Phytochemical components and GC–MS analysis of Petiveria alliaceae L. fractions and volatile oils

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Phytochemical components and GC–MS analysis of *Petiveria alliaceae* L. fractions and volatile oils

Abstract: Phytochemical constituents are important in the determination of plant activities. Their presence in medicinal plants gives their therapeutic values. These phytoconstituents possesses pharmacological activities that include antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and several other activities. These activities can be determined by the identification of the phytochemicals present in medicinal plants. *Petivera alliaceae* L. is one of the medicinal plants in the family of phtytolaccaceae used traditionally as an anti-rheumatic, analgesics, antimicrobial, anticancer and immunomodulators. It is believed to possess rich phytoconstituents especially sulphur containing a broad-spectrum antimicrobial activity. Although the root extracts is been explored but there are limited publication to its root fractions. Therefore, the study is aimed at screening phytochemicals present in the fractions and volatile oil of the root parts of *P. alliaceae* using qualitative tests and Gas Chromatography–Mass Spectrometry (GC–MS) analysis. Fresh root parts of *Petivera allliaceae* plant were collected and air-dried. The dried root parts was macerated in absolute methanol for 72 h. The solution was extracted and dried. The dried root methanol extract was partitioned into *n*-hexane (PAH) and methanol fractions (PAM), while volatile oil (PAO) was extracted using Clevenger-type

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hydrodistillation method. The result shows that alkaloids, saponins and flavonoids were present while cardiac glycosides, phenol, terpenoids and anthraqunione glycosides were absent in both fractions. The GC–MS analysis identified 18 compounds in PAH, 19 compounds in PAM and 28 compounds in PAO. The fractions and volatile oils of *P. alliaceae* roots are rich in phytochemical constituents and compounds should be isolated from the fractions and explored for their potentials.

Keywords: GC–MS; *Petivera alliaceae* L.; phytochemical constituents.

1 Introduction

To protect themselves from attacks from a relatively large number of external predators or annoyances, including herbivorous mammals, insects, and microbes, plants constantly produce chemical compounds known as phytoconstituents [1]. Many isolated compounds from medicinal plants have pharmacological, therapeutic, and microbiological potentials, including those for anti-bacterial, anti-viral, anti-inflammatory, antiseptic, anti-fungal, insect repellant, expectorant, detoxification, fever reduction, anti-HIV, antioxidant, and analgesic properties [2–8]. These actions of medicinal plants are caused by phytoconstituents, which include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. [9]. In many parts of the world, traditional understanding of plant functions and qualities is transmitted forward from generation to generation. This information has had a significant impact on the creation of many traditional medicines [10].

Petiveria alliaceae, which belongs to Phytolaccaceae family, is one of such plant with abundant phytoconstituents [11]. The name of the plant varies depending on where it is found; for instance, it is called “guine” in parts of Latin America, “anamu” in parts of South America, “awogba arun” in parts of Yoruba land, “mapurite” in parts of Trinidad, and “guinea hen weed,” “apacin,” and “mucura” in some other parts of the world [12–14].

It is a flowering plant that is indigenous to the Caribbean, Central America, the United States, Mexico, and tropical South America [15]. The plants are herbaceous shrubs with small green flowers and a deep root system that can reach heights of 1 m. The leaves and roots have a strong aroma resembling that of garlic [16]. It is a well-known medicinal herb in many tropical regions. It is occasionally grown in gardens for easy access to medicinal purposes, and it is also occasionally sold for that purpose in local markets. It is also traded online in capsule form [17]. Although this plant is common, its known danger is that if handled carelessly, the sharp fruit spines can easily and painfully pierce the skin [15]. Furthermore, it can store nitrates, leading to nitrate poisoning in cattle [18].

Sulfur is a compound that is frequently found in this plant, but flavonoids, triterpenes, and steroids as well as tannins, saponins, terpenoids, steroids, glycosides, and alkaloids have all been found in various plant parts [1]. Benzylhydroxymethyl sulfide,

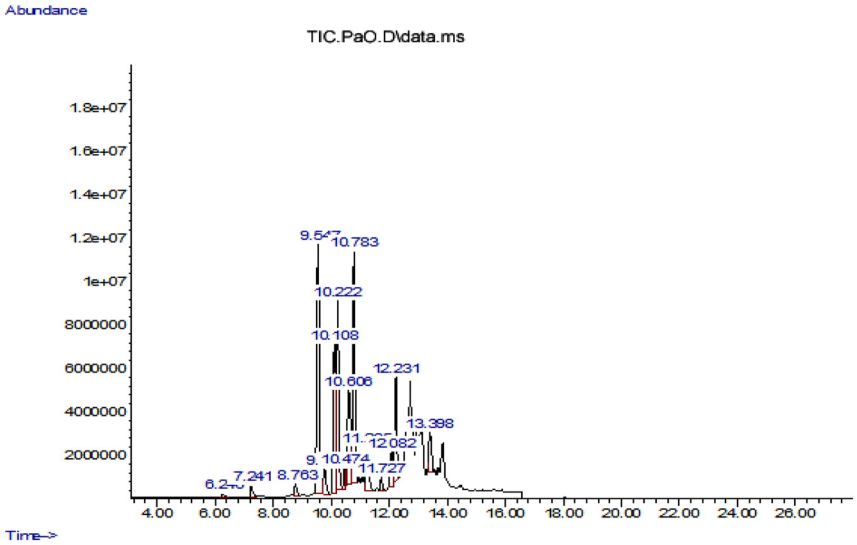

Figure 1: Chromatogram of *P. alliaceae* volatile oil.

dipropyl disulphide, dibenzyl sulphide, dibenzyl disulphide, dibenzyl trisulphide, dibenzyl tetrasulphide, and di (benzyltrithio) methane have reportedly been discovered in the leaves [19]. From Gas Chromatography–Mass Spectrometry (GCMS) analysis of the volatile oil from *P. alliaceae* leaves, this medicinal plant contains benzenecarbothioic acid, 1,2,3-trithiolane, 1,2,5 trithiepane, and 1,2,5,6-tetrathiocane as well as sulfur heterocyclic compounds [10]. As rich as this plant is it has not been fully explored for its therapeutic potentials, especially from its fractions. Also, it has been observed that some plant compounds are activated when they are fractionated [20–22]. Therefore, documentation of other phytoconstituents in this plant is necessary.

Hence, the aim of this research is to explore the phytochemical constituents of *n*-hexane and methanol fractions as well as volatile oil from the roots of *P. alliaceae* (Figures 1–3).

2 Materials and methods

2.1 Materials

Conical flask (Pyrex, 500 mL), absolute Ethanol (Analar),Cotton swab, water-bath, Methanol, separating funnel (Pyrex, 250 mL), *n*-Hexane, Ferric chloride ($FeCl_3$) reagent, Chloroform, 10% Hydrochloric acid (HCl), Ethylacetate, Columns, Dragendorff reagents, Concentrated Tetraoxosulphate (VI) acid (H_2SO_4), Gas Chromatography Mass Spectroscopy (GCMS) machine (Agilent, USA), Clevenger-type apparatus.

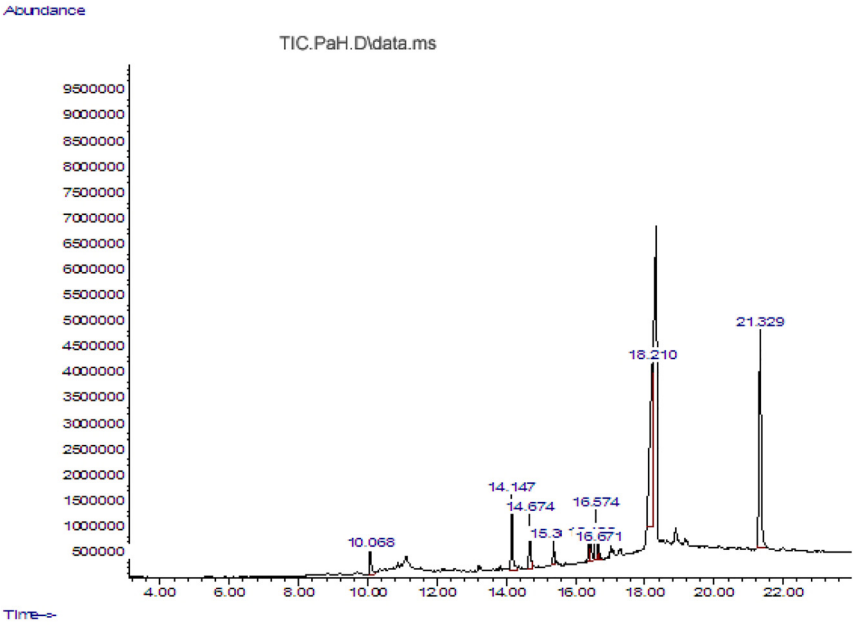

Figure 2: Chromatogram of *P. alliaceae* hexane fraction.

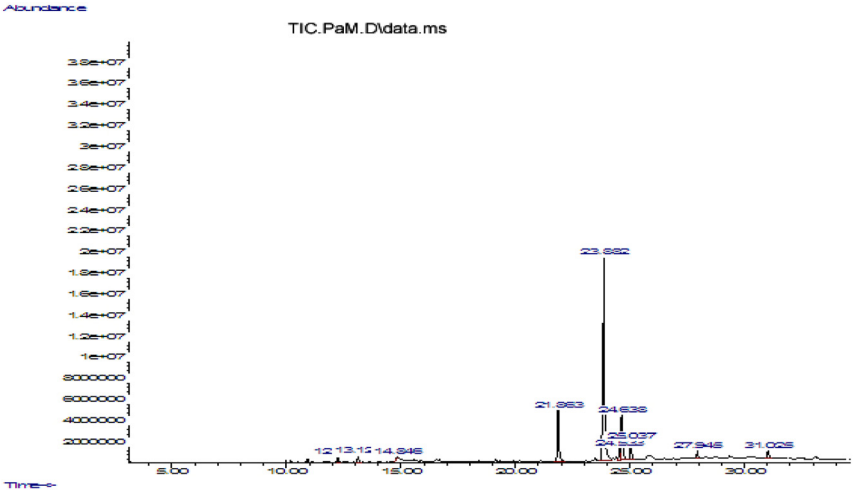

Figure 3: Chromatogram of *P. alliaceae* methanol fraction.

2.2 Methods

2.2.1 Plant collection and preparation: *Petiveria alliacea* root part was collection on the field at the Olabisi Onabanjo University main campus and taken to laboratory. The root was cleaned to remove sand and chopped to increase the surface area, thereafter was dried under shade in an aerated room (inside the laboratory) for two weeks. The plant was then pulverized, weighed and macerated in absolute methanol for five days. Extraction was done by means of first sieving the solvent from the shaft and concentrated in a rotary evaporator to obtain a concentrate that was stored at 40 °C until further use. A fractionation of the *P. alliacea* methanol extract was done using a separating funnel in ratio of 20:30:50 to make up to 200 mL constitution of water: methanol: hexane so as to obtain hexane and methanol fractions respectively. The percentage yields of the extracts were calculated as;

$$\%\ \text{yield} = \left(\frac{\text{weight of extract}}{\text{weight of dry sample}}\right) \times 100$$

2.2.2 Phytochemical screening: According to Sofowora [23] and Trease and Evans [24], phytochemical screening was done on the plant samples to check for alkaloids, anthra-quinone glycosides, cardiac glycosides, flavonoids, saponins, terpenoids, steroids, phenol, and tannins using conventional techniques.

1. Alkaloid: 10 mL of 10% Hydrochloric acid (HCl) were used to extract 1 g of the sample for 5 min in a water bath. After filtering, the extract was allowed to cool. Through the use of litmus paper and 10% ammonia, the pH was brought down to roughly 6–7. Wagner's and Dragendorff reagents were applied in little amounts to 5 mL of the filtrate in separate test tubes before being inspected. Alkaloids were detected by the presence of turbidity or precipitation.
2. Anthraquinone glycoside (The Borntrager's test): After boiling for 5 min and filtering while still hot, 1 g of the material was extracted with 2 mL of 10% Hydrochloric acid (HCl), then allowed to cool. With an equal volume (aliquot) of chloroform, the filtrate was divided, then gently shaken. The lower layer of chloroform was transferred to a clean test tube, to which an aliquot of a 10% ammonia solution was added, and the test tube was gently shaken. Anthraquinones glycosides were present because the test solution had a fine film of rose-pink colour on it.
3. Cardiac glycoside (Keller-Kelani test): A drop of the ferric chloride reagent was added to 2 mL of glacial acetic acid after one g of the sample had been extracted with water. To create an underlayer, 1 mL of concentrated Tetraoxosulphate (IV) acid (H_2SO_4) was then slowly added. The presence of cardiac glycoside was indicated by a brown, purple, or reddish-brown ring that developed at the interface and by the acetic acid layer's green colour.
4. Flavonoids
   i. Three drops of ferric chloride ($FeCl_3$) solution and one g of sample were added to 10 mL of ethanol. The presence of flavonoids was suggested by the dark green colour that was seen.
   ii. Ethyl acetate was used to extract one g of the plant material, followed by 3 min of boiling. 10% ammonia (NH_3) was used to treat the residue. The presence of flavonoids was suggested by the yellow colour that was seen.
   iii. A gram of the material was heated for 3 min and extracted with distilled water. After allowing it to cool, 2 mL of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added; the presence of a yellow color that vanishes upon standing indicated a successful reaction.
5. Tannins (The Braemer's test): 1 g of the powdered material was decocted by boiling for 10 min with 10 mL of distilled water, filtered while still hot, and then allowed to cool. The filtrate received 0.1% ferric chloride reagent addition. Tannins are present when a precipitate is blue-black, green, or blue-green.
6. Saponins

i. Frothing: A test tube holding 10 mL of distilled water and a gram of the sample was then filled, boiled for 5 min, and then filtered. The filtrate was forcefully shaken before being examined. The existence of saponins was detected by the formation of the froths.
ii. Emulsification: Three drops of olive oil were added to the foam and vigorously shaken; the appearance of emulsions reveals the existence of saponins in the sample.

7. Phenols: Three drops of phenol solution and 10 mL of ethanol were added to one g of the plant material. The presence of phenol was indicated by the dark green colour that was seen.
8. Steriods: After extracting the sample with 0.5 g of ethanol and 2 mL of H_2SO_4, 2 mL of acetic anhydride were added. In a sample, the colour transitioned from violet to blue or green, indicating the presence of steroids.
9. Terpenoids (Salkowski test): 2 mL of chloroform were added to 5 mL of ethanol extract, and 3 mL of pure H_2SO_4 were carefully added to create a layer. The inter-phase created had a reddish brown coloring, which was indicative of the presence of terpenoids.
10. Phloba-tanins: 1 g of the material was put into a test tube with 10 mL of distilled water, heated for 5 min, and then filtered. Add 5 mL of 1% HCl to the filter and boil it for 5 min. The presence of a yellow precipitate is a positive result.

2.2.3 Extraction of volatile oils: Fresh root of *P. alliaceae* were collected and chopped into pieces. These pieces were kept in a round bottom flask of a Clevenger-type apparatus and steamed for 4 h. After which oils were collected from the distilling vial by trapping it with hexane solvent and stored in the fridge (Figures 4 and 5).

2.2.4 Gas chromatography–mass spectrometry analysis: The plant samples PAM, PAH and PAO were analyzed using GC–MS machine, GC (Agilent technologies 7890, USA) coupled with an MS (Agilent, technologies 5975, USA). The HP5MS column length is 30 m, has internal diameter 0.320 mm and thickness 0.25 µL. The oven temperature is 80 °C held for 2 min at 12°/min to the temperature of 240° held for 6 min. The samples (1 µL) were injected while the interface temperature between GC and MS is 250 °C. The scan ranges from 50 to 500 and the mode of analysis is split less (Figures 6–8).

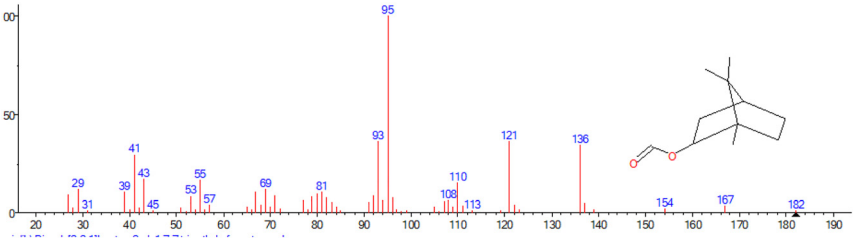

Figure 4: Bicyclo [2.2.]heptan-2-ol, 1,7,7-trimethyl-,formate,endo.

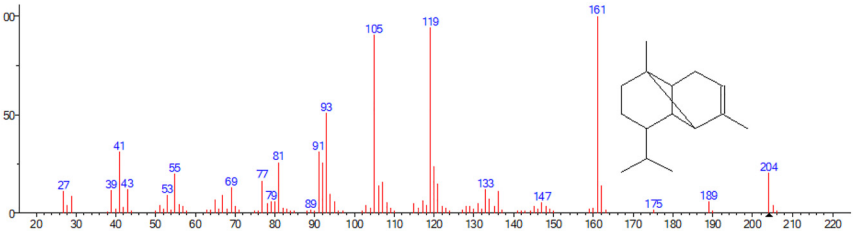

Figure 5: Copaene.

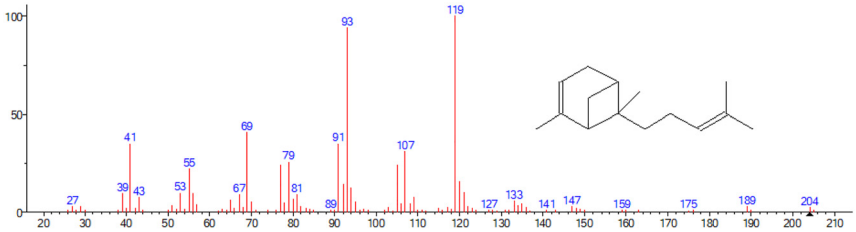

Figure 6: trans-α-Bergamotene.

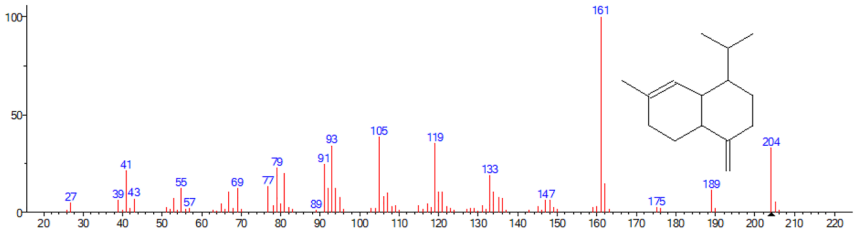

Figure 7: γ-Muurolene.

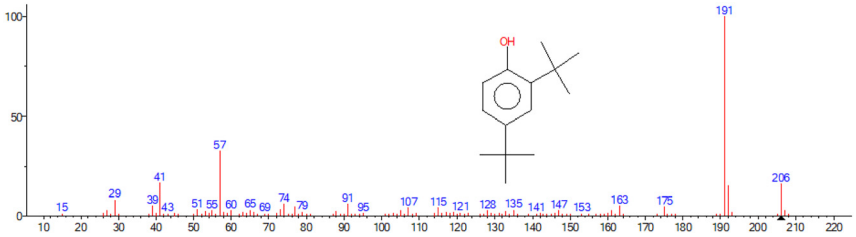

Figure 8: 2,4-Di-tert-butylphenol.

3 Results and discussion

The fractions' and extracts' percentage yields are presented below in Table 1 which hexane fraction gave a percentage yield of 3.64% while methanol fraction gave 60.78%. The results from the table indicated that more yield were obtained from the methanol

Table 1: Percentage Yield of *P. alliacea* extract and fractions.

Sample	Weight of dry sample (g)	Weight of extract (g)	% Yield
Methanol crude extract	820	10.30	1.26
Hexane fraction	10.30	0.38	3.64
Methanol fraction	10.30	6.26	60.78

Table 2: Results of phytochemical screening.

	Test	Hexane fraction	Methanol fraction
1.	Alkaloid	+	+
	a. Wagner	++	++
	b. Dragendorff		
2.	Anthraquinone glycoside	–	–
3.	Cardiac glycoside	–	–
4.	Saponins	+	++
	a. Frothing	+	+++
	b. Emulsification		
5.	Phenol	–	–
6.	Flavonoids	+	+
	a. Water	++	–
	b. Ethanol	++	–
	c. Ethylaeatate		
7.	Tanins	+	–
8.	Phyloba-tanins	+	–
9.	Terpenoids	–	–
10.	Steroids	+++	–

–, absent; +, trace; ++, Moderate; +++, Abundant.

fraction than the hexane fraction. Methanol has been reported to have high yield of extraction [25–27].

Table 2 reported the phytochemical screening of both hexane and methanol fractions and from it we noticed that alkaloids, saponins and flavonoids were present in them. But tannins, phylobatanins and steroids were present only in the hexane fraction while cardiac glycosides phenol, terpenoids and anthraqunione glycosides were absent in both fractions. These were also observed in Genus Acalypha and in some Nigerian savannah plants [28, 29].

The analysis from Table 3 of PAH reveals that 18 compounds were identified in which most of it were fatty acids. Others class phytoconstituents identified are carboxylic acids and terpenes. 19 compounds were identified in PAM and these compounds also belongs to fatty acids and terpenes classes Then PAO also revealed 28 compounds, which are majorly sesquiterpenes. Castellar et al. [30] identified constituents from *P. alliacea* and reported that this plant is rich in sulfur compounds such as bis(phenyl-methyl)-disulfyde, iso-thiazole (1,2-thiazole), 2-thiopropane, dimethyl sulphyde, ethylene disulfyde, and 2,3-dimethyl-thiirane (Figures 9 and 10).

Also, Sathiyabalan et al. [31] identified 14 compounds from ethanol extract of *P. alliacea* whole plants. This compounds includes Asarone, 3-(4-methoxy phenyl), 2-propenonic acid Phytol, Ester, a-D-glucopyranose-4-O-a-D-galactopyranosyl, Heptadecane 2, 6, 10, 14 tetramethyl-, Z,Z,-2-5-pentadecadien-1-ol, Squalene, as well as Vitamin E. Furthermore, Medina-Santos et al. [32] reported dichloromethane and methanol extract having several common chemical constituents with abundance

Table 3: Phytoconstituents present in PAH, PAM and PAO using GC–MS.

Fraction	Compounds names	Area (%)	RT
PAH	2,4-Di-tert-butylphenol	3.02	10.068
	1,2-Benzenedicarboxylic acid, phthalic acid, decyl isobutyl ester	8.20	14.147
	Hexadecanoic acid, methyl ester	5.78	14.674
	Hexadecanoic acid, ethyl ester	2.18	15.361
	Methyl 9-cis,11-trans-octadecadienoate, 9,12-octadecadienoic acid (Z,Z)-, methyl ester, 8,11-octadecadienoic acid, methyl ester	2.03	16.379
	9-Octadecenoic acid (Z)-, methyl ester, cis-13-octadecenoic acid, methyl ester, 11-octadecenoic acid, methyl ester	2.24	16.436
	Phytol	5.06	16.574
	Methyl stearate	1.44	16.671
	Hexadecane, 1-iodo-, heneicosane, tricosane, 2-methyl-	35.87	18.210
	Bis(2-ethylhexyl) phthalate, diisooctyl phthalate	34.19	21.329
PAM	Germacrene D, 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a. alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	1.24	12.242
	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-1-isopropyl-4,7-dimethyl-1,2,3,5,6, 8a-hexahydronaphthalene	1.42	13.129
	Diethyl phthalate	0.27	14.845
	Hexadecanoic acid, ethyl ester, undecanoic acid, ethyl ester	13.42	21.861
	Phytol	60.59	23.880
	Linoleic acid ethyl ester, 9,12-octadecadienoic acid, ethyl ester, linoleic acid ethyl ester	2.01	24.533
	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-, Ethyl oleate, (E)-9-octadecenoic acid ethyl ester	12.74	24.636
	Octadecanoic acid, ethyl ester	4.59	25.036
	Eicosanoic acid, ethyl ester, methyl 19-methyl-eicosanoate, undecanoic acid, ethyl ester	1.46	27.943
	Docosanoic acid, ethyl ester, hexadecanoic acid, undecanoic acid	2.24	31.027
PAO	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-, Ethyl (1R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl carbonate, Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	0.37	6.245
	Bornyl acetate, acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester, Bicyclo [2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	0.91	7.241
	Copaene, .alfa.-Copaene	0.88	8.763
	Caryophyllene	22.67	9.547
	trans-.alpha.-Bergamotene, 1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (Z,E)-,	2.18	9.765
	Humulene	12.04	10.108
	Alloaromadendrene, (1R,9R,E)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0] undec-4-ene,	12.98	10.222
	gamma.-Muurolene	1.34	10.474
	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	10.20	10.606
	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-, trans-.alpha.-Bergamotene	17.39	10.783
	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-, 1-isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene, .alfa.-Copaene	4.05	11.235

Table 3: (continued)

Fraction	Compounds names	Area (%)	RT
	Caryophyllenyl alcohol, 4-pyridinol-1-oxide, 4-(2-methyl-3-oxocyclohexyl)butyric acid, ethyl ester	2.75	11.727
	Caryophyllene oxide	7.61	12.231
	Aromandendrene, naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)], alloaromadendrene	3.73	13.398

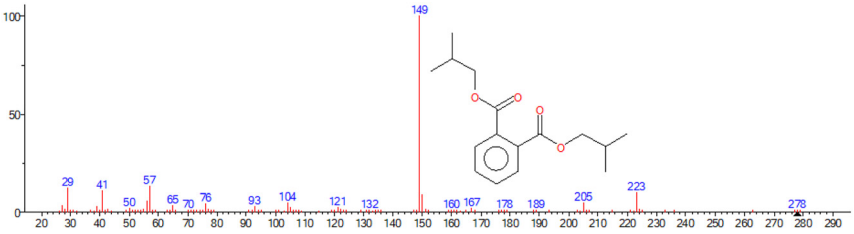

Figure 9: 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester.

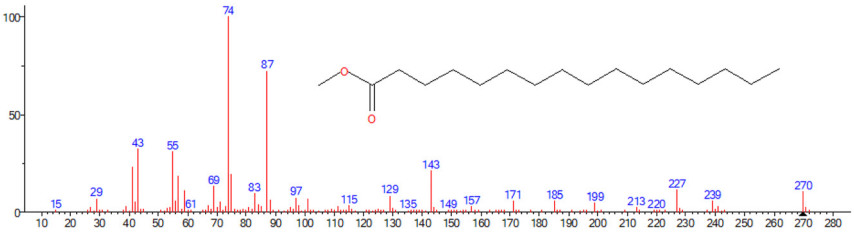

Figure 10: Hexadecanoic acid, methyl ester.

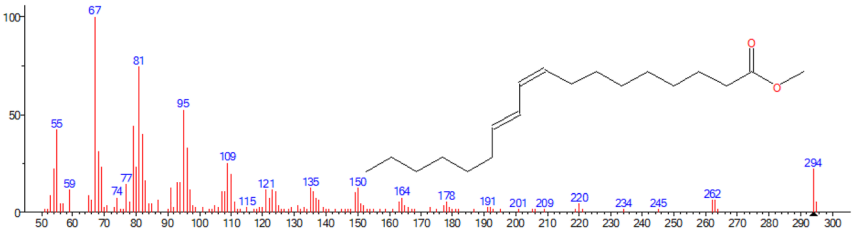

Figure 11: Methyl 9-cis, 11-trans-octadecadienoate.

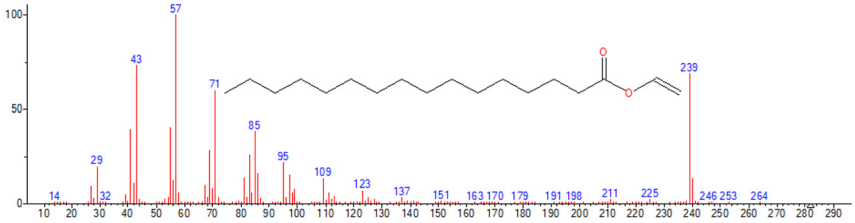

Figure 12: Palmitic acid vinyl ester.

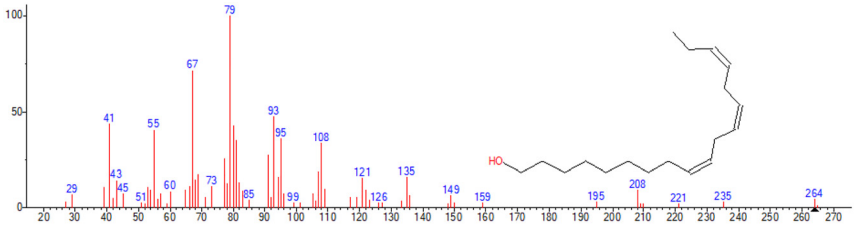

Figure 13: 9, 12, 15-octadecatrien-1-ol(Z,Z,Z).

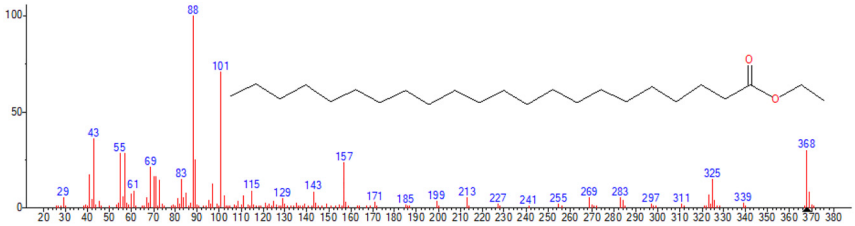

Figure 14: Docosanoic acid, ethyl ester.

compounds being phytol, phytol, 4-vinyl-2-methoxy-phenol, and hexadecanoic acid (Figures 11 and 12).

From a pentane extract identified, 13 components were identified with most abundant compounds been benzaldehyde, dibenzyl disulfide,e dibenzyl trisulfide and cis- and trans-stilbene [33]. The root and flower oils of *P. alliacea* showed that the benzenoids, such as benzaldehyde, benzyl alcohol, and (Z) 3 hexeny benzoate, as the main constituents [34]. These compounds have also been found from the study conducted (Figures 13 and 14).

4 Conclusions

Natural plant products currently represent a potential source for the discovery of new drugs to treat a variety of disorders. Among such plant is *P. alliacea*. Its different parts extracts, fractions and volatile oils are with several bioactive properties such as

antimicrobial, anticancer, immune-regulator, anti-inflammatory and several others activities. *P. alliaceae* root fractions and volatile oil are rich in phytochemical constituents as several research conducted as indicated. Hence, the exploration of these constituents can help with drug discovery in natural product and nutriceuticals. To clarify the synergistic/antagonistic mechanisms by which the compounds present in the plant extracts act, additional research is required. For instance, fractions and volatile oils may interact to produce pharmacological activities.

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