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Investigations on the Chemical Composition and Antimicrobial Potency of *Eucalyptus camadulensis* (River Red Gum) Leaf Extracts

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Abstract: The search for new antimicrobials has been prompted by the serious global health concern of antimicrobial resistance. Strong anti-infective compounds are thought to be abundant in plants. Based on its ethnomedical use, examining the chemical components and antibacterial potency of Nigerian *Eucalyptus camaldulensis* was the aim of this investigation. The air-dried, powdered leaves of *E. camaldulensis* were extracted with methanol to get the crude extract. To test for phytochemicals, the extract was screened using standard protocols. The crude extract was separated into fractions that were soluble in hexane, chloroform, ethylacetate, and methanol-chloroform. The crude extract's and its fractions' activity was evaluated against *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Aspergillus niger,* and *Candida albicans.* The chemical components of the crude extract were identified by GC-MS analysis. Phytochemical analysis showed that phenols, carbohydrates, terpenoids, alkaloids, flavonoids, saponins, tannins, steroids/sterols, and volatile oils were present. The broadest range of antibacterial activity was shown by the crude extract. With a MIC value of 12.5 mg/ml, the hexane-soluble fraction, however, exhibited the highest activity against *Salmonella typhi*. *A. niger* and *C. albicans* were not inhibited by the crude extract or its fractions. The main compounds identified by the GC-MS analysis of the crude extract were long chain saturated and unsaturated carboxylic acids, esters, fatty acids, and fatty acid esters, with recognised biological activity. These findings demonstrate *Eucalyptus camaldulensis's* potential as a natural source of antibacterial compounds and validate its traditional use.

Keywords: *Eucalyptus camaldulensis, Phytochemicals, Antibacterial Activity.*

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I. INTRODUCTION

Prior to the development of science, plants have been utilised for medical purposes. In order to adapt to varying environmental conditions, higher plants can generate secondary metabolites through their regular metabolic activities. They have a large number of compounds that can be use to utilised in drug synthesis [1]. Medicinal plants produce phytochemicals that are pharmacologically active substances [2]. Terpenoids are widely recognised for their anti-inflammatory, anti-cancer, anti-bacterial, antiviral, antihelminthic, and antimalarial activities. According to reports, glycosides have antibacterial and antifungal properties. Shakya [2] claims that alkaloids have diuretic, antispasmodic, antimalarial, analgesic, and properties, whereas saponins have been demonstrated to have antiviral, plant defence, , and anti-inflammatory properties.

Medicinal herbs are the sole source of medication for 80% of the world's population, especially in developing countries like Nigeria [3]. A medicinal plant can be used in its entirety or in its many sections, such as its roots, leaves, fruits, seeds, and flowers. Medicinal plants have been identified as possible therapeutic candidates due to their drug-like qualities [4].

Since ancient times, infectious diseases have affected the quality of life of mankind. Over the years, various kinds of antibiotics have been used to combat these illnesses. Human life is still at risk due to the advent of microorganisms that are resistant to several drugs. It is estimated that half of clinical infection-related deaths in Europe are caused by bacteria that are resistant to multiple drugs [5]. Some bacterial infections are very difficult to treat. Antibiotic discovery is vital, even though some are still not resistant to a variety of new and old antibiotics. Modern medicine's

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survival depends on the ongoing search for novel antibiotics [6].

Originating in Australia, *Eucalyptus camaldulensis*, also referred to as red gum or eucalyptus, is found all over the world. It is one of the most commonly planted eucalypts worldwide and has the largest native range [7] A perennial tree with a single stem and a large trunk, eucalyptus often reaches a height of 20 to 50 meters [7,8]. Usually, this tree grows well by riverbanks where water is available year-round or sometimes.[8]. In addition, the tree possesses antioxidant, analgesic, anti-inflammatory, immune-stimulatory, and spasmolytic properties. Vapour inhalation or the oral route can be used to treat respiratory diseases that are purulent or non-purulent, including bronchitis, asthma, and chronic obstructive pulmonary disease [9]. *E. camaldulensis* is employed in the synthesis of medications for a number of conditions, such as respiratory, digestive, and throat conditions.

From previous studies, the phytochemical contained in the *E. Camaldulensis* plant include , flavonoids, phenols, quinones, terpenoids, tannins. [10, 12] carbohydrates and fat [13]. The aqueous extract of all plant sections contained steroids, anthraquinones, glycosides, tannins, and saponins, but not terpenoids, flavonoids, or alkaloids, according to a study's qualitative screening. The ethanolic extract of the entire plant contained tannins and steroids, but only a tiny portion of the plant contained saponins, alkaloids, flavonoids, and terpenoids. However, neither glycosides nor anthraquinone were present in any of the ethanolic extracts. [11].

Phytochemicals such as flavonoids, phenols, saponins, terpenes, and tannins are abundant in E. camaldulensis extracts, according to earlier research. Quinones, saponins, carbohydrates, tannins, phenols, flavonoids, and lipids were all present in the methanolic extract [12, 13]. Alkaloids, flavonoids, pigments, phenolics, terpenes, starches, steroids, and essential oils are the main active ingredients in these plants. [8]. *E. Camaldulensis* extracts have been found to be active multidrug-resistant Nematodes, *Acinetobacter baumannii, Candida albicans and Candida parapsilosis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus [8]*.

The study intends to explore the chemical components and antibacterial qualities of the Nigerian *E. Camaldulensis* leaf extracts used in traditional medicine.

II. MATERIALS AND METHODS

➢ *Plant Material Collection and Identification*

In March 2025, leaves of Eucalyptus camadulensis were collected from Veritas University in Abuja. A specimen with the voucher number NIPRD/H/7496 was deposited after the leaves were verified at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu. After being weighed, the leaves were cleaned with running distilled water. For two weeks, they were kept at room temperature and out of the sun on a lab bench to dry. An electric blender was then used to grind the dried plant material into a powder.

➢ *Extraction of Dried Powdered Plant*

42.0 L of methanol was used to cold macerate the dried powdered leaf of *Eucalyptus camadulensis in* a glass container for 48 hours while being shaken occasionally. A glass funnel and filter paper were then used to filter the extract. The filtrate was dried by evaporating it in a rotary evaporator set to 40 °C. The result was a solid residue that was dark green in colour. The best component separation was seen using four spots on a thin-layer chromatography (TLC) plate, which is an aluminium sheet that has been pre-coated with silica gel. The crude extract was spotted on the plate using a 1:5 ethyl acetate/hexane mobile phase.

➢ *Fractionation*

Hexane (2 x 150 cm3) was added to a clean, weighed 250 ml beaker containing the crude methanolic extract, and the mixture was allowed to stand for a few minutes. The hexane-soluble fraction was then extracted by decanting it. After hexane was removed from the residue, it was successively extracted using chloroform (2x150 cm^3), ethyl acetate (2x150 cm^3), and 50% methanol-chloroform (2x150 cm^3) to givethe chloroform-soluble, ethylacetate-soluble, and methanol-chloroform-soluble fractions (Figure 1). Each fraction was dried by evaporation, and the residue was then weighed.

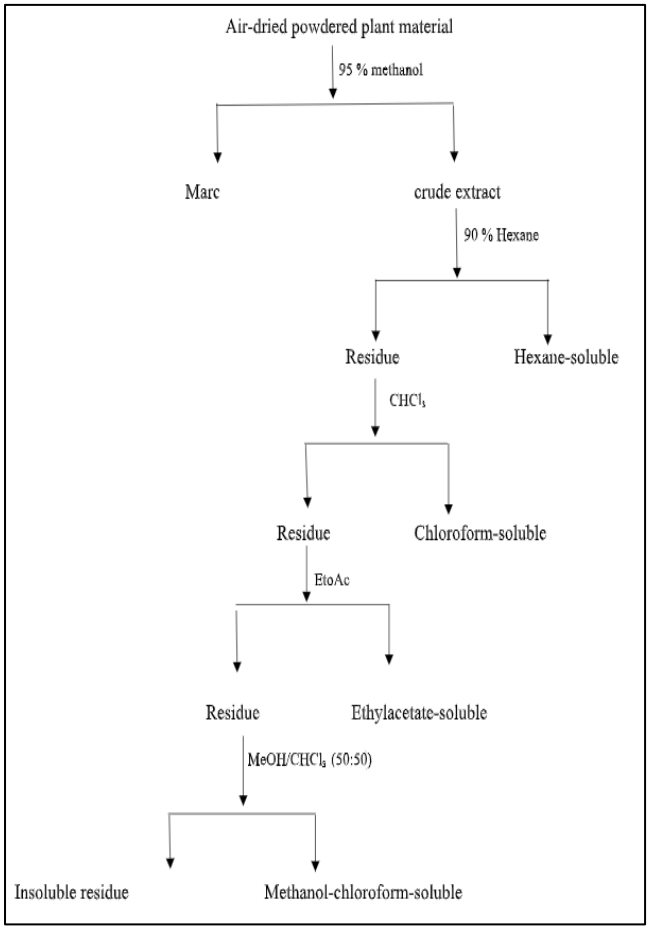

Fig 1 Scheme for Fractionating *Eucalyptus camadulensis* Crude Extract

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➢ *Preliminary Phytochemical Screening*

A preliminary qualitative screening of phytochemicals was conducted on a crude methanolic extract of *E. camadulensis*. Nine secondary metabolites were identified from the crude extract using the technique by Obi & Okwute [14]. These included phenols, volatile oils, flavonoids, sterols/steroids, alkaloids, tannins, carbohydrates, saponins, and terpenoids.

➢ *Antimicrobial Screening*

A standard protocol was used to conduct the antimicrobial screenings, which included sensitivity tests, minimum inhibitory concentration (MIC), and minimum bacteriocidal concentration (MBC) [14].

➢ *Sensitivity Test for Determining Inhibitory Activity*

The sensitivity test was done by the agar well diffusion method. The standardised inocula of the bacterial and fungal isolates were streaked onto sterilised Mueller Hinton and Potatoe dextrose agar plates, respectively, with a sterile swab stick. Using a sterile cork borer, four wells were punched into each inoculated agar plate. The wells were properly labelled based on the concentrations of the extract prepared (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively). The extract (approximately 0.2 mL) was added to each well. For about an hour, the inoculated plates with the extract were placed on the bench to allow the extract to permeate the agar. After that, the incubated plates were checked for any signs of inhibition, which showed up as a clear zone of inhibition encircling the wells.

A clear ruler that was calibrated in millimetres was us ed to measure these inhibitory zones' diameter.

➢ *Minimum Inhibitory Concentration (MIC) Determination*

In order to ascertain the extracts' minimal inhibitory concentration, Mueller Hinton Broth was used as a diluent in the tube dilution method. The extract was serially diluted to the lowest concentration that inhibited each organism during the sensitivity test in a test tube containing Mueller Hinton broth. The standardised organisms were introduced to each tube that contained the broth and extract. The infected tubes were incubated at 37 °C for 24 hours. At the end of the incubation period, the tubes were examined for growth using turbidity as a criterion. By selecting the lowest concentration in the series that exhibited no growth or obvious symptoms, the minimum inhibitory concentration (MIC) was ascertained.

➢ *Minimum Bacteriocidal concentration (MBC) Determination*

The MBC of the extract was determined using the results of the MIC test. A sterile wire loop was used to fill the test tubes that showed no turbidity (clear) in the MIC test, and a loopful was streaked across sterile nutrient agar plates. The plates were incubated at 37 °C for 18 to 24 hours. After the incubation period, the plates were checked for any growth. This evaluation aimed to determine if the extract's antibacterial activity was bacteriostatic or bacteriocidal.

➢ *GC-MS Analyses of Crude Methanolic Extract of E. camadulensis*

A mass-selective detector was connected to an Agilent gas chromatographic column (30 m x 0.25 mm x 0.25 mm). Methanol and 1-pentanol were used as internal standards to dilute the crude extract sample. Three microlitres of the diluted crude sample were then added to the GC-MS for examination. The ion source's temperature was 230 °C, and the injector's was 250 °C. The oven's temperature ranges from 50 to 160 °C. The MS had a scanning range of 30 to 400 amu.

III. RESULTS AND DISCUSSION

➢ *Extraction and Fractionation*

A dark green colouration was obtained from all the extracts. The cold maceration of 520.83 g of dried leaves produced 76.52g of crude, a yield of about 13.42 % relative to the plant material. Fractionation of the crude methanolic extract yielded hexane-soluble, chloroform-soluble, ethylacetate-soluble, and methanol-chloroform-soluble fractions. The chloroform soluble fraction had the highest yield among the fractions (6.1 g, 7.97 %) (Table 1).

Table 1 Physical Features and Extractive Yields of *E. camadulensis* Leaves

Extractives	Yield in (g) (%) of crude and relative to crude	Colour and Consistency
Crude	(76.52) (13.42)	Dark green solid
Hexane-soluble	(1.60) (2.09)	Dark green solid
Chloroform-soluble	(6.1) (7.97)	Dark green solid
Ethylacetate-soluble	(1.16) (1.52)	Dark green solid
Methanol-Chloroform- Soluble (50:50)	(1.73) (2.26)	Dark green solid

➢ *Phytochemical Analysis*

According to a phytochemical analysis of the leaf, phenols, volatile oils, terpenoids, flavonoids, alkaloids, tannins, sterols, and steroids were all present (Table 2). With the exception of alkaloids, which have sporadically been reported to be found in the plant, this discovery is in line with other accounts. The wide range of biological activity exhibited by these phytochemicals is well-known. Alkaloids, terpenes, and phenolic chemicals are the main sources of anti-inflammatory and antimalarial substances [14]. Additionally, previous research has demonstrated the antibacterial properties of volatile oil against a range of pathogens, including *Escherichia coli*, *Bacillus cereus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, and various fungal species [15, 16, 17].

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Table 2 Phytochemical Screening of the Crude Methanolic Extract of the Leaf of *E. camadulensis*

Secondary Metabolites	Result
Tannins	+
Alkaloids	+
Carbohydrate	+
Sterols/Steroids	+
Flavonoids	+
Volatile oils	+
Terpenoids	+
Saponins	+
Phenols	+

(+) represents present

➢ *Antimicrobial Screening*

When screened for antimicrobial activity, the crude extract and fractions of *E. camadulensis* showed antibacterial activity against both gram-positive and gram-negative bacteria(Table 3).The crude extract had the widest spectrum of anti-bacterial activity. However, it was inactive against B. *subtilis.* The wider spectrum of antibacterial activity of the crude extract, when compared to its fractions, may be ascribed to the combined effect of the fractions in the crude. Additionally, the hexane-soluble fraction inhibited *S. typhi* at a MIC value of 12.5 mg/ml, but the crude extract shown reduced activity against the bacteria at a MIC value of 50 mg/ml (Table 3). Similarly, the inhibition zone of the crude extract (16 mm) against *P. aeruginosa* at 100 mg/ml is less than those of the chloroform-soluble (20 mm) and ethylacetate-soluble (18 mm) fractions at the same concentration. Fractionation has therefore aided in the crude extract's distribution and increased activity against *S. typhi* and *P. aeruginosa*.

Notably, the fungi *A. niger* and *C. albicans* were not sensitive to the crude extract or its fractions. Although *E. camadulensis* has been shown to exhibit antifungal properties in previous research [18, 21, 22], this contradictory result could be because of differences in soil composition and environmental conditions. Additionally, the antibacterial properties of the crude extract and its fractions was weaker than that of the control or standard. According to certain research, combining essential oils and their extracts with antibiotics (beta-lactams, fluorochinolones, aminoglycosides, and polymyxins), antivirals (acyclovir), and plant extracts can increase their antibacterial potency [21].

Table 3 Inhibitory activity of the crude extract and fractions of *E. camadulensis*

Zone of Inhibition (mm) at different extract concentration (mg/ml)																
TEST ORGANISM	HEX				$CHCl_3$				EtOAC				MeOH-$CHCl_3$				CRUDE				
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	C
S. aureus	18	16	12	-	-	-	-	-	16	14	12	-	14	12	10	-	19	16	14	10	34
B. subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	12	10	-	33
S. typhi	22	16	12	10	-	-	-	-	-	-	-	-	-	-	-	-	13	10	-	-	25
P. aerugi - nosa	-	-	-	-	20	18	15	12	18	15	13	12	-	-	-	-	16	13	11	10	45
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40

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KEY: (-) = No Activity

Hex = Hexane-soluble fraction

$CHCl_3$ = Chloroform-soluble fraction

EtOAc = Ethylacetate-soluble fraction

MeOH-$CHCl_3$ = Methanol-chloroform-soluble fraction

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) / Fungicidal Concentration (M.F.C) of the Extracts

TEST ORGANISMS	MIC					MBC				
	Crude	Hex	$CHCl_3$	EtOAc	MeOH-$CHCl_3$	Crude	Hex	$CHCl_3$	EtOAc	MeOH-$CHCl_3$
S. aureus	25	25	-	50	50	50	50	-	#	#
B. subtilis	-	-	-	-	-	-	-	-	-	-
E. coli	50	-	-	-	-	50	#	-	-	-	-
S. typhi	50	12.5	-	-	-	50	#	25	-	-	-
P. aeruginosa	25	-	25	25	25	25	50	-	50	50	50
C. albican	-	-	-	-	-	-	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-

KEY: #= No M.B.C. The extract is bacteriostatic against the Organisms.

Hex = Hexane-soluble fraction

$CHCl_3$ = Chloroform-soluble fraction

EtOAc = Ethylacetate-soluble fraction

MeOH-$CHCl_3$ = Methanol-chloroform-soluble fractionC = Control

➢ *Analysis of Crude Methanolic Extract Using GC-MS*

A 95% quality of characterisation and a 1% area percentage indicated sixteen peaks in the gas chromatogram of the crude extract of *Eucalyptus camadulensis* (Figure 2). The sample's mass spectra were compared to database standards in order to identify the compounds' type and structure (Figure 3). The major compounds identified include: n-Hexadecanoic acid, 9-Octadecenoic acid, Hexadecanoic acid, methyl ester, trans-13-Octadecenoic acid, methyl ester, 9, 12-Octadecadienoic acid, methyl ester, and Ethyl Oleate. The identified compounds are mainly long chain saturated and unsaturated fatty acids, fatty acid esters, carboxylic acids, and esters. Numerous fatty acids and their methyl esters have the ability to kill bacteria [19, 22]. n-hexadecanoic acid, methyl ester, and 9, 12-octadecadienoic acid, show antibacterial properties [20], and hexadecanoic acid, methyl ester have antifungal properties [14, 20]. The most frequent site of action for fatty acids, despite their unclear antibacterial mechanisms, is the cell membrane. The electron transport chain is disrupted, oxidative phosphorylation is uncoupled, enzymatic activity and nutritional intake are inhibited, and fatty acids increase permeability and promote cell lysis. Fatty acids can alter the expression of virulence genes, interfere with the metabolic functions of bacteria, and prevent DNA/RNA replication in addition to their effects on cell membranes [24]. Hexadecanoic acid, methyl ester appear to have the ability to reduce blood cholesterol. Due to its inhibition of the cyclooxygenase II enzymes, it demonstrates a specific anti-inflammatory effect. In addition to its antibacterial and antifungal properties, it is an antioxidant [14]. These compounds may therefore be the cause of the antibacterial properties of *Eucalyptus camadulensis* extracts.

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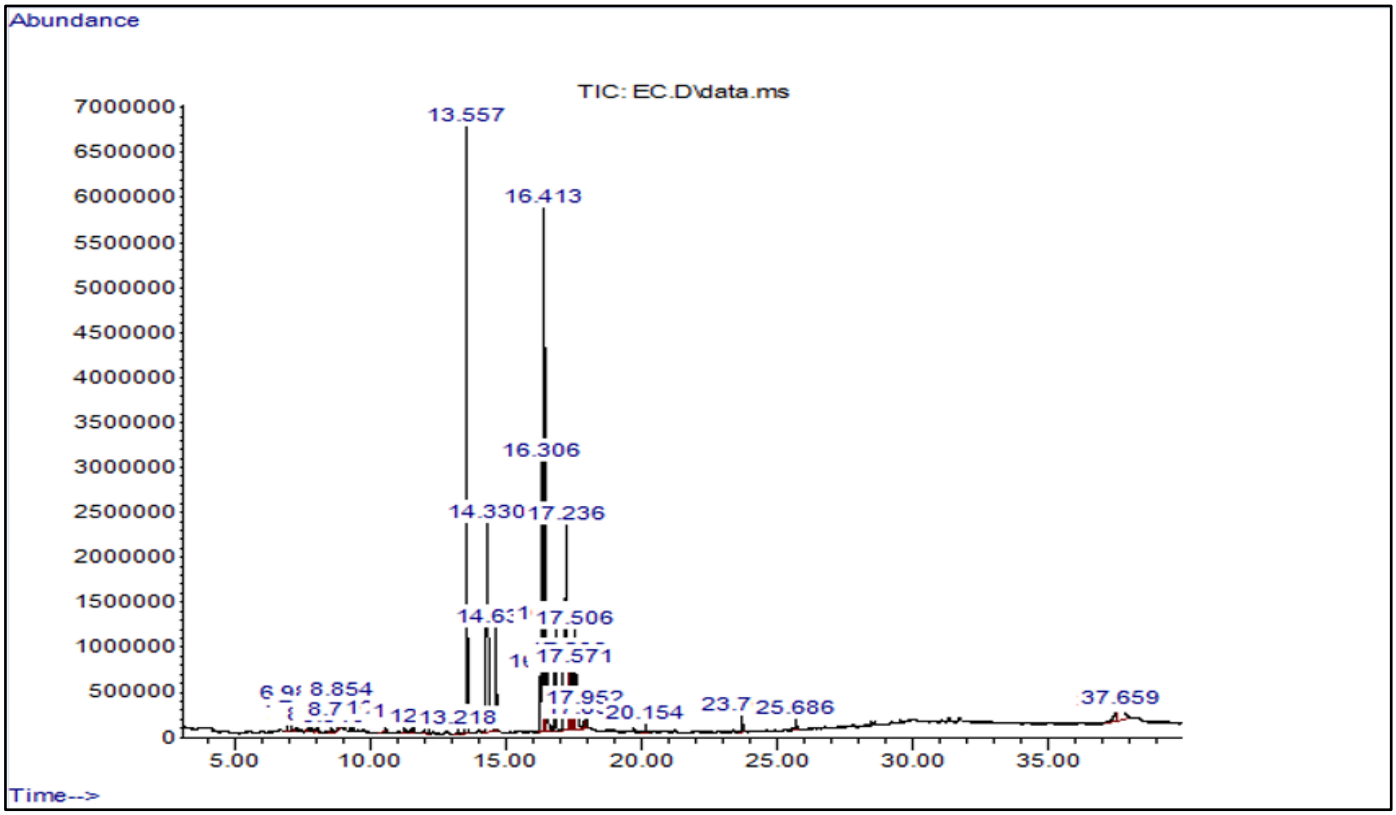
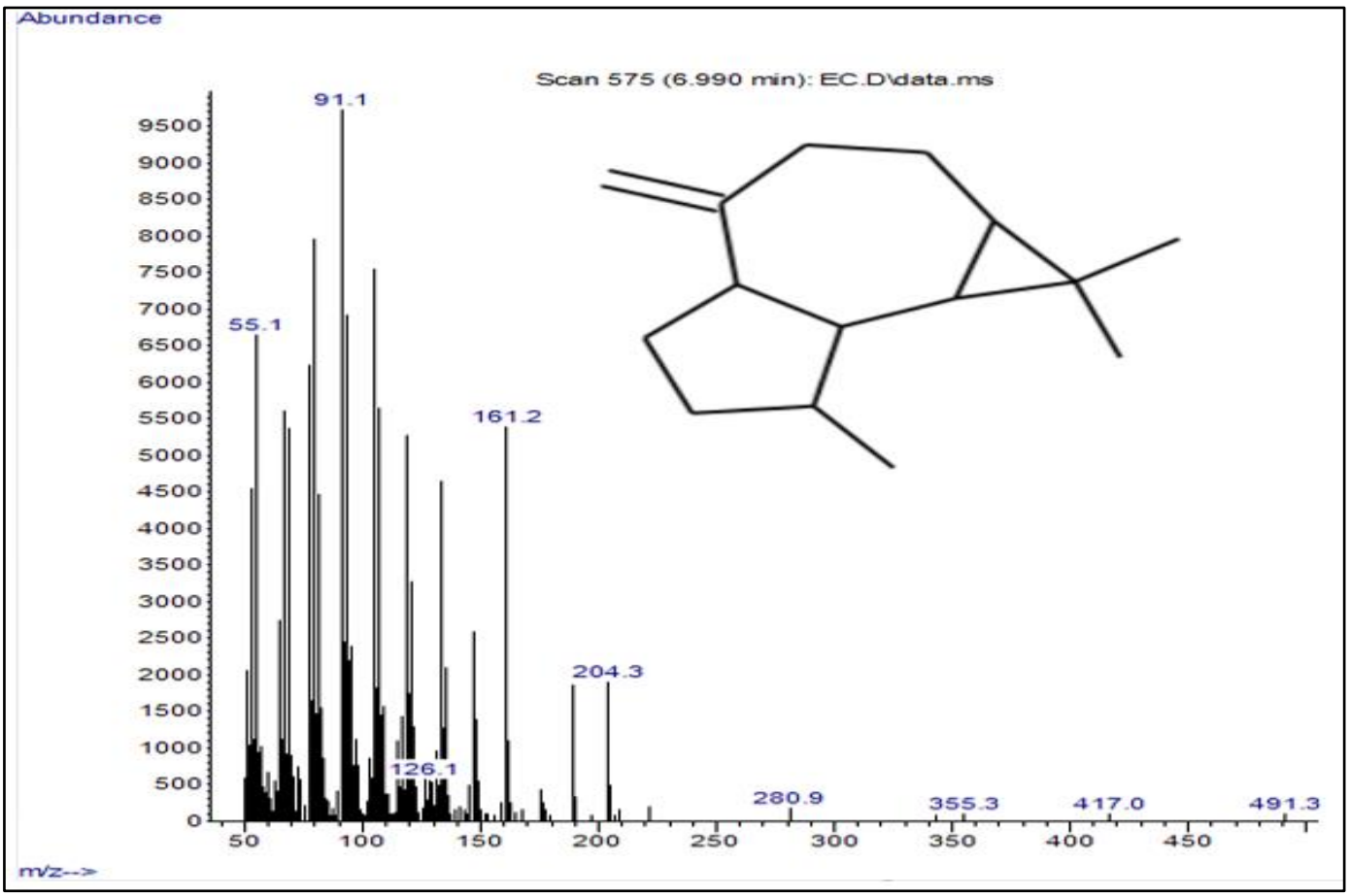


Fig 2 Gas Chromatogram for Crude Methanolic Extract of *E. camadulensis*

➢ *MS Spectra and Structures of Some Components of the Crude Methanolic Extract E. camadulensis*



Aromadendrin

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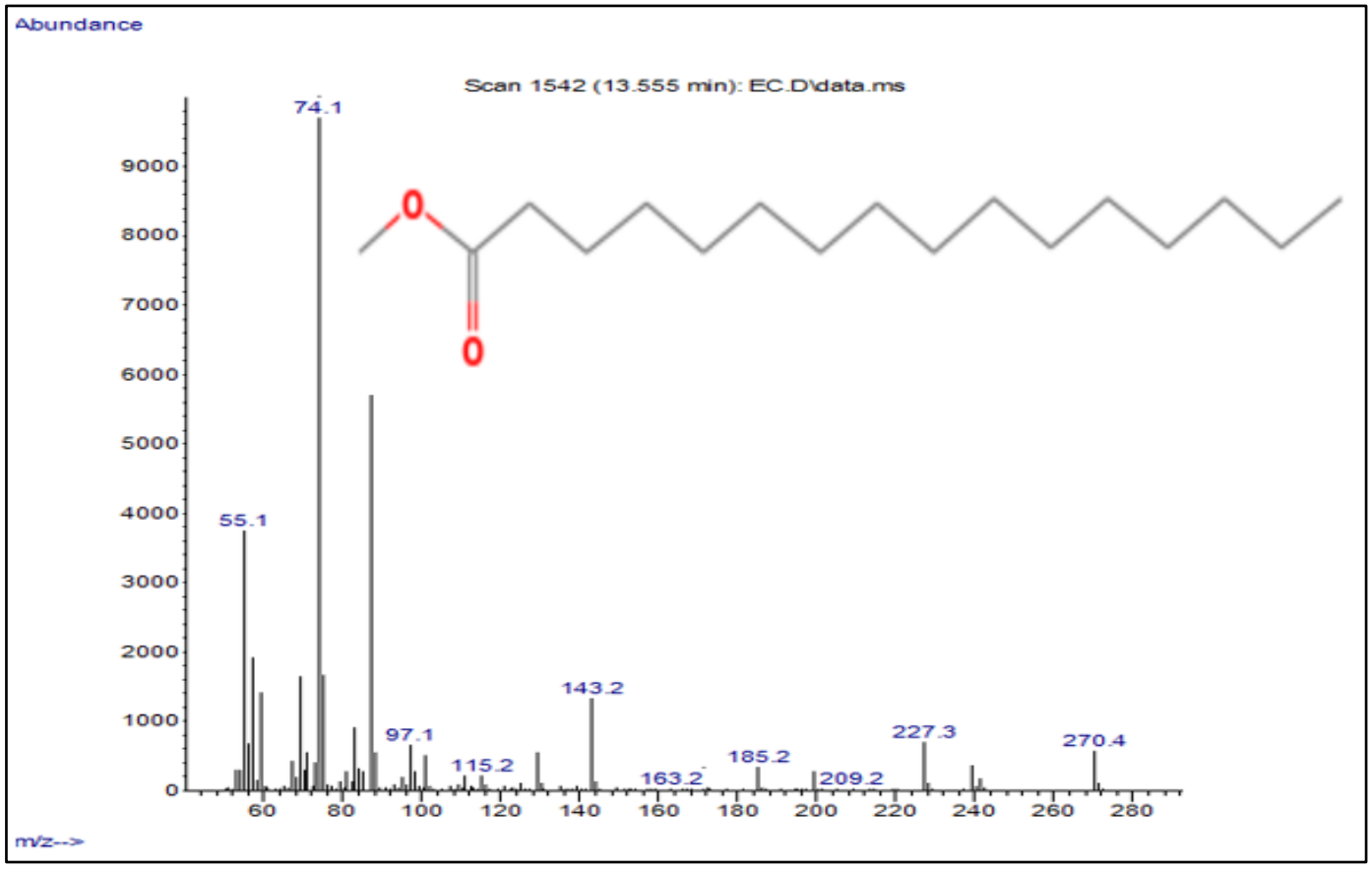
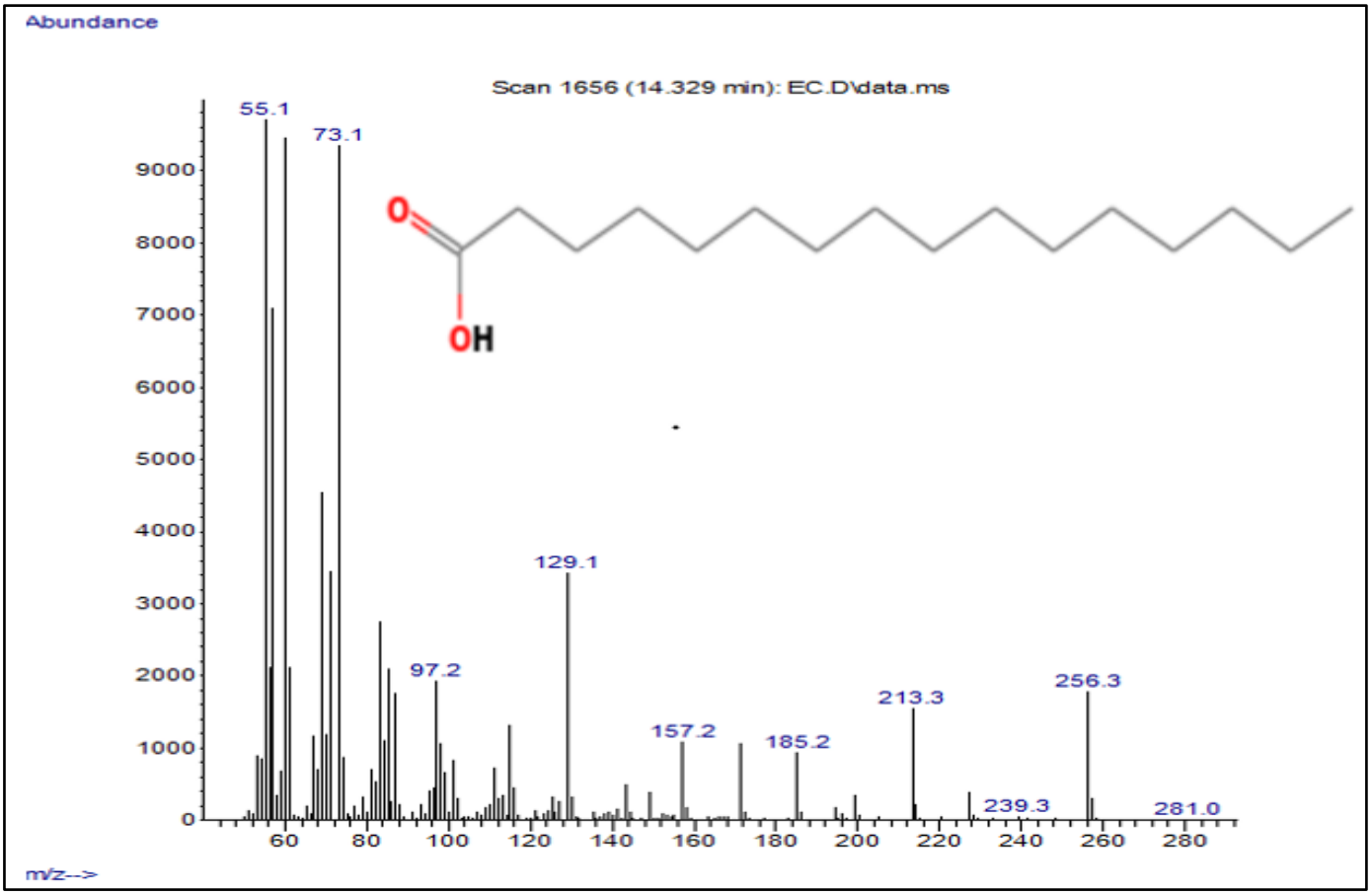


Hexadecanoic acid, methyl ester



n-Hexadecanoic acid

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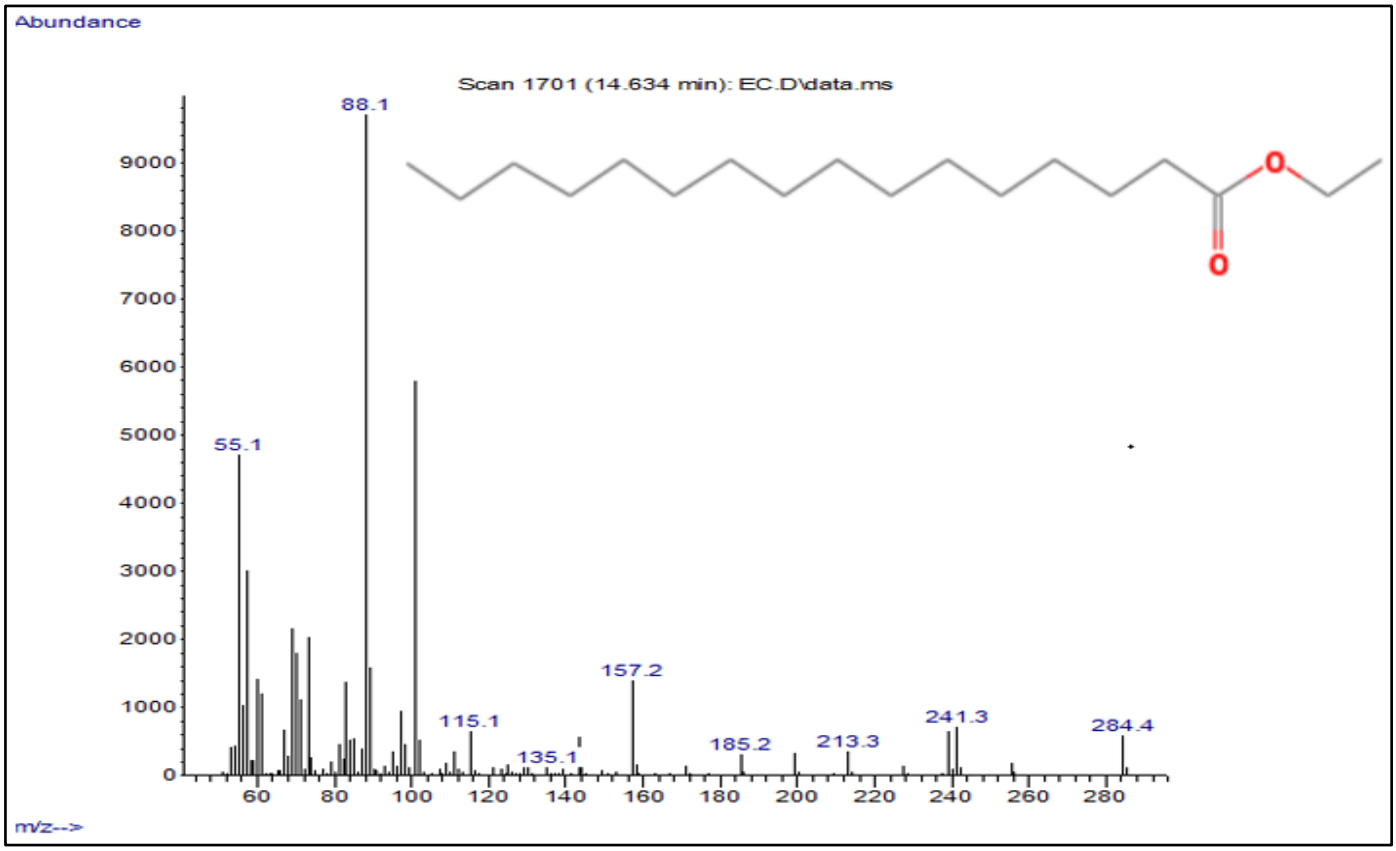
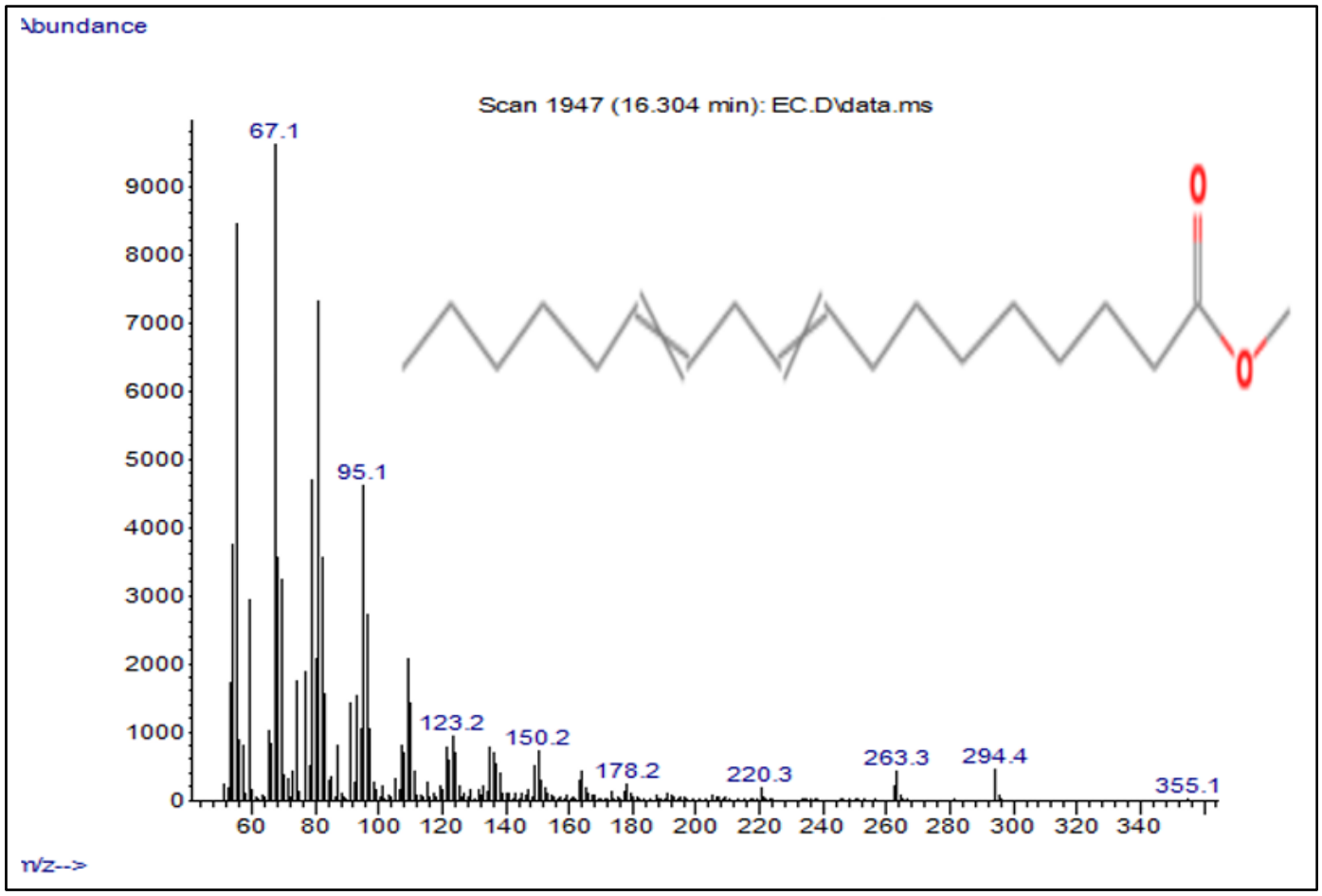


Hexadecanoic Acid Ethyl Ester



9, 12-Octadecadienoic Acid, Methyl Ester

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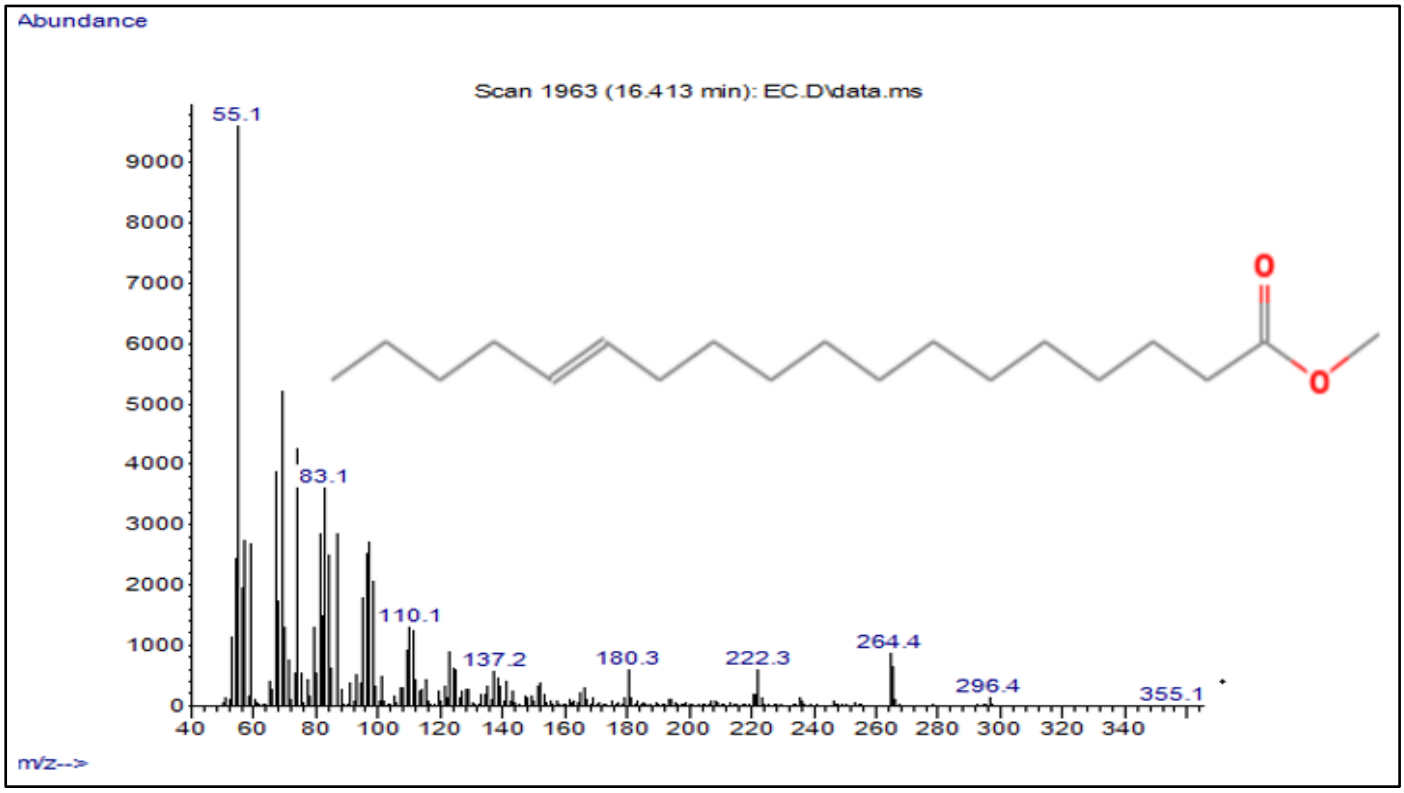

Trans-13-Octadecenoic Acid, Methyl Ester

Table 5 The GC-MS Analysis of the Crude Methanolic Extract of *E. camadulensis*, Showing its Major Compounds

Name	Retention time (mins)	Area (%)	Quality	Molecular weight (g/mol)	Molecular Formula
Aromandendrene	6.987	1.49	97	204.35	$C_{15}H_{24}$
Hexadecanoic acid, methyl ester	13.557	16.61	97	270.45	$C_{17}H_{34}O_2$
n-Hexadecanoic acid	14.330	11.43	99	256.42	$C_{16}H_{32}O_2$
Hexadecanoic acid ethyl ester	14.637	3.02	98	284.47	$C_{18}H_{36}O_2$
9, 12-Octadecadienoic acid, methyl ester	16.306	8.73	99	294.47	$C_{19}H_{34}O_2$
trans-13-Octadecenoic acid, methyl ester	16.413	17.08	99	296.48	$C_{19}H_{36}O_2$
9-Octadecenoic acid (Z)-, methyl ester	16.508	2.84	99	296.48	$C_{19}H_{36}O_2$
Methyl stearate	16.829	3.12	98	298.50	$C_{19}H_{38}O_2$
9-Octadecenoic acid	17.236	15.04	99	282.50	$C_{18}H_{34}O_2$
cis-Vaccenic acid	17.298	2.67	96	282.50	$C_{18}H_{34}O_2$
Linoleic acid ethyl ester	17.414	2.29	99	308.49	$C_{20}H_{36}O_2$
Ethyl Oleate	17.506	3.26	98	310.5	$C_{20}H_{38}O_2$
Octadecanoic acid	17.571	3.14	99	284.47	$C_{18}H_{36}O_2$
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	37.659	2.13	95	356.53	$C_{21}H_{40}O_4$

IV. CONCLUSION

These results demonstrate the potential of Eucalyptus camadulensis as a natural source of antibacterial compounds and offer scientific support for the plant's traditional usage in herbal medicine. A smaller range of antifungal potential was shown by the extracts' lack of activity against *Aspergillus niger* and *Candida albicans.*

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