinCiocalteu reagent and 500 μ l of distilled water were added to 100 μ l of the extract and incubated for 6 min at room temperature. The volume was made to 3 ml using water, after adding 1.25 ml of 7% sodium carbonate. The absorbance was recorded at 760 nm, after incubation for 90 min and the result was expressed as mg Gallic acid equivalents (GAE) per gram dry weight (DW) of the plant material (Udayaprakash *et al.*, 2015).

Total flavonoid content (TFC): The solvent in 200 μ l of the extract was evaporated and 5 ml of aluminium chloride (0.1 M) was added to the residue. After incubation for 40 min, the absorbance was measured at 415 nm. The TFC was expressed with reference to Quercetin equivalents (QE) per gram dry weight (DW) of the plant material (Udayaprakash *et al.*, 2015).

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: As adopted by Hamma *et al.* (2024) varying concentrations of *A. muricata* extracts (100, 200, 300, 400, & 500 μ g/ml) were made up to 1 ml. To this, 1 ml of 0.01 mM DPPH was added and incubated in the dark for 30 min, after which the absorbance was measured at 517 nm.

Ferric reducing antioxidant power (FRAP) assay: As adopted by Hamma *et al.* (2024) 2.5 ml of 0.2 M phosphate buffer (pH 7) and 2.5 ml of 1% potassium ferricyanide were added to the different concentrations of the extract (100, 200, 300, 400, & 500 μ g/ml) and maintained at 50 C for 30 min. Additional, 10% trichloroacetic acid (2.5 ml) was added and centrifuged for 10 min at 6500 rpm. To the supernatant, 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added and the absorbance was measured at 700 nm.

The percent of inhibition exhibited by different concentrations of the extract in both DPPH and FRAP was calculated as given below:

$$\text{Percent inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

Data were expressed as Mean $\pm$ SEM of three replicates. Statistical analyses was carried out using SPSS version 20. One-way ANOVA was used to compare the values of samples with control. P value < 0.05 will be deemed as indicating significant differences.

Quantitative Phytochemical Analysis

Annona muricata leaves were subjected to quantitative screening by standard methods and procedures. The methanol extract of the leaves of the plant were analysed for Total flavonoids and phenolics. The results obtained from the quantitative analysis of the methanol extract showed the presence of phytochemicals from highest to least extent. The table 1 result clearly indicated that the highest amount is reported in Phenolics with (325.84 µg/mg extract) while Total flavonoid has the least with (182.46 µg/mg extract) as shown in table 1.

Table 1: Quantitative Phytochemical Analysis of Leaves of *Annona muricata*

Phytochemical parameters	Quantitative composition of the leaves
Total Phenolics (Gallic Acid Eqv)	325.84±0.19
Total Flavonoids (Quercetin Eqv)	182.46±0.34

Values are expressed as mean ± Standard Error Mean (SEM) of replicate determinations

FT-IR Spectral Analysis of Fraction 2: τ -Cadinol and 9-octadecenoic acid methyl

Fraction 2 isolated as a pale yellow, non-crystalline oil comprised majorly τ -cadinol and (Z)-9-octadecenoic acid methyl ester with minor lipophilic impurities. The FTIR spectrum shows a weak band in the 3500–3300 cm⁻¹ range suggests trace hydroxyl groups, while strong absorptions at 2957, 2920, and 2851 cm⁻¹ indicate the presence of long hydrocarbon chains typical of terpenoids. A sharp peak at 1735 cm⁻¹ confirms ester carbonyl stretching, distinguishing esters from free carboxylic acids. The 1664 cm⁻¹ absorption relates to C=C stretching, and bands at 1509–1448 cm⁻¹ pertain to CH₃ bending. Additionally, strong band in the 1012 cm⁻¹ range indicate C–O stretching of ester linkages **Fig 1** (Atiko *et al.*, 2025).

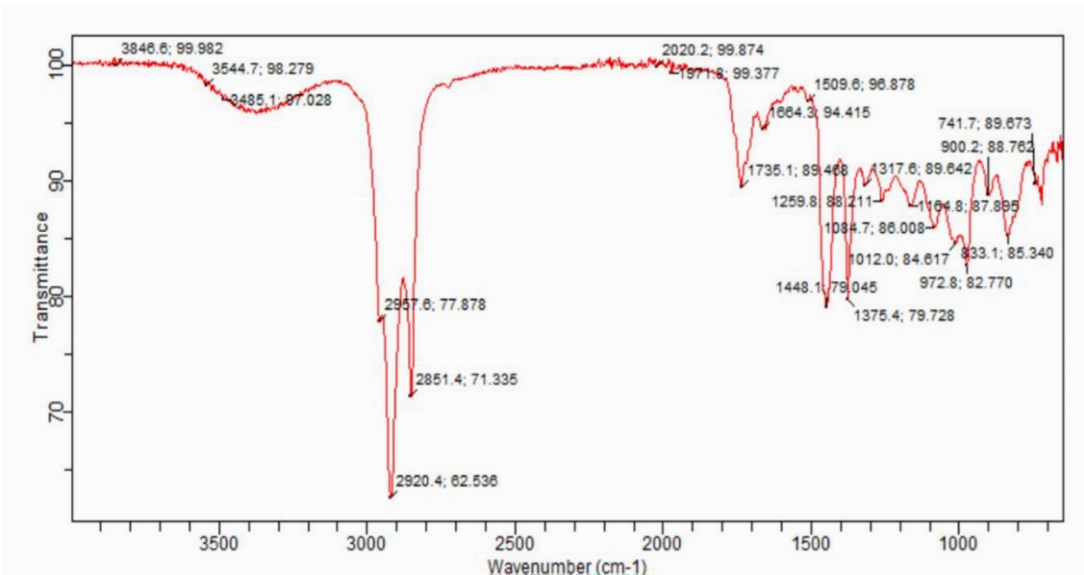


Fig 1 FTIR Spectrum of Fraction 2

Mass Spectrum and Fragmentation of τ -Cadinol ($C_{15}H_{26}O$, $[M]^+$ - m/z 222,)

GC-MS analysis identified τ -cadinol in the extract based on its retention time and electron impact (EI) mass spectrum, matching the NIST Chemistry WebBook reference (Fig. 2). The molecular ion at m/z 222 corresponds to the sesquiterpene alcohol formula $C_{15}H_{26}O$. Initial fragmentation involves dehydration of the tertiary alcohol, losing H_2O (18 amu) to form the stable m/z 204 ($C_{15}H_{24}^+$) ion (Fig. 3). This energetically favored process enhances π -bond character in the bicyclic cadinane skeleton, aligning with spectra of cadinane sesquiterpenes (National Institute of Standards and Technology, 2023; Rabe *et al.*, 2016).

From m/z 204, α -cleavage (allylic) eliminates the isopropyl radical (C_3H_7 , 43 amu), yielding the diagnostic m/z 161 ($C_{12}H_{17}^+$) ion. This even-electron carbocation is stabilized by conjugation in the bicyclic system, serving as a marker for cadinane frameworks (Wen *et al.*, 2009). Further rearrangement of m/z 161 promotes aromatization, losing C_2H_3 (27 amu) to give m/z 134 ($C_{10}H_{14}^+$), a resonance-stabilized benzene-type radical cation. Sequential benzylic cleavages produce stable endpoints at m/z 119 (loss of CH_3 , 15 amu) and m/z 105 (loss of C_2H_5 , 29 amu). Final breakdown yields m/z 67 ($C_5H_7^+$) via C_4H_4 neutral loss, reflecting terpenoid aromatization patterns.

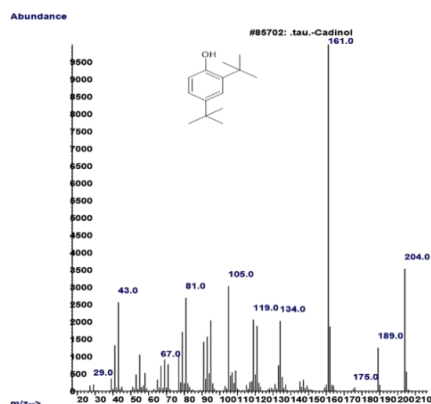


Fig 2: Mass spectrum of tau-Cadinol

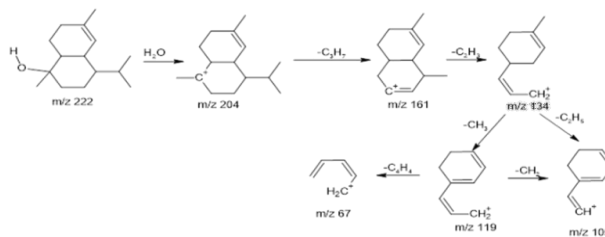


Fig: 3 Fragmentation pathways of tau-cadinol

Mass Spectrum and Fragmentation Methyl Oleate ($C_{19}H_{36}O_2$, $[M]^+$ - m/z 296)

GC-MS analysis identified 9-octadecenoic acid (Z) methyl ester (methyl oleate) based on its retention time and electron impact (EI) mass spectrum, matching NIST Chemistry WebBook reference data and fatty acid methyl ester studies (National Institute of Standards and Technology, 2003; Boshra, 2023). The molecular ion at m/z 296 aligns with the formula $C_{19}H_{36}O_2$ for this monounsaturated ester (Fig. 4). Initial fragmentation features methanol elimination (CH_3OH , 32 amu) from the ester, yielding m/z 264 ($C_{18}H_{32}O^+$), a resonance-stabilized acylium cation. This primary pathway, common in aliphatic esters, enhances π -delocalization near the carbonyl (JEOL Ltd., 2016).

Subsequent cleavages, influenced by the 9,10-double bond, involve allylic β -cleavage, losing a C_5H_8 fragment (68 amu) to form m/z 222 ($C_{13}H_{26}O^+$). This conjugated acylium/allylic cation reflects chain

truncation at the olefinic site (**Fig. 5**). Further β -cleavages shorten the chain: loss of C_3H_6 (42 amu) from m/z 222 gives m/z 180, a delocalized acylium ion. Additional rearrangement yields m/z 137 via C_4H_7 loss, forming an oxygenated carbocation typical of ladder patterns in unsaturated esters (Kiuchi *et al.*, 2025). Low-mass fragmentation culminates in the base peak at m/z 55 ($C_4H_7^+$) a stable conjugated allylic cation from extensive chain breakdown, hallmarking methyl oleate spectra.

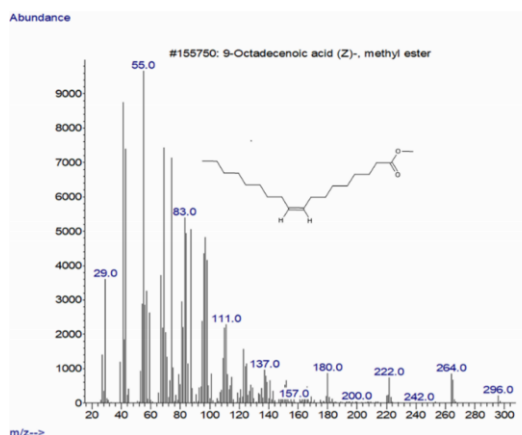


Fig 4: Mass spectrum of 9-Octadecenoic Acid (Z) Methyl Ester

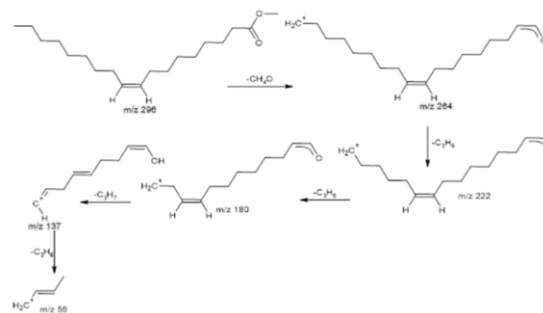


Fig 5 Fragmentation pathways 9-Octadecenoic Acid (Z) Methyl Ester

FT-IR Spectral Analysis of Fraction 3: Pentacyclic Triterpene Acetates

FT-IR analysis of the triterpene acetate fraction revealed a broad, weak O–H stretch at 3400–3300 cm^{-1} , indicative of trace unesterified alcohols or moisture in triterpene mixtures. Strong aliphatic C–H stretches at 2930–2850 cm^{-1} reflect the saturated pentacyclic skeleton. The diagnostic intense band at 1733 cm^{-1} confirms ester C=O stretching from C-3 acetylation, distinguishing acetates from free alcohols (**Fig. 6**). Olefinic C=C stretch appears at 1634 cm^{-1} , characteristic of oleanane/ursane frameworks. CH_3 bending/skeletal modes occur at 1455–1369 cm^{-1} , while C–O stretches at 1245–1020 cm^{-1} further validate ester functionality.

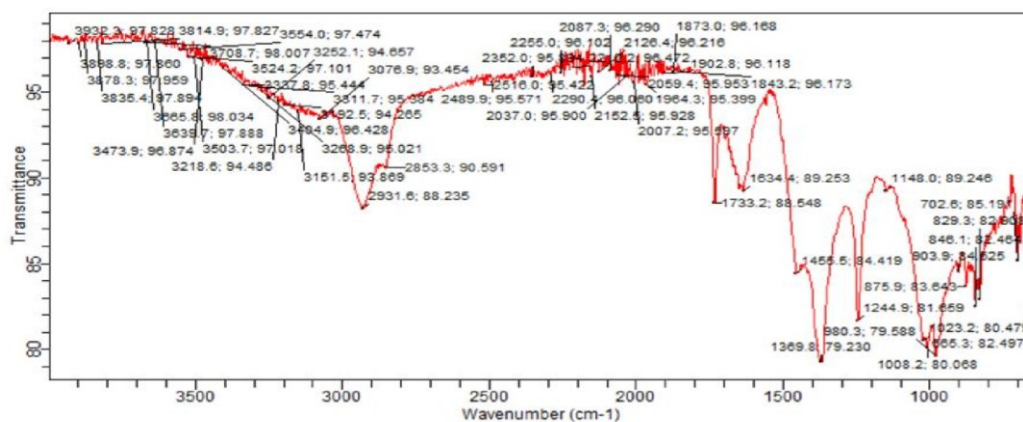


Fig 6: FTIR of Fraction 3

Mass Spectrum and Fragmentation of Lupeol Acetate ($C_{32}H_{52}O_2$, $[M]^+$ - m/z 468)

Fraction 3, isolated as a white to off-white amorphous solid, comprised predominantly pentacyclic triterpene acetates—lupeol acetate, olean-12-en-3-ol acetate, and urs-12-en-3-ol acetate—as determined by GC-MS. These structurally similar, low-polarity compounds co-eluted during chromatography, forming the fraction's major constituents with trace impurities, rendering it ideal for spectrometric and bioactivity studies. Lupeol acetate ($C_{32}H_{52}O_2$), a lupane-type triterpenoid with C-3 acetylation, exhibits a molecular ion at m/z 468 (**Fig. 7**). Initial fragmentation cleaves the acetyl group, losing C_2H_3O (43 amu) to yield m/z 425 ($C_{30}H_{49}^+$), a triterpenyl cation typical of triterpene esters (Silverstein *et al.*, 2015).

Subsequent demethylation and side-chain losses, facilitated by 1,2-methyl shifts, produce m/z 393 (loss of CH_4O 30 amu) and m/z 357 (loss of $C_7H_{11}O$, 111 amu), reflecting stabilized allylic carbocations in the lupane skeleton (**Fig. 8**). Further elimination of C_3H_8O (60 amu) from m/z 357 affords m/z 298, a compact polycyclic ion. Final breakdown loses an C_8H_{13} fragment (109 amu), generating the diagnostic m/z 189 conjugated cyclopentenyl cation, indicating lupeol acetate and validating the fragmentation pathway.

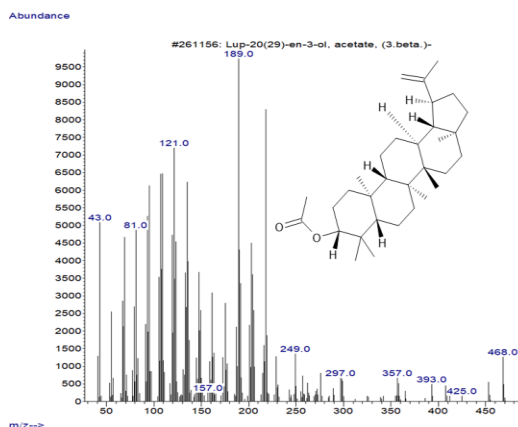


Fig 7: Mass spectrum of Lupeol Acetate

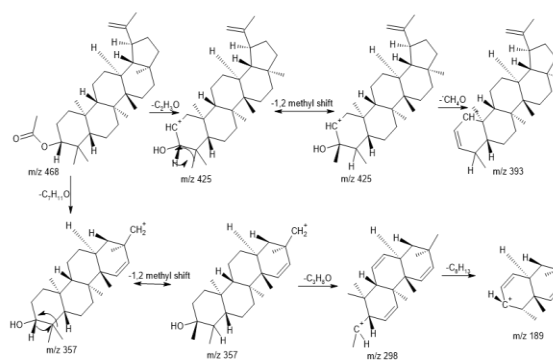


Fig 8 Fragmentation pathways of Lupeol Acetate

Mass Spectrum and Fragmentation of Olean-12-en-3-yl Acetate ($C_{32}H_{52}O_2$, $[M]^+$ - m/z 468,)

Olean-12-en-3 β -yl acetate, an oleanane-type pentacyclic triterpenoid ($C_{32}H_{52}O_2$) exhibits characteristic EI fragmentation with major ions at m/z 468, 425, 393, 313, and 257, consistent with oleanane ester mechanisms (**Fig. 9**) (Doshi *et al.*, 2015). The molecular ion at m/z 468 undergoes initial acetyl loss (C_2H_3O , 43 amu), yielding m/z 425 ($C_{30}H_{49}^+$). Subsequent 1,2-methyl shift stabilizes a tertiary carbocation, facilitating CH_4O elimination (32 amu) to form m/z 393, a consistent with that of oleanane triterpenoids (Uddin *et al.*, 2022). Rearrangement of m/z 393 promotes ring/side-chain cleavage, losing C_6H_8 (80 amu) to generate m/z 313, an oleanane core ion. Further alkyl cleavage via C_4H_8 loss (56 amu) affords m/z 257, a conjugated carbocation (**Fig. 10**). Progressive cleavages

yield low-mass ions at m/z 218, 177, 147, and 107 through competitive ring junction and substituent breakdowns, matching reference spectra and validating the oleanane skeleton (Doshi *et al.*, 2015).

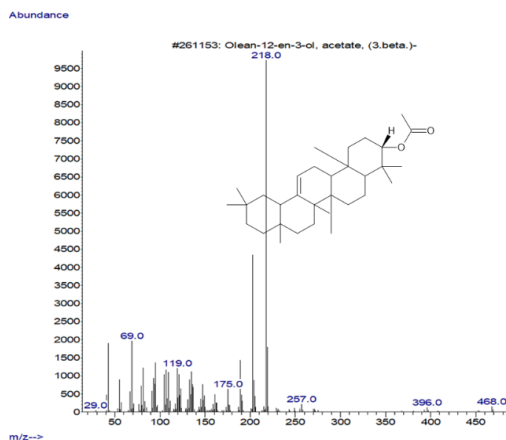


Fig 9: Mass spectrum of Olean-12-en-3β-yl Acetate

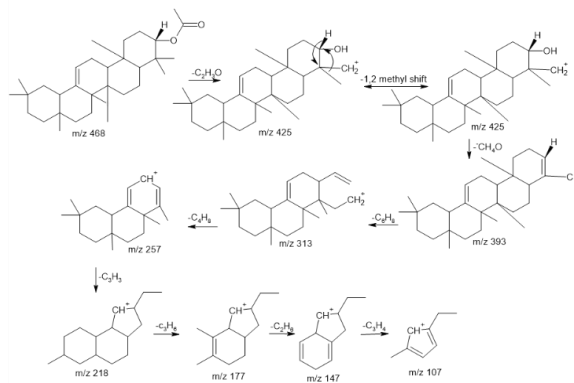


Fig 10 Fragmentation pathways of Olean-12-en-3β-yl Acetate

Mass Spectrum and Fragmentation of Urs-12-en-3-yl Acetate ($C_{32}H_{52}O_2$, $[M]^+$ - m/z 468)

Urs-12-en-3β-yl acetate ($C_{32}H_{52}O_2$), an ursane-type pentacyclic triterpenoid acetate, displays characteristic EI fragmentation with a stable molecular ion at m/z 468, followed by high-mass fragments from side-chain and acyl cleavages, matching reference ursane spectra (Fig. 11); Van Doelen, 1999). Initial loss of a C_3H_7 unit (43 amu) from the molecular ion yields m/z 409, reflecting demethylation or alkyl elimination with intact 3-acetoxy functionality. Subsequent cleavage of C_5H_8 (68 amu) from m/z 409 produces m/z 325, an ursane core ion with truncated side chain (Fig. 12). Further degradation loses C_4H_7 (55 amu) to form m/z 270, indicative of progressive alkyl stripping. Elimination of C_4H_4 (52 amu) generates m/z 218, a compact cyclic carbocation post-ring contraction. Final steps involve C_4H_8 loss to m/z 161, then C_6H_6 elimination yielding m/z 83 ($C_6H_7^+$), a common allylic fragment in triterpenoid spectra, validating the ursane skeleton.

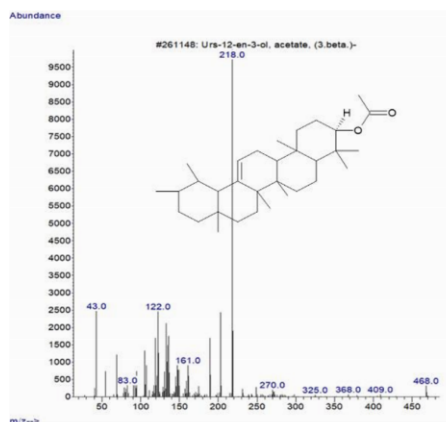


Fig 11: Mass spectrum of Urs-12-en-3β-yl acetate

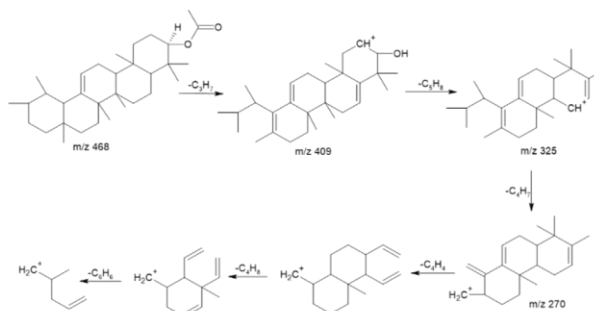


Fig 12 Fragmentation pathways Urs-12-en-3β-yl acetate

FT-IR Spectral Analysis of Fraction 6: Sterically Hindered Phenol and Ethyl Linolenate

FT-IR analysis revealed broad O-H stretches at 3500–3200 cm^{-1} , indicative of hydroxyl groups. Intense CH (sp^3) stretches at 2924.1 and 2853.3 cm^{-1} confirm extensive methylene and methyl chains in aliphatic hydrocarbons. Bending modes at 1459.3 cm^{-1} (CH_2 scissoring) and 1377.3 cm^{-1} (CH_3) support saturated alkyl structures (**Fig. 13**). A sharp carbonyl stretch at 1736.9 cm^{-1} signifies ester or saturated carboxylic acid functionality. Weak C=C at 1664.3 cm^{-1} indicates minor unsaturation. C-O stretch at 1164.8 cm^{-1} , combined with the carbonyl, confirms ester presence. The isolated fraction was obtained as a light yellow oily to semi-solid mixture, comprising predominantly 2,4-di-tert-butylphenol and 9,12,15-octadecanoic acid ethyl ester, as confirmed by GC–MS analysis.

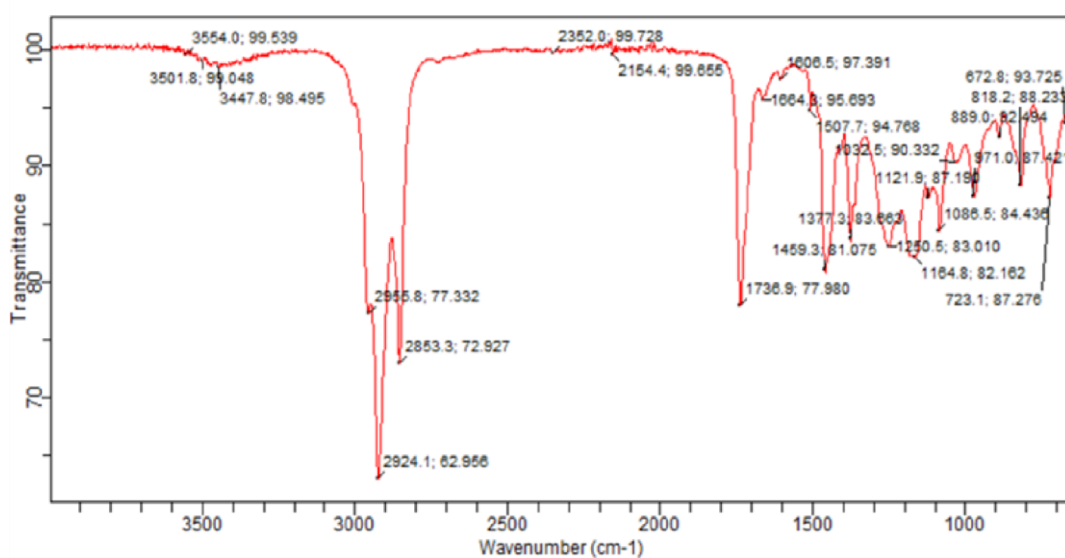


Fig 13 FTIR Spectrum of Fraction 6

Mass Spectrum and Fragmentation of 2,4-Di-tert-butylphenol ($\text{C}_{14}\text{H}_{22}\text{O}$, $[\text{M}]^+$ - m/z 206)

Hindered phenol, 2,4-Di-tert-butylphenol $\text{C}_{14}\text{H}_{22}\text{O}$ exhibits a molecular ion at m/z 206 in EI mass spectrometry, with characteristic fragments at m/z 191, 163, 135, 91, 57, and 41 from stepwise alkyl cleavages, matching NIST reference spectra (**Fig. 14**) (NIST, 2013). Initial α -cleavage loses a methyl radical (CH_3 15 amu) from a tert-butyl group, yielding the base peak benzylic cation at m/z 191. A 1,5methyl shift stabilizes this ion (**Fig. 15**). Subsequent β -cleavage eliminates C_2H_4 (28 amu) to form m/z 163, followed by another C_2H_4 loss producing m/z 135 (mono-tert-butylphenol cation). From m/z 135, competing pathways generate m/z 91 (benzyl/tropylium via $\text{C}_2\text{H}_4\text{O}$ loss), m/z 57 (allylic oxonium or butyl cation) via benzene (C_6H_6 , 78 amu), and further breakdown to m/z 41 and 15 via propene elimination. These patterns reflect typical branched alkylphenol fragmentation (Doshi *et al.*, 2015).

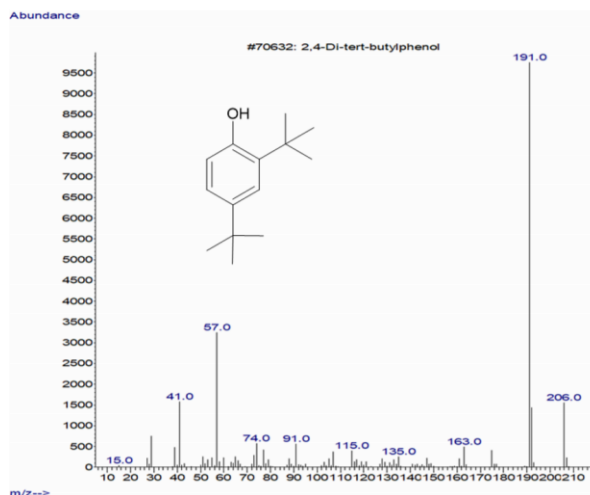


Fig 14: Mass spectrum of 2,4-Di-Tert-Butylphenol

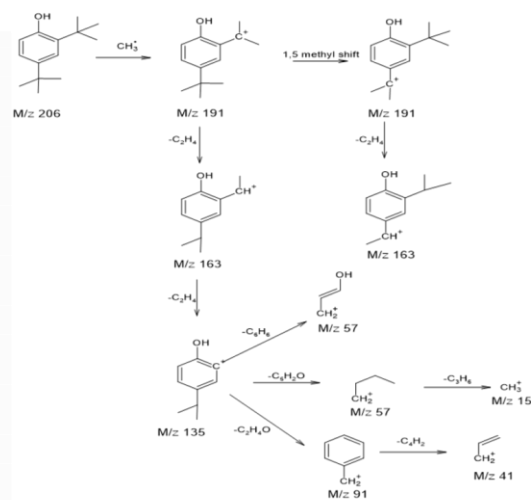


Fig 15 Fragmentation pathways of 2,4-Di-Tert-Butylphenol

Mass Spectrum and Fragmentation of 9,12,15-Octadecatrienoic Acid, Ethyl Ester, (Z,Z,Z) ($C_{20}H_{34}O_2$, $[M]^+$ m/z 306) The EI mass spectrum of 9,12,15-octadecatrienoic acid ethyl ester ($C_{20}H_{34}O_2$) shows a molecular ion at m/z 306, matching reference data **Fig. 16** (NIST Chemistry WebBook, 2015). The prominent m/z 261 arises from α -cleavage at the ester carbonyl (C_2H_5O 45 amu), forming an acylium ion typical of polyunsaturated fatty acid ethyl esters. Successive allylic cleavages along the triene chain yield high-mass fragments at m/z 191 and 163, reflecting shortened conjugated cations (**Fig. 17**). Medium-mass ions at m/z 163 and 135 result from hydride shifts and charge migration to stable allylic species. Lowmass peaks at m/z 79 ($C_6H_7^+$), 55 ($C_4H_7^+$), and 29 ($C_2H_5^+$) emerge from progressive β -cleavages, characteristic of unsaturated ester fragmentation (Doshi *et al.*, 2015).

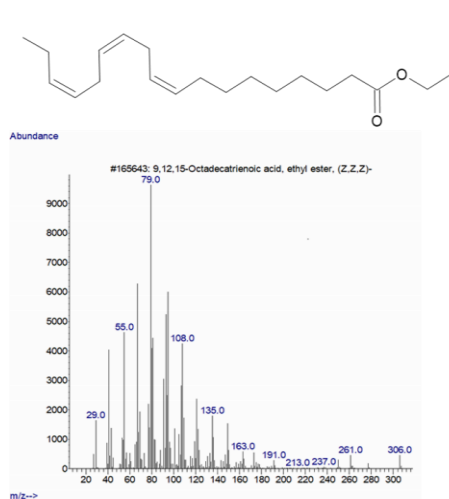


Fig 16: Mass spectrum 9,12,15-Octadecatrienoic Acid, Ethyl Ester, (Z,Z,Z)

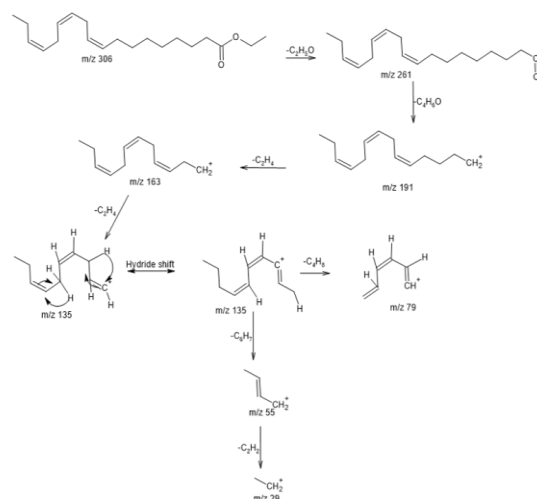


Fig 17 Fragmentation pathways of 9,12,15-Octadecatrienoic Acid, Ethyl Ester, (Z,Z,Z)

DPPH radical scavenging assay

The percentage inhibition produced by the methanol leaves extract was significantly different ($P < 0.05$) from that of Ascorbic acid (the standard antioxidant). The Scavenging effect of the extract occurred in a dose dependent manner as presented in (Fig. 18) below.

Ferric Reducing antioxidant power (FRAP)

The percentage inhibition produced by the methanol leaves extract was significantly different ($P < 0.05$) from that of Trolox standard (the standard antioxidant). The extract showed concentrationdependent inhibition. This was evident in the transformation of ferric ions to its ferrous form at 700 nm as shown in (Fig. 19).

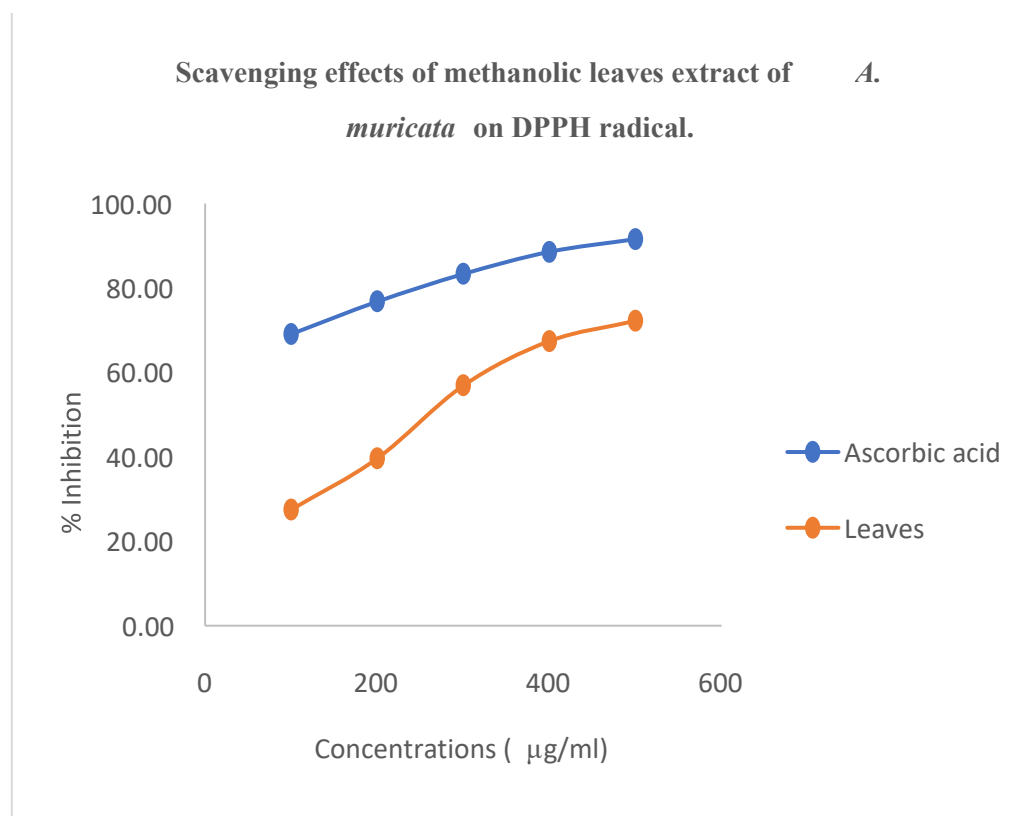


Fig. 18: Scavenging activity of methanol extract of *A. muricata* leaves on DPPH radical.

Table 2. IC₅₀ of DPPH radical.

Samples	IC ₅₀ (µg/mL)
<i>A. muricata</i> Leaves Extact	260.17
Ascorbic Acid	72.38

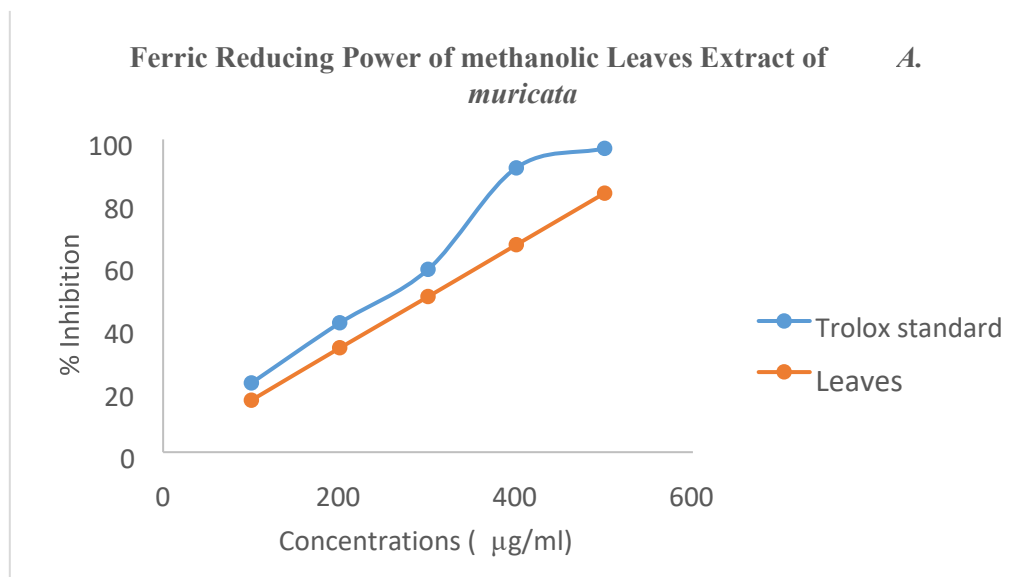


Fig. 19: Ferric reducing ability of methanol extract of *A. muricata* leaves.

Table 3. IC₅₀ of Ferric Reducing antioxidant Power

Samples	IC ₅₀ (µg/mL)
Trolox Standard	251.00
<i>A. muricata</i> Leaves Extract	302.00

CONCLUSION

In conclusion, the results highlight Soursop leaves as a promising source of natural bioactive compounds. The plant-based bio-active compounds have the effective dosage response with minimal side effects, when compared to the synthetic compounds. The quantitative study conducted on *A. muricata* leaves revealed the reasonable quantitative of total phenolics and flavonoids which enhances its antioxidant activity, as evidenced by decreased IC₅₀ values. The presence of this secondary plant product is responsible for their healing effects. It further reflects a hope for the development of many novel therapeutic agents from the plant which in future may serve to produce synthetically improved therapeutic drugs. The data obtained from this study implies that the methanol extract of *A. muricata* leaves and is rich in antioxidants. It is reasonable to speculate that the chemical constituents are responsible for the potent antioxidant properties exhibited by the plant. However, the mechanism of action of these compounds should be revealed.

Acknowledgement

The authors acknowledged and express their sincere gratitude to Tertiary Education Trust Fund (TETFund) for their support in sponsoring our research project through the institution based research (IBR) funding. This funding has been instrumental in advancing our research goals and contributing to

the academic and intellectual growth of our institution. We would also like to appreciate our Rector and the ICR for given us the opportunity to carry out this research work.

Reference

- Anwar S, Shamsi A, Shahbaaz M, Queen A, Khan P, Hasan G.M, Islam A, Alajmi M.F, Hussain A, Ahmad F. (2020). Rosmarinic acid exhibits anticancer effects via MARK4 inhibition. *Science*10, 1–13.
- Atiko, R., Musba'u, I. B., Kwaji, A., Nyakuma, B. B., Pius, P., Umar, I. M., & Muhammad, A. I. (2025). Cytotoxicity and Isolation of Betullinic Acid from *Anthonothea noldeae* Stem Bark. *Biology Medicine & Natural Product Chemistry*, 14(1), 193–197. <https://doi.org/10.14421/biomedich.2025.141.193-197>
- Bhatia K, Bhumika and Das A. (2020). Combinatorial drug therapy in cancer new insights. *Life Science*. 258, 118134. doi:10.1016/j.lfs.2020.118134
- Bray F, Ferlay J, Soerjomataram I, Siegel R.L, Torre L.A, Jemal A. (2018). Global cancer Statistics – GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer Journal for Clinicians* 68, 394–424.
- Coria-Téllez, A.V.; Montalvo-González, E.; Yahia, E.M.; Obledo-Vázquez, E.N. (2018). Annonamuricata: A Comprehensive Review on Its Traditional Medicinal Uses, Phytochemicals, Pharmacological Activities, Mechanisms of Action and Toxicity. *Arab. Journal of Chemistry*. 2018, 11, 662–691. [CrossRef]
- Ding Y, Hou R, Yu J, Xing C, Zhuang C, Qu Z. (2023). Dietary Phytochemicals as Potential Chemopreventive Agents against Tobacco-Induced Lung Carcinogenesis. *Nutrients*:15, 491.
- Doshi, G. M., Adsule, P. G., Banerjee, K., & Oulkar, D. P. (2015). Mass spectroscopy. *Pharmacognosy Research*, 7(3), 282-289. <https://www.phcogres.com/sites/default/files/PharmacognRes-7-3-282.pdf>
- Gavamukulya, Y.; Wamunyokoli, F.; El-Shemy, H.A. (2017). Annona muricata: Is the Natural Therapy to Most Disease Conditions Including Cancer Growing in Our Backyard? A Systematic Review of Its Research History and Future Prospects. *Asian Pacific Journal Tropical Medicine*, 835–848. [CrossRef]
- Gyamfi, K.; Sarfo, D.K.; Nyarko, B.J.B.; Akaho, E.K.H. (2011). Assessment of Elemental Content in the Fruit of Graviola Plant, Annona muricata, from Some Selected Communities in Ghana by Instrumental Neutron Activation Analysis. *Elixir Food Science*, 41, 5676– 5680.
- Hamma I. I., U. M. Jajere and Gimba A.M (2024). Phytochemical Composition and Antioxidant Activity of Maerua angolensis used for Cancer Treatment in Funakaye Local Government, Gombe State, Nigeria. *Bima Journal of Science and Technology*, Vol. 8(1A). DOI: 10.56892/bima.v8i1.602
- Hartwig A, Arand M, Epe B, Guth S, Jahnke G, Lampen A, Martus H.J, Monien B, Rietjens IMCM, Schmitz-Spanke S, Schriever-Schwemmer G, Steinberg P, Eisenbrand G. (2020). Mode of action-based risk assessment of genotoxic carcinogens. *Arch. Toxicology*. 94, 1787–1877. doi:10.1007/s00204-020-02733-2
- JEOL Ltd. (2016). *Integrated analysis of fatty acid methyl esters using GC–EI–MS* (MSTips 301). JEOL Application Note.
- Kiuchi, T., Gerbaulet, M., Möllerke, A., Harig, T., Dinter, A., Beuerle, T., & Schulz, S. (2025). Determination of Methyl Group Positions in Long-Chain Aliphatic Methyl Ethers and Alcohols by Gas Chromatography/Orbitrap Mass Spectrometry. *Analytical chemistry*, 97(31), 17150–17157. <https://doi.org/10.1021/acs.analchem.5c03083>
- Khaltaev N, and Axelrod S. (2020). Global lung cancer mortality trends and lifestyle modifications: preliminary analysis. *Chinese Medical Journal*. 133(13): 1526–1532. doi:10.1097/Cm 9.0000000000000918
- Mbele M, Hull R and Dlamini Z. (2017). African medicinal plants and their derivatives: Current efforts towards potential anti-cancer drugs. *Experimental Molecular Pathology* 103, 121– 134. [CrossRef]
- Mir T.u.G, Wani A.K, Singh J, Shukla S. (2022). Therapeutic Application and Toxicity associated with Crocus Sativus (Saffron) and Its Phytochemicals. *Pharmacology Research in Modern Chinese Medicine* 4, 100136. <https://doi.org/10.1016/j.prmcm.2022.100136>.
- Moghadamtousi, S.Z.; Fadaeinasab, M.; Nikzad, S.; Mohan, G.; Ali, H.M.; Kadir, H.A. (2015). Annona muricata (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *International Journal of Molecular Science*. 16, 15625–15658. [CrossRef] [PubMed]

- Najmi A, Javed S.A, Al Bratty M and Alhazmi H.A (2022). Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents. *Molecules*, 27, 349. <https://doi.org/10.3390/molecules27020349>
- National Institute of Standards and Technology (2003). 9-Octadecenoic acid (Z)-, methyl ester. In *NIST Chemistry WebBook*, SRD 69.
- National Institute of Standards and Technology. (2023). α -Cadinol mass spectrum. In *NIST Chemistry WebBook* (SRD 69). <https://webbook.nist.gov>
- NIST Chemistry WebBook. (2015). 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) (C₂₀H₃₄O₂). National Institute of Standards and Technology. <https://webbook.nist.gov/cgi/cbook.cgi?ID=C1191419>
- NIST. (2013). 2,4-Di-tert-butylphenol – Mass spectrum (electron ionization). NIST Chemistry WebBook. Retrieved December 18, 2025, <https://webbook.nist.gov/cgi/cbook.cgi?ID=C96764&Mask=200#Mass-Spec>
- Pinto, L.C.; Cerqueira-Lima, A.T.; Suzarth, S.D.S.; de Souza, R.; Tosta, B.R.; da Silva, H.B.; Pires, A.D.O.; Queiroz, G.D.A.; Teixeira, T.O.; Dourado, K.M.C.; et al. (2018). Annona muricata L. (Soursop) Seed Oil Improves Type 1 Diabetes Parameters in Vivo and in Vitro. *Pharma Nutrition* 6, 1–8. [CrossRef]
- Rabe, P., Lüdeke, K. A. J., & Dickschat, J. S. (2016). The EIMS fragmentation mechanisms of the sesquiterpenes corvol ethers A and B, epicubebol and isodauc-8-en-11-ol. *Beilstein Journal of Organic Chemistry*, 12, 1339–1351. <https://10.3762/bjoc.12.132>
- Rai P.K, Lee S.S, Zhang M, Tsang Y.F, and Kim K. (2019). Heavy metals in food crops: Health risks, fate, Mechanisms, and Management. *Environment International*, 125:365–385 <https://doi.org/10.1016/j.envint.2019.01.067>
- Ranjan A, Ramachandran S, Gupta N, Kaushik I, Wright S, Srivastava S, Das H, Srivastava S, Prasad S, Srivastava S.K. (2019). Role of phytochemicals in cancer prevention. *International Journal of Molecular Science*. 20, 4981.
- Sanford N.N, Sher D.J, Xu X, Ahn C, D’Amico A.V, Aizer A.A and Mahal B.A. (2020). Alcohol use among patients with cancer and survivors in the United States, 2000-2017, *Journal of the National Comprehensive Cancer Network* 18, 1; doi:10.6004/jnccn.2019.7341
- Sharma R, Aashima, Nanda M, Fronterre C, Sewagudde P, Ssentongo A.E, Yenney K, Arhin N.D, Oh J, Amponsah-Manu F. (2022). Mapping Cancer in Africa: A Comprehensive and Comparable Characterization of 34 Cancer Types Using Estimates From GLOBOCAN 2020. *Frontier Public Health* 10, 839835. [CrossRef]
- Silverstein, R. M., Webster, F. X., Kiemle, D. J., & Bryce, D. L. (2015). Spectrometric identification of organic compounds (8th ed.). Hoboken, NJ: John Wiley & Sons.
- Siegel R.L, Miller K.D, Fuchs H.E and Jemal A. (2022). Cancer statistics. *CA Cancer Journal of Clinicians* 72, 7–33. [CrossRef] [PubMed]
- Sofowora A. (2008). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 3rd Edition. 200-202.
- Sung H, Ferlay J, Siegel R.L, Laversanne M, Soerjomataram I, Jemal A and Bray F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer Journal Clinicians* 2021, 71, 209– 249. [CrossRef]
- The Global Cancer Observatory (2021). Nigeria. International Agency for Research on Cancer, World Health Organization. <https://gco.iarc.fr/today/data/factsheets/populations/566nigeria-fact-sheets.pdf> Google Scholar
- Uddin, J., Muhsinah, A. B., Imran, M., Khan, M. N., & Musharraf, S. G. (2021). Structure–fragmentation study of pentacyclic triterpenoids using electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-QTOFMS/MS). *Rapid Communications in Mass Spectrometry*, 36(4), e9243. <https://doi.org/10.1002/rcm.9243>
- Van Doelen, G. A. (1999). *Molecular studies of fresh and aged triterpenoid varnishes* (Doctoral thesis). University of Amsterdam.
- Wen, W., Li, F., Ji, N. Y., Li, X. M., Cui, C. M., Li, X. D., Zhang, L. N., Xue, Q. Z., & Wang, B. G. (2009). A new cadinane sesquiterpene from the marine brown alga Dictyopteris divaricata. *Molecules (Basel, Switzerland)*, 14(6), 2273–2277. <https://doi.org/10.3390/molecules14062273>