DRUG DISCOVERY

16(38), 2022

GC-MS Analysis of Bioactive Composition of *Averrhoa carambola* Leaf and Fruit Extracts and Acute Toxicity Study in Albino Mice

Uju OP, Ukairo ID, Emejulu AA

To Cite:
Uju OP, Ukairo ID, Emejulu AA. Gc-ms analysis of bioactive composition of *Averrhoa carambola* leaf and fruit extracts and acute toxicity study in Albino Mice.*Drug Discovery*, 2022, 16(38), 86-93

Author Affiliation:
Department of Biochemistry, School of Biological Sciences, Federal University of Technology, Owerri, P.M.B 1526 Imo State, Nigeria

***Corresponding author**
Department of Biochemistry, School of Biological Sciences, Federal University of Technology, Owerri, P.M.B 1526 Imo State, Nigeria
Email: ujujennifer70@gmail.com

Peer-Review History
Received: 20 September 2022
Reviewed & Revised: 24/September/2022 to 25/November/2022
Accepted: 29 November 2022
Published: 03 December 2022

Peer-review
External peer-review was done through double-blind method.

ABSTRACT

Averrhoa carambola is one of the ornamental plants that are becoming increasingly popular because of its medicinal properties. This study assesses the bioactive components of *Averrhoa carambola* ethanol leaf and fruit extracts using Gas Chromatography-Mass Spectrometer. Acute toxicity study was also carried out on wistar strain albino mice using standard procedures. The varying doses of the extracts were administered orally to the male wistar albino mice and signs accompanying toxicity and possible death of animals were monitored. Eight bioactive phytocomponents were identified in the GC-MS analysis of *Averrhoa carambola* ethanol leaf extract, with Bis (2-ethylhexyl) phthalate having the highest percentage concentration (24.31%) quantitatively. The ethanol fruit extract had six bioactive components identified, with citral having the highest concentration of (20.59%). Acute toxicity studies revealed that, the ethanol leaf extract had a median lethal dose (LD_{50}) value of 3605.55mg/kg body weight. Conversely, the ethanol fruit extract of *A. carambola* showed no lethal signs of morbidity and mortality even at doses up to 5000mg/kg body weight. It is therefore concluded that *Averrhoa carambola* leaf and fruit ethanol extracts contain some essential photochemicals which seem to justify their use in ethno medicine and may be relatively safe. To determine their safety in certain bodily organs, however, further studies is advised.

Keywords: *Averrhoa carambola*, GC-MS, lethal dose, phytochemicals.

1. INTRODUCTION

In the treatment of diseases, plants have been used as a prospective source of medicinal compounds. Many newly discovered drugs nowadays are made from natural ingredients or from chemically modified versions of such items (Prasad and Koch, 2014). Due to natural products, efficacy in preventing and treating illnesses with minimal to no adverse effects, many have recently gone back to conventional treatment, including natural products (Hong, 2011). The world health organization (WHO) supports the

DISCOVERY SCIENTIFIC SOCIETY

use of traditional medicine provided they are proven to be efficacious and safe (WHO, 1985). Numerous bioactive substances produced by secondary metabolic pathways are found in plants. These components serve as therapeutic and preventative agents, as well as raw materials for the creation of pharmaceutically active molecules (Mohomned *et al*., 2020). The most effective technique for identifying the bioactive elements of long-chain alcohols, acids and hydrocarbons present in plants etc is GC-MS (Sermakkani and Thangapandian, 2012). Gas chromatography (GC) and Mass spectroscopy (MS), along with specific detection methods, have therefore developed into sophisticated methods for analyzing a variety of substances (Vinodh *et al*., 2013). *Averrhoa carambola*, commonly referred to as star fruit and Chinese gooseberry, has been used in traditional Chinese medicine to cure ailments like cough, jaundice and skin rashes (Sheth and Ashok, 2005). *Averrhoa carambola* has been shown to have analgesic qualities, anti-inflammatory impact and hypoglycemic potential (Ahmed and Das, 2015). Additional properties of *Averrhoa carambola* include anthelmintic, antioxidant, hypolipidemic, antibacterial, hypotensive, anti-ulcer, and anti-tumor actions (Shah *et al*., 2011; Soncini *et al*., 2011). Vitamin C, protein, carbs, dietary fibre, calcium, iron, phosphorus, potassium, carotenes, B- vitamins and amino acids are all nutrients found in *Averrhoa carambola* (star fruit) (Patrick and Augustus, 2019). Although many therapeutic plants may have pharmacological effects that are advantageous to human health, they may also have hazardous consequences when consumed without scientific backing. For this reason, it is crucial to screen natural products for possible toxicity (Rosidah *et al*., 2009). Despite the numerous ways in which plants are utilized in traditional medicine, there is no any comprehensive research that can explain the toxicity of all plants (Prabu *et al*., 2013). This research is aimed at examining the safety of the *Averrhoa carambola* leaf and fruit extracts in albino mice through acute toxicity study and identifying the possible bioactive chemical components present in both the leaf and fruit extracts using modern scientific analytic technique (Gas Chromatography-Mass Spectrometry).

2. MATERIALS AND METHODS

Collections and Identification of *Averrhoa carambola* Leaves and Fruits

The plant materials (Fresh leaves and matured ripe fruits) of *Averrohoa carambola* were collected from Umueme Obike in Ngor Okpala Local Government Area in Imo State, Nigeria. Botanical identification and authentication were performed by Dr. Hyginus. C. Ogbuchi of Crop Science and Biotechnology, Faculty of Agriculture and Veterinary Medicine Imo State University where a voucher (001/CSB/IMSU/2021) specimen was assigned at herbarium for reference.

Experimental Animals

A total of thirty-six (36) male mice weighing 22 to 28g were used for the acute toxicity study (LD_{50}) of leaf and fruit ethanol extracts. All animals were purchased from the faculty of Veterinary Medicine, University of Nigeria Nsukka. They were transported to the animal house Department of Biochemistry Federal University of Technology Owerri, Imo State, Nigeria and maintained in a clean environment of about 27^{o}C and fed with standard feed and allowed free access to water throughout the study period.

Processing and Extraction of Plant Material

Fresh leaves and ripe fruits from *Averrohoa carambola* tree were properly cleaned with purified tap water and allowed to air dry for about two weeks at room temperature. After that, each was independently blended into a coarse powder. Two conical flasks were filled with 10g of the coarse powder (leaf and fruit) separately, which was then suspended in 100 ml of 100% ethanol and let to stand for 48 hours. After that, muslin cloth was used to filter the suspension. Whatman no1 filter paper was used to filter the filtrate once more. The subsequent filtrates were dried in a vacuum desiccator after being evaporated in a water bath at 50^{o}C. The extracts were put into an airtight bottle and kept in a 4°C refrigerator until they were needed.

GC-MS Analysis of *Averrhoa carambola* Leaf and Fruit Extracts

Averrhoa carambola fruit and leaf ethanol extracts were analyzed using a thermal Gas Chromatography Mass Spectrometry (GCMS) device (model: QP2010 plus Shimadzu, Japan). The apparatus was run in electron impact mode with the following operating conditions: Ionization voltage of 70 eV, injector temperature of 230^{o}C and detector temperature 280^{o}C. 1 ml of the samples were injected while using helium as the carrier gas, which had a purity of 99.9% at a flow rate of 1ml/min. The oven temperature was initially programmed at 80^{o}C /5min and then increased to 200^{o}C at 5/min and finally increased to 220^{o}C at 5/min. The spectrum of the unknown components was compared with the spectrum of known components which is stored in the National institute of Standards and Technology (NIST) library. The name, molecular weight, molecular formula and percentage concentration were ascertained.

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

Lethal Dose (LD_{50}) Determination

The lethal doses of ethanol leaf and fruit extracts of *Averrhoa carambola* were done according to the method of Lorke's *et al*., (1983) in two different phases for leaf and fruit extracts.

Phase I

Three groups of mice containing three mice each were used. The crude extracts were administered orally at concentrations of 10mg/kg body weight, 100mg/kg and 1000mg/kg body weight to group I, group II and group III respectively. They were monitored for 24 hours for abnormal reactions or death.

Phase II

In this phase, three groups of three mice each were used. Group 1 received 1900mg of the extract per kilogram body weight. Group 2 received 2600mg of the extract per kilogram body weight while group 3 received 5000mg of extract per kilogram body weight. They were monitored for 24 hours and observations were recorded. The extract's lethal dosage (LD_{50}) was calculated by taking the geometric mean of the greatest dose with 0% mortality and the minimum dose with mortality.

LD_{50} = Maximium dose with 0% mortality × Minimium dose with mortality

3. RESULTS

The GC-MS analysis of *Averrhoa carambola* leaf extract identified the presence of eight bioactive phytocomponents with Bis (2-ethyhexyl) phthalate having the highest percentage concentration (24.31%) quantitatively. While triethyl citrate was found to have the lowest percentage composition of (0.56%) with retention time 17.785. The GC-MS analysis of *Averrhoa carambola* ethanol fruit extract showed the presence of six bioactive phytocomponents. Citral was found to have the highest percentage concentration of (20.59%) while oleic acid was the lowest with percentage concentration of (2.33%).

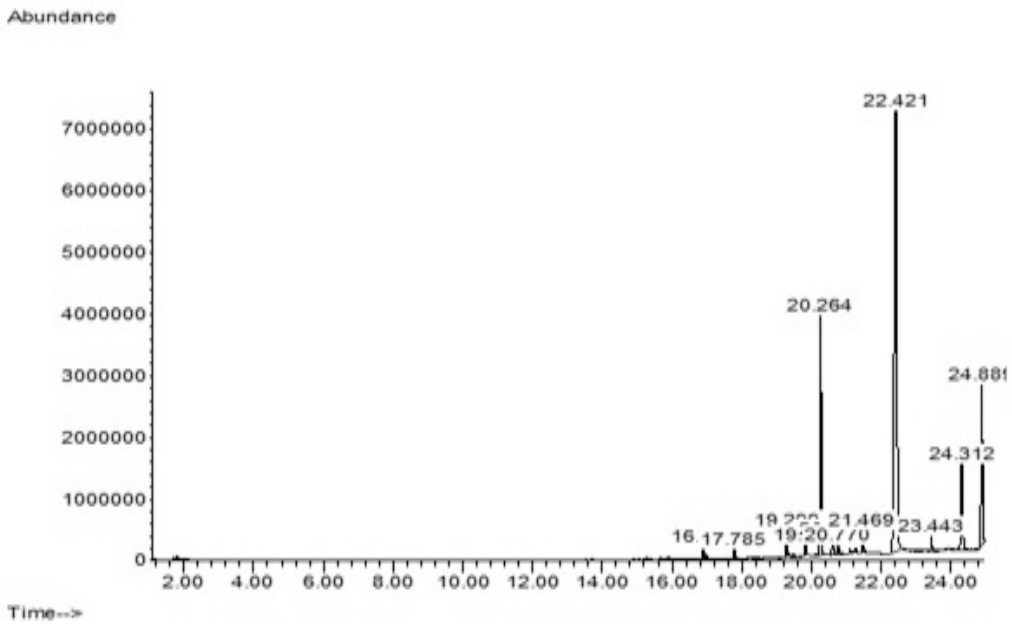

Figure 1: Gas Chromatographic Profile of *Averrhoa carambola* Leaf Extract.

Table 1: Bioactive Phytocompotents Identified in GC-MS Analysis of *Averrhoa carambola* Ethanol Leaf Extract

S/N	Compound name	Retention Time	Molecular Weight	Total area %	Molecular Formula
1	1-Hexadecene	16.894	224.425g/mol	0.67	$C_{16}H_{32}$

2	Triethyl citrate	17.785	276.283g/mol	0.56	$C_{12}H_{20}O_7$
3	1-Octadecene	19.299	252.478g/mol	1.35	$C_{18}H_{36}$
4	Dibutyl phthalate	20.264	166.132g/mol	14.57	C_6H_6O
5	Behenic alcohol	21.469	326g/mol	1.08	$C_{22}H_{46}O$
6	Bis 2-ethylhexyl phthalate	22.421	390.564g/mol	24.31	$C_{24}H_{38}O_2$
7	1-docosene	23.443	308.6g/mol	10.31	$C_{22}H_{44}$

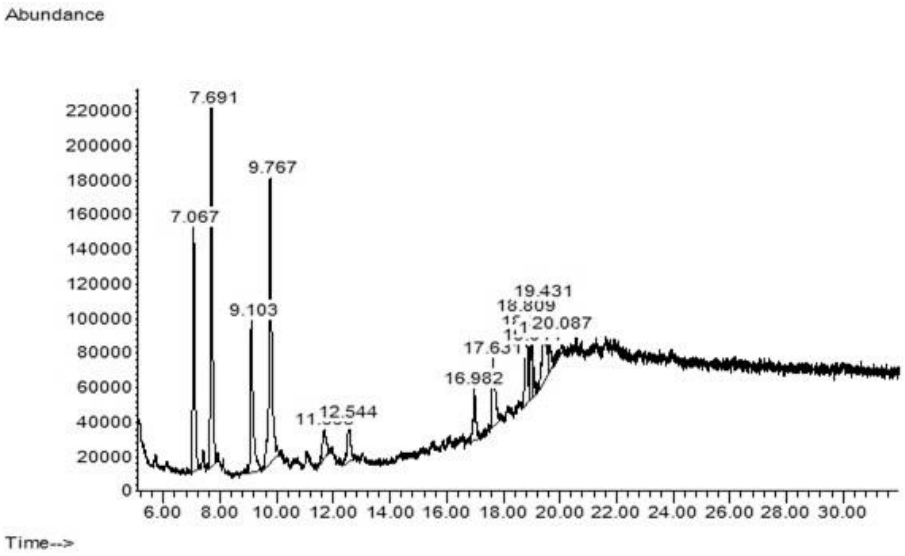

Figure 2 : Gas Chromatographic Profile of *Averrhoa carambola* Fruit Extract.

Table 2: Bioactive Phytocomponents Identified in GC-MS Analysis of *Averrhoa carambola* Ethanol Fruit Extract.

	Compound Name	Retention time	Molecular weight	Total area %	Molecular formula
1	Citral	7.691	152.23g/mol	20.59	$C_{10}H_{16}O$
2	Trans -2,7 -dimethyl -4,6-octadien-2-ol	9.767	152.23g/mol	18.92	$C_{10}H_{18}O$
3	Hexadecanoic acid	16.982	256.430g/mol	3.75	$C_{16}H_{32}O_2$
4	n-Hexadecanoic acid (palmitic acid)	17.631	256.424g/mol	5.39	$C_{16}H_{32}O_2$
5	Cis-vaccenic acid	18.809	9.767	4.93	$C_{18}H_{34}O_2$
6	Oleic acid	19.605	282.468g/mol	2.33	$C_{18}H_{32}O_2$

Table 3: Acute Toxicity Study of *Averrhoa carambola* Leaf and Fruit Ethanol Extracts

Phase I				
Groups	No. of mice	Treatment	Mortality in Leaf Extract	Mortality in Fruit Extract
I	3	10mg/kg	-	-
II	3	100mg/kg	-	-
III	3	1000mg/kg	-	-

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

Phase II				
IV	3	1900mg/kg	-	-
V	3	2600mg/kg	-	-
VI	3	5000mg/kg	2 (LD_{50}) =3605.55mg/kg	-

The result of the acute toxicity test (LD_{50}) presented in Table 3 showed no death recorded within 24hours in all the groups in phase 1 after oral administration of ethanol leaf and fruit extracts of *Averrhoa carambola*. However, in phase 2, after 24 hours of oral administration of *Averrhoa carambola* leaf extract the animals displayed visible clinical signs such as decreased movement and clumping together. Mortality was recorded in the group given 5000mg/kg body weight of *Averrhoa carambola* leaf extract while no death was recorded in all the groups administered ethanol fruit extract. Therefore, the acute toxicity test (LD_{50}) of *Averrhoa carambola* leaf extract was calculated to be 3605.55mg/kg according to Lorke's method (1983) that was used while the plant fruit extract may be regarded safe at the study level.

4. DISCUSSION

Eight bioactive components were found in the ethanol leaves extract of *Averrhoa carambola* during GC-MS analysis. The found chemicals have a wide range of biological characteristics. For instance, triethyl citrate a colourless and odourless liquid with chemical formula $C_{12}H_{20}O_7$ have been reported to possess antiulcer and anti-inflammatory activities (Al-marzogi, 2015). Previous research has demonstrated that the anticancer, antioxidant and antibacterial properties of higher plants and algae are due to the presence of 1-octadecene and 1-hexadecene (Lee *et al*., 2007); hence the presence of 1-octadecene and 1-Hexadecene in *Averrhoa carambola* leaf would impact these biomedical properties and make them useful in pharmaceuticals. Yogeswari *et al*., (2012) also reported the antibacterial properties of 1-docosene, 1-octadecene and 1-hexadecene. The analysis also identified the presence of behenic alcohol which has been reported to have antimicrobial activity (Ranganathan, 2014). Quinoline act as an intermediate in the manufacture of pharmaceuticals and veterinary drugs, it is used to produce nicotinic acid and its derivative niacin or vitamin B, antimalarial medicines (Chloroquine, Quinine). Quinoline yellow is also used as a food additive (Lava *et al*., 2012; Abbey *et al*., 2013).

In the study on the chemical composition and bioactivity of *Buchholzia coriacea*engler's seed (fruit) extract, Ojinnaka *et al*., (2015) noted that bis2 (ethyhexyl) phthalate has anti-oxidant, anti-tumor, anti-viral, anti-fungal and anti-bacterial characteristics. When compared to other research findings, the results of the current study showed that bis (2-ethylhexyl) phthalate is the predominant (24.31%) bioactive component present in the leaf extract of *Averrhoa carambola*, validating its antibacterial, antifungal and antioxidant capabilities (El-sayed *et al*., 2012; Biswas *et al*., 2017; Naseer *et al*., 2018). Other bioactive compounds found in the *Averrhoa carambola* ethanol leaf extract are 1,2-benzenedicarboxylic acid and dibutyl phthalate. Both have antimicrobial and antifouling properties (Watanuki *et al*., 2003).

Among the phytoconstituent identified using GC-MS in *Averrhoa carambola* ethanol fruit extract as shown in Table 2, hexadecanoic acid, n-hexadecanoic acid and oleic acid are all nematicides, pesticides, hemolytic 5-alpha reductase inhibitors, antioxidants and hypercholesterolemic agents (Abubaka and Majinda, 2016; Tulika and Male, 2017). In addition to possessing anti-inflammatory, antibacterial, antiviral and antimalarial activities, n-hexadecanoic acid has been demonstrated to inhibit phospholipase A(2) in a competitive way, promote transdermal absorption, prevent and treat cardiovascular disease and have hypoglycemic qualities (Vasudevan *et al*., 2012; Melting *et al*., 2020).

Cis-vaccenic acid is an omega 7 fatty acid that has been found to have health benefits such as boosting HDL cholesterol levels and decreasing LDL cholesterol levels, enhancing insulin sensitivity and avoiding cardiovascular disease among others. They are widely used in cosmetics due to their moisturizing properties for health of the skin and mucous membrane (Nunes and Rafacho, 2017; Tulika and Mala, 2017).

Trans-2,7-dimethyl-4,6-octadiel-2-ol is an essential oil belonging to the hydrocarbon monoterpenoid with a molecular formula $C_{10}H_{18}O$ and molecular weight of 154.25g/mol. In the prevention and treatment of illnesses including cancer, cardiovascular disorders including atherosclerosis and thrombosis, essential oils and their volatile components are frequently employed. They also exhibit antimicrobial, antiviral, antioxidant and antidiabetic properties (Hamid *et al*., 2011). The presence of these phytocomponents in *Averrhoa carambola* leaf and fruit ethanol extract might be responsible for their therapeutic effects and their uses in pharmaceuticals (Table 4 and 5).

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

Table 4: Summary of the Bioactive Phytocomponent Found in the Leaf Ethanol Extract of *Averrhoa carambola*

S/N	Compound	Compound Group	Molecular Formula	Molecular Weight	Total area %	Biological Activity
1	1-Hexadecene	Alkene	$C_{16}H_{32}$	224.425 g/mol	0.67	Hypocholesterolaemic, Hypotensive, Anti cancer, anti-inflammatory
2	1-Octadecene	Alkene	$C_{18}H_{36}$	252.478 g/mol	1.35	Antibacteria, Antioxidant, Anticancer
3	Dibutyl phthalate	Aromatic dicarboxylic Acid	C_6H_6O	166.132 g/mol	14.57	Antimicrobial, Anti inflammatory, Anti-diuretic and Anti cancer
4	Behenic alcohol	Alcohol	$C_{22}H_{46}O$	326g/mol	1.08	Antimicrobial
5	Triethyl citrate	Tricarboxylic Acid	$C_{12}H_{20}O_7$	276.283 g/mol	0.56	Antiulcer and Anti inflammatory agent
6	1-docosene	Unsaturated fatty acid	$C_{22}H_{44}$	308.6 g/mol	58.31	Antimicrobial, Strong Radical scavengers, Antioxidant
7	Quinoline	Heterocyclic-aromatic organic compound	C_9H_7N	129.16 g/mol	7.67	Antimicrobial, use in the production of vitaminB_3, pharmaceuticals and veterinary drugs, food addictive.
8	Bis(2-ethylhexyl) phthalate	Diester of phthalic acid	$C_{24}H_{38}O_2$	390.564 g/mol	58.31	Antioxidant, antitumor, Antiviral, antifungal and Anti bacterial properties

Table 5 Summary of the Bioactive Phytocomponents Found in the Fruit Ethanol Extract of *Averrhoa carambola*

S/N	Compound	Chemical group	Molecular formula	Molecular weight	Total area %	Biological activity
1	Citral	Terpenoid	$C_{10}H_{16}O$	152.23 g/mol	20.59	Antimicrobial, Anticancer, Anti inflammatory
2	Hexadecanoic Acid	Saturated fatty acid	$C_{16}H_{32}O_2$	256.430 g/mol	3.75	Antioxidant, Hypocholesterolemic
3	n-Hexadecanoic acid	Saturated fatty acid	$C_{16}H_{32}O_2$	256.424 g/mol	3.75	Antioxidant, Hypocholesterolemic
4	Cis-Vaccenic Acid	Trans fatty acid	$C_{18}H_{34}O_2$	282.461 g/mol	4.93	Cancer prevention Hypolipidemic, insulin sensitivity, helps wound healing.
5	Oleic acid	Carboxylic acid	$C_{18}H_{32}O_2$	282.468 g/mol	2.33	Hypocholesterolaemic, Anticancer Antiinflammatory, antibacterial, antitumour, antimicrobial

ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

6	Trans -2,7 -dimethyl -4,6-Octadien-2-ol	Monoterpenoid alcohol	$C_{10}H_{18}O$	152.23 g/mol	18.92	Anti-inflammatory, Antimicrobial, Anticonvulsant. antiviral, antioxidant antidiabetic and Improves endocrime system

5. CONCLUSION

The presence of bioactive compounds in *Averrhoa carambola* leaf and fruit ethanol extracts justifies the use of the leaf and fruit parts for various ailments by traditional practitioners. The results also showed that the extract of *Averrhoa carambola* fruit was safe at high dose. However, higher doses of the leaf extract should be avoided and users should not rule out completely the possibility of chronic toxicity which might develop with the continuous usage of the extract. Further studies are actually ongoing in our laboratory to actually substantiate effects on body organs.

Authors' Contribution

All authors contributed equally for the successful completion of the research work. Prof. Mrs. Emejulu designs the work. Data collection and processing was done by Uju Philippa O., Dr. Mrs. Ukairo did the final correction. Finally, all authors read and approve the final work.

Ethical Approval

The ethical guidelines for plants and animal materials are followed in the study for sample collection and identification. *Averrohoa carambola* were collected from umueme Obike in Ngor Okpala Local Government Area in Imo State, Nigeria. Botanical identification and authentication were performed by Dr. Hyginus. C. Ogbuchi of Crop Science and Biotechnology, Faculty of Agriculture and Veterinary Medicine Imo State University where a voucher (001/CSB/IMSU/2021) specimen was assigned at herbarium for reference. The Animal ethical guidelines are followed in the study for phase I & II.

Funding

The study has not received any external funding.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and Materials Availability

All data associated with this study are present in the paper.

REFERENCES

1. Abbey J, Fields B, O'Mullane M, Tomaska LD. Food Additives Colorants in: Hazard and Diseases. Encyclopedia of Food Safety, Academic Press 2013; 2:459-465.
2. Abubakar MN, Majinda RT. GC-MS Analysis and Prilimary Antimicrobial Activity of *Albizia adianthifolia* (Schamach) and *Pterocarpus angiolensis. De Medicines*2016; 3(3):1-9.
3. Ahmed M, Das BN. Analgesic Activity of Fruit Extract of *Averrhoa carambola. Internal Journal Life Science and Pharm Res* 2015; 1(3):22-26.
4. Al-Marzogi AH, Hameedi IH, Idan SA. Analysis of Bioactive Chemical Components of Two Medicinal Plants (*Coriandrum satium* and *Melia azedarach*) Leaves Using Gas Chromatography Mass Spectrometry (GC-Ms). *Afr J Biotechnol* 2015; 14(40):2812-2830.
5. Biswas B, Rogers K, Mclaughlin D, Daniels, Yadav A. Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava L*) on Two Gram Negative and Gram Positive Bacteria. *Int J Microbial* 2017;10:1155-746165.
6. El-Sayed MH. Bis(2-Thylhexyl) Phthalate A Major Bioactive Metabolite with Microbial and Cytotoxic Activity Isolated from the Culture Filtrate of Newly Isolated Soil Streptomyces (*Streptomyces mirabilisstrain* NSQU-25). *World Applied Sci* J 2012; 20:1202-1212.

7. Hamid AA, Alyelaagbe OO, Usman LA. Essential Oils: Its Medicinal and Pharmacological Uses. *Int J Curr Res* 2011; 3(2):086-098.
8. Hong JY. Natural Product Diversity and its Role in Chemical Biology and Drug Discovery. *Curr Opin Chem Biol* 2011; 15:350-354.
9. Lava K, Eurard Y,Vanttecke K, Van Meevvelta L, Binnemans K. Studies on Guinoline Type, Dyes and their Characterization Studies on Acrylic Fabric Colour. *Technol* 2012; 128(3):192-240.
10. Lee SH, Chang KS, S U MS, Huang YS, Jang HD. Effects of Some Medical Plant Extracts on Different Fungi. Food Control 2007; 18:1547-155.
11. Locke's D. A New Approach to Practical Acute Toxicity Testing. *ArchToxicol* 1983; 54:275-287.
12. Melting WU, Lin NI, Haixiao LU, Huiyou XU. Terpenoids and their Biological Activities from Cinnamomum. A review 2020; 2(1):1-10.
13. Mohomned B, Abdelfattah EL, Ahmed B, Riaz U, Hafiz MM. Phytochemical Identification Acute and Sub Acute Toxicity Studies of the Foliar Extract of *Withania frutescens*. *Molecules* 2020; 25(4528):103390-25194528.
14. Naseer S, Hussain MS, Naeem N, Pervalz M, Rahman M. The Phytochemistry and Medicinal Value of *Psidium guajava* (Guava).*Clin Phytosci* 2018; 4(1):40816.
15. Nunes EA, Rafacho A. Implication of Palmitoleic Acid (Palmitoleate) on Glucose Hemeostasis Insulin Resistance and Diabetes. *Curr Drug Targets* 2017; 186:619-628.
16. Ojinnka CM, Nwachukwu KI, Ezediokpu MN. The Chemical Constituents and Bioactivity of the Seed (Fruit) Extracts of *Buchhollzia engler* (*Capparaceae*). *Journal Applied Sci Environ Manage* 2015; 19(1):795-801.
17. Patrick EA, Augustus UA. Evaluation of the Possible Hepatotic and Nephrotoxic Potentials of the *Averrhoa carambola* Juice Extract in Female Albino Rats. *J Basic Clin Physiol Pharmacol* 2019; 7(13):167-172.
18. Prabu PC, Panchapakesan S, Raj CD. Acute and Sub-Acute Oral Toxicity Assessment of the Hydroalcoholic extract of *Withania somnifera* Roots in Wistar Rats. *Phyther Res* 2013; 27:1169–1178.
19. Prasad R, Koch B. Antitumor Activity of Ethanolic Extract of *Dendrobium formosum*in T-Cell Lymphoma: An in Vitro and in Vivo Study. *Biomed Res Int* 2014.
20. Ranganathan D. Phytochemical Analysis of *Carallum anilagiriana* Using Gc-Ms. *Int J Pharmacogn Phytochem Res* 2014; 3(1):155-159.
21. Rosidah Yam MF, Sadikun A, Ahmad M, Akowuah GA, Asmawi M. Toxicology Evaluation of standardized Methanol Extract of *Gynura procumbens. J Ethnopharmacol* 2009; 123:244–249.
22. Sermakkani M, Thangapandian V. Gc-ms Analysis of *Cassia italic* Leaf Methanol Extract. *Asian J Pharm Clin* Res2012; 5(2):0974-24441.
23. Shah NA, Raut BA, Baheti A, Kuchekar BS. In Vitro Anthelmintic Activity of Leaf Extract of *Averrhoa carambola* Against Pheretima poshuma. *Pharmaco*2011; 1:524-780.
24. Sheth A, Ashok K. The Herbs of Ayurveda. Ahmedabed Sheth Publisher 2005; 1:140.
25. Soncini R, Santiato MB, Moraes GO, Peloso AL. Hypotensive Effect of Aqueous Extract of *Averrhoa carambola* L (Oxiladacea) In Rats: An in-Vivo and Invitro Approach. *J Ethnopharmacol* 2011; 133:353-700.
26. Tulika-Tyagi, Mala Agarwal. Phytochemical Screening and GC-MS Analysis of Bioactive Constituents in the Ethanol Extract of *Pistia stratiotes L* and *Eichhornia crassipes*. *J pharmacogn phytochem* 2017; 6(1):195-206.
27. Vasudevan A, Dileep V, Predeep M, Ponnuras K. Anti Inflammatory Property of N-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. *Chem Biol Drug Des* 2012; 80(3):434-523.
28. Vinodh KS, Natarajan A, Devi K, Senthilkumar B. Chemical Composition of Aqueous Leaf Extract of *Murraya koenigii*. *Int J Pharm Biol Archiv* 2013; 4:493–700.
29. Watanuki H, Gushiken Y, Sakai M. In Vitro Modulation of Common Compound *Lyprinuscarpio* L Phagocytic Cells by Di-N-Butyl Phthalate and Di-2 Ethyl Phthalate. *AguateToxical* 2003; 63(119):126-2003.
30. Yogeswari S, Ramalashmi S, Neelavathy R, Muthumary S. Identification and Comparative Studies of Different Volatile Fractions from *Monochaeta Kansensis* by Gc-MS. *Global J Pharamacol* 2012; 6(2):65-71.

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS