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Investigation of Bioactive Phytochemical Compounds from the Chloroform Extract of the Leaves of *Phyllanthus amarus* by GC-MS Technique

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Abstract

The chloroform extract of the leaves of *Phyllanthus amarus* yielded dark oily substance (5.68 g). The extract was subjected to GC-MS analysis. Ten phytochemicals were identified with 9,12,15-octadecatrienoic acid(57.05%) constituting the bulk of the oil followed by L-(+)-ascorbic acid 2,6-dihexadecanoate (22.54%). Other compounds identified include hexadecanoic acid, 1-methylethyl ester (5.39%), methanesulfonic acid, 2-(2-hydroxy-hexahydropentalen-3a-yl)-ethyl ester (3.24%), tetradecanoic acid (2.90%), dodecanoic acid (2.53%), 1-nonadecene (2.17%), 1-heptadecene (1.86%), hexadecanoic acid methyl ester (1.25%) and tetradecanoic acid,1-methylethyl ester(1.07%). The presence of these phytochemicals might have contributed to the reasons why the plant extract is used in herbal medicine for the treatment of health disorders, diseases and infections in Eastern Nigeria.

Keywords: GC-MS analysis, *Phyllanthus amarus*, Phytochemicals, Herbal medicine, Chloroform extract

Contents

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1. Introduction

Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications in pharmaceuticals, insecticides, dyes, flavours and fragrance [1]. Phytochemical compounds comprise a large group of molecules derived from a variety of plant sources [2]. *Phyllanthus amarus* belongs to the family *Euphorbiaceae*. It is an annual many branched, erect herb up to 80 cm high. It has numerous small leaves on lateral branches of the stem that give the plant the appearance of having pinnate leaves up to 70 cm high [3]. It produces from seeds. The stem is rounded, woody at the base, horizontally branched, smooth and greenish. The leaves are alternate, elliptic-oblong, 5-10 mm long and 3-4 mm wide, pale beneath and with short petioles. The inflorescence is axillary and consists of one male flower and one female flower

in each axil [3]. The flowers are greenish and rather small, up to 1.5 mm in diameter. The fruit is a round capsule, brownish, 1.5-2 mm wide and occurs in leaf axils on the lower side of the lateral branches. Each capsule contains six small seeds [3]. *P. amarus* is a widespread tropical plant commonly found in coastal areas well known for its medicinal properties and widely used worldwide. This herb is common weed that can be found in most parts of tropical countries in fields, cultivated fields of cotton, maize, rice, coffee, banana plantation, and gardens and on waste ground. It is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen [4]. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhoea, menorrhagia and other genital affections. It is also useful in gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds [4].

In Eastern Nigeria, *P. amarus* whole plant is used in the treatment of malaria. A clinical study of the plant indicated that it may reduce the levels of urinary calcium [5]. The plant has also been used in Brazil and Peru as an herbal remedy for kidney stones. *P. amarus* root and leaf extracts show significant hepatitis C antiviral activity [6]. The whole plant root, leaves tender aerial parts and latex can be used to treat all kinds of jaundice as single remedy and as a cholerectic, liver protectant, inflammation, diarrhea, dyspepsia, diabetes, fever, sprained hoof, haematuria, night blindness, colic, stoppage of urination, appetite stimulant, diuretic and frequent menstruation. The leaves have been shown to reduce blood sugar, exhibit antibacterial, antifungal and antiviral activity [7]. *P. amarus* whole plant extract can be used in products like hair tonic, hair shampoo, body lotion, body massage oil and dry powder used to control skin diseases. It is used for cystitis, prostatitis, tuberculosis, venereal diseases and urinary tract infections [8]. *P. amarus* is also widely used in local medicine in Africa and Asia with antiviral, anti-HIV, anti-inflammatory, antioxidant, antibacterial and antidiabetic activities. *P. amarus* contains ligans, flavonoids, several tannins, alkaloids and sterols. The plant also has many physiologically active alkaloids in the fruits, leaves and roots. The ligans phyllanthin and hypophyllanthin have been shown to be hepatroprotective against carbon tetrachloride induced hepatotoxicity in primary cultured hepatocyctes [8].

2. 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 80-200℃ held at 80℃ for 1 minute, rate 5℃/min and at 200℃ for 20 minutes, FID temperature 300℃, injection temperature 250℃, carrier gas nitrogen at a flow rate of 1 mL/min, split ratio 1: 75. GC-MS (Gas chromatography mass spectrometry) analysis was conducted using GCMS-QP 2010 Plus Shimazu Japan with injector temperature of 230 ℃ and carrier gas pressure of 100 Kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 mL/min. The eluents were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 KV and sampling rate of 0.2 seconds. 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

Phyllanthus amarus leaves were harvested from an abandoned farm land located at Ubakala, Umuahia South Local Government Area of Abia State, Nigeria. The leaves were then dried on the laboratory bench 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 chloroform (8hrs/3 times/30℃). The extract was concentrated under reduced pressure and the supernatant extract was decanted (5.68g) 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.

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 [9].

3. Results and Discussion

The chloroform extract of the leaves of *Phyllanthus amarus* showed ten peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of ten compounds (1-10) 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 hydrocarbon (4.03%), fatty acids (62.48%), sulfonic acid ester (3.24%), fatty acid esters (7.71%) and vitamin C (22.54%).

Compound **1** was identified as 1-heptadecene and has molecular formula of $C_{17}H_{34}$ (m/z 238) with base peak at m/z 43. The base peak was as a result of the detachment of C_3H_7 group from the compound. The compound comprised 1.86% of the extract. Compound **2** was a fatty acid named dodecanoic acid. It has molecular formula of $C_{12}H_{14}O_2$

(m/z 200) and base peak at m/z 73 which resulted because of the cleavage of $C_3H_5O_2$ group from the compound. The compound comprised 2.53% of the extract. Compound **3** was identified as methanesulfonic acid, 2-(2-hydroxy-hexahydropentalen-3a-yl)-ethyl ester with a molecular formula of $C_{11}H_{20}O_4S$ (m/z 248) and a base peak at m/z 109. The base peak was due to the loss of $C_2H_5O_3S$ group from the compound. The compound comprised 3.24% of the extract. Compound **4** was identified as 1-nonadecene with a molecular formula of $C_{19}H_{38}$ (m/z 266) and a base peak at m/z 97 due to the loss of a C_7H_{13} group from the compound. The compound comprised 2.17% of the extract. Compound **5** was a fatty acid molecule named tetradecanoic acid. It has a molecular formula of $C_{14}H_{28}O_2$ (m/z 228) and a base peak at m/z 73 due to the loss of $C_3H_5O_2$ group from the compound.

The compound comprised 2.90% of the extract. Compound **6** was identified as tetradecanoic acid, 1-methylethyl ester with a molecular formula of $C_{17}H_{34}O_2$ (m/z 270) and a base peak at m/z 43. The base peak was as a result of the cleavage of C_3H_7 group from the compound. The compound comprised 1.07% of the extract. Compound **7** was identified as hexadecanoic acid, methyl ester with a molecular formula of $C_{17}H_{34}O_2$ (m/z 270) and a base peak at m/z 74 due to the cleavage of $C_3H_6O_2$ group from the compound. The compound comprised 1.25% of the extract. Compound **8** has a molecular formula of $C_{38}H_{68}O_8$ (m/z 652). It was identified as 1-(+)-ascorbic acid 2,6-dihexadecanoate and comprised 22.54% of the extract. It has a base peak at m/z 57 resulting from the detachment of C_4H_9 group from the molecule. Compound **9** was a fatty acid ester identified as hexadecanoic acid, 1-methylethyl ester with a molecular formula of $C_{19}H_{38}O_2$ (m/z 298) and a base peak at m/z 102 which occurred as a result of the cleavage of a $C_5H_{10}O_2$ fragment from the compound. The compound comprised 5.39% of the extract. Compound **10** comprised 57.05% of the extract. It was identified as 9, 12, 15-octadecatrienoic acid with a molecular formula of $C_{18}H_{30}O_2$ (m/z 278) and a base peak at m/z 41. The base peak occurred as a result of the detachment of C_3H_5 fragment from the compound.

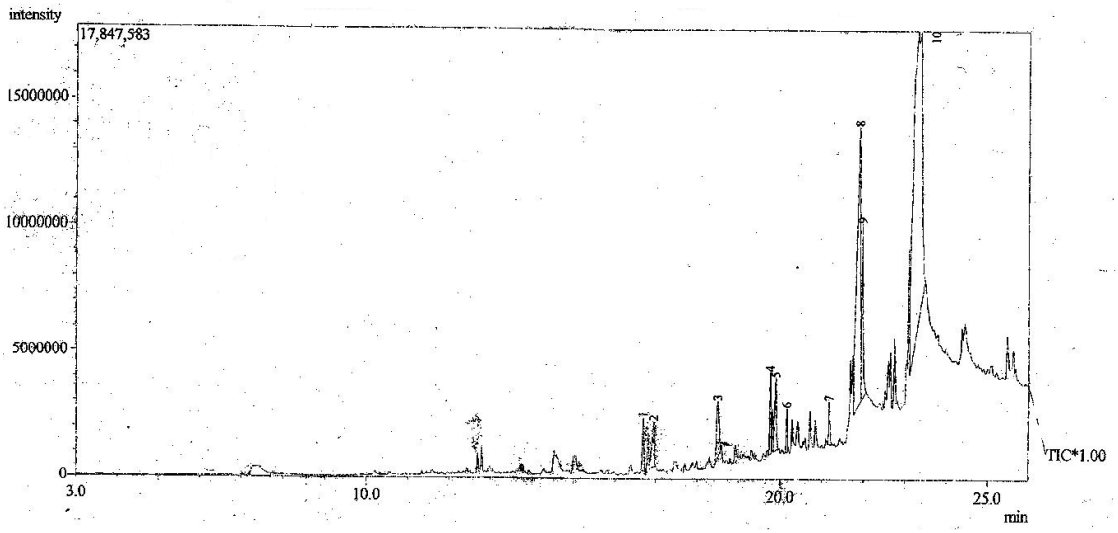


Fig. 1: GC-MS chromatogram of chloroform extracts of Phyllanthus amarus

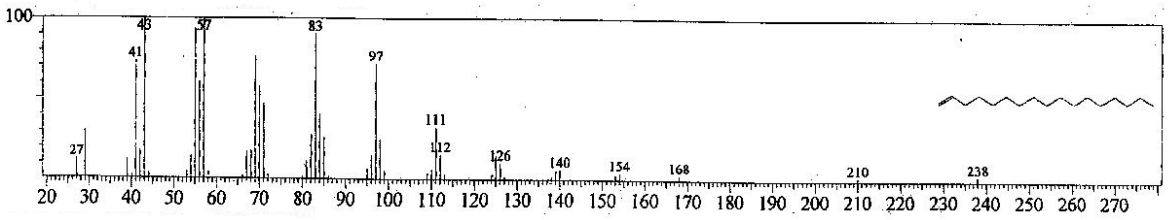


Fig. 2a: 1-Heptadecene

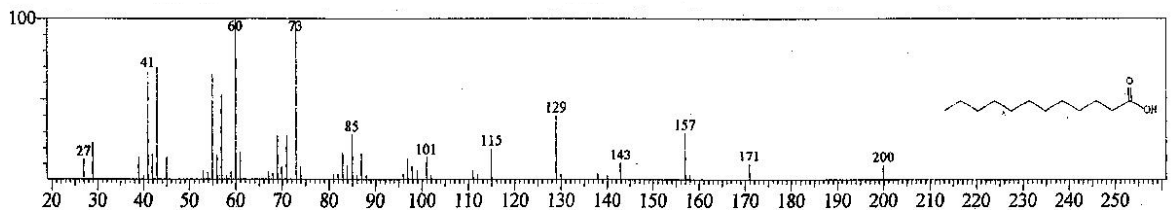


Fig. 2b: Dodecanoic acid

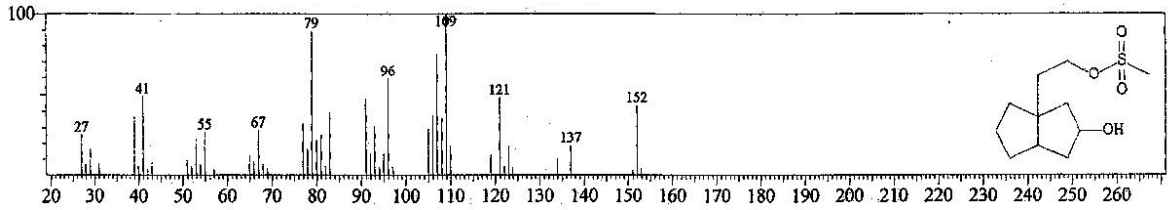


Fig. 2c: Methanesulfonic acid, 2-(2-hydroxy-hexahydropentalen-3a-yl)-ethyl ester

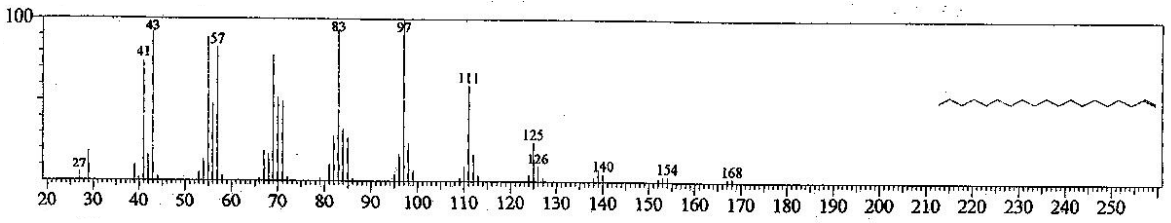


Fig. 2d: 1-Nonadecene

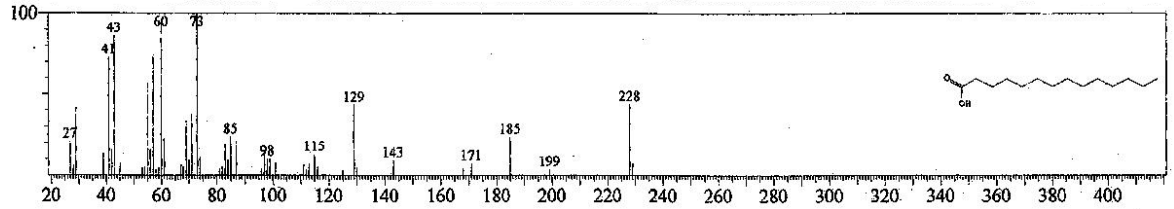


Fig. 2e: Tetradecanoic acid

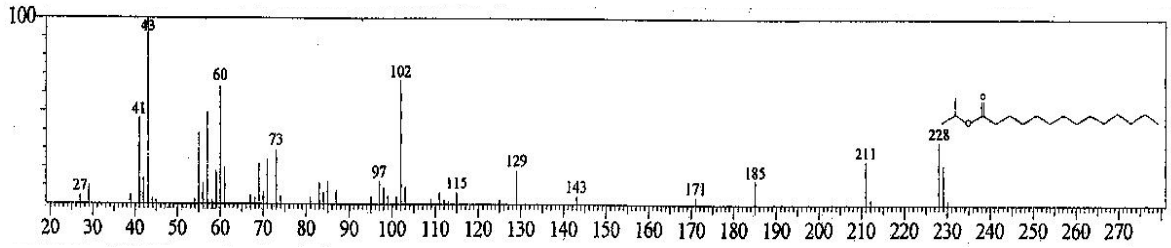


Fig. 2f: Tetradecanoic acid,1-methylethyl ester

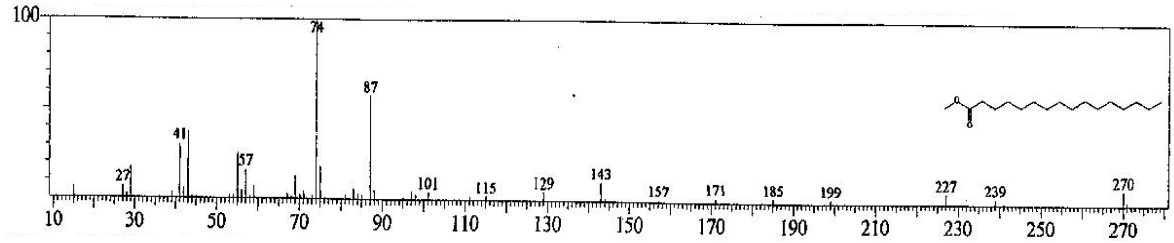


Fig. 2g: Hexadecanoic acid, methyl ester

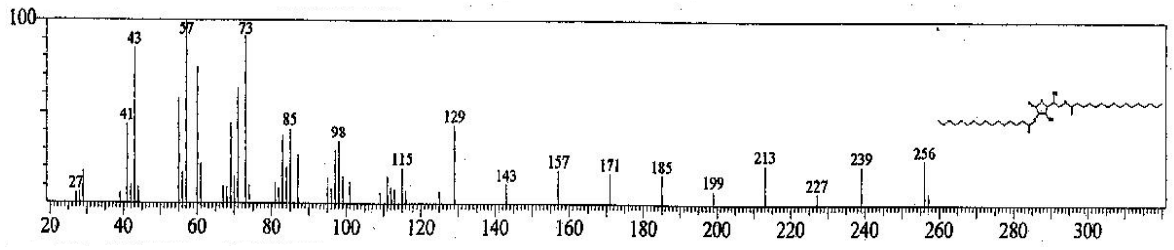


Fig. 2h: L-(+)-Ascorbic acid 2,6-dihexadecanoate

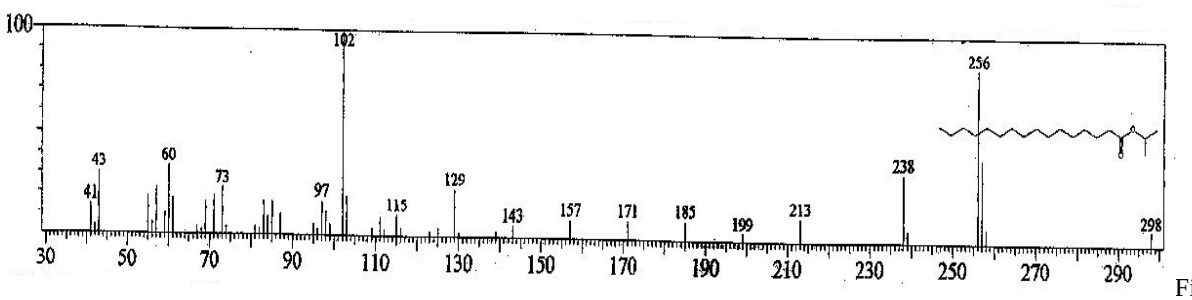


Fig. **2i: Hexadecanoic acid, 1-methylethyl ester**

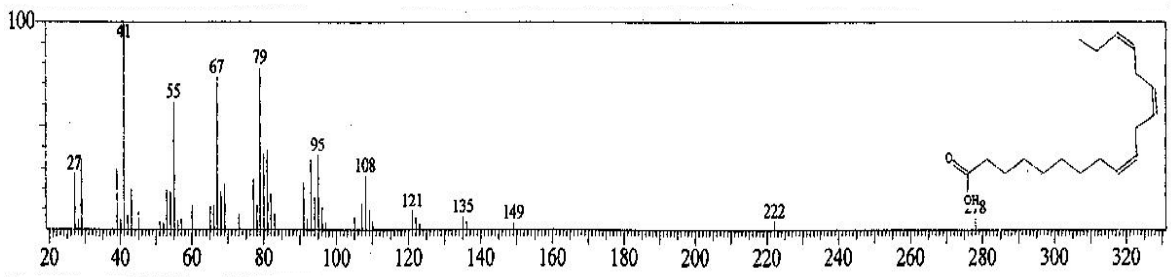


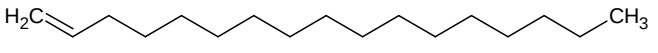
Fig. 2j: 9,12,15-Octadecatrienoic acid

L-(+)-ascorbic acid 2,6-dihexadecanoate was the second highest of the ten components of the extract of the leaf of *P. amarus*. It is a vitamin C compound. Ascorbic acid is often used for preventing and treating common cold, gum disease, acne and other skin infections, bronchitis, stomach ulcers, tuberculosis, dysentery, boils and wounds [10]. Ascorbic acid is also used to prevent glaucoma, cataracts, gallbladder disease, dental cavities, constipation, hay fever, asthma, arthritis, back pain, diabetes, chronic fatigue syndrome, osteoporosis and boosting the immune system [10]. Ascorbic acid acts as antioxidant in the skin by scavenging and quenching free radicals generated by ultraviolet radiation [11]. The use of *P. amarus* leaves in the treatment of diarrhoea, dysentery, stomach problems, ulcers, wound, fever and for ophthalmopathy in herbal medicine could be as a result of the presence of ascorbic acid 2,6-dihexadecanoate in the leaf of the plant. Organosulphur compounds (OSCs) prevent or slow down the carcinogenic process induced by a variety of chemical carcinogens. These include inhibition of the carcinogens, dermatitis and other minor wounds [12]. Compound **3** which is an organosulphur compound could have a contributory role in the use of *P. amarus* for the treatment of disease and infection in herbal medicine.

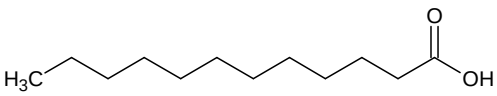
Dodecanoic acid also known as lauric acid has been claimed to have antimicrobial properties [13,14]. Lauric acid has been found to increase total cholesterol the most of all fatty acids. But most of the increase is attributable to an increase in high-density lipoprotein (HDL) 'good' cholesterol. As a result, lauric acid has a more favourable effect on total HDL cholesterol than any other fatty acid, either saturated or unsaturated [15], a lower total/HDL cholesterol ratio suggests a decrease in atherosclerotic risk [16]. Tetradecanoic acid known as myristic acid is commonly added co-translationally to the penultimate, nitrogen-terminus, glycine in receptor-associated kinases to confer the membrane localisation of the enzyme [17].

The myristic acid has a sufficiently high hydrophobicity to become incorporated into the fatty acyl core of the phospholipid bilayer of the plasma membrane of the eukaryotic cell. In this way, myristic acid acts as a lipid anchor in bio-membranes and may be used in cosmetics [17]. Isopropyl myristate (compound **6**) is used in cosmetics and topical medicinal preparations where good absorption through the skin is desired. It is also used as a pesticide-free treatment against head lice which works by dissolving the wax that covers the exoskeleton of head lice, killing them by dehydration [18]. The extract from *P. amarus* could be used in combating head lice and also be incorporated in cosmetic products.

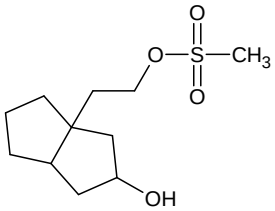
The constituent with the highest quantity in the leaf extract of *P. amarus* was 9,12,15-octadecatrienoic acid having a composition of 57.05%. Unsaturated fatty acids are important to every cell in the body for normal growth, especially of blood vessels and nerves and to keep the skin and other tissues youthful and supple through their lubricating quality [9]. They are nutrients which are invaluable for the production and movement of energy throughout the body, regulation of transportation of oxygen and are vital in maintaining the integrity of cell structure as well as the unique ability to lower cholesterol levels of the blood [9].



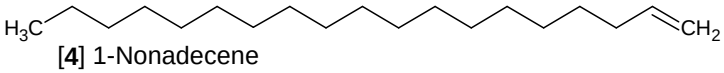
[**1**] 1-Heptadecene



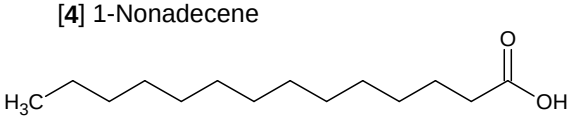
[**2**] Dodecanoic acid



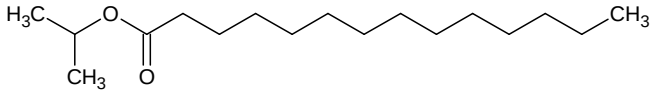
[**3**] 2-(2-Hydroxy-hexahydropentalen-3a-yl)-ethyl ester



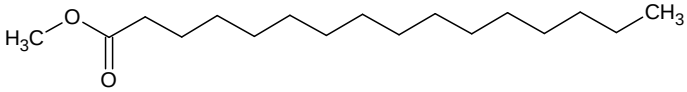
[**4**] 1-Nonadecene



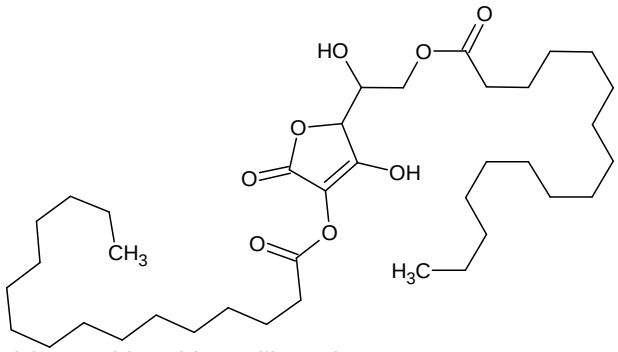
[**5**] Tetradecanoic acid



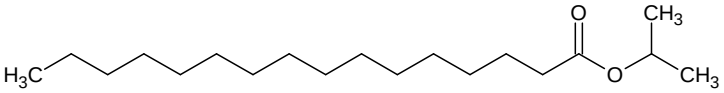
[**6**]Tetradecanoic acid,1-methylethyl ester



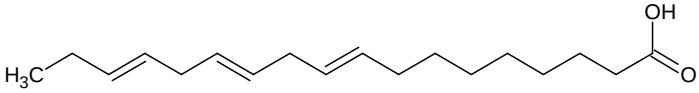
[**7**] Hexadecanoic acid, methyl ester



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



[**9**] Hexadecanoic acid, 1-methylethyl ester



[**10**] 9,12,15-Octadecatrienoic acid

Figure 3: Structures of the phytochemicals from the chloroform leaf extract of *Phyllanthus amarus*

Table 1: Phytochemicals identified in the chloroform leaf extract of *Phyllanthus amarus* by GC-MS

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time(min)	Peak area(%)	Nature of compound
1	1-heptadecene	$C_{17}H_{34}$	238	16.700	1.86	Hydrocarbon
2	Dodecanoic acid	$C_{12}H_{24}O_2$	200	16.958	2.53	Fatty acid
3	Methanesulfonic acid, 2-(2-hydroxy-hexahydropentalen-3a-yl)-ethyl ester	$C_{11}H_{20}O_4S$	248	18.500	3.24	Sulfonic acid ester
4	1-nonadecene	$C_{18}H_{38}$	266	19.767	2.17	Hydrocarbon
5	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	19.900	2.90	Fatty acid
6	Tetradecanoic acid,1-methylethyl ester	$C_{17}H_{34}O_2$	270	20.167	1.07	Fatty acid ester
7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	21.183	1.25	Fatty acid ester
8	L-(+)-ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	21.908	22.54	vitamin
9	Hexadecanoic acid, 1-methylethyl ester	$C_{19}H_{38}O_2$	298	21.975	5.39	Fatty acid ester
10	9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	278	23.375	57.05	Fatty acid

4. Conclusion

The medicinal values of *Phyllanthus amarus* in herbal medicine could be as a result of the contributory role of each of the phytochemical and/or a synergistic effect of these phytochemicals. It is worthy to also conclusively state that the phytochemicals observed in the leaves of *P. amarus* were with respect to chloroform as a choice of solvent for different solvents would manifest slightly different phytochemicals. This research therefore explores these plant chemicals thereby giving insight on the reasons behind the therapeutic properties of *P. amarus* when used in herbal medicine.

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