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Screening of phytocompounds in the leaf extract of *Moringa oleifera* by gas chromatography-mass spectroscopy

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Abstract

Background and objective: Numerous studies have documented the existence of antimicrobial compounds within different plant components, including leaves, bark, fruit, root, and flowers. Moringa species have been extensively studied and documented as plant herbs, primarily due to their exceptional nutritional and medicinal characteristics. This study aimed to carry out phytocompound screening of *Moringa oleifera* extract using gas chromatography-mass spectroscopy technique.

Materials and methods: For extraction, 100 ml of ethanol per gram of plant leaf powder was used. The ethanolic extract was subjected to vacuum drying at 40 °C. Then, 10 mg of dry extract was solubilized in 1 ml of ethanol. The phytochemicals extracted from *Moringa oleifera* leaves were analyzed using gas chromatography-mass spectrometer.

Results and conclusion: Eleven compounds were detected through the gas chromatography-mass spectrometry (GC-MS) analysis of the leaf extract of *M. oleifera*. Among these compounds, the most prevalent were methyl (11E)-11-octadecanoate, accounting for 30.15% of the identified compounds, and cis octadecanoic acid, which constituted 19.16% of the total compounds. This study has demonstrated that the leaf extract of *M. oleifera* possesses phytochemical compounds that have the potential to serve as substitutes for antibiotics, antihelminthics, and antivirals against various infectious agents. Additionally, they can function as nutritional supplements for non-infectious diseases. Moreover, they exhibit antioxidant properties and can be utilized as flavor enhancers.

Keywords: Medicinal properties, bioactive compounds, Rigasa, Nigeria

1. Introduction

Moringa oleifera, a member of the Moringaceae family, is a genus of rapidly growing tropical deciduous plants. These plants are characterized by their thick, tuberous roots, light green leaves, and prolific flowering, which results in elongated, pendulous fruits and seeds [1]. The crop in question is indigenous to the northern regions of India, although it can also be found in other areas such as southwest Asia, southwest and northwest Africa, and Madagascar. The utilization of this practice has been deeply ingrained in the field of traditional horticulture for a considerable period. Its primary application has been in the realm of ornamental purposes within urban areas situated along the Pacific coast of Mexico. Additionally, it has found implementation in planta-

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tions located in Bolivia, Argentina, and various other regions across the globe [2]. The plant exhibits resilience to extended periods of aridity, thriving in regions characterized by arid and semi-arid conditions. Based on the findings of [3], it has been determined that the plant exhibits tolerance towards soils characterized by a pH range of 4.5 to 8. However, it is worth noting that a neutral or slightly acidic pH level is considered more advantageous for its growth and development. The species under consideration exhibits a high degree of adaptability, with a typical lifespan of approximately 20 years. Within a relatively brief timeframe, this species is capable of attaining a height ranging from 5 to 10 meters, with a remarkable growth rate of 4 meters achieved within a span of 6 months. The plant is widely recognized for its versatility, as it possesses an exceptional capacity to yield edible sustenance. The components of the plant encompass a range of structures, including leaves, pod shells, stems, flowers, fruits, and seeds. The various constituents of these botanical elements encompass a diverse array of advantageous compounds and nutrients, such as phenolic compounds, fatty acids, carbohydrates, dietary fiber, minerals, vitamins, and functional peptides. According to [4], substantial potential exists for the utilization of these entities within the food industry.

The nutritional value of Moringa is attributed to the diverse range of essential phytochemicals found in its leaves, pods, and seeds. *M. oleifera*, commonly known as *M. oleifera*, has been frequently described as a panacea with the potential to treat over 300 ailments. The utilization of Moringa in traditional herbal medicine has been prevalent among Indian and African populations for an extended period of time. The inclusion of phytochemicals renders it a favorable therapeutic agent. The utilization of *M. oleifera* leaves in ethnomedicine has been observed among indigenous healers as a means of addressing a range of health conditions, including gastric discomfort, stomach ulcers, diarrhea, dysentery, and skin infections. According to [3], Moringa has been found to have potential to stabilize sugar levels and arterial tension in specific instances of diabetes. According to [3], the leaves have been discovered to exhibit various medicinal properties, including anti-tumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, and antioxidant effects.

M. oleifera exhibits a rich composition of glucosinolates, flavonoids, phenolic acids [5], carotenoids [6], and tocopherols [7]. The leaf of *M. oleifera* contains a variety of bioactive compounds such as alkaloids, saponins, tannins, steroids, phenolic acids, carotenoids, polyphenols, isothiocyanates, phytates, glucosinolates, flavonoids, and terpenes [8]. The glucosinolate known as benzyl 4-O-(α-L-rhamnopyranosyloxy)-glucosinolate, commonly referred to as glucomoringin, is the most prevalent compound within the glucosinolate family. The leaves of the plant contain a total of 11 phenolic acids, namely gallic acid, caffeic acid, chlorogenic acid, o-coumaric acid, p-coumaric acid, ellagic acid, gentisic acid, sinapic acid, and syringic acid [9]. Additionally, the leaves also contain derivatives of these phenolic acids, such as coumaroyl quinic acids and their isomers, as well as feruloylquinic and caffeoylquinic acids. Furthermore, the leaves contain a total of 26 flavonoids, primarily in the form of flavonol and glycoside compounds. These flavonoids include quercetin, rhamnetin, campferol, apigenin, and myricetin [8]. The class of compounds known as flavonoids encompasses various types of flavonol glycosides, including glycosides, rutinosides, and malonyl glycosides, which consist of quercetin, kaempferol, and isorhamnetin. Additionally, lignans such as isolariciresinol, medioresinol, epipinoresinol glycosides, and secoisolariciresinol are also included in this group [2]. Moreover, there exists a variation based on geographic location indicating a greater concentration of phenolic compounds in Pakistan compared to India, Thailand, Nicaragua, and the United States [10-11]. Concentration of flavonoids in *M. oleifera* leaves is significantly greater than the seeds, with levels ranging from 2000 to 12,200 mg per dl [12].

The seeds of *M. oleifera* are known to contain phytosterols, with sitosterol, stigmasterol, and campesterol being the most prevalent among them [13]. Alkaloids, saponins, phytates, tannins [14], and phenolic compounds such as quercetin and p-hydroxybenzoic acid [14] are also present in the sample. It is, therefore, imperative for the phytocompounds properties of this plant to be assayed for its medicinal and pharmaceutical applications. The objective of this study was to analyze the chemical composition of ethanol extracts derived from *M. oleifera* using GC-MS analysis. This investigation was conducted to identify the bioactive compounds present in *M. oleifera* leaf extracts with potential antibacterial activity against pathogens causing urinary tract and enteric infections. Ethanol extracts were used for extraction in this study due to their minimum toxicity and medium polarity in extracting both polar and nonpolar phytochemicals.

2. Materials and methods

2.1. Collecting the plant materials

The leaves of *M. oleifera* were gathered from a house in the Rigasa community and transported immediately to the Department of Biological Sciences at Kaduna State University in Kaduna, where they were subjected to identification by a qualified botanist. The leaves were dried in a shaded environment, followed by grinding into a powdered consistency using a sterile mortar and pestle. The powder was stored further in an airtight plastic container until analysis.

2.2. Extraction

Approximately, 100 ml of ethanol per gram of plant leaf powder was employed. The ethanol extract was subjected to vacuum drying at 40 °C. The desiccated extract was preserved in aseptic containers for subsequent utilization. Before conducting the experiment, 10 mg of the dry extract was solubilized in 1 ml of ethanol [15].

2.3. GC-MS analysis

The phytochemicals isolated from *M. oleifera* leaves were analyzed by gas chromatography-mass spectroscopy (GC-MS) (Agilent Technologies-5977MSD) equipped with a capillary column (Agilent Technologies GC-MS, HP-5MS). The dimensions of the column were 30 m × 0.25 mm ID, with a film thickness of 0.25 m. The column composition consisted of 5% diphenyl and 95% dimethyl polysiloxane. The system utilized an electron ionization with ionizing energy of 70 ev. The carrier gas was helium with purity of 99.99%. It was maintained at a constant flow rate of 1 ml/min. The injection volume was 1 ml, and a split ratio of 50:1 was applied. The injector temperature was 60 °C, and the ion source temperature was 250 °C. The mass spectra were acquired with a voltage of 70 ev. The relative percentage of each component was determined by comparing its average peak area to the total areas using the MassHunter software [16].

3. Results and discussion

Table 1 presents GC-MS analysis of the leaf extract of *M. oleifera*, including retention time (RT), Peak area, molecular weight, and chemical formula. The following figures show the chromatograph of the compounds presented in the sample. The values on the vertical axis show the peak area of the analyzer, while the values on the horizontal axis show the temperatures at which the compounds were detected (Figures 1-11).

Table 1- GC-MS analysis of *Moringa oleifera* leaf extracts

Compound	Retention time	Peak area	Molecular weight	Molecular formula
Octadecanoic acid	23.104	4.23	284.47	$C_{18}H_{36}O_2$
cis-Octadecenoic acid	22.423	19.16	282.46	$C_{18}H_{34}O_2$
Methyl hexadecenoate	21.896	7.64	268.43	$C_{17}H_{32}O_2$
Methyl (11E)-11-octadecenoate	21.657	30.15	296.48	$C_{19}H_{36}O_2$
Methyl linolelaidate	20.915	9.21	294.47	$C_{19}H_{34}O_2$
n-Hexadecanoic acid	19.629	7.46	256.42	$C_{16}H_{32}O_2$
Undecanoic acid	18.21	0.98	186.29	$C_{11}H_{22}O_2$
Methyl undecanoate	16.93	0.57	200.31	$C_{12}H_{24}O_2$
Methyl-14-methyl-pentadecanoate	9.298	17.67	270.45	$C_{17}H_{34}O_2$
Methyl tetradeca-8,10, trienoate 12-	9.090	2.08	236.34	$C_{17}H_{26}O_2$
Ethyl hexadecanoate	0.214	0.86	284.47	$C_{18}H_{36}O_2$

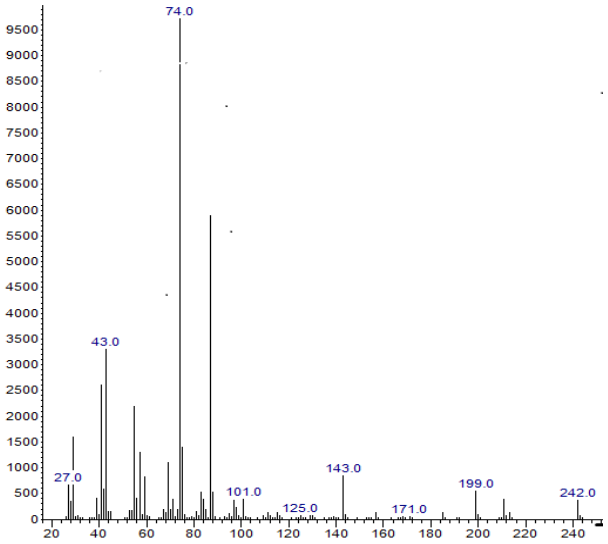

Figure 1- Octadecanoic acid

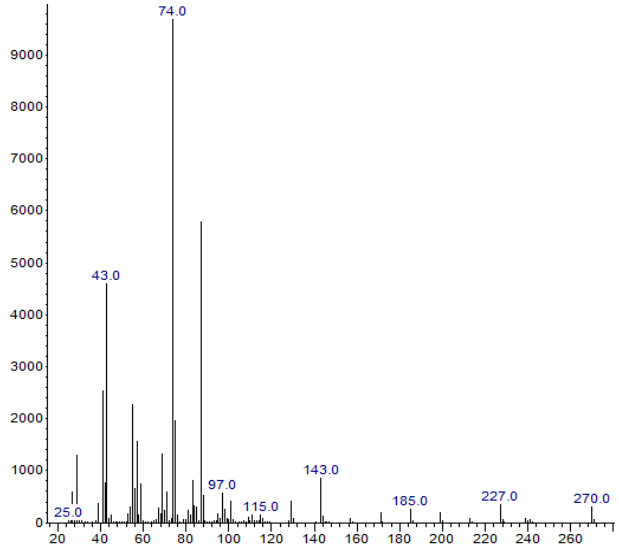

Figure 2- cis-Octadecenoic acid

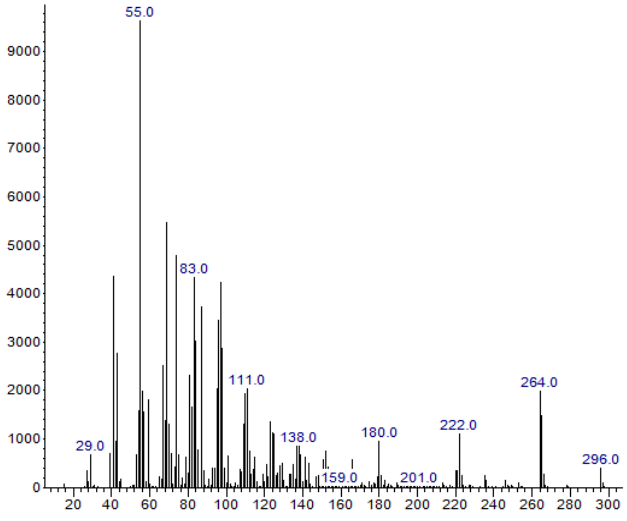

Figure 3- Methyl hexadecenoate

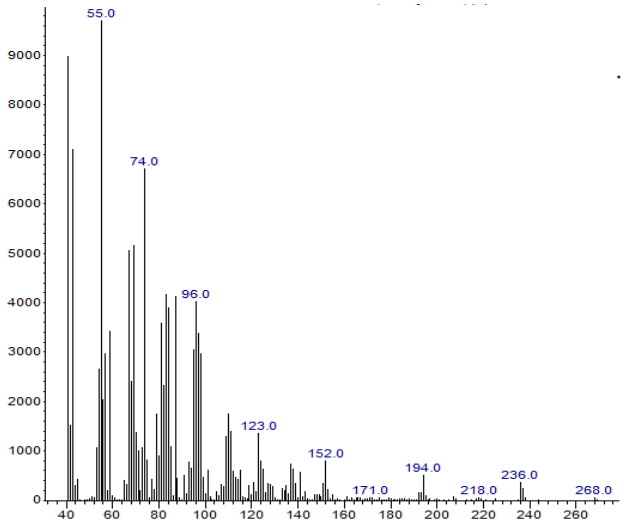

Figure 4- Methyl (11E)-11-Octadecenoate

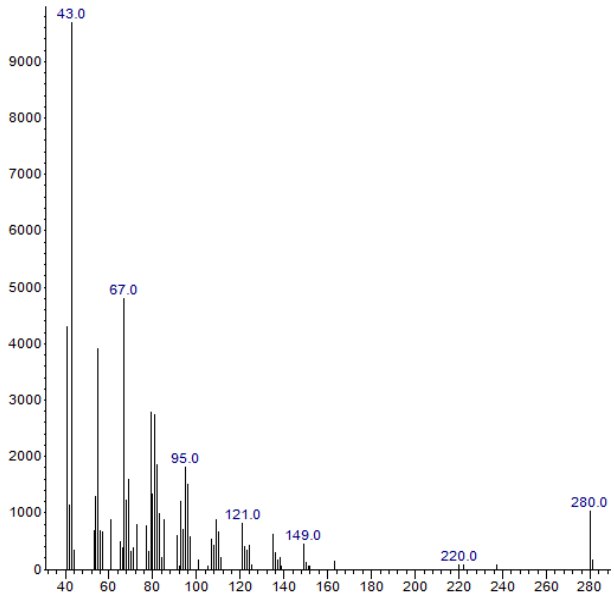

Figure 5- Methyl linolelaidate

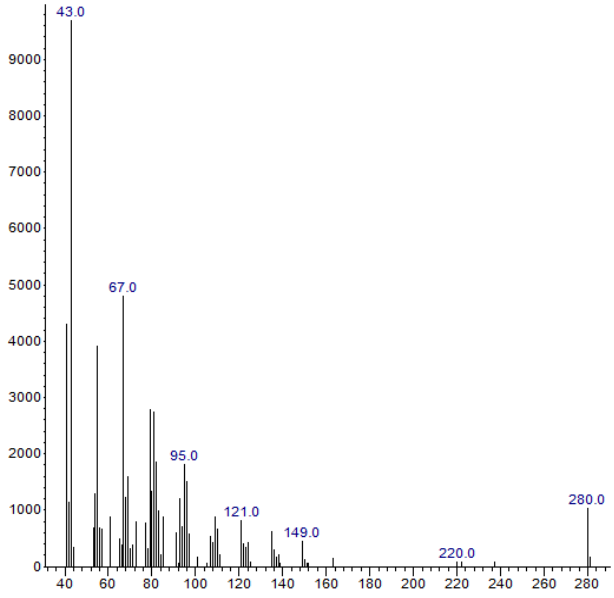

Figure 6- n-Hexadecanoic acid

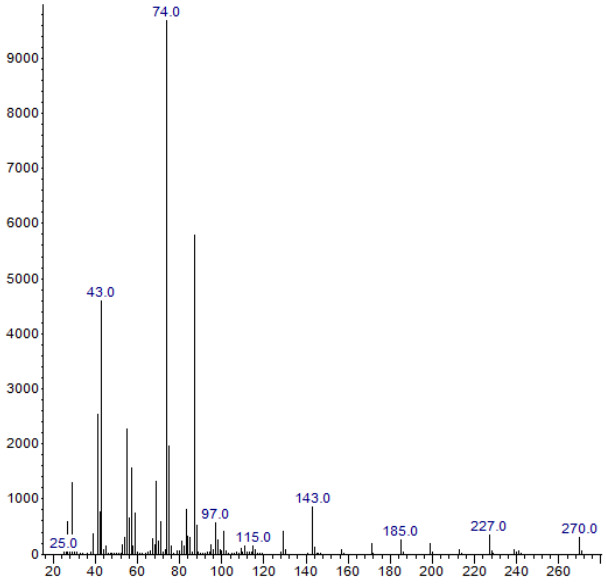

Figure 7- Undecanoic acid

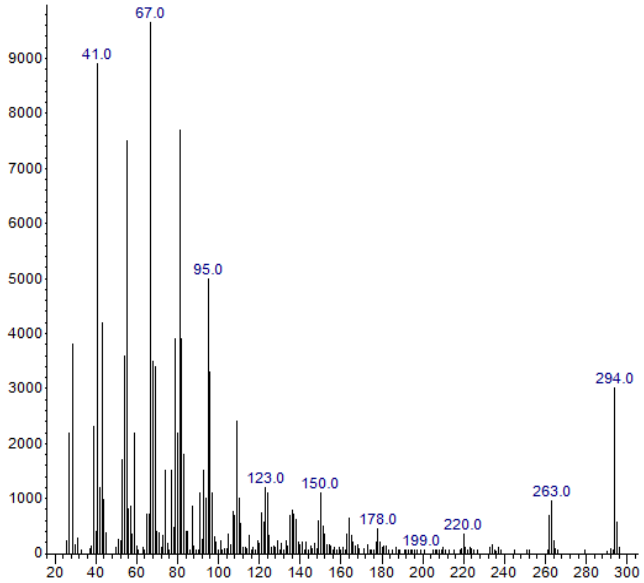

Figure 8- Methyl undecanoate

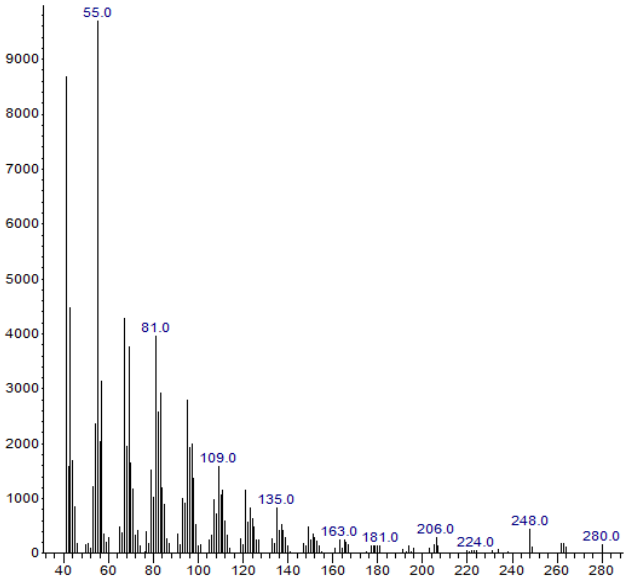

Figure 9- Methyl-14-methyl-pentadecanoate

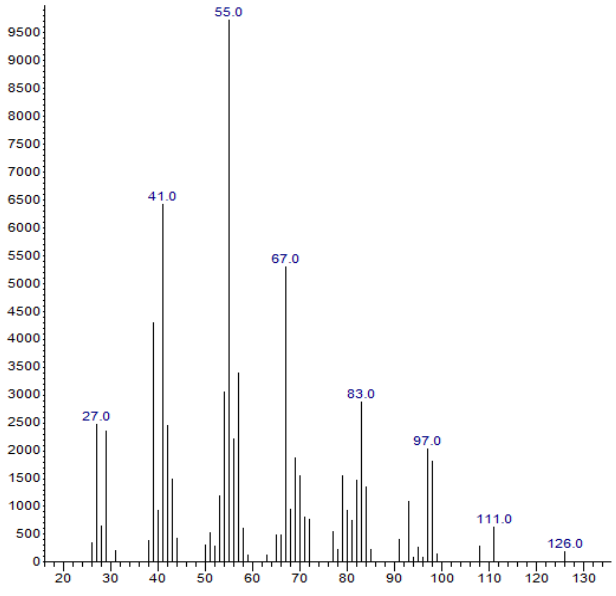

Figure 10- Methyl tetradeca-8,10, trienoate 12-

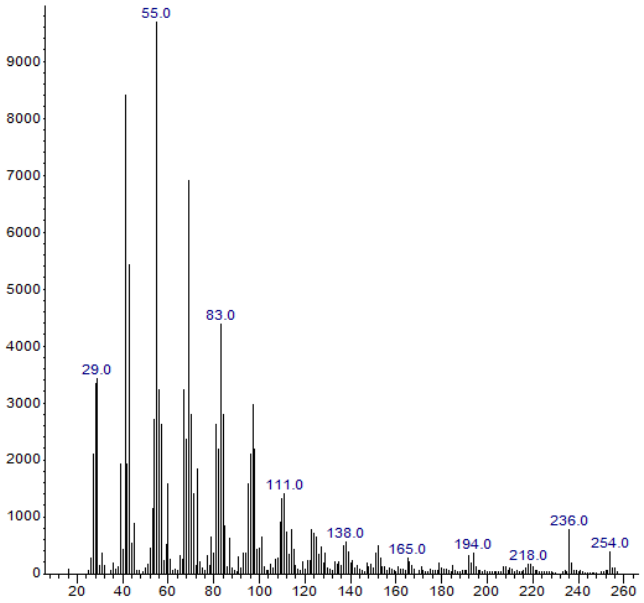

Figure 11- Ethyl hexadecanoate

Plants possess inherent abundance of bioactive chemicals that hold significant therapeutic value. The presence of bioactive compounds in plants has been a significant motivator for scientific research, as it offers the potential to utilize the abundant compounds found in plants for various purposes, including developing drugs for disease prevention and treatment, among other applications. The current study's findings demonstrate that the GC-MS analysis of the leaf extract of *M. oleifera* revealed the presence of bioactive chemicals with significant therapeutic capabilities. For example, *M. oleifera* contains

octadecanoic acid (stearic acid) $C_{18}H_{36}O_2$ and cis-octadecenoic acid (cis-oleic acid) $C_{18}H_{34}O_2$. These compounds have been found to exhibit hypolipidemic activity, potentially due to their ability to act as inhibitors of 5-alpha reductase. This inhibition may have resulted in blocking HMG-CoA reductase, a key enzyme involved in the biosynthesis of cholesterol [17]. According to [17], many compounds such as methyl (11E)-11-octadecenoate and methyl undecanoate have demonstrated inhibitory effects on specific bacterial strains, including intestinal bacteria. Additional studies on the bioactive constituents in extracts derived from *M. oleifera* have also demonstrated the presence of methyl (11E)-11-octadecanoate and cis octadecanoic acid. These compounds have been found to exhibit bioactivities associated with mitigating metabolic syndrome and reducing cardiovascular risk factors. Specifically, they have been shown to effectively lower levels of cholesterol and triglycerides [18,19]. Consequently, these findings highlight the potential therapeutic value of targeting cholesterol and triglyceride reduction in managing metabolic syndrome.

4. Conclusion

Phytocompounds are of medical and pharmaceutical importance as most synthetic antibiotics are derivatives of these plant extracts. In this study, eleven compounds were identified. The most abundant phytochemicals in the leaf extract of *M. oleifera* were methyl (11E)-11-octadecanoate (30.15%) and cis-Octadecenoic acid (19.16%). These compounds have high medicinal implications, and full exploitation of the plant is highly recommended for its nutritional uses.

5. Conflict of Interest

There are no competing interests to be declared.

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