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Int. J. Biol. Chem. Sci. 17(3): 1199-1207, April 2023

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Gas Chromatography-Mass Spectrometry analysis of bioactive compounds of *Curcuma longa* leaves extract

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Received: 11-01-2023 Accepted: 18-04-2023 Published: 30-04-2023

ABSTRACT

*Curcuma Longa (*Turmeric) is a unique plant that combines the properties of a spice, colorant, cosmetic, and drug. As a medicinal plant, it is used by traditional healers in the treatment of ailments such as diabetic wounds, rheumatism arthritis, multiple sclerosis etc. This study was carried out to determine the presence of potential bioactive compounds present in *Curcuma longa* leaves. The bioactive compounds were extracted using the Soxhlet method of extraction and was analyzed using Gas Chromatography-Mass Spectrometry. The presence of eleven chemical constituents was identified in the GC-MS analysis of *Curcuma longa* leaves aqueous extract. This includes Cis-13- octadecenoic acid, methyl ester (28.40%), Cis- vaccenic acid (17.14%), Hexadecanoic acid, methyl ester (16.40%), n- Hexadecanoic acid (15.59%), Heptadecanoic acid, 16-methyl-, thylester (6.03%), Oleic Acid (6.02%) as well as compounds which were identified at low level. The GC-MS analysis of *Curcuma longa* leaves extract showed the presence of bioactive constituents which could have important medicinal properties. As a result, the presence of these bioactive constituents could be responsible for the therapeutic properties of the plant which can be used to develop new antimicrobials.

Keywords; Chemical constituents, turmeric, medicinal, antimicrobial, mixture, concentration

INTRODUCTION

Curcuma longa (turmeric) is a plant in the Zingiberaceae family native to south Asian region. *Curcuma longa* has been utilized as the main component in recipes from Nigeria, India, and Bangladesh because of its color, flavor, and taste (Shahiq and Navid, 2013). The rhizomes of *Curcuma longa* contain Curcuminoids which are used as a food additive to promote health. (Shahiq and Navid, 2013). The yellow hue of curcuma longa is due to the presence of three primary curcuminoids in the rhizome which include curcumin, demethoxycurcumin and bis-demethoxycurcumin (Johnson et al., 2022). It is widely used as an Indian folk medicine and for treating ailments such as cough, wounds, rheumatism, sinusitis, digestive disorders and various eye diseases (Aggarwal et al., 2007). In West Africa, it is mainly used as a dye to color products such as cotton cloth, tanned leathers, plant fibers and thread to a golden yellow. It

DOI : https://dx.doi.org/10.4314/ijbcs.v17i3.34

has been used as anti-inflammatory, anti-bacterial, anti-oxidant, anti-fungal, anti-schistosomal, antidepressant agent (Braga et al., 2003). The yellow color of turmeric rhizome and other plant derivatives is increasingly being used as a dye, with the goal of replacing synthetic chemicals with natural substances (Johnson et al., 2022).

C. longa is used in traditional Chinese medicine to treat diseases that cause abdominal pain. Turmeric is still used in religious ceremonies in a variety of ways. *Curcuma longa* has traditionally been used as an antimicrobial and insect repellant (Rudrappa and Bais, 2008). Several studies have demonstrated that Curcumin has broad-spectrum antimicrobial activity, including antibacterial, antiviral, antifungal, and antimalarial properties. The scientific community's interest in medicinal plants has recently increased due to an increase in antibiotic resistance as well as growing concerns about the modern medicine's side effects. The use of medicinal plants and their bioactive compounds is included in the modern field of phytosciences as well as scientific knowledge about them (Anand et al., 2007). Gas chromatography mass-spectrometry (GC-MS) is a useful technique for identifying bioactive compounds with high accuracy. Previous studies have shown the presence of important bioactive compounds in rhizomes of *Curcuma longa.* Subramanian et al (2019) reported the presence of various bioactive constituents in the methanolic rhizome extract of *Curcuma longa* using GC-MS. Shagufta et al (2010) and Abdul et al (2015) reported the presence of essential oils and bioactive compounds present from *Curcuma longa* rhizome through GC-MS. *Curcuma longa* rhizomes have been extensively researched on, showing the bioactive components that are present. However, there is little information on the chromatographic analyses of Curcuma longa leaves extracts. As a result, the aim of this study was to identify bioactive compounds present in *Curcuma longa* leaves and utilize the medicinal properties of these compounds by developing new antimicrobials for the treatment of infectious diseases.

MATERIALS AND METHODS

Plant collection and extraction of materials

Fresh leaves of *Curcuma longa (*Turmeric) were purchased from a local market in Owerri, Imo state, Nigeria. The leaves were identified and authenticated by a Plant taxonomist in the Department of Crop Science, Federal University of Technology, Owerri, Imo state, Nigeria. The leaves were thoroughly washed with running water to remove all traces of soil and dirt. The preparation of the plant extract was carried out using Soxhlet method as described by Jensen (Jensen, 2007). The extracts were dried in 500ml clean boiling flasks for 30 minutes in an oven set at 105 – 110 degrees Celsius. After that, it was placed in a desiccator and allowed to cool. 100g of the sample was weighed and poured into the soxhlet thimble. The extraction thimble was lightly plugged with cotton wool to aid in extract filtering, and the boiling flask was filled with 300ml of ethanol. The soxhlet apparatus was assembled and allowed four hours of reflux at 600 degrees Celsius. The thimble was removed with caution and the extract was poured into a volumetric flask and allowed to cool. The volumetric flask contents were then transferred to a rotatory evaporator to separate the solvent from the oil.

Extraction of phytochemicals

The extract (1 g) was weighed and placed in a test tube, followed by 25 ml of ethanol. The mixture was allowed to react for 90 minutes on a hotplate set at 600 degrees Celsius. The test tube which contained the reaction product was transferred to a separator funnel following the reaction time. The tube was successfully washed with 20 ml of ethanol, 10ml of cold water, 10 ml of hot water, and 3 ml of hexane, all of which were added to the funnel. This extract was combined and washed three times with a 10ml aqueous solution of 10% v/v ethanol. The solvent was evaporated after being dried with anhydrous sodium sulfate. 1000 ul of pyridine was used to dissolve the sample, of which 200 ul was transferred to a vial for analysis.

Gas Chromatography–Mass Spectrometry (GC-MS)

The GC–MS analysis of bioactive compounds from the *Curcuma longa* extracts was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The starting temperature was set at 50 –150°C with a 3°C/min increase rate and holding time of about 10 minutes. Finally, the temperature was raised to 300 °C at 10 °C/min. In a splitless mode, one microliter of the prepared 1% of the extracts diluted with respective solvents was injected. Based on the peak area produced in the chromatogram, the relative quantity of the chemical compounds present in the extracts *was* expressed as a percentage.

Identification of chemical constituents

Bioactive constituents extracted from *Curcuma longa* leaves were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems) (Buss and Butler, 2010).

RESULTS

A total of 11 peaks were identified from the GC-MS analysis of aqueous fraction of *Curcuma longa* leaves extract indicating the presence of 11 bioactive compounds. The chromatogram is presented in Fig.one, while the bioactive compounds with their retention time (RT), peak areas (%) molecular formula and molecular weight (MW) are presented in Table 1. The mass fragmentation of a few of the bioactive compounds with high concentrations are presented in Figure 2. The following bioactive compounds were present in the GC-MS analysis of *Curcuma longa* leaves; 10- undecen-4- 0ne, 2,2,6,6-tetramethyl, 2,6- Octadienal, 3-7 Dimethyl-(E), Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Cis-13- octadecenoic acid, methyl ester, Fumaric acid, pent-4- en-2-yl tride cylester, Heptadecanoic acid 16-methyl-, thylester, Cis- vaccenic acid, Oleic Acid, 12-Methyl-E, E-2, 13- Octadecadien-1- ol and 9,17- Octadecadienal (Z).

Table 1: Bioactive Compounds Identified in *Curcuma longa* Leaves Extract by GC-MS

S.NO	Name of Compound	RT (retention time)	Peak area (%)	Molecular formula	Molecular weight
1	10- undecen-4- 0ne, 2,2,6,6- tetramethyl	7.081	2.61	$C_{15}H_{28}O$	224
2	2,6- Octadienal, 3-7 Dimethyl-(E)	7.691	4.30	$C_{10}H_{16}O$	152
3	Hexadecanoic acid, methyl ester	16.993	16.40	$C_{17}H_{37}O$	270
4	n- Hexadecanoic acid	17.641	15.59	$C_{17}H_{34}O_2$	270
5	Cis-13- octadecenoic acid, methyl ester	18.808	28.40	$C_{19}H_{36}O_2$	297
6	Fumaric acid, pent-4- en-2-yl tride cylester	18.974	0.88	$C_{22}H_{38}O_4$	366
7	Heptadecanoic acid, 16-methyl-, thylester	19.050	6.03	$C_{19}H_{38}O_2$	298
8	Cis- vaccenic aci d	19.448	17.14	$C_{18}H_{34}O_2$	282
9	Oleic Acid	19.647	6.02	$C_{18}H_{34}O_2$	282
10	12- Methyl-E, E-2, 13- Octadecadien-1- ol	19.699	2.12	$C_{19}H_{36}O$	281
11	9,17- Octadecadienal (Z)	19.799	0.52	$C_{19}H_{36}$	264

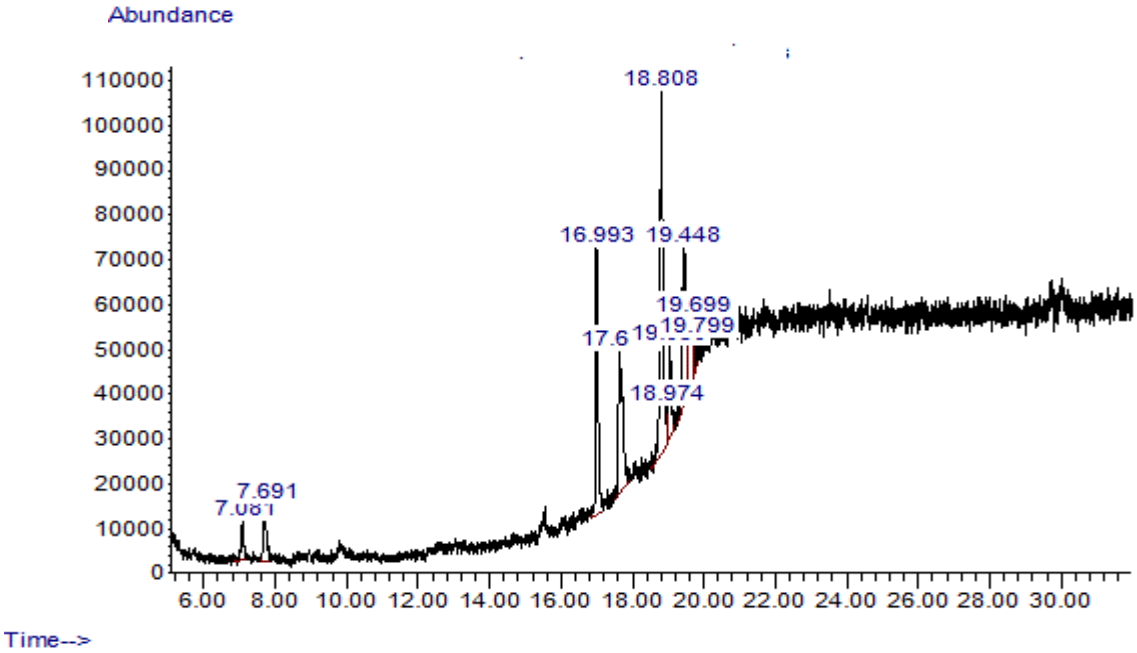
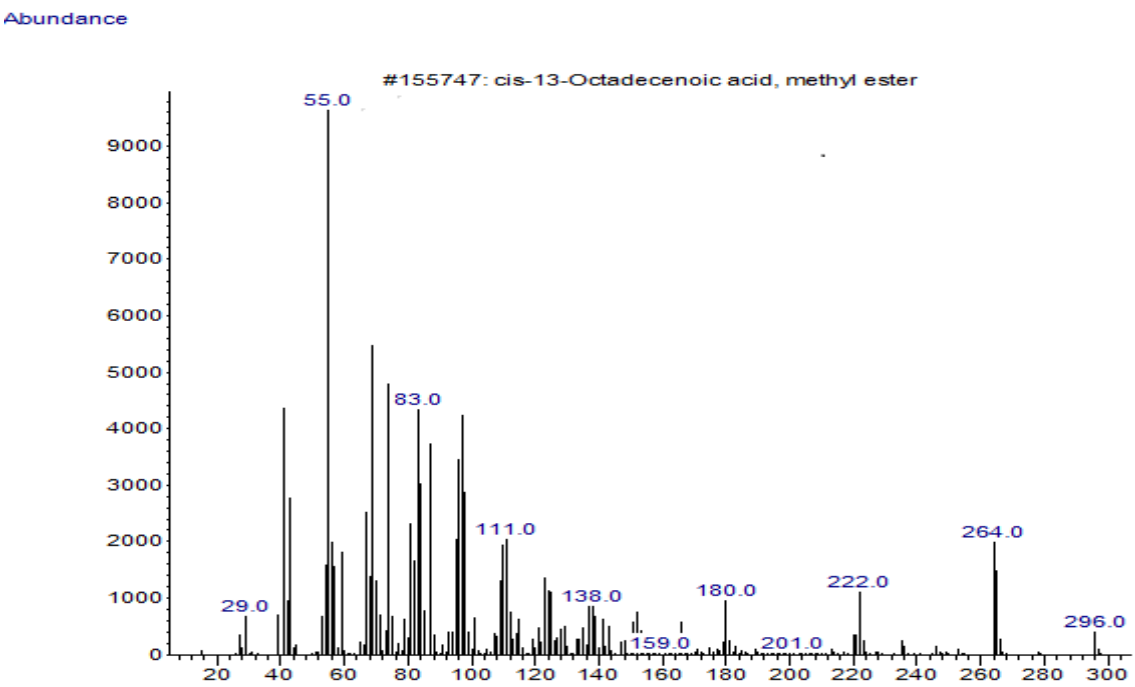
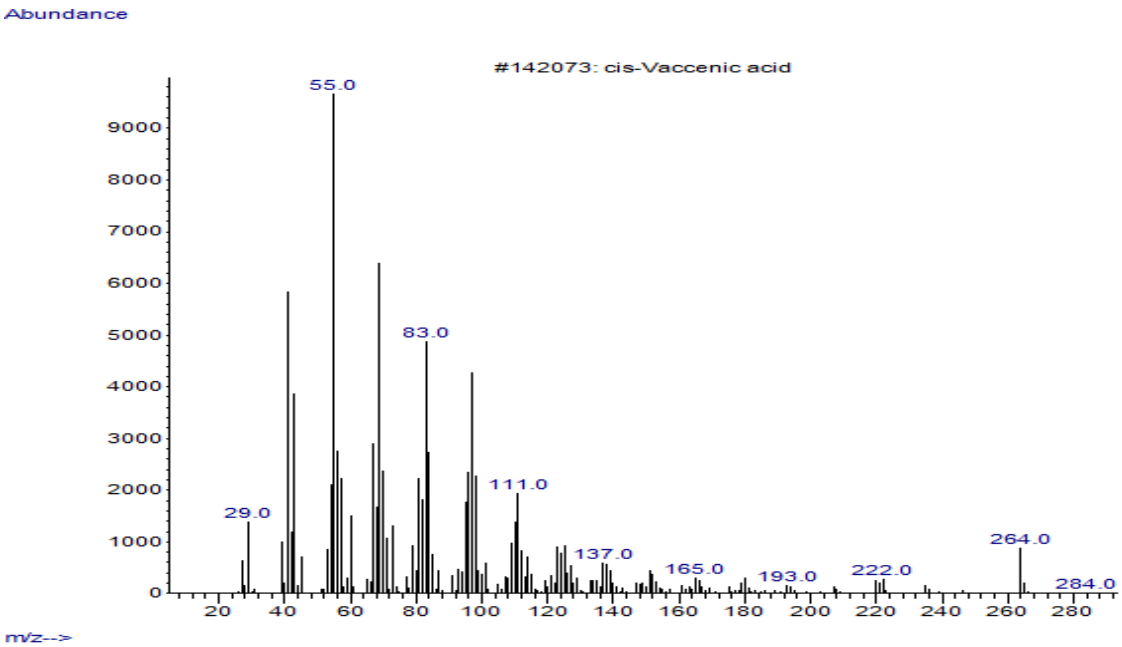


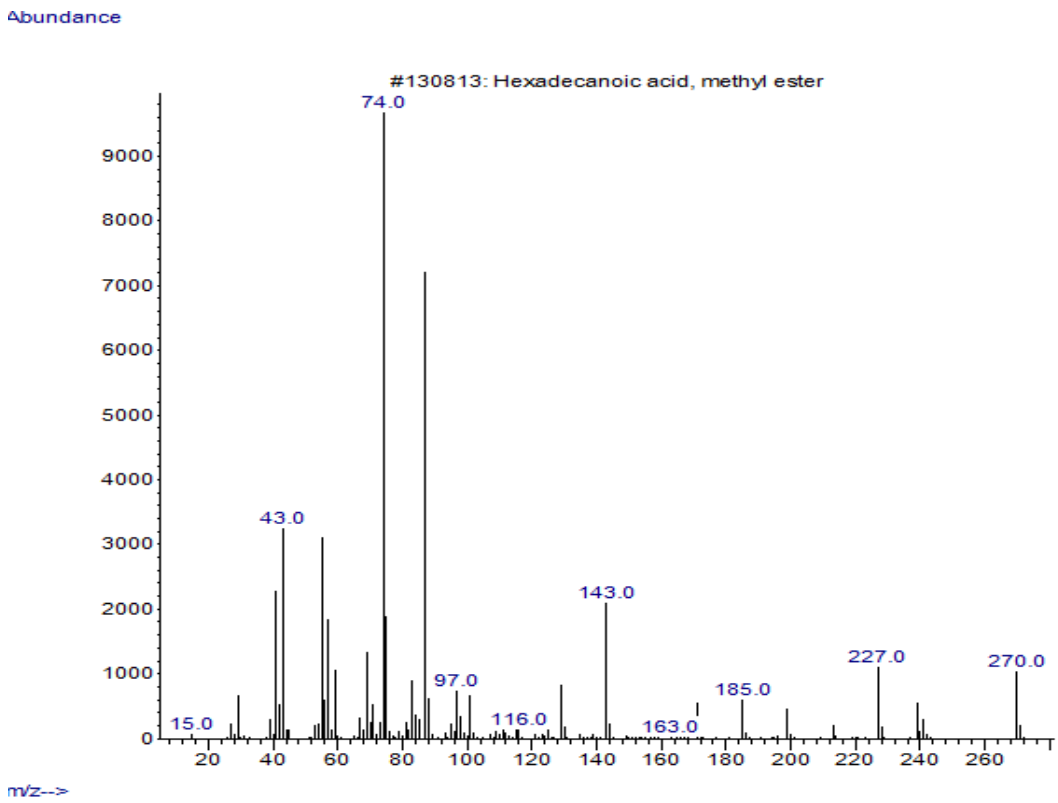
Figure 1: GC-MS analysis of *Curcuma longa* leaves extract of aqueous extract: the GC-MS chromatogram showed eleven peaks, indicating the presence of eleven compounds.



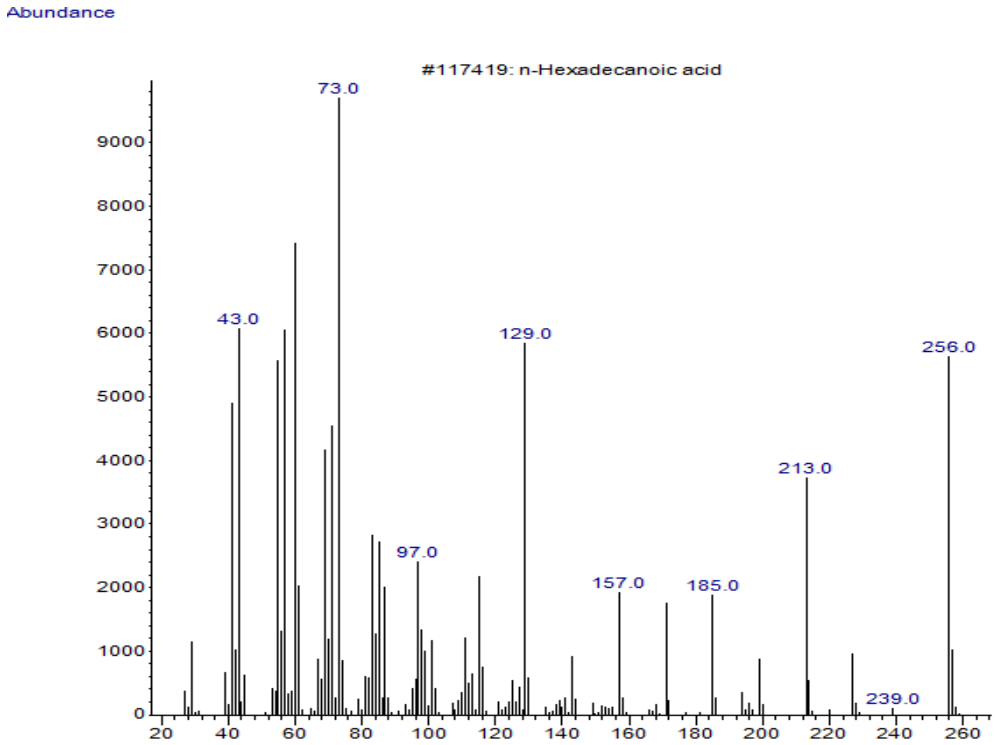
A. Cis-13 Octadecenoic acid, methyl ester



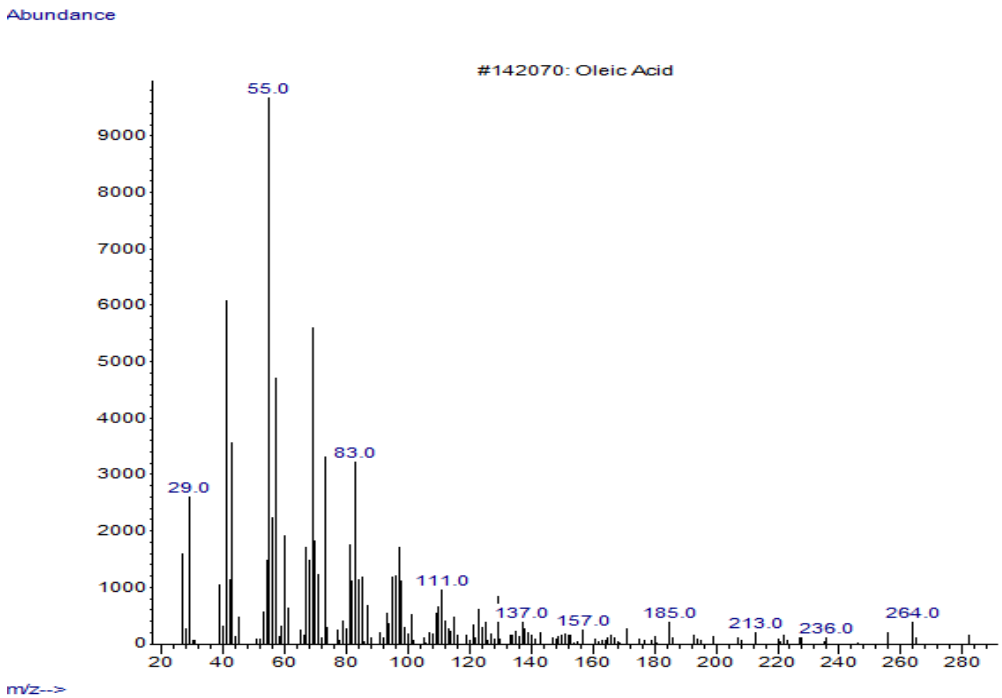
B. Cis-Vaccenic acid



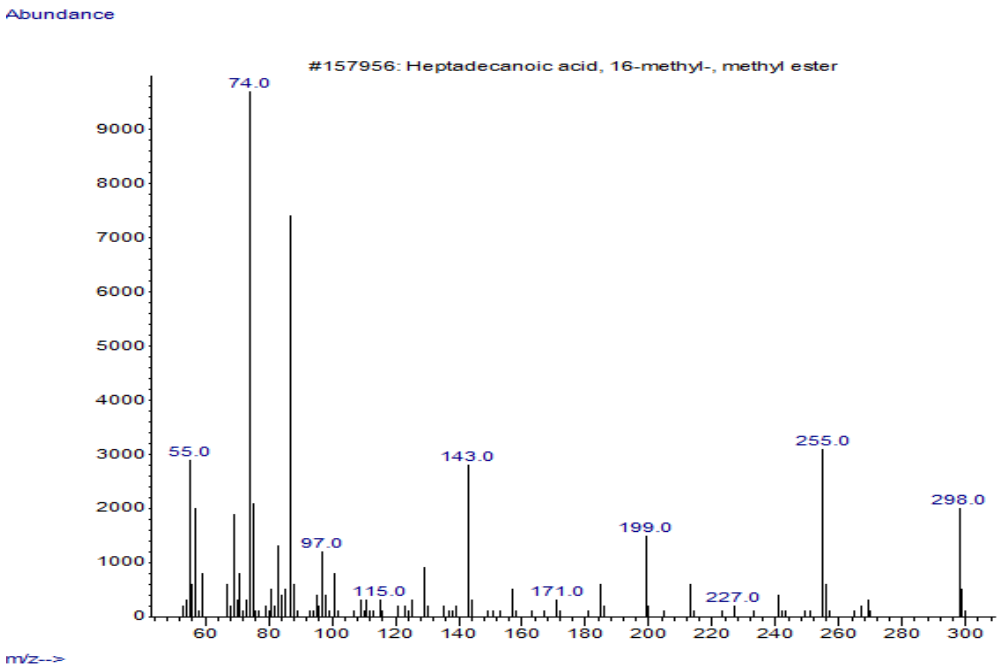
C. Hexadecanoic acid, methyl ester



D. n-Hexadecanoic acid



E. Oleic acid



F. Heptadecanoic acid, 16-methyl- methyl ester

Figure 2: (A,B,C,D,E,F): The following charts represent mass fragmentation of bioactive components with the highest percentage peak area identified in the aqueous extract of *Curcuma longa* and listed in Table 1 respectively.

DISCUSSION

Gas Chromatogarpghy-Mass Spectrometry analysis of *Curcuma longa* aqueous extracts showed the presence of eleven bioactive compounds. Among the identified bioactive components, Cis-13-octadecenoic acid, methyl ester, Cis- vaccenic acid, Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Heptadecanoic acid, 16-methyl-, thylester and Oleic acid had the highest percentage peak area while other compounds were identified as low level. However, two of the components were identified for the first time which included 10-undecen-4- 0ne, 2,2,6,6- tetramethyl and Fumaric acid, pent-4- en-2-yl tride cylester. The discovery of the two new compounds could be as a result of the geographical location of the plant collection. Also, the bioactive compounds present in the leaves of *Curcuma longa* are different from the bioactive compounds present in the rhizomes of *Curcuma longa* as seen in a study by Subramanian et al (2019). These constituents have a number of pharmacological actions such as antibacterial, anti-oxidant, anti-inflammatory and anti-cancer properties, amongst others. Cis-13-Octadecenoic acid, methyl ester possesses anti-inflammatory activity (Diab et al., 2021), cis vaccenic acid possesses anti-bacterial activity (Hamazaki et al., 2016) whereas Heptadecanoic acid has several benefits which includes a key player in the metabolic benefits of dairy products in humans, its use can help prevent elevated ferritin and associated complications such as diabetes and autoimmune diseases. Hexadecanoic acid and n-Hexadecenoic acid possesses antioxidants properties (Sera et al., 2021), n-Hexadecenoic acid also possess hypocholesteromic, nematicide and pesticide activity (Sheela and Utaayaukumari, 2018). Oleic acid exhibits anti-cancer, anti-inflammatory and anti-bacterial activity (Paul

et al., 2011). Other compounds with low concentrations possess biological activity such as 3, 7 dimethyl-2,6 octadienal, which is used in food industries as a flavoring agent (Liao et al., 2015). Modern pharmacological studies focusing on the chemical nature of turmeric, its consequences for various parameters, and investigations of the mechanisms of the observed biological actions and molecular research can validate comprehensive traditional knowledge on turmeric (Ashraf and Sultan, 2017). Considering the above-mentioned properties of *Curcuma longa* leaves, it is clear that with its availability in pure form, and possessing a broad spectrum of biological functions, it can be applied in the development of various medicinal products that can help in the treatment of various diseases in the near future.

Conclusion

This current work was done to establish the various GC-MS parameters present in *Curcuma longa* which could serve as important and commercially interesting parameters in both research institutes and pharmaceutical companies for the manufacturing of innovative drugs. From the GC-MS analysis, it showed that *Curcuma longa* contained bioactive compounds with medicinal potentials which are responsible for their therapeutic effects. Further investigation is required for the development of newer drugs using some of these compounds found in *Curcuma longa.*

COMPETING INTERESTS

The authors declare that there is no competing interests.

AUTHORS' CONTRIBUTIONS

All the authors participated fully in the design of the study. IIA and FCI sourced the plant materials while NUN dried and extracted the plant materials. All authors contributed in the chromatographic analysis of the plant extract. CIC, ESA and IIA contributed in the GC-MS evaluation of the plant samples. IIA and FCI participated in the interpretation of results. All authors contributed in assessing the manuscript before it was finally approved for submission.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the plant taxonomist who authenticated the plant materials and the Department of Crop science, Federal University of Technology Owerri, Imo state, Nigeria for their time and assistance.

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