CHARACTERIZATION OF BIOACTIVE SESQUITERPENES, ORGANIC ACIDS AND THEIR DERIVATIVES FROM THE LEAVES OF *PSIDIUM GUAJAVA* LINN

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Authors Contribution

This work is a collective contribution of two authors. Author OUI[1] designed the research and carried out the analyses. Author TA[2] assisted in interpreting the GC-MS spectra and drawing the structures with Chemsketch software.

ABSTRACT

The isopropanolic extract of the leaves of *Psidium guajava* Linn was analyzed by Gas chromatography-mass spectrometry (GC-MS). Fourteen different phytochemical compounds have been characterized, including 1,3,3-trimethyl-2-oxabicyclo[2.2.2] octane, (2.06%), 4,11,11-trimethyl-bicyclo[7.2.0] undec-4-ene(6.26%), (E,E,E)-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene (1.05%), eudesma-4(14),11-diene(5.07%), guaia-1(10),11-diene(4.63%), 3,7,11-trimethyl-1,6,10-dodecatriene-3-ol(4.01%), 1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.0(2,7)]dec-3-ene (1.66%), 1,1,4,7-tetramethyl-decahydro-1H-cycloprop[e]azulen-4-ol (11.00%), hexadecanoic acid methyl ester(1.53%), 4,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol(2.78%), L-(+)-ascorbic acid 2,6-dihexadecanoate(20.43%), 7-octadecenoic acid methyl ester(3.81%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol(2.24%) and 9-octadecenoic acid (33.47%). The presence of these bioactive compounds in the leaves of *Psidium guajava* Linn could be the reason behind its use for the treatment of diseases and infections in herbal medicine in Nigeria.

Keywords: GC-MS analysis, *Psidium guajava*, Bioactive compounds, Herbal medicine.

INTRODUCTION

P. guajava familiarly known as Guava belongs to *Myrtaceae* family. The plant is a small tree with quadrangular branch lets, oval to oblong leaves about 7.6 cm in length, and four-petaled white flowers about 2.5 cm broad [1] . It is cultivated in many tropical and subtropical countries [1]. From preliminary medical research in laboratory models, extracts from *P. guajava* leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain [2,3,4]. Essential oils from *P. guajava* leaves have been reported to display anti-cancer activity in vitro [5]. *P. guajava* leaves are used in herbal medicine as a remedy for diarrhoea and as well as the bark, for their supposed antimicrobial properties and as an astringent [6]. *P. guajava* leaves or bark are used in traditional herbal

medicine against diabetes [7,8]. It has also been reported that the young leaves are used to make a tea in Trinidad which is used for the treatment of diarrhoea, dysentery and fever [9].

It has recently been reported that by using the agar dilution streaking method, the extract of *P. guajava* showed strong inhibition against *Staphylococcus aureus* and *Escherichia coli* collected as clinical isolates from the urine sample obtained from patients at the University medical centre of Michael Okpara University of Agriculture, Umudike, Abia, Nigeria [10]. *P. guajava* leafy part is used as a medicinal plant by indigenes of south-eastern Nigeria as antibacterial remedies in treatment of tropical diseases [10]. The present study was aimed at identifying the phytochemicals of the leaves of *P. guajava* with isopropanol as a choice of solvent.

MATERIALS AND METHODS

Experimental

GC analyses were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 70-280 ^{o}C held at 70 ^{o}C for 3 minutes, and at 160 f ^{o}C or 4 minutes (rate 4 ^{o}C/min) and finally at 280 ^{o}C for another 4 minutes (rate 15 ^{o}C /min) , injection temperature 250 ^{o}C. GC-MS (Gas chromatography mass spectrometry) analysis was conducted using GCMS-QP 2010 Plus Shimazu Japan with column oven temperature of 70 ^{o}C, injection mode was split, flow control mode was linear velocity, carrier gas pressure was 104.1 Kpa, total flow was 6.2 mL/min, column flow was 1.59 mL/min, linear velocity was 46.3 cm/sec, purge flow was 3.0 mL/min and split ratio was 1.0. Also, ion source temperature was 200 ^{o}C, in terface temperature was 250 ^{o}C, solvent cut time was 2.5 min., detector gain was 10.0 KV, detector gain mode was relative and the threshold was 2000. For the mass spectrum, the start time was 3.0 min., the end time was 26.0 min, the event time was 0.5 sec, the scan speed was 1250, and the start m/z was 50 while the end m/z was 600.The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge Germany was used. Solvents were all of analytical grade and were procured from Merck, Germany.

Plant Materials

P. guajava leaves were harvested from the tree plant located at Ubakala, Umuahia South Local Government Area of Abia State, Nigeria. The leaves were then dried for 30 days and thereafter milled into a uniform and fine powder by a mechanically driven attrition mill.

Extraction of Plant Materials

The powdered plant sample (300 g) was successfully extracted with 2 L of isopropanol (8hrs/3 times/60 ^{o}C). The extract was concentrated under reduced pressure and the supernatant extract was decanted (5.42g) after complete removal of the solvent. The extract was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant extract was subjected to systematic GC-MS analysis [11].

Components Identification

The components of the extracts were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature [11].

RESULTS AND DISCUSSION

The isopropanol extract of the leaves of *P. guajava* showed fourteen peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of fourteen compounds (1-14) in the extract (Figs. 2 and 3). The molecular formulae, percentage compositions and molecular masses of the compounds are shown in Table 1. These compounds comprise ether (2.06%), sesquiterpenes (18.67%), sesquiterpene alcohols (17.79%), diterpene alcohol (2.24%), fatty acid esters (5.34%), ascorbic acid (20.43%) and fatty acid (33.47%).

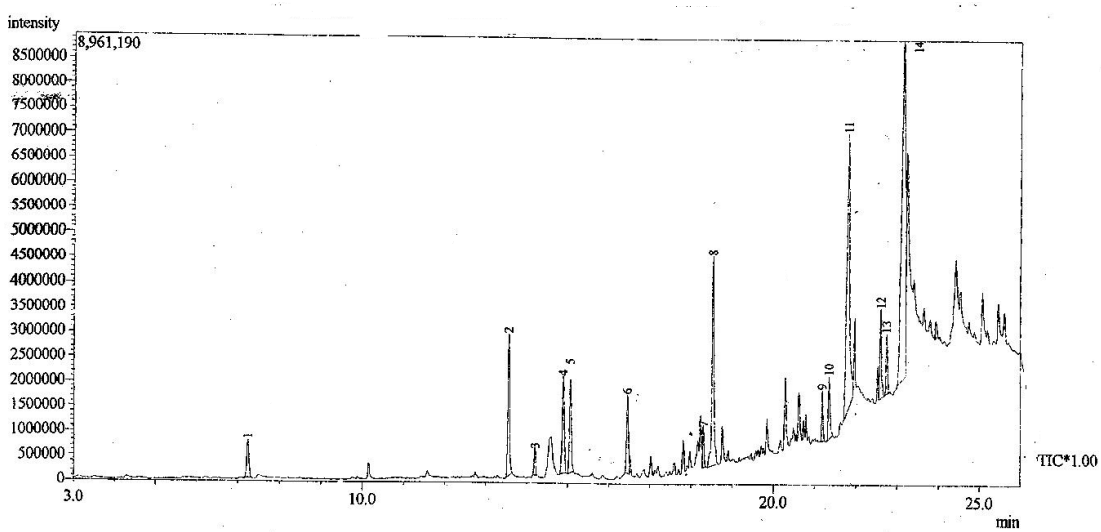


Fig. 1: GC-MS chromatogram of isopropanolic extract of *P. guajava*

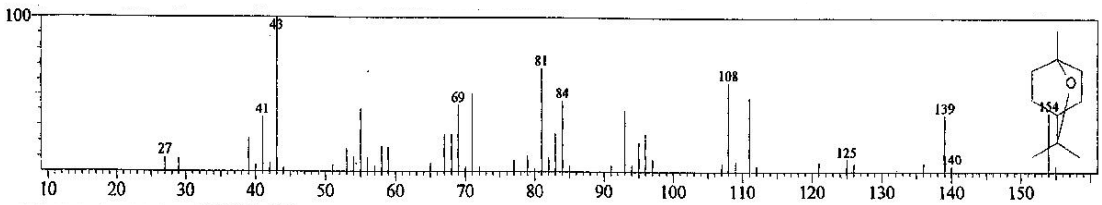


Fig. 2a: 1,3,3-trimethyl-2-oxabicyclo[2.2.2] octane,

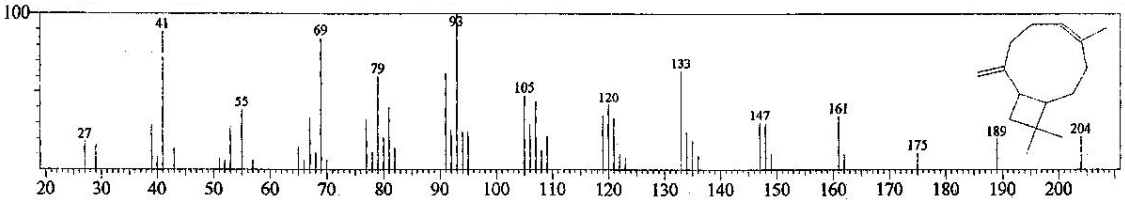


Fig. 2b: 4,11,11-trimethyl-bicyclo[7.2.0] undec-4-ene

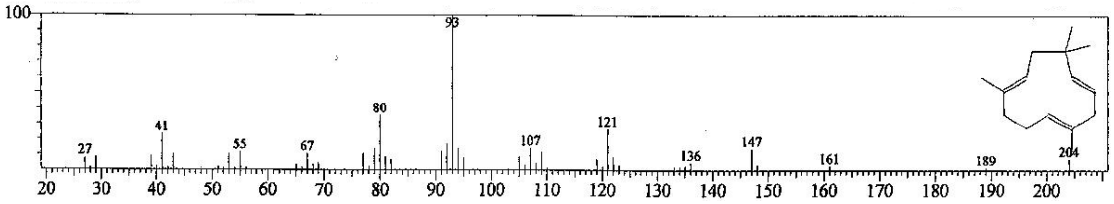


Fig. 2c: (E,E,E)-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene

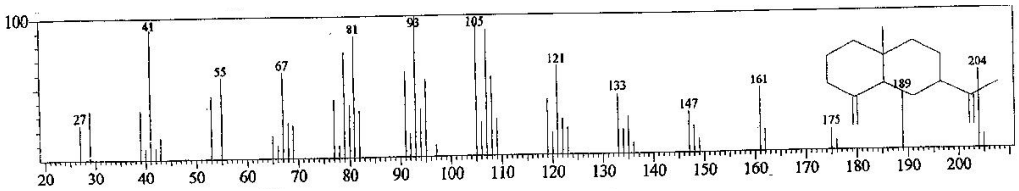


Fig. 2d: Eudesma-4(14),11-diene

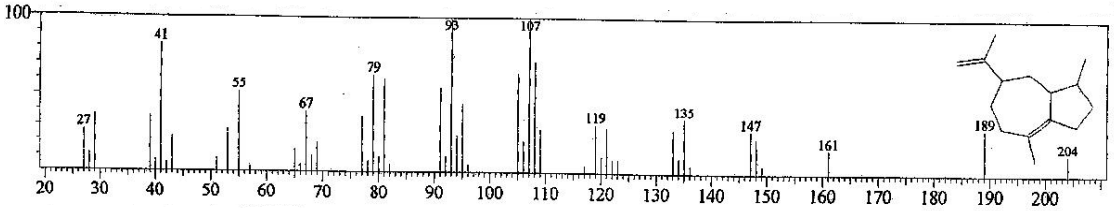


Fig. 2e: Guaia-1(10),11-diene

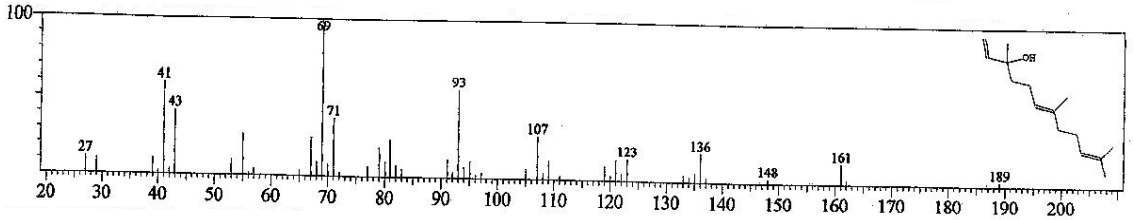


Fig. 2f: 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol

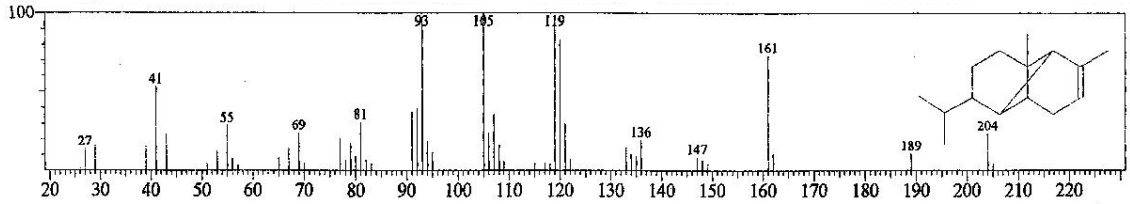


Fig. 2g: 1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.0(2,7)]dec-3-ene

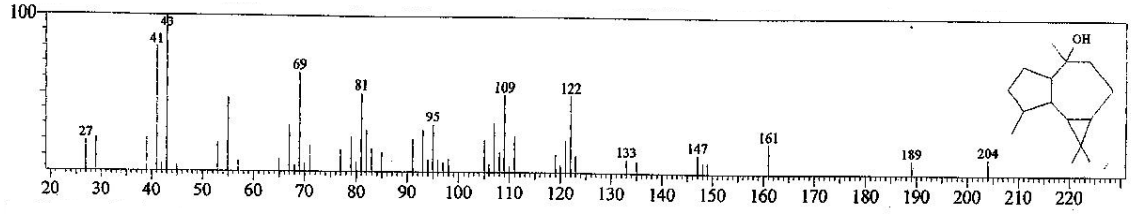


Fig. 2h: 1,1,4,7-tetramethyl-decahydro-1H-cycloprop[e]azulen-4-ol

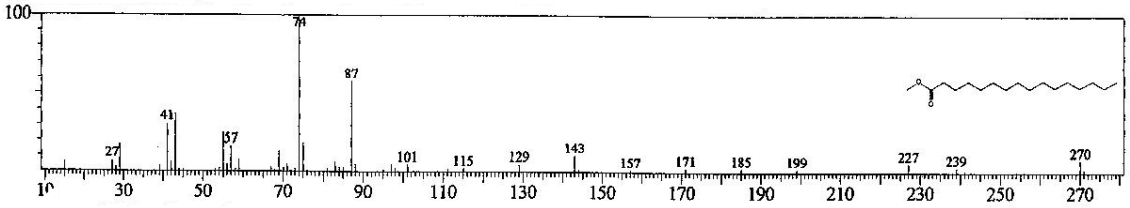


Fig. 2i: hexadecanoic acid methyl ester

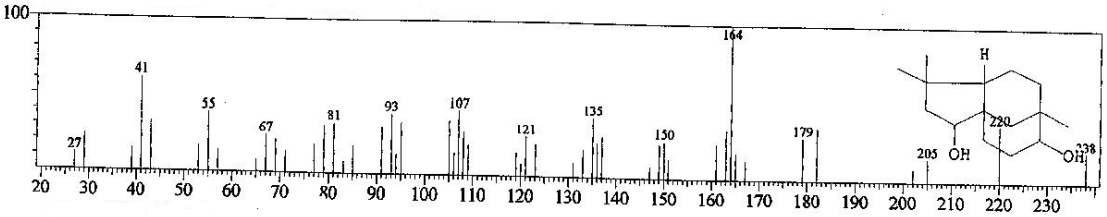


Fig. 2j: 4,4,8-trimethyltricyclo[6.3.1.0(1,5)] dodecane-2,9-diol

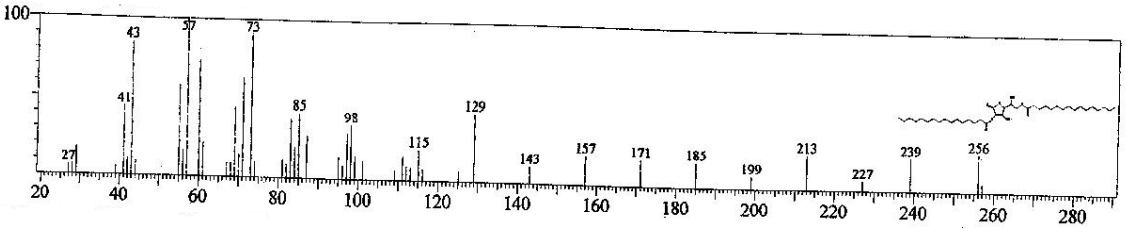


Fig. 2k: l-(+)-ascorbic acid 2,6-dihexadecanoate

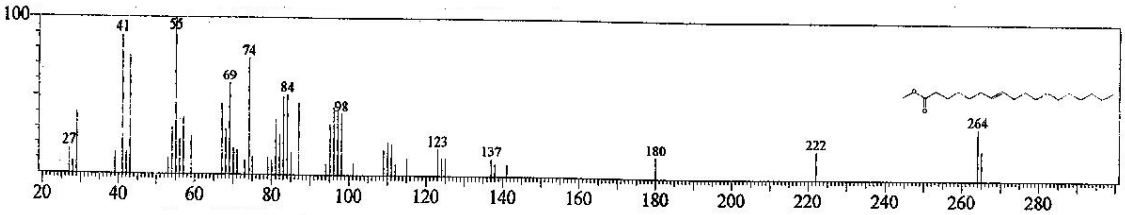


Fig. 2l: 7-octadecenoic acid methyl ester

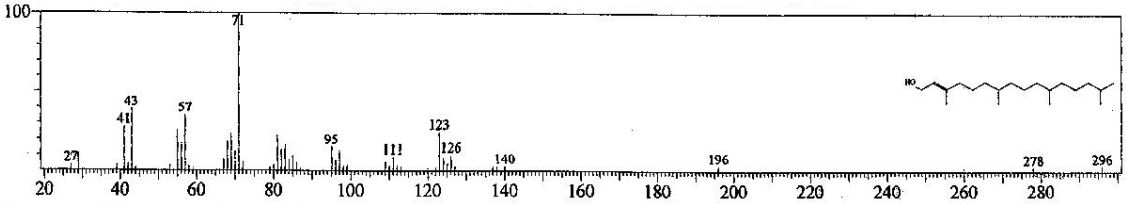


Fig. 3,7,11,15-tetramethyl-2-hexadecen-1-ol

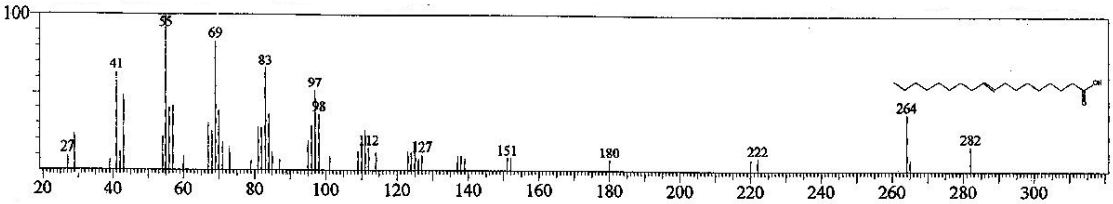


Fig. 2n: 9-octadecenoic acid

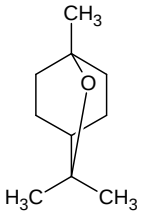
Compound **1** also known as Cineole or eucalyptol is used as component in pharmaceutical preparations to relieve the symptoms of influenza and colds, in products like cough sweets, ointments and inhalants. It has antibacterial effects on pathogenic bacteria in the respiratory tract [12]. Inhaled cineole-based oil vapour is a decongestant and treatment for bronchitis [13]. Cineole controls airway mucus hyper-secretion and asthma via anti-inflammatory cytokine inhibition [14]. Cineole oil also has anti-inflammatory and analgesic qualities as a topically applied liniment ingredient [15,16]. It is also used in personal hygiene products for antimicrobial properties in dental care and soaps. It can also be applied to wounds to prevent infection [17].

It is worthy to note that compounds **2**,**3**,**4**,**5** and **7** are sesquiterpene isomers. The rearrangement of carbocations by hydride shift played a role in the formation of these sesquiterpenes. They underwent a series of rearrangements in the course of construction to generate the most stable carbocations. Terpenes and their derivatives are generally used as flavouring and medicinal agents and in perfumery. They are used by the food industry to give flavour to drinks and foods and are also a component for the pharmaceutical industry for the preparation of drugs, soaps, perfumes and other cosmetics as well as for home cleaning products [18]. Also, compounds **6**,**8** and**10** are isomers but sesquiterpene alcohols. Compound **13** also known as phytol is used to increase energy and to fight infection and are natural alternatives to use for hypertension and cancer [19]. Phytol has been reported to have anti-mycobacterial activity against mycobacterium tuberculosis [20].

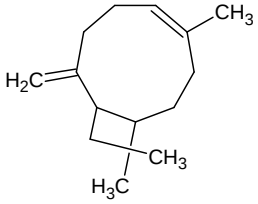
L-(+)-ascorbic acid 2,6-dihexadecanoate (compound **11**) was the second largest constituent of the extract with 20.43%. Ascorbic acid is used in the treatment of cold, scurvy, skin infections, bronchitis, stomach ulcers, dysentery, boils and wounds. It is also used in the prevention of arthritis, back pain, fatigue and boosting the immune system [21]. Ascorbic acid is an important antioxidant and may protect the body against free radicals and oxidative damage and may also play an important role in sperm maturation [21]. Hexadecanoic acid methyl ester (compound **9**) has been reported to possess anti-inflammatory activities and also being used as a fragrance [22]. The use of *P. guajava* extract in the treatment of disease and infection in herbal medicine could be as a result of the synergistic effect of these phytocompounds found in the extract as revealed by GC-MS method.

Table 1: Phytoconstituents identified in the isopropanolic leaf extract of P. guajava by GC-MS

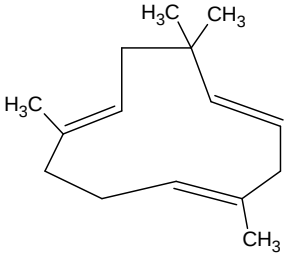
Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time(min)	Peak area(%)	Nature of compound
1	1,3,3-trimethyl-2-oxabicyclo[2.2.2] octane,	$C_{10}H_{18}O$	154	7.240	2.06	Ether
2	4,11,11-trimethyl-bicyclo[7.2.0] undec-4-ene	$C_{15}H_{24}$	204	13.555	6.26	Sesquiterpene
3	(E,E,E)-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene	$C_{15}H_{24}$	204	14.201	1.05	Sesquiterpene
4	Eudesma-4(14),11-diene	$C_{15}H_{24}$	204	14.892	5.07	Sesquiterpene
5	Guaia-1(10),11-diene	$C_{15}H_{24}$	204	15.067	4.63	Sesquiterpene
6	3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	$C_{15}H_{26}O$	222	16.458	4.01	Sesquiterpene alcohol
7	1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.0(2,7)]dec-3-ene	$C_{15}H_{24}$	204	18.308	1.66	Sesquiterpene
8	1,1,4,7-tetramethyl-decahydro-1H-cycloprop[e]azulen-4-ol	$C_{15}H_{26}O$	222	18.533	11.00	Sesquiterpene alcohol
9	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	21.183	1.53	Fatty acid ester
10	4,4,8-trimethyltricyclo[6.3.1.0(1,5)] dodecane-2,9-diol	$C_{15}H_{26}O_2$	238	21.350	2.78	Sesquiterpene alcohol
11	L-(+)-ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	21.808	20.43	Ascorbic acid
12	7-octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	22.600	3.81	Fatty acid ester
13	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	22.733	2.24	Diterpene alcohol
14	9-octadecenoic acid	$C_{18}H_{34}O_2$	282	23.133	33.47	Fatty acid



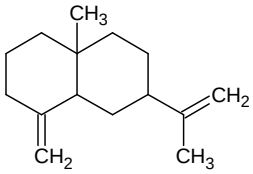
[**1**] 1,3,3-trimethyl-2-oxabicyclo[2.2.2] octane



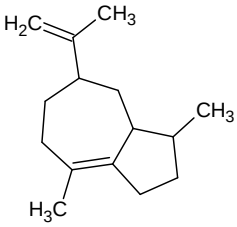
[**2**] 4,11,11-trimethyl-bicyclo[7.2.0] undec-4-ene



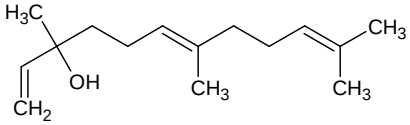
[**3**] (E,E,E)-2,6,6,9-tetramethyl-4,4,8-cycloundecatriene



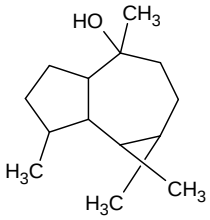
[**4**] eudesma-4(14), 11-diene



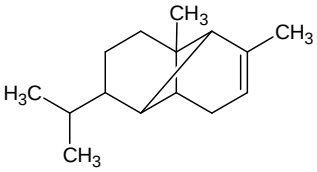
[**5**] guaia-1(10), 11-diene



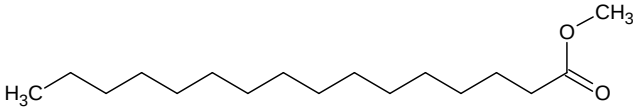
[**6**] 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol



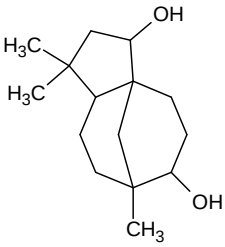
[**8**] 1,1,4,7-tetramethyl-decahydro-1H-cycloprop[e]azulen-4-ol



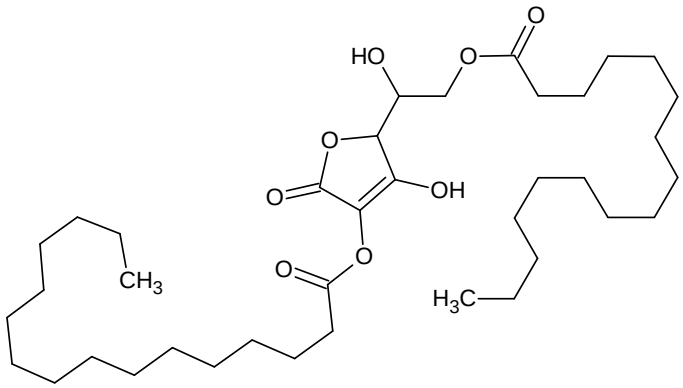
[**7**] 1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.0(2,7)]dec-3-ene



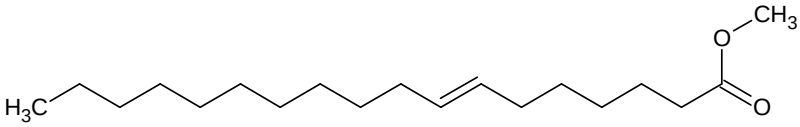
[**9**] hexadecanoic acid methyl ester



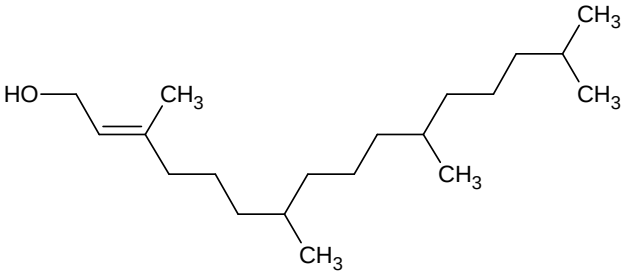
[**10**] 4,4,8-trimethyltricyclo[6.3.1.0(1,5)] dodecane-2,9-diol



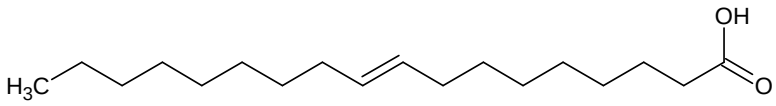
[**11**] L-(+)-ascorbic acid 2,6-dihexadecanoate



[**12**] 7-octadecenoic acid methyl ester



[**13**] 3,7,11,15-tetramethyl-2-hexadecen-1-ol



[**14**] 9-octadecenoic acid

Figure 3: Structures of the phytochemicals from the leaf extract of *P. guajava*

CONCLUSION

P. guajava is widely used as herbal medicine. This work describes the GC-MS analysis results of the isopropanolic extract of the leaves of *P. guajava,* and fourteen bioactive components have been characterized. This research reveals that the major components of the extract are sesquiterpenes and their derivatives. The compounds observed could also be as a result of the use of isopropanol as a choice of solvent which has a particular polarity and eluant power. The synergistic effect of these bioactive compounds in the leaves of *P. guajava* could be the reason behind its use for the treatment of diseases and infections in herbal medicine in Nigeria.

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