Scientific African 3 (2019) e00059

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

GC–MS analysis of bioactive compounds from *Melastomastrum capitatum* (Vahl) Fern. leaf methanol extract: An anticancer plant

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ARTICLE INFO

Article history:
Received 10 January 2019
Revised 21 March 2019
Accepted 29 March 2019

Keywords:
GC–MS
Melastomastrum capitatum
Bioactive compounds
Column chromatography

ABSTRACT

Traditional and herbal medicines are the most and easily available health care remedy in most African countries. The use of medicinal plants began centuries due to their accessibility. *Melastomastrum capitatum* is a plant use in Mambila plateau in Taraba State, Nigeria for treating many diseases notably ovarian cancer in traditional medicine. Decoction of leaf of the plant is mostly used in this case. This study was carried out in order to determine the bioactive compounds present in the leaf methanol extract of *M. capitatum* by using the GC–MS machine. Eight hundred gram of powdered leaves was extracted in methanol using Soxhlet apparatus and concentrated *in vacuo* in a rotary evaporator. The crude extract was purified in a silica gel filled column chromatography. Fractions were grouped based on their TLC similar profiles into six, and analyzed in an Agilent 7890A GC system coupled with a 5975C VL mass spectrum detector. GC–MS analysis revealed the presence of the following phyto-compounds: Methyl tetradecanoate (Z; 242.3975, RT: 14.171; 1.93%), 9-dodeoenoic acid methyl ester E (Z; 296.495, RT: 16.853, 1.01%), Hexadecanoic acid methyl ester (Z; 270.45, RT: 17.475; 86.31%), 9, 12-octadecadienoic acid methyl ester (Z; 294.479, RT: 20.514; 100.00%), Methyl stearate (Z; 298.504, RT: 20.989; 7.66%) and Methyl 18-nonadecanoate (Z;326.557, RT: 24.500; 0.71%). These compounds are the first isolated compounds from the leaf, and unarguably play very crucial roles in the health care system especially in cancer treatment and other diseases. The study showed that the presence of these compounds in the leaves of *M. capitatum* might be responsible for its biological activities in traditional medicine. It is therefore a possible important plant of medical and pharmaceutical importance from which drug can be discovered.

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https://doi.org/10.1016/j.sciaf.2019.e00059
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Introduction

Melastomastrum capitatum is a shrubby plant that grows up to 1·25 m high found in dry situations and stream banks in Nigeria especially in Mambila Plateau, Taraba State [1]. It is locally called "*Belkon*" by the Fulani tribe in Mambila Plateau Nigeria who use the leaf to treat cancer and other diseases in traditional medicine. A large part of the plant has sweet to sour taste. The leaf-sap diluted into a little water is used in Ivory Coast as a sedative. Its leaf can reduce cholesterol, acts as an analgesic, and cleans the blood vessels in traditional medicine. In South-south Nigeria, it has been used to heal many wounds. The leaves are somewhat distinctive, being opposite decussate and usually contain sweet and sour tastes. Research on *M. capitatum* is still new and no compounds have been isolated from any part of the plant, but preliminary phytochemical screening of the leaf methanol extract has been reported to reveal the presence of glycosides, alkaloids, and carbohydrates [2–5].

The plant usually grows throughout the year in the edges of water cannels and valleys in Mambila plateau Sarduana Local Government Area of Taraba, and it is one of the most dominant shrubs in the area with occasionally colored leaves. The presence of water in this area and cold climate essentially favours their flourish. Apart from the Mambila plateau, the plant also grows well in Ogurugu Uzo-Uwani Local Government Area of Enugu State; where it is majorly found in mash land and wet areas especially in shallow streams. They also found in Ibaji Local Government Area of Kogi State, Borgu Local Government Area of Niger State, Southern part of Kaduna State, Edo State, South-west Nigerian States of Ondo, Ekiti, Lagos Oyo and Osun [6].

The leaves are the major part use in traditional medicine where it is used for blood purification, as a remedy for stomach ache, as an antitumor and anticancer agent by the Fulani tribe (these claims have not been scientifically proven or ascertained yet). The leaf has been shown to display analgesic and anti-inflammatory properties, and antihypercholesterolemic in mice [7]. Other uses of the plant are not documented or known yet since research activities on the plant is so scanty due to its virginity in plant science and pharmacognosy.

This present study was therefore carried out to determine the major bioactive compounds abound in *M. capitatum* leaf methanol extract using gas chromatography/mass spectrophotometer (GC–MS). This is the first time an attempt was made to isolate compounds from the leaf of *M. capitatum*, hence the justification for the study (Plate 1).

Materials and methods

Materials

Some of the chemicals and apparatus used in this research are: n-hexane, ethylacetate, n-butanol, methanol, GEMINI-20 portal milligram scale (AWS, China), 1000 mL capacity separating funnels, rotary evaporator, TLC plates silica gel 60 F2S4 20 × 20 cm (6Merck. Germany), 35 × 950 mm glass column, GC–MS (7890 GC system; Agilent technologies), among others.

Methods

Collection and identification of plant

Fresh leaves of *Melastomastrum capitatum* were collected from Mambila plateau Sarduana Local Government Area, Taraba State, Nigeria, in the evening hour to correlate with the time the traditional medical practitioners collect the plant for treatment purposes. It was authenticated with a voucher specimen number of "*ABU2761*" at the herbarium of Department of Botany, Ahmadu Bello University, Zaria by a taxonomist Mr. Namadi Sunusi.

Preparation of plant material

The fresh leaves of *M. capitatum* were air-dried under shade for two weeks and were pulverized into a fine powder using an electronic blender (Model 5000 MH, Japan). The powder was then sieved using sieve number 20 mesh to obtain a fine powder and remove any unwanted debris and was weighed on a scale balance to know the initial weight of the powder. The powdered plant material was then kept in a clean and dried bag for further use.

Extraction of plant material

Twenty and a half kilogram (2.5 kg) of powdered leaves of *M. capitatum* was defatted with petroleum ether (60–80 °C) to remove fat and latex. The defatted plant residues were extracted using Soxhlet apparatus in methanol for 12 h at a temperature of 40 °C, to obtain the methanol extract (MCME). Methanol was used because it has the ability to extract both polar and non-polar constituents in the leaf. The solvent was regularly changed until no coloration was observed. The collected extract was filtered through Whatman filter paper No. 1. Finally, the filtrate was concentrated *in vacuo* using rotary evaporator. The final weight of the extract was then noted and the percentage yield was calculated with reference to the initial weight of the powder as given below:

$$\%\,Yield = \frac{Final\ weight\ of\ extract}{The\ initial\ weight\ of\ the\ powdered\ drug} \times 100 \quad (1)$$

It was then stored in a clean dried bottle and kept in a desiccator for further use.

Plate 1. Pictorial view of *M. capitatum* in its natural habitat. Source: Ukwubile et al. [22].

Liquid to liquid partitioning of M. capitatum leaf extract

Methanol leaf extract of *M. capitatum* was partitioned repeatedly with solvents in increasing order of their polarities in this order: n-hexane, ethyl acetate, n-butanol, and methanol. Three hundred and fifty gram (350 g) of leaf extract was dissolved in 300 mL of methanol and 700 mL of distilled water in a 1000 mL separating funnel. The content was shaken vigorously until the extract properly dissolved. It was then partitioned with 300 mL each of the above solvents for three times to get the filtrates. The obtained filtrates were then concentrated *in vacuo* in a rotary evaporator to obtain a dark jelly-like extract. The extracts were then weighed and stored in clean sample bottles after labelling them.

Purification of extract by column chromatography

Five grams of methanol extract was subjected to silica gel column (35 × 950 mm), because from the previous works by Ukwubile et al., methanol portion of the extract posses with anticancer activity on ovarian cancer cell line OV7 with IC_{50} value of 22.12 ± 0.2. The selective index of this extract against veroE6 cell line was high as compared to other fractions investigated. The elution was gradient with ethyl acetate: methanol in 95:5, 90:10, 80:20, 70:30, 50:50, and 0:100. A total of 50 fractions were collected and grouped into six (I–VI) according to their profiles on TLC plates. Each of the groups was further purified on a short column to obtain final purified portions tagged as ME I, ME II, ME III, ME IV, ME V, and ME VI. Each isolate was concentrated to dryness, weighed and were stored in ALS vial for further use [8–10].

GC–MS analysis of methanol fractions

ME I-ME VI was dissolved in methanol and injected in an Agilent 7890A GC system coupled with an MS (Agilent technologies) by author injection at the Central Research Laboratory, University of Lagos, Akoka, Lagos Nigeria. The operating conditions of the GC–MS set for the analysis were as follows: oven temperature 50 °C for 2 min then 100 °C at 10 °C/ min and finally increased to 200 °C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out 70 eV. The total running time of the GC was 24.50 min [11].

NIST14.L library (2018) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST14.L) [12].

Results and discussion

GC–MS analysis of methanol leaf fractions of M. capitatum

Gas chromatography-mass spectrometry (GC–MS) analysis of M. capitatum leaf methanol extract fractions revealed the presence of six major bioactive compounds as shown in Table 1; Fig. 1. From the results from GC–MS spectra, 9,12-octadecadienoic acid, methyl ester (Z, Z), hexadecanoic, methyl ester, and methyl stearate are the most abundant in occurrence while methyl tetradecanoate, 9-dodecanoic acid, methyl ester E and Methyl-18-methyl -nonadecanoate are the least. These bioactive compounds has been reported to play crucial roles in disease and general metabolisms of humans. For example, hexadecanoic acid, methyl ester has antioxidant, anti-inflammatory, antihyperlipidemic as well as antimicrobial in functions (Figs. 2–7) [13,14].

Table 1
Phytochemical components of *M. capitatum* leaf methanol fraction using GC–MS.

S/no	RT (min)	Name of compound	MF	MW(g/mol)	PA(%)
1.	14.171	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.3975	1.93
2.	16.853	9-dodecanoic acid, methyl ester E	$C_{19}H_{36}O_2$	296.495	1.01
3.	17.475	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	270.45	86.31
4.	20.514	9,12-octadecadienoic acids, methyl ester (Z, Z)	$C_{19}H_{34}O_2$	294.479	100.00
5.	20.989	Methyl stearate	$C_{19}H_{36}O_2$	298.504	7.66
6.	24.500	Methyl-18-methyl-nonadecanoate	$C_{21}H_{42}O_2$	326.5570	0.71

RT = retention time; MF = molecular formula; MW = molecular weight; PA = peak area.

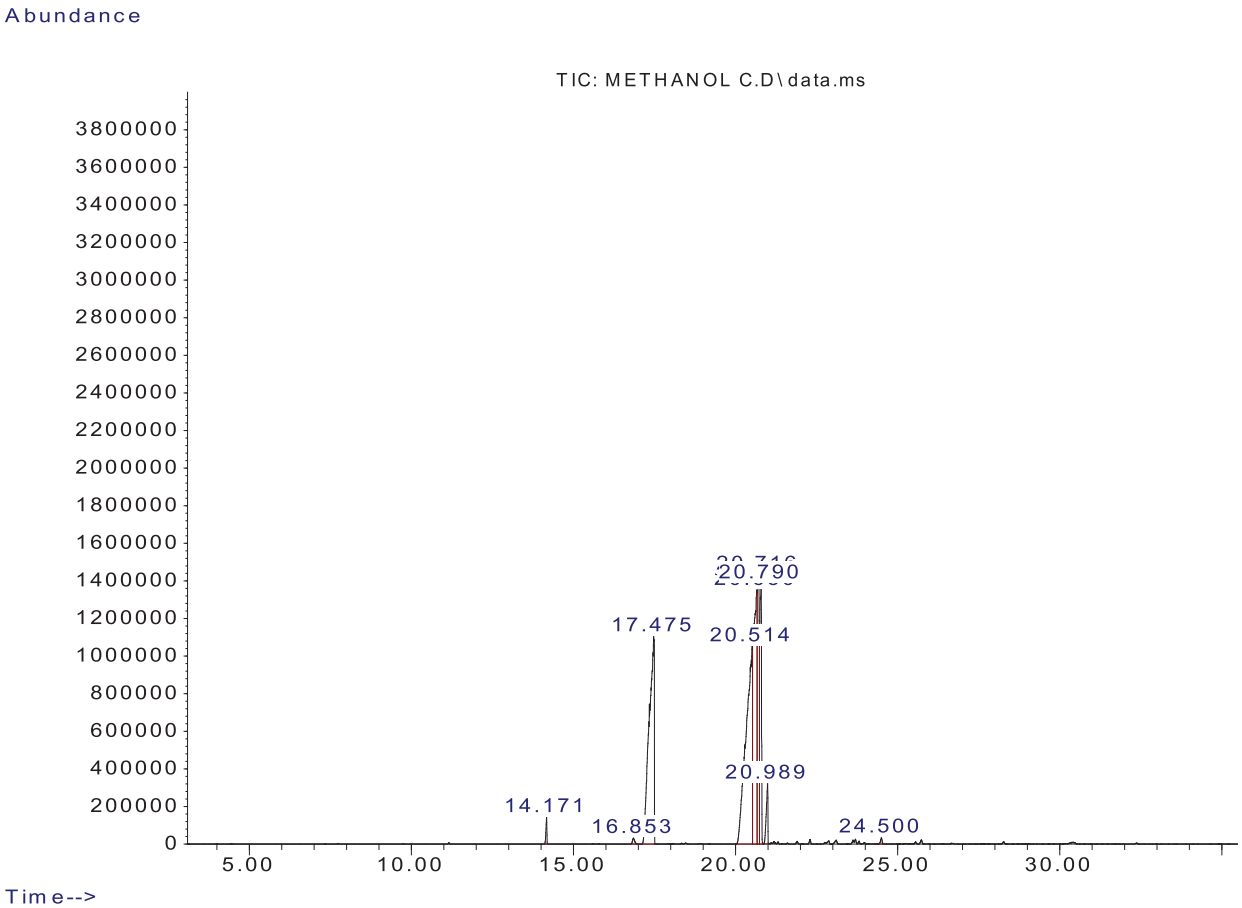

Fig. 1. GC–MS profile of crude leaf methanol extract of *Melastomastrum capitatum*.

In a similar vein, 9-dodecanoic acid, methyl ester E and 9, 12-Octadecenoic acid methyl ester (Z, Z) has been reported to show anticancer activity against prostate cancer cell line with an IC50. It value of 32.11 ± 0.41 [14], antitumor and antioxidant activities. 9-dodecanoic acid, methyl ester E is also used as dermatitigenic flavor and exists in human blood and urine where it serves as endogenous peroxisome proliferator-activated receptor ligand. 9-Octadecenoic acid, methyl ester (E) possess antioxidant and anticancer activities [14,15], and it has been isolated firstly from the leaves of *Physalis angulata* (Solanaceae,) as well as other plant families.

Methyl stearate is used as a solvent and lipid carrier in agriculture. 9,12-octadecadienoic acid, methyl ester (Z, Z) has been reported be possessed strong anticancer activity against human lung cancer cell line A549 with an IC_{50} value of 10.60 ± 1.12 [15], while methyl tetradecanoate is a membrane stabilizer, energy source by storing energy, and source of nutrient to the protoplasm [16–20]. Till date, researchers have not been able to state categorically the biological roles of methyl-18-methylnonadeanoate (Fig. 7). This is the first time this compound was isolated from any plant family. It might account for the major reason in which its biological activities were not known or documented in the literature. This class of fatty acid, however, might also play a crucial role in the metabolism of the plant as well as other health benefits to humans in traditional medicine. Methanol leaf fractions of *M. capitatum* showed the presence of compounds with documented

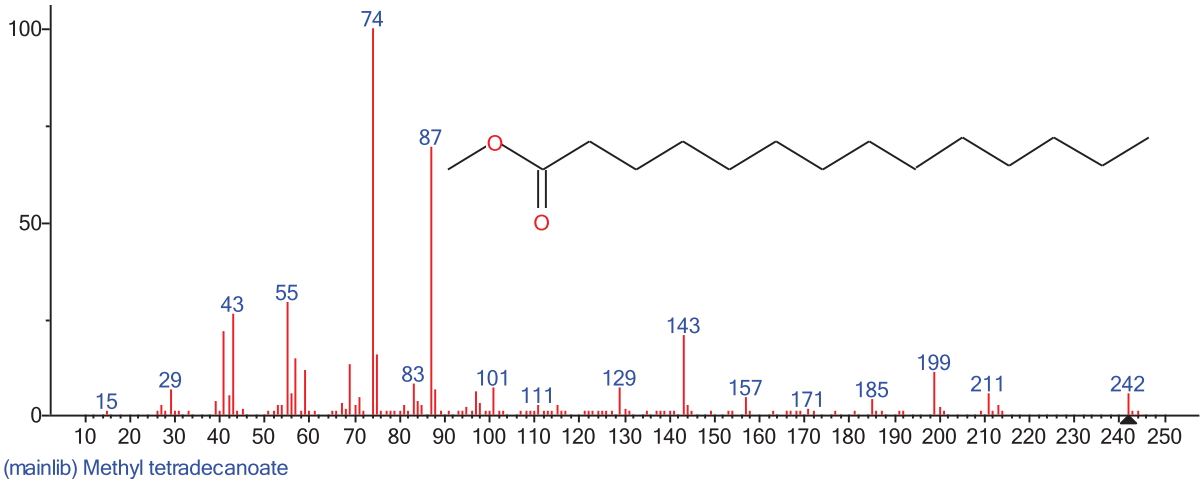

Fig. 2. GC–MS spectra of methyl tetradecanoate(1.93%, RT:14.171) from *M. capitatum* leaf extract.

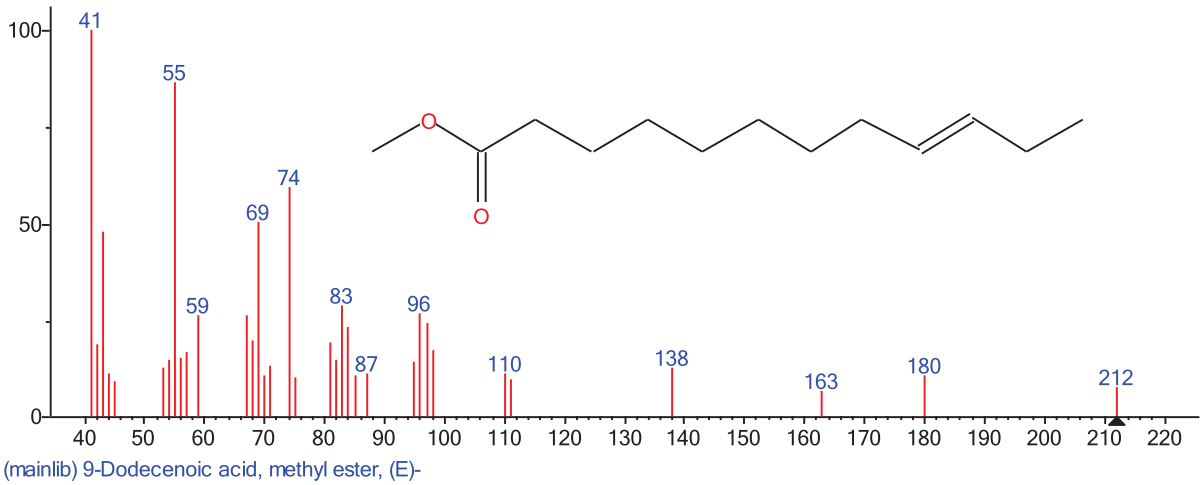

Fig. 3. GC–MS spectra of methyl 9-dodecanoic acid methyl ester (E) (1.01%, RT:16.853) from *M. capitatum* leaf extract.

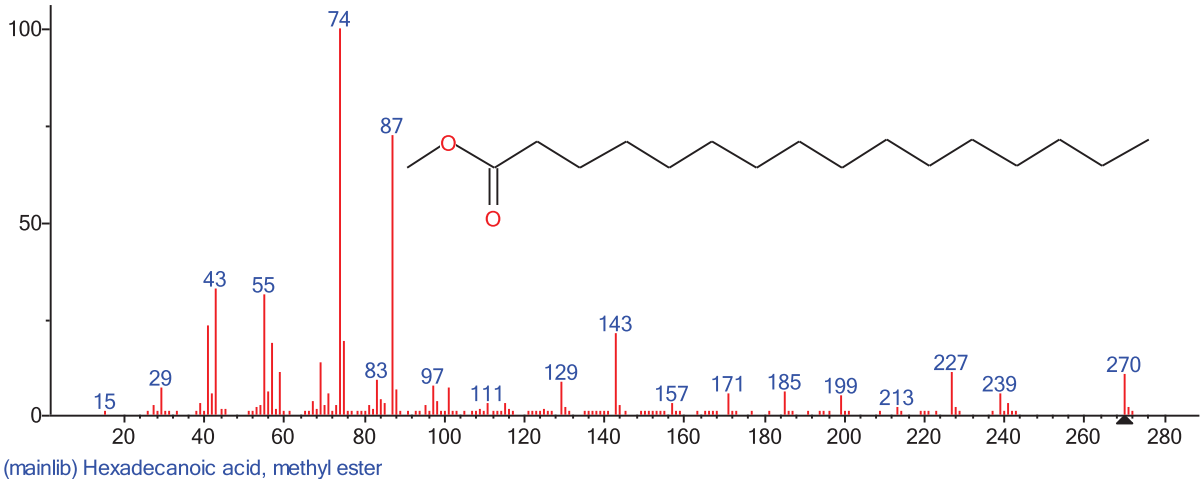

Fig. 4. GC–MS spectra of hexadecanoic acid methyl ester(86.31%, RT: 17.475) from *M. capitatum* leaf extract.

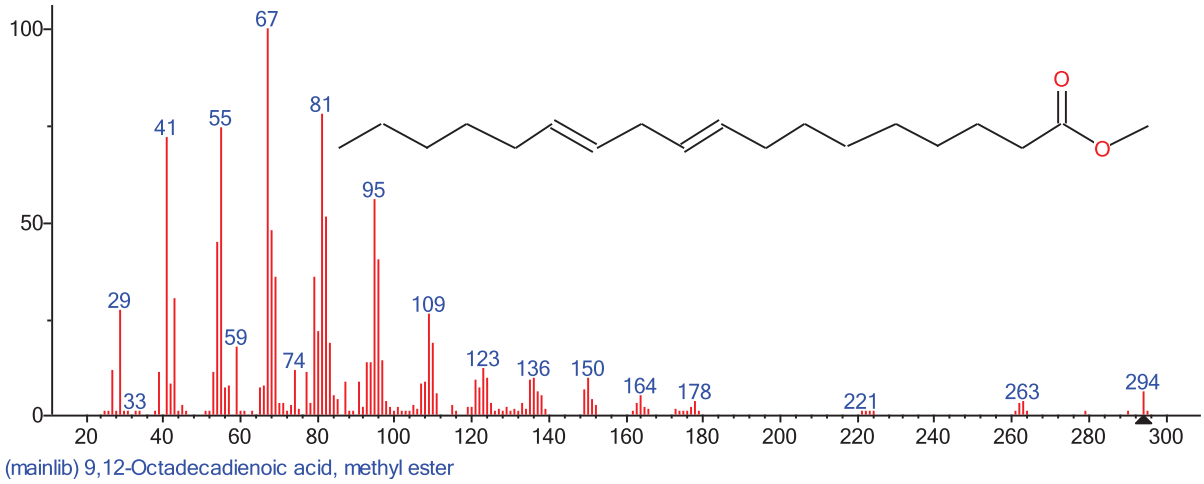

Fig. 5. GC–MS spectra of 9, 12-Octadecadienoic methyl ester(100.00%, RT: 20.514) from *M. capitatum* leaf extract.

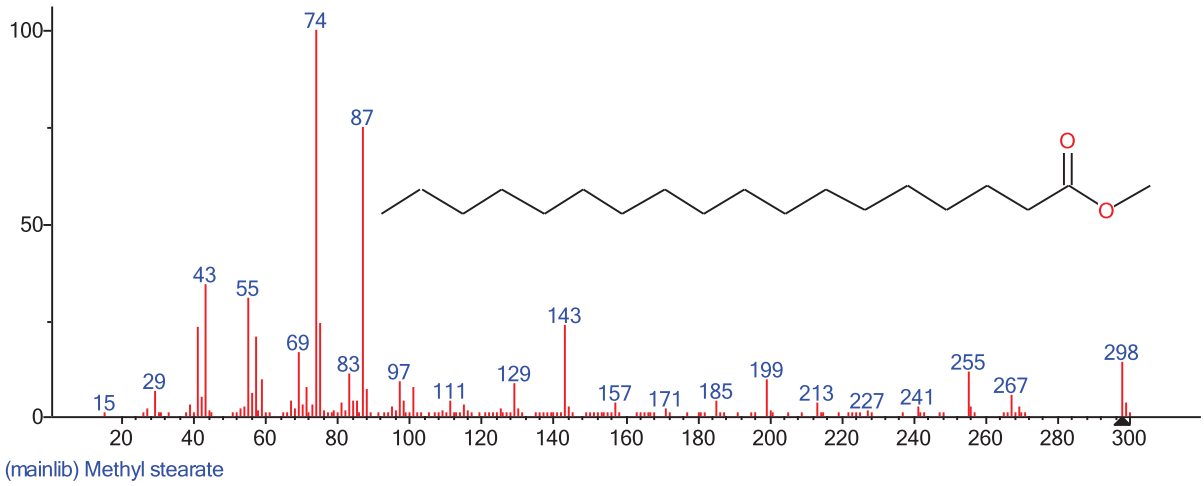

Fig. 6. GC–MS spectra of methyl stearate(7.66%, RT: 20.989) from *M. capitatum* leaf extract.

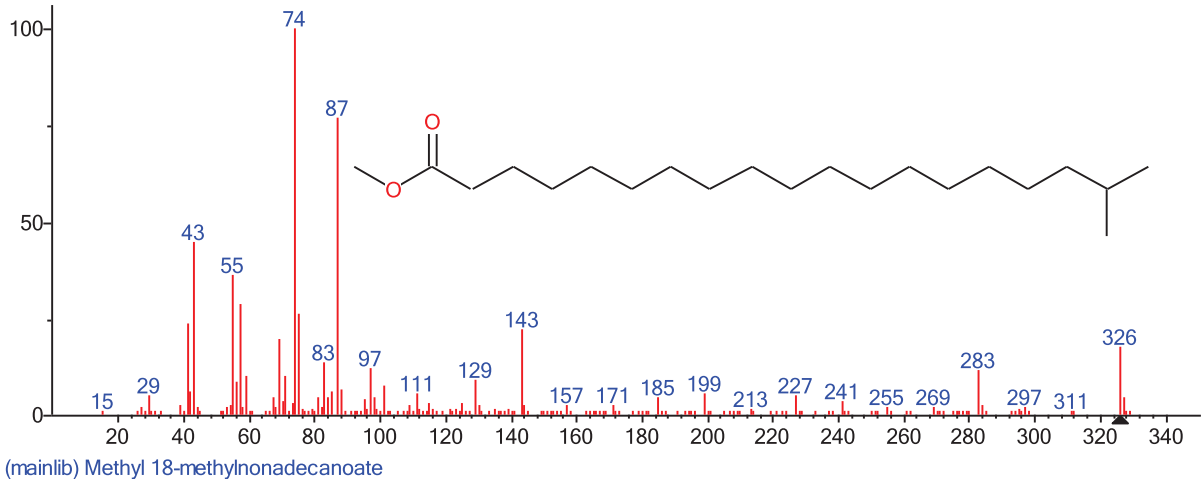

Fig. 7. GC–MS spectra of methyl 18-methylnonadecanoate(0.71%, RT: 24.500) from *M. capitatum* leaf extract.

anticancer activities, but their percentages in this present study might not be enough to activate quantum cancer therapy when administered orally due to the activities of gastro-intestinal tract enzymes [21]. For proper action of drugs on specific site in the body, there is the need for a chemotherapeutic agent to be enough in terms of availability to disease tissues of interest [21].

Hence, in our ongoing research, we are comparing the activities of these bioactive compounds with those obtained from ethyl acetate fractions of *M. capitatum*. From the study, methyl-18-methylnonadeanoate was isolated as a new compound in which its biological activities were not known or documented. Finally, this present was the first time an attempt was made to isolate compounds from the leaf or any other morphological parts of *M. capitatum*. Literature on the biological activities of the plant is also limited. Therefore, more research is recommended on this novel plant with regards to the isolation of its compounds from leaves using different solvents, and evaluation of its acclaimed anticancer activity on ovarian cancer as well as other biological activities in traditional medicine. Furthermore, there is the need to investigate the biological activities of these isolated compounds to know exactly which of the compounds is responsible for its acclaimed anticancer activity on ovarian cancer and to further enrich the literature on the plant.

Conclusion

This present study identified six fatty acid compounds. Out of these compounds, one compound identified as methyl-18-methylnonadeanoate was isolated for the first time from this plant, and there was no documented evidence on its biological activities. Furthermore, the presence of these compounds might be responsible for the acclaimed biological activities of *M. capitatum* in traditional medicine. It is, therefore, recommended that further research is necessary to determine the biological activities of these isolated compounds with regards to their anti-ovarian cancer activities in order to ascertain this claim scientifically. Finally, further research on this plant will further provide adequate literature on the plant because the available literature is very scanty due to the virginity of *M. capitatum* in Pharmacognosy and Medicinal chemistry researches.

Acknowledgments

The authors are grateful to Mr. Julius Nyiayem of Department of Chemistry, Federal University of Agriculture Makurdi, Nigeria for his technical advice during this work.

Conflict of interests

We have no conflict of interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2019.e00059.

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