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Gas chromatography-mass spectrometry profile and acute toxicity studies of *Annona muricata* leaf ethanol extract.

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Abstract

Medicinal plants are primary source of bio-active compounds applied in the management of diseases. The present work focuses on determining the acute toxicity of *Annona muricata* leaf ethanol extract and the volatile organic constituents using Gas Chromatography-Mass Spectrometry. The acute toxicity test of the extract of *A. muricata* was determined using standard method. The GC-MS profiling of the extract obtained 13 compounds at different retention times. The dominant chemical constituents in the extracts of *A. muricata* leaf include: Linoelaidic acid (30.69%), Oleic acid (12.98%), 6-octadecenoic acid (10.99%), n-Hexadecanoic acid (9.28%), Octadecanoic acid (7.73) and 10-Octadecanoic acid (6.69%). Acute toxicity studies showed that LD_{50} was >5000 mg/kg body weight. The extracts of *A. muricata* are rich in a battery of bioa-active compounds that may be pharmacologically responsible for its known medicinal properties and considered generally safe.

Keywords: Phytochemicals; Gas Chromatography; Mass Spectrometry; Acute toxicity.

1. Introduction

Medicinal herbs and other plant-derived products form the bedrock for development of modern therapeutic interventions. In Africa and Asia, indigenous herbs are used as readily available medicinal interventions for treatment of a wide variety of ailments have proven effective; and also guided drug discovery efforts through identification of these pharmacological active plants. Medicinal plants have historically been used as powders, tinctures, teas, and poultices, then as formulations, and finally as pure chemicals (Kumar *et al.,* 2015).

Plants are a novel source of natural bioactive compounds, of which numerous are yet to be identified or effectively isolated. Their adaptation against bacteria, insects, fungus, and extreme weather, are responsible for the production of distinctive secondary metabolites with a variety of structural features (Fellows and Scofield, 1995). They have been the source of the majority of pharmaceuticals' active components. Recently, modern techniques of synthetic chemistry applied in drug discovery hold the dominance in drug development; however this does not erode the potential of plants to provide new and novel products for disease treatment (Raskin *et al.,* 2002).

Plant phytochemicals have played a significant part in the pharmaceutical discovery process during the last century. Significant research interest in the biological activities of phyto-constituents was driven by the medicinal value of these

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chemicals (Moghadamtousi *et al.*, 2013; Asiwe *et al.,* 2021; Asiwe *et al.,* 2023, Ezirim *et al*., 2024). In all, majority of plant species have not been exhaustively studied for their activity and possible mechanism of action. Plants with a long held historical application in ethno-medicine is a direct pointer to abundance of useful phyto-constituents. *Annona muricata* is commonly identified with names such as sour sop (English), '*Ebo'* or '*Apekan* in Yoruba, '*Shawshopu'* or '*Sawansop'* in Igbo, '*Tuwon Biri'* or '*Mama'* in Hausa, 'Graviola' (Portuguese). *Annona muricata* is part of over 130 genera and 70 species belonging to the *Annonaceae* family. (Moghadamtousi *et al.,* 2015; Makeri *et al*., 2015; Opara *et al.,* 2024).

A. muricata is cultivated widely throughout the West Indies, and in some countries such as China, Australia, India, Southern and Northern America, Africa and it is becoming invasive in tropical climates throughout the world (Opara *et al.,* 2021). The plant is grown for its fruit and other allied uses throughout the Southern Nigeria. The different parts of *A. muricata* (fruits, leaf, stem bark, root and root bark) has been studied and documented for their benefits; which has significantly improved and elevated the primary health care system and interest in plant pharmacology, particularly because these plant extracts are thought to be safer than synthetic drugs. According to Okigbo and Mmeka (2006), the main shortcomings of ethno-medicine are imprecise diagnosis and lack of precision in dosing, which are particularly problematic for chronic and complex illnesses.

Several medicinal properties have been attributed to the leaves of *A. muricata*; according to Arthur *et al.,* (2011), these include anti-parasitic, anti-diarrhoeal, anti-rheumatic, anti-neuralgic, anti-spasmodic, astringent, hepato-protective, gastro-protective, anti-diabetic, gastric upsets, jaundice, and kidney ailments. Infusion of the leaves has also been used to treat fever, respiratory illness, malaria, hypotension, and cancer (Arthur *et al.,* 2011; Coria-Tellez *et al*., 2016). The annonaceousacetogenins including murihexocin and annocuricin, annopentocin A, B and C, (2,4-cis)-annomuricin-D-one, murihexocin A and B, (2,4-trans)-annomuricin-D-one, 4-acetyl gigantetrocin and cis-gigantrionin, muricatocin A, B and C, and annohexocin are found in the leaves of *A*. *muricata*. These chemical constituents hold high potency, selectivity, biological activity, and effectiveness against microbial resistance may form the bed-rock of the next class of antitumor, immunomodulators, anti-spasmodic, anti-malarial and bio-pesticidal agents (Arthur *et al.,* 2011; Usunobun and Okolie, 2015; Coria-Tellez *et al*., 2016). The works of Adeyemi *et al.,* (2009) and Opara *et al.,* (2021) described the hypoglycemic and hypolipidemic effect in alloxan-induced diabetes.

Phytochemicals including alkaloids, flavonoids, phenolic compounds, glycosides, saponins, tannins, terpenoids, and phytosterols have been identified as various components of *A. muricata* (Santhi and Sengottuvel, 2016; Opara *et al.,* 2021). The present study aims to identify using GC-MS, the chemical components of ethanol leaf extract of *A. muricata* and its acute toxicity.

2. Materials and methods

2.1. Sample Collection, Identification, and Preparation of *Annona muricata* Leaf

The apparently fresh and healthy leaves of *A. muricata* were collected from a garden in Umunnemochie Akabo, Ikeduru L.G.A of Imo State, Nigeria. The plant materials were identified, deposited and assigned herbarium number NAUH-004B by Mr. Finan Iroka, the taxonomist at the Department of Botany, Nnamdi Azikiwe University, Awka. The leaves were separated from the stalk and washed thoroughly in clean portable water; and air-dried to constant weights at room temperature (28 ± 2 ^{0}C). The dried leaves were milled to fine powder using a mechanical grinder (Corona).

2.2. Experimental Animals

The animals used in the study were twelve (12) male Wistar albino rats (*Rattus norvegicus*) averaging 80-100 g in body weight. They were purchased from the animal house at the Biological Sciences Faculty, University of Nigeria Nsukka, Enugu State. All animals were acclimatized for 7 days prior to the study; and housed under standard conditions of light, temperature, and humidity, with access to standard commercial rat pellets and portable water *ad libitum.* The study was carried out at the animal house of Anatomy Department, Imo State University Owerri

2.3. Ethical Clearance

The entire study, plant collection, processing and animal handling strictly followed Ethical guidelinesby National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals (Pub. No. 85-23 Revised 1985) approved by the Nnamdi Azikiwe University, Awka Ethical Committee for the use of Laboratory Animals.

2.4. Extraction from Plant Leaf

The extraction of the plant materials followed the method described by Usunobun *et al*., (2015) and Sanni *et al.,* (2014) respectively. This was by maceration of 100 g portion of the dry powdered plant leaves in 1000 ml of 70% ethanol solution at room temperature for 48 hrs. The extracts were filtered using a muslin cloth, and then through Whatman filter paper No.4. The filtrate was concentrated at 55 ^{o}C using a water bath to obtain a slurry sediment. The crude extracts of the leaves were then stored at 4 ^{o}C in the refrigerator and applied in the studies. An aliquot portion of the crude plant extract was weighed and used for GC-MS profiling while the portion for acute toxicity study was reconstituted in distilled water.

2.5. GC-MS Analysis

The analysis followed standard procedure described by Kanthal *et al.,* (2014). The investigation of ethanol extracts of the leaf of *A. muricata* was performed on Thermo GC-TRACE ultra version: 5.0, Thermo MS DSQ II. The following experimental conditions prevailed: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. The flow rate of the mobile phase (carrier gas: Helium) was set at 1.0 ml/min. In the gas chromatography part, the experimental conditions were temperature: 250 °C raised at 5 °C/min, injection volume (1 µl), holding temperature: 280 ^{o}C raised at the rate of 20 ^{o}C/min, holding time: 8minutes,detection temperature: 250 ^{o}C, split ratio and ionization voltage were 110:1 eV respectively. The unknown components present in the extract, were identified by matching the mass spectral peak value with the National Institute of Science and Technology database.

2.6. Acute Toxicity Testing

The median lethal dose (LD_{50}) of *A. muricata* leaf ethanol extract was determined in rats using the method of Lorke (1983), with modifications as described by Aroma and Enegide (2014). The study was segmented into two phases. The first phase consisted of nine (9) rats divided into three (3) groups of three (3) rats each and administered extract doses of 10, 100, and 1000 mg/kg body weight respectively by intubation. After administration, the animals were observed at regular intervals for 24 hours for the onset of adverse effects, time of death, or time of recovery. The second phase, consisted of three (3) rats were divided into three (3) groups each, and administered extract doses of 1600, 2900, and 5000 mg/kg body weight of the extract. They were observed for 24 hours for possible toxicity symptoms as well as for possible delayed toxicity symptoms for 14 days. The lethal dose was calculated using the formular:

$LD_{50} = \sqrt{(C_o \times C_{100})}$

Where C_o = highest with no mortality

C_{100} = lowest with mortality

3. Results and discussion

3.1. GC-MS profile of *A. muricata* leaf ethanol extract

The GC-MS analysis results showed that a total of 20 peaks were obtained in the chromatogram with different retention times. About 13 compounds were identified using their retention time, the abundance, molecular formular and weight are presented in table 1. The constituents: Linoelaidic acid (30.69%), Oleic acid (12.98%), 6-octadecenoic acid (10.99%), n-Hexadecanoic acid (9.28%), Octadecanoic acid (7.73) and 10-Octadecanoic acid (6.69%) were the most abundant in the extract.

Table 1 Volatile constituents of *A. muricata* leaf ethanol extract

SN	RT (mins)	Compound name	Abundance %	Molecular formular	Molecular weight (*g/mol*)
1	9.840	5-Octadecene	1.36	$C_{18}H_{36}$	252.48
2	13.113	Dodecanoic acid (Lauric acid)	2.60	$C_{12}H_{24}O_2$	200.3178
3	15.408	Tetradecanoic acid (Myristic acid)	2.10	$C_{14}H_{28}O_2$	228.37
4	16.952	Hexadecanoic acid (Palmitic acid)	1.49	$C_{16}H_{32}O_2$	256.43
5	17.538	n-Hexadecanoic acid	9.28	$C_{16}H_{32}O_2$	256.43

6	18.734	10-Octadecanoic acid	6.69	$C_{18}H_{34}O_2$	282.46
7	19.301	Linoelaidic acid	30.69	$C_{18}H_{32}O_2$	280.45
8	19.497	Octadecanoic acid	7.73	$C_{18}H_{36}O_2$	284.48
9	19.753	9,12-0ctadecadienoic acid (Z,Z)-oleic acid	2.90	$C_{18}H_{32}O_2$	280.45
10	19.869	9,12-0ctadecadienoic acid (Z,Z)-linoelaidic acid	3.47	$C_{18}H_{32}O_2$	280.45
11	20.900	6-Octadecenoic acid	10.99	$C_{18}H_{34}O_2$	282.46
12	30.931	Oleic Acid	12.98	$C_{18}H_{34}O_2$	282.46
13	31.429	Ethyl Oleate	0.99	$C_{20}H_{38}O_2$	310.51

3.2. Median lethal dose (LD_{50}) of the extract

Oral administration of a single dose of the extract at doses 10, 100, 1000, 1600, and 2900mg/kg body weight did not produce any mortality in the rats, while dose 5000mg/kg body weight produced discomfort and made the animals to be sluggish in movement with loss of appetite during 72 hrs of observation. (Table 2). The calculated LD_{50} for the extracts of *Annona muricata* at the end of 72 hours of acute toxicity test was >5000mg/kg body weight.

Table 2 Median Lethal Dose (LD_{50}) of the Extracts

Group	Dose (mg/kg) Body weight	Number of Animals	Number of Death	% Mortality
A	10	3	0	0
B	100	3	0	0
C	1000	3	0	0
D	1600	1	0	0
E	2900	1	0	0
F	5000	1	0	0

4. Discussion

The phytochemical screening carried out in this study revealed compounds with significant interest which have been documented to have both therapeutic and industrial uses.

Hexadecanoic acid also known as palmitic acid has been documented to serve as a lubricant, with antiandrogenic, antioxidant, 5- alpha-reductase inhibitory activity (Choudhary *et al.,* 2019). Similarly, n-Hexadecanoic acid free radical scavenging, anticancer properties, hypocholesterolemic, nematicidal, pesticidal, antiandrogenic, anti-plasmodial, antimicrobial and hemolytic potentials have been reported (Gopalakrishnan and Vadivel 2011; Olowofolahan *et al*., 2022; Olasiehinde *et al.,*2022).

The phyto-constituents in the leaf of *Annona muricata* in this study, is in agreement with the findings of Olasehinde *et al.,* (2022), in that the ethanolic leaf extract of *A. muricata* contains n-Hexadecanoic acid, 9,12-Octadecadienoic acid methyl esters, Octadecanoic acid, Oleic Acid while 5-Octadecene, Dodecanoic acid, Tetradecanoic acid, Linoelaidic acid, Ethyl Oleate were conspicuously absent. Furthermore, this study is in agreement with the study carried out by Ezirim *et al.,* (2024) in that the ethanol extracts of *A. muricata* contains Dodecanoic acid, Tetradecanoic acid, Oleic Acid, n-Hexadecanoic acid while ethyl Oleate, Linoelaidic acid, 5-Octadeene, 6-Octadecanoic acid were conspicuously absent, this could be as a result of geographical variations where the leaf samples were collected.

Myristic acids are saturated fatty acids present in the leaf extract of *Annona muricata*. Myristic acid or Tetradecanoic acid (2.10%) has been reported to have cancer-preventive, antioxidant, antifungal, and antibacterial properties (Choudhary *et al.,* 2019). Dodecanoic acid also known as lauric acid a medium-chain saturated fatty acid, has been reported to have anticancer activity in reproductive system cancer, some digestive tract cancers, and liver cancer (Chen *et al.,* 2020), it has also been reported to have antibacterial properties (Rajeswari and Rani, 2014)

Oleic acid or 9-Octadecanoic acid, a monounsaturated fatty acid present in the leaf extract of *Annona muricata* (12.98%) has been reported to have anti-inflammatory, cancer preventive, anti-androgenic, 5-alpha reductase inhibitor, anemia genic, insectifuge, dermatitigenic and hypocholesterolemic properties. (Choudhary *et al.,* 2019; Oganez *et al.,* 2022), Moreover, it is also used in aerosol products as an emulsifying or solubilizing agent (Choudhary *et al.,*2019). However, Octadecanoic acid is a potent inhibitor of lipoxygenase activity and interferes with Na^+ K^+ flux across membranes (Herrell and Stimers, 2002).

9,12-Octadecadienoic acid (Z, Z)-oleic acid also known as linoleic acid (3.47%) present in the leaf extract of *Annona muricata* is reported to possess anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, anti-histaminic, anti-eczemic, antiacne, 5-alpha reductase inhibitor, anti-androgenic, anti-arthritic and anti-coronary activities (Jegadeeswari *et al.,* 2012). Studies also show that they are important in maintenance of bone health, homeostasis, and stimulation of skin and hair growth (Campi *et al.,* 2023). However, octadecenoic acid, which has been found to be a mechanism-based inhibitor of lipoxygenase; reported to be toxic in humans by blocking both the Na^+ current and the transient outward K^+ current (Herrell and Stimers, 2002); consequently, metabolic and ionic variations in tissue cultures are often linked to tumor cell proliferation, malignant characteristics, and loss of apoptosis (Yu *et al.,* 2005). These bioactive constituents found in the leaves of *Annona muricata* thus confer to it pharmacological properties which are beneficial to humans to a large extent.

The determination of mean lethal dose of *A. muricata* leaf ethanol extracts on experimental rats showed that the extract was not lethal to the animals at dose of 5000mg/kg, and this is in agreement with the study of Arthur *et al*. (2011) and Olowofolahan *et al*., (2022). Their study similarly found that no severe adverse changes was inflicted on the experimental animals from the critical 24hours post administration to the end of the seventh day, and that the LD_{50} was estimated to be >5000mg/kg.

However, contrasting findings were made in the study of Adewole and Ojewole (2009), Agu *et al.,* (2017), and Alphonse *et al.,* (2018)*;* they reported that high dose of aqueous leaf extract of *A. muricata* were found to be toxic and lethal to the animals. The discrepancy in toxicity may be attributed to differences in the extracting solvents. Polarity differences of extracting solvents are important determinant of extraction yield, phytochemical content, and antioxidant properties (Ngo *et al.,* 2017; Nawaz *et al.,* 2020; Alozie *et al.,* 2022).

Furthermore, the ethanol leaf extracts of *A. muricata* are considered to have mild toxicity in rats by oral route according to the classification of Diezi, (1989) as reported by Alphonse *et al.,* (2018) thus caution is required in its consumption. This may be attributed to the presence of oxalate, annonaceous acetogenins, acetophenone, and octadecenoic acid methyl ester derivatives which studies have shown to possess deleterious effect at high dose/concentrations (Opara *et al.,* 2021; Coria-Tellez *et al*., 2016; Yu *et al.,* (2005).

5. Conclusion

The present study has demonstrated that *A. muricata* leaf contain a battery of volatile organic compounds of with known pharmacological benefits. Also, the extracts may be considered to be generally safe judging from the lack of serious alteration in the functional and behavioral pattern of the animals following the administration of the extract.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical clearance was obtained and granted by the Nnamdi Azikiwe University-Animal Research Ethics Committee and all animal studies were conducted in compliance with the National Institutes of Health (NIH) Guide for Care and Use of

Laboratory Animals (Pub. No. 85-23 Revised 1985) as approved by the Nnamdi Azikiwe University, Awka Ethical Committee for the use of Laboratory Animals.

References

[1] Adeyemi, D.O., Komolafe, O.A., Adewole, O.S., Obuotor, E.M. and Adenowo, T.K. (2009) Anti hyperglycemic activities of *Annona muricata* (Linn). *African Journal of Traditional Complements and Alternative Medicine*. **6**:62–69.

[2] Agu, K.C, Okolie, N.P, Eze, I., Anionye, J.C. and Falodun, A. (2017). Phytochemical analysis, toxicity profile, and hemomodulatoryproperties of *Annona muricata* (Soursop). *Egyptian Journal of Haematology*; 42:36-44

[3] Alozie, E. U., Iheanacho, K. M., Alisi, C. S. , Asiwe, E. S.,Nwosu, C. J. , and Iheanacho, J. N. (2022). Antidiabetic and hypolipidemic properties of *Vernonia amygdalina* aqueous, ethanol, methanol, toluene and benzene extracts in Alloxan-Induced diabetic rats. *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB).* 8(1), 12-22*.*

[4] Alphonse, S., Natacha, A., Melanie, A., and Behanzin, J. (2018). Evaluation of the toxicity of *Annona muricata* leaf extracts on liver and kidney function and investigation of acute and subacute toxicity in Wistar rats. *American Journal of PharmTechResearch* 8(1): 189-217.

[5] Aroma, D. and Enegide, C. (2014). The Importance of Toxicity Testing. *Journal of Pharmaceutical and Biosciences,* 4(2013): 146-148.

[6] Arthur, F.K.N, Woode, E., Terlabi, E.O. and Larbie, C. (2011) Evaluation of acute and sub-chronic toxicity of *Annona muricata* (Linn) aqueous extract in animals. *European Journal of Experimental Biology* 1 (4): 115-124.

[7] Asiwe, E. S. , Igwe , C. U., Iheanacho , K. M. E., Onyeocha, I. O., and Onwuliri, V. A. (2021). Bioactive Composition and Acute Oral Toxicity Studies on *Persea americana* Seed Ethyl Acetate Fraction. *Asian Journal of Research in Biochemistry, 8(4): 10-17.*

[8] Asiwe, Emeka S., Ejiogu, Prince C., Enenebeaku, Uchechi E., Ugenyi, Assumpta U., Opara, Peter O., and Mbakwe, Ikechukwu E. (2023). Phytochemicals and volatile composition of *Justicia carnea* leaf ethanol extract. *World Journal of advanced research and reviews*. 20 (3), 115-128.

[9] Campi, M., Mancuello, C., Maubet, Y., Cristaldo, E., Veloso, B., Ferreira, F., Thomton, L., and Robledo, G. (2023). Biochemical, nutritional, and toxicological properties of the edible species *Phlebopus beniensis* with ethnomycological notes from Paraguay. *Brazilian Journal of Food Technology,* 26, e2022126.

[10] Chen, X., Zhao, X., Deng, Y., Bu, X., Ye, H., and Guo, N. (2020) Antimicrobial potential of myristic acid against Listeria monocytogenes in milk, *Journal of Antibiotics,* 72(5):298–305.

[11] Choudhary, D, Shekhawat, J.K and Kataria, V. (2019). GC-MS Analysis of Bioactive Phytochemicals in Methanol Extract of Aerial Part and Callus of *Dipterygium glaucum* Decne. *Pharmacognosy Journal*; 11(5):1055-63.

[12] Coria-Tellez, A.V., Montalvo-Gonzalez, E., Yahia, M.E. and Obiedo-Vazquez, E.N. (2016), *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of Chemistry*. 1:1-2.

[13] Diezi, J. (1989) Toxicology: basic principles and clinical implications. In: Pharmacology: from fundamental principles to therapeutic applications. Éditions Slatkine-Genève, Pp 33–44.

[14] Ezirim, A.U., Ezekwesili-Ofili, J.O., Igwilo, I.O., Iheme, C.I. and Ukairo, D.I. (2024) GC-MS Analysis, Pharmaceutical and Industrial Significance of Phytochemicals Present in *Annona muricata* from Eziobodo, Imo State. *GSC Biological and Pharmaceutical Sciences,* 27(01), 174-180.

[15] Fellows, L., and Scofield, A. (1995) Chemical diversity in plants. In Intellectual Property Rights and Biodiversity Conservation-An Interdisciplinary Analysis of the Values of Medicinal Plants; University Press: Cambridge, UK. Pp 453-462.

[16] Gopalakrishnan, S. and Vadival, E. (2011). GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* linn. *International Journal of Pharmacy and Biological Sciences.* 2(1):313-320.

[17] Harrell, M.D., and Stimers, J.R. (2002) Differential effects of linoleic Acid metabolites on cardiac sodium current. *Journal of Pharmacology and Experimental Therapeutics*, 303(1):347-55.

[18] Jegadeeswari, P., Nishanthini, A., Muthukumarasamy, S and Mohan, V.R. (2012). GC-MS Analysis of bioactive compounds of *Aristolochia krysagathra* (*Aristolochiaceae*). *Journal of Current Chemical and Pharmaceutical Sciences*, 2(4):226-232.

[19] Kanthal, L.K., Dey, A., Satyavathy, K. and Bhojaraju, P. (2014). GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. *Pharmacognosy Research*, 6(1):58-61.

[20] Kumar, S, Paul, S, Walia, Y.K, Kumar, A and Singha, P (2015) Therapeutic Potential of Medicinal Plants: A Review; *Journal of Biological and Chemical Chronicles*, 1(1), 46-54.

[21] Lorke, D. (1983) A new Approach to practical Acute Toxicity Testing. Archives of Toxicology, 54:275-287

[22] Makeri, H.K, Maikai, V.A and Muhammad, A (2015), Evaluation of Antibacterial Activities of Graviola (*Annona muricata*) Leave and Stem Bark Extracts against Clinical Isolates of *Salmonella spp*. and *Escherichia coli*. *Bayero Journal of Pure and Applied Sciences (Bajopas)*, 8(1):1143-148.

[23] Moghadamtousi, S.Z., Fadaeinasab M., Nikzad S., Mohan G., Ali H.M. and 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.

[24] Moghadamtousi, S.Z., Goh B.H., Chan C.K., Shabab T. and Kadir H.A., (2013) Biological activities and Phytochemcials of *Swietenia macrophylla. King Molecule* 18:10465-10483.

[25] Molyneux, R.J., Lee S.T., Gardner D.R., Panter K.E. and James L.F. (2007) Phytochmical: the Good, the bad and the ugly?" *Phytochemsitry*, 68(22-24):2973-2985.

[26] Nawaz, H. Shad, M., Rehman, N., Andaleeb, H., and Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences,* 56. 10.1590/s2175-97902019000417129.

[27] Ngo Van, T., Scarlett, C., Bowyer, M., Ngo, P., and Vuong, Quan. (2017). Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of *Salacia chinensis* L.. *Journal of Food Quality,* 1-8, 10.1155/2017/9305047.

[28] Oganezi, N.C., Okoronkwo, C.U, Ebere, U.C., and Peter, P.O. (2022) Evaluation of Bioactive Compounds in *Afrostyrax Lepidophyllus* Seeds Using Gas Chromatography-Mass Spectrometry. *International Journal of Food Science and Agriculture*, 6(4), 403-408.

[29] Okigbo, R.N., and Mmeka, E.C. (2006) An Appraisal of Phytomedicine in Africa. *KMITL Science and Technology Journal*, 6:(2) 83-94.

[30] Olasehinde, O.R., Afolabi, O.B., Owolabi, O.V., Akawa, A.B., and Omiyale, O.B., (2022). GC–MS analysis of phytochemical constituents of methanolic fraction of *Annona muricata* leaf and its inhibition against two key enzymes linked to type II diabetes, *Scientific African*, 16:1-9.

[31] Olowofolahan A.O, Dare H.M, Adeoye Y.D and Olorunsogo O.O (2022), *Annona muricata* Linn Stem Bark Protects against Uterine Proliferative Disorder Induced by Estradiol Benzoate in Female Rat. *European Journal of Medicinal Plants.* 33(9): 48-61.

[32] Opara, P.O, Enemor, V.H.A, Eneh, F.U, and Emengaha, F,C. (2021). Blood glucose-lowering potentials of *Anonna muricata* leaf extract in alloxan-induced diabetic rats. *European Journal of Biology and Biotechnology,*2(2):106-113.

[33] Opara, P.O, Enemor, V.H.A, Eneh, F.U, Emengaha, F.C, Asiwe, E.S, and Duru, M.K.C. (2024) Acute Toxicity Studies and *In Vivo* Antioxidant Potential of *Annona muricata* Leaf in Alloxan-Induced Diabetic Rats. *Tropical Journal of Natural Product Research.* 8(2):6465-6470.

[34] Rajeswari, J. and Rani, S. (2014). GC-MS Analysis of Whole Plant of *Leptadenia reticulata*. *InternationalJournal of PharmTech Research*. 6(7): 2043-2050.

[35] Raskin, I., Ribnicky, D., Komarnytsky, S., Ilic, N., Poulev, A., and Borisjuk, N. (2002) Plants and human health in the twenty-first century. *Trends In Biotechnology*.; 20(12):522-531.

[36] Sanni, O., Obidoa, O., and Omale, J. (2014). Toxicity, Anti-Lipid Peroxidation, *In vitro* and *In vivo* Evaluation of Antioxidant Activity of *Annona muricata* Ethanol Stem Bark Extract. *American Journal of Life Sciences.* 2(5):271-277.

[37] Santhi, K., Sengottuvel, R. (2016) Qualitative and Quantitative Phytochemcal analysis of *Moringa concanensis Nimmo*. *International Journal of Current Microbiology and Applied Sciences* 5(1): 633-640.

[38] Sneader, W. (1996) Drug Prototypes and Their Exploitation. Wiley, UK, P 536.

[39] Usunobun, U., and Okolie, N.P., (2015), Phytochemical Analysis and mineral composition of *Annona muricata* leaves. International Journal of Research and Current Development. 1(1):38-42.

[40] Yu, F.R., Lian X.Z., Guo, H.Y., Mcguire, P.M., Li, R.D., Wang, R., and Yu, F. H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui (Euphorbiaceae)* and their inhibitory effects on the human SGC-7901 cells. *Journal of Pharmaceutical Science.* 8(3):528-535.