Characterization of Acetone-Water Extract, Fractionated with Ethyl Acetate Isolated from *Phyllanthus amarus* (Schum and Thonn).

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ABSTRACT

Preliminary phytochemical screening of *Phyllanthus amarus* (Schum and Thonn) showed the presence of saponins, cardiac glycosides, flavonoids, phenols, and tannins and the absence of anthraquinone. The aim of this study was to identify and characterize the bioactive principle from water soluble portion of *Phyllanthus amarus* leaf extract obtained from acetone and water fractionated with ethyl acetate. Spectroscopic studies (IR, UV, MS) showed the presence of unsaturated fatty acid identified as 9,12,15-octadecatrienoic acid. This compound may contribute to the medicinal properties of this plant.

(Keywords: *Phyllanthus amarus*, isolation, 9, 12, 15 – octadecatrienoic acid, Abraka, Delta State, Nigeria)

INTRODUCTION

The plant *Phyllanthus amarus* belongs to the family Euphorbiaceae. There are more than 300 genera and 500 species in this family over the globe.

Phyllantus amarus Schum and Thonn also called carry me seed, is of great importance in ethnomedine. The plant is important in Indian Ayurvedic system of medicine. Application of this plant include problems of genitourinary system, spleen, liver, kidney, diarrhea and dysentery (Patal *et al.,* 2011). The plant possess secondary metabolites like alkaloids, flavonoids, triterpenes, sterols, polyphenols, and volatile oil (Shuckla *et al.,* 2011).

Natural products play a prominent role in the development of novel drug leads for treatment and prevention of diseases (Newman *et al.*, 2003, Yesh Raj *et al.,* 2012). This study was undertaken to identify compounds which may have therapeutic potentials against micro-organisms.

MATERIALS AND METHODS

Phyllanthus amarus plants were harvested from the wild in the Abraka area of Delta State and were sent to the Department of Botany, Delta State University for identification. The plant leaves were washed in distilled water and dried at ambient temperature. The dried plant material was milled into a powder.

A total of 750g of *Phyllanthus amarus* leaves were percolated three times in an acetone water mix (1:1) (2.5L) for 48h: at room temperature. The combined solvent was removed by simple distillation to yield a brown-colored solid (extract 1).

The water-soluble portion was further extracted successively with ethyl acetate (1.25L) (extract 2), and n- butanol (1.25L) (extract 3). Each of the extracts (1-3) were separately dissolved in n-hexane and the undissolved portions were removed by filtration.

Separation of Ethyl Acetate Soluble Portion

The ethyl acetate soluble extract was reduced to solid by reduced pressure and the residue was chromatographed over silica gel (60-120 mesh) using different solvent systems. The elusion of the column was done using petroleum ether, petroleum ether-ethyl acetate in the ratio of 4:1, 3:1, 2:1. Fractions having similar peaks were bulked together based on their TLC profile. Further separation on TLC was not carried out on extracts 1 and 3.

GCMS Analysis

The GCMS analysis was carried out on fractions using GC-MS- Q 2010 plus Shimazu (Japan) with an injector temperature of 250^{o}C and carrier gas pressure of 108kpa.

Spectroscopic Analysis – FT-IR

Fourier transform infrared spectroscopy was used to analyze the compound obtained from *Phyllanthus amarus*. The spectrum was focused on IR range between 500-4500 CM^{-1} by the KBr pellet technique.

UV-Vis Spectroscopic Analysis

The compound obtained from *Phyllanthus amarus* was also analyzed using UV-Visible Spectrometer (UV-2500, Japan), at a range of 200 nm to 800 nm, to detect the characteristic wavelength.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of *Phyllanthus amarus* (Schum and Thonn), showed the presence of saponins, cardiac glycosides, flavonoids, phenols, and tannins and the absence of anthraquinone (Table 1). The IR for this compound showed absorption at CM^{-1} 3413, 2935, 2088, 1640, 1456, 1247, 1112, 490 and the suggested assignment were OH, CH_2, CH_3, O=C-O ester, CH_2, CH_3 or OH bending, C- stretching, respectively (Table 2).

The UV spectrum of the compound shows absorption bands of 409nm (absorbance 3.520) or 343nm (absorbance 1.706). The GC of the compound gave a peak with retention time of 21.217 minutes. The mass spectra library data suggest the compound 9, 12, 15-octa decatrienoic acid which has a molecular peak of 278m/z with a base peak of 42m/z.

The compound has the following fragmentation pattern m/z 278, 222, 149, 135, 121, 108, 79, 67, 65, 41, 27. The compound, 9,12,15-octadecantrienoic acid (Linolenic acid) as identified by GC-MS and IR is a fatty acid which possess different characteristics such as anti-inflammatory, cancer prevention, hepatoprotective, nematicide, anticoronary, antieczemic, antiacne, 5-alpha reductase inhibitor (Sermakkani and Thangoypandioan, 2012) 9, 12, 15, octadecantrienoic and other compounds like 1-pentadecane, 1, Nonadecane, tetradecanoic acid, Hexadecanoic acid, 1 methyl ester was isolated from chloroform extract of leaf of *P. amarus* (Igwe and okwunodulu, 2014) and from other plant sources (Vinoth et al., 2011). 9, 12 – octadecatrienoic acid is an unsaturated fatty acid, unsaturated fatty acids are essential for normal growth of cell within the body especially nerves and blood vessels which enhance and keep tissues including skin supple and young through their lubricating characteristics (Igwe and Okwu 2013, Igwe and Okwunodulu 2014).

Unsaturated fatty acids regulate and transport oxygen, maintains the integrity of cell structure and lowers cholesterol levels in the blood. Energy production and transportation are other function of unsaturated fatty acid, (Igwe and Okunodulu 2014).

General and Physical Property

- Insoluble in water,
- liquid (not solid),
- and colorless

Table 1: Phytochemical Screening *Phyllanthus amarus* Schum and Thonn.

Compound	Presence in Leaf
Alkaloids	+
Anthraquinone	-
Flavonoids	+
Tannins	+
Reducing Sugar	+
Phenols	+
Steroids	+
Terpenoid	+
Cardiac glycoside	+
Phlobatanin	+
Saponins	+

Key + (Present)

- (Absent)

Table 2: FTIR Spectroscopic Data of *Phyllanthus amarus* Leaf.

1	O-H	3413.15
2	CH_2, CH_3	2935.76
3	O=C-O ester	2088.01
4	O=C-O ester	1640.51
5	CH_2, CH_3 (M) or O-H bonding	1456. 30
6	O-H bending	1247.99
7	C-O stretching	1112.00
8	C-O stretching	1027.13
9	Finger print region	490. 90

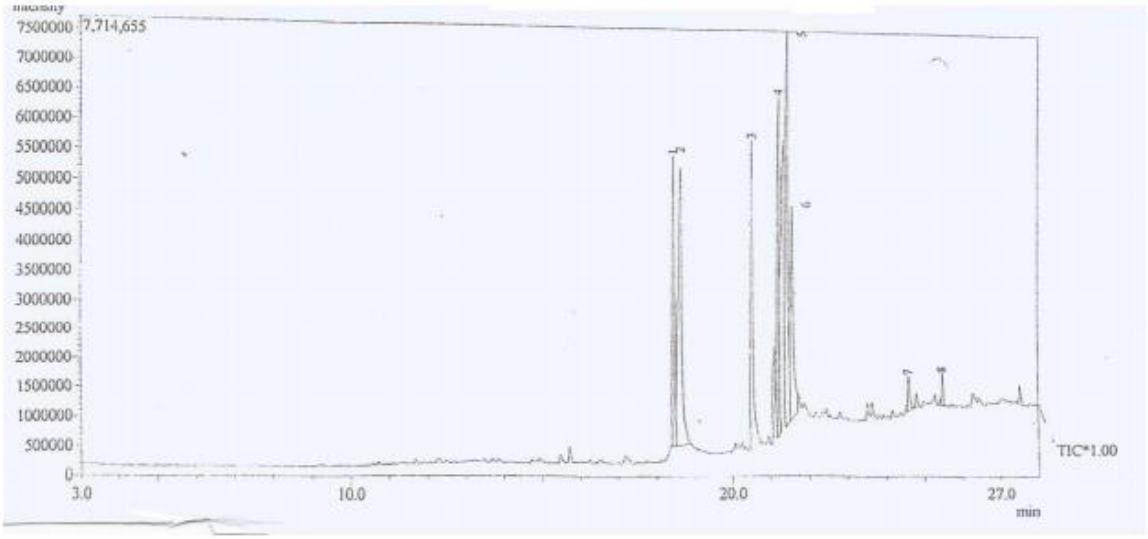

Figure 1: GC-MS Spectra of Acetone-Water Extract Fractionated with Ethyl Acetate Isolated from *P. amarus*

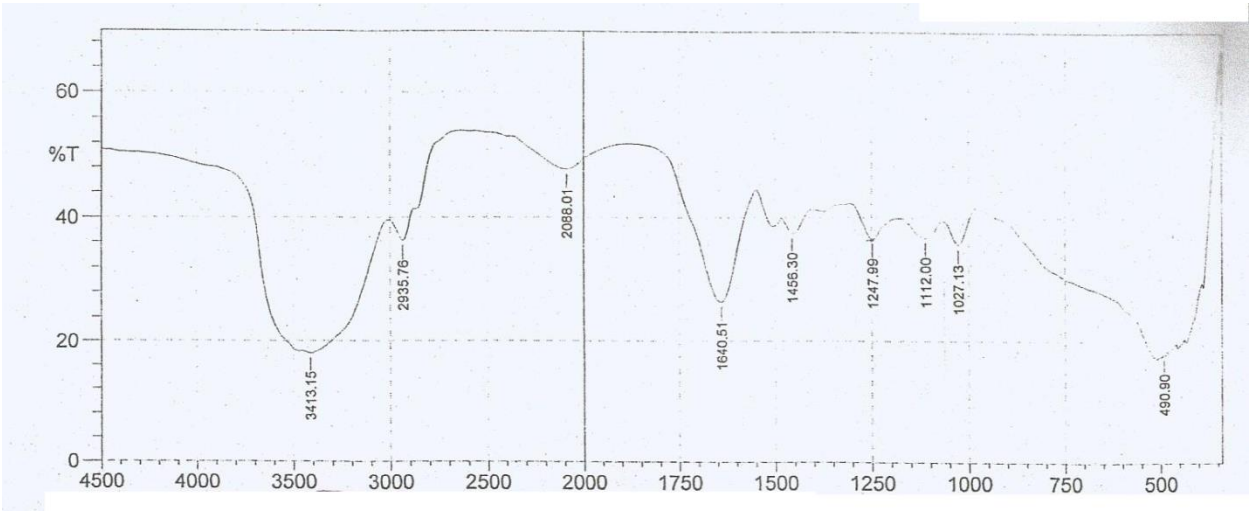

Figure 2: FT-IR Spectra of Acetone-Water Extract Fractionated with Ethyl Acetate Isolated from *P. amarus*

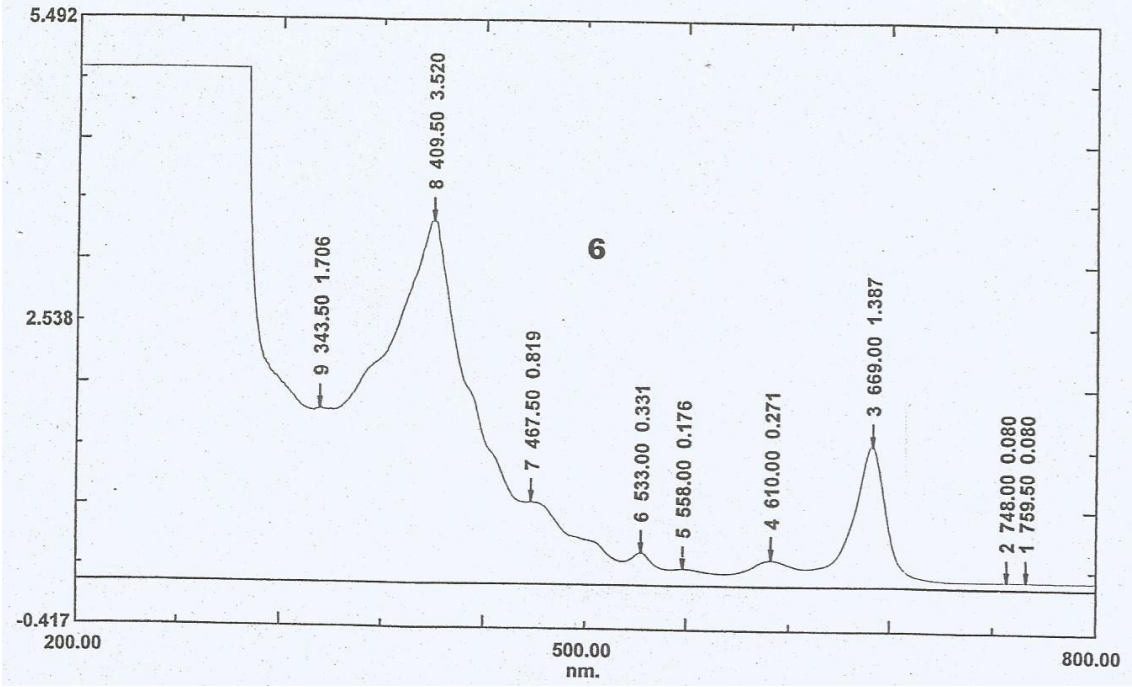

Figure 3: UV-Vis Spectra of Acetone-Water Extract, Fractionated with Ethyl Acetate Isolated from *P. amarus*.

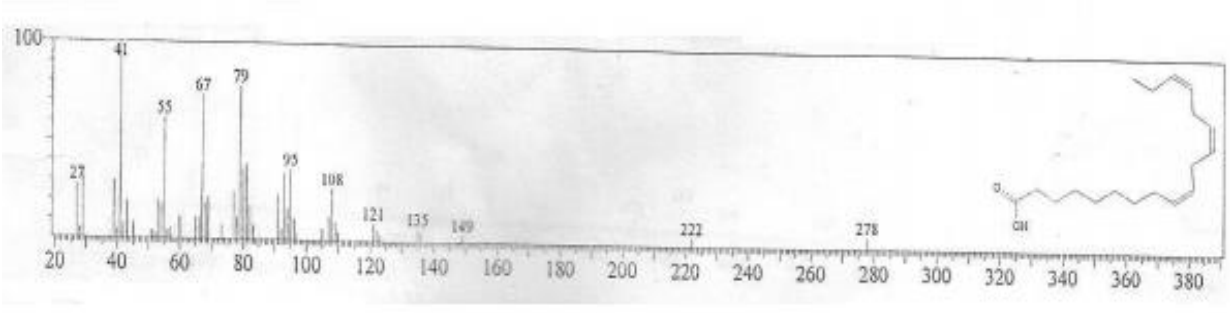

Figure 4: 9,12-15-octadecatrienoic acid

CONCLUSION

From the above study, 9,12,15 octadecatrienoic acid (linolenic acid) was isolated from acetone water extract of *Phyllanthus amarus* fractionated with ethyl acetate. Linolenic acid may contribute to the medicinal properties of *Phyllanthus amarus*. There is need to determine the antimicrobial and plasmodial activity of linolenic acid. Possible antimicrobial effect of compound shall be carried out.

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