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Phytochemical Characterization Of Vernonia Amygdalina.Del Ethanolic Extract Fraction And Contractile Response On Isolated Uterine Tissue In Female Albino Wistar Rats

K.K..Igwe[1], I..I.Ijeh [2]., P..N. Okafor [3]. S.M Anika [4]

[1]Departmemt of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike, NIGERIA. [1]E-mail: kkigwe191@gmail.com

[2,3]Departmemt of Biochemistry, Michael Okpara University of Agriculture Umudike, NIGERIA .

[2]E-mail:ijehirene@yahoo.com

[3]E-mail:pnokafor@yahoo.com

[4]Departmemt of Veterinary Physiology, Pharmacology and Biochemistry, University of Nigeria, Nsukka . .NIGERIA

[4]E-mail:smanika@yahoo.com

Corresponding Author* kkigwe191@gmail.com

ABSTRACT

The aim of the research is to analyze the phytocomponent in the contractile fraction of Vernonia amygdalina on uterus using GC-MS analysis. In the present research, extract for leaf of Vernonia amygdalina was screened for contractile potential by standard test procedures and this study was further extended by fractionation and analyzing the potent bioactive compounds in the ethanolic extract fraction of Vernonia amygdalina leaves using GC-MS analysis. Using physiograph uterine tissue contractile amplitudes were determined at 0.25 mg/ml, 0.3 mg/ml, 0.7 mg/ml, 1.0mg/ml, 1.25mg/ml and 1.5mg/ml for the different fractions. Fraction F5 had the best contractile response on isolated uterine tissue in the presence of agonist acetylcholine (ACh). F5 was used for characterization by GCMS analysis. In the quantitative phytochemical characterization using various extracts of the plant, it was found that most of the biologically active phytochemicals were present in the ethanolic extract of Vernonia amygdalina leaves. Gas Chromatography-Mass Spectrometry (GC-MS) of F5 revealed the presence of eleven bioactive compounds which includes, 3, 5-bis 1, 1 dimethylethyl (Phenol) ; Tetradecanoic acid ; 1, 2-epoxyhexadecane(Oxirane) ; Methylhexadecanoate (Palmitic acid) ; Hexadecanoic acid (Eicosanoic acid); 9, 12-octadecadienoic acid (Linoleic acid); 3, 7-dimethyldodecan-1-ol (Phytol) ; 6-octadecenoic acid(Oleic acid); octadecanoic acid(Stearic acid); Cholest-5, 3-ol, 5-acetate (Cholestane) and 1,2-Benzenedicarboxylic acid (Di-n-octyl phthalate). Results confirmed the presence of contractile potent compounds in the leaf extract of Vernonia amygdalina

Key Word: Vernonia amygdalina, Phytochemicals, GC-MS, Uterine tissue.

1.INTRODUCTION

Vernonia amygdalina is a shrub or small tree usually branched near the base, 2-10 m high, bark rough with dense black streaks and grows under a range of ecological zone in Africa. It belongs to the family compositae [1]. Vernonia amygdalina has the common name "bitter leaf" and is used mainly in soup making in the tropics. Vernonia amygdalina has long history of use in ethnomedicine as a digestive tonic, appetizer, and febrifuge and also as an anti-parasitic agent [2]. It is also used in obstetrics, gynaecology and in the management of diabetes [2] the composition of the vegetable, Vernonia amygdalina has been shown to effect uterine contraction [3, 4]. It sesquiterpene lactones and stigmastane type steriodal glycoside are believed to be cytotoxic to cancer cells [5]. The bitter taste is due to anti-nutritional factors like saponins, tannins and glycosides [6]. It contains 18% protein, 8.5% fibre in a dry matter and a good composition of macroelements and microelements [7]

Phytochemical screening is of importance in identifying new source of therapeutically and industrially valuable compound having medical significance from natural product. The present research was carried out to determine the possible phytochemical components from crude fraction of Vernonia amygdalina responsible for contraction using GC-MS analysis. Recently interest for characterization of organic compounds from plants has increased therefore it is important to screen and isolate the bioactive compounds, evaluate the bioactive potential and characterize them by GC-MS analysis.

2.MATERIAL AND METHODS

2.1 Plant materials

The fresh leaves of Vernonia amygdalina was harvested from University Farm, Michael Okpara University of Agriculture, Umudike, Nigeria. The plant sample was identified by Prof M. C. Dike at the taxonomy section, Forestry Department of the University. Voucher specimen was deposited at the Department of Vet Pharmacology and Biochemistry Herbarium of the University.

2.2 Preparation of Plant Extract

The plant materials (leave) of Vernonia amygdalina was collected and was air dried on the laboratory bench for 10 days. The dried leaves were milled and ground into coarse powder using Wiley machine (model 5 USA) at the National Root Crop Research Institute, Umudike. The coarse powder plant materials was dried and stored in air tight bottle for chemical analysis. The powdered plant sample, 300 g was soaked in 2000 ml of ethanol for 24 hours, thereafter it was filtered using Whatmann No 1 filter paper of 185 mm size. The ethanolic extract was concentrated using rotary evaporator. From the 300 g powdered leaves 25 g crude extract was obtained which represented 8.33% yield. The extract was then freeze dried in a lyophilizer (Vacuubrad, GM BH Germany)

2.3 Solvent Fractionation

For the in vitro test, 12 g of the crude extract was fractionated by means of column chromatography by using silica gel of size 0.05 - 0.25 (50 -200 mesh size) as stationary phase while a gradient

solvent system comprising of petroleum ether, chloroform and methanol was used as the mobile phase. Fractions were collected and examined by thin layer chromatography (TLC). Fractions having similar compounds were pooled together using their Resolution front (R_f) values.

From the 12 g of crude extract, 1.4 g, 2.2 g, 2,4 g, 1.8 g, 3.5 g, 0.7 g, of F1, F2, F3, F4, F5 and F6 respectively were obtained.

2.4 Screening extract fraction for bioactivity

The different fractions were subjected to in vitro contractile screening to provide preliminary observations necessary to elect the plant extract with the best contractile potential for further investigation.

Fractions F1, F2, F3, F4, F5 and F6, were subjected to in vitro contractile experiment using uterine strip on physiograph to find fraction with the best contractile response in the presence of agonist, acetylcholine (ACh). The amplitude of contractile of each fraction was recorded. The crude extract fraction F5 of Vernonia amygdalina was screened as the fraction with the best contractile activity.

2.5 Gas Chromatography- Mass Spectrometry (GC-MS) analysis of contractile (F5) fraction of Vernonia amygdalina.The characterization of the Phytochemical in the F5 fraction of Vernonia amygdalina (fraction with the best contractile potential) was done at the National Research Institute for Chemical Technology (NARICT) Federal Ministry of Science and Technology, Zaria using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5).The initial column temperature was 80^{o}C for 1min, and then increased linearly at 70^{o}C min^{-1} to 220^{o}C, held for 3 min followed by linear increased temperature 10^{o}C min^{-1} to 290^{o}C for 10 min. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

2.6 Identification of Phytocomponents

The identification of the components in the contractile fraction (F5) of Vernonia amygdalina was achieved by the comparing their retention indices and mass spectra fragmentation pattern with those stored in the GC-MS computer Library in National Research Institute for Chemical Technology (NARICT) and also with published literature. NIST 08. LIB [8]; WILEY 8. LIB [9]; PESTE 1-3. LIB and FA-ME. LIB. Library sources were matching the identified components from the plant material. The name, molecular weight and structure of compounds in the

contractile fraction of Vernonia amygdalina were ascertained.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.11 Result of in vitro contraction of rat uterine tissues exposed to different fractions of Vernonia amygdalina

The result of the screening of the different fractions of Vernonia amygdalina F1, F2, F3, F4, F5, and F6 for the peak uterotonic activity revealed that F5 had the highest amplitude of contraction among the other fractions when compared to the control agonist acetylcholine figure-1. At 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml and 1.5 mg/ml the amplitude of contraction was 38 mm, 40 mm, 45 mm, 48 mm, 50 mm and 54 mm respectively as compared to acetylcholine, 42 mm, 41 mm, 46 mm, 50mm, 53 mm and 58 mm. F5 was therefore selected for further studies of their phytochemical components involved in the uterine contraction.

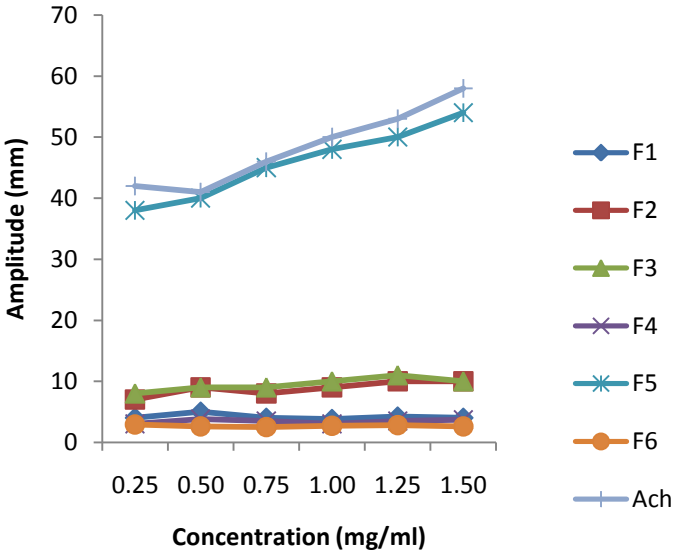


Figure-1:Shows contractile amplitude of different fractions of Vernonia amygdalina on isolated rat uterus at 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml and 1.5 mg/ml compared to the control agonist acetylcholine.

3.12 Result of GC-MS chromatogram and identification of phytocomponents in the F5 fraction of Vernonia amygdalina

GC-MS Chromatogram of F5 fraction of Vernonia amygdalina revealed eleven peaks Figure-2 showing that eleven different phytocompounds were present. The name and molecular weight of the compounds in F5 fraction of Vernonia amygdalina is shown in Table-1. Activities and nature of Phyto-components identified in F5 fraction of Vernonia amygdalina by GC-MS analysis is shown in Table-2. The structures of compounds in F5 fraction of Vernonia amygdalina is shown in Figure-3.

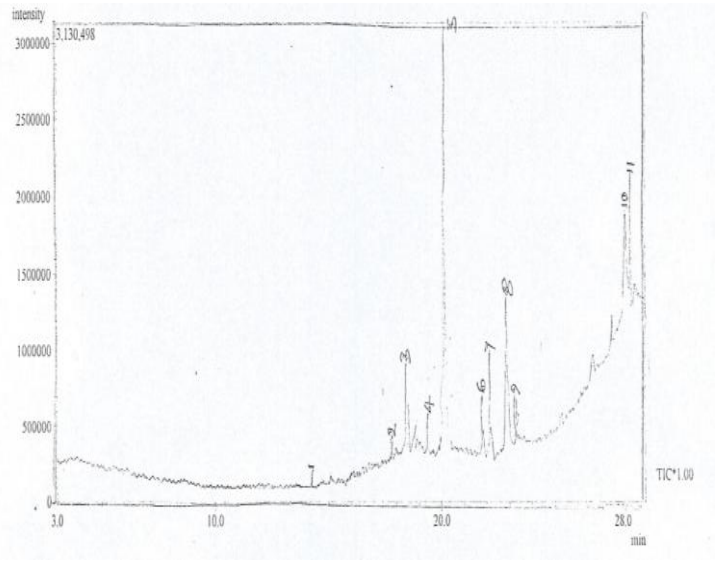
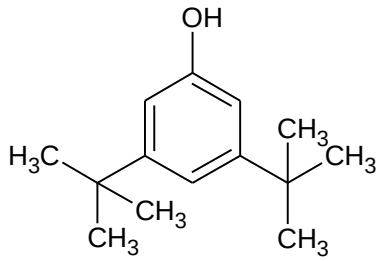


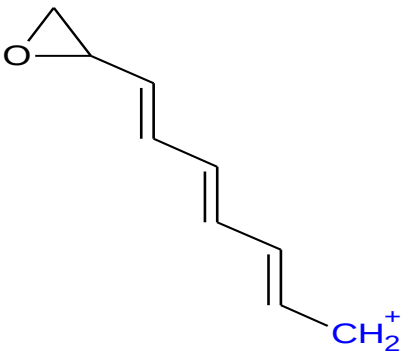
Figure-2. GC-MS chromatogram of the ethanol extract of contractile fraction of Vernonia amygdalina

Table-1. GC-MS Analysis of F5 Fraction of Vernonia amygdalina

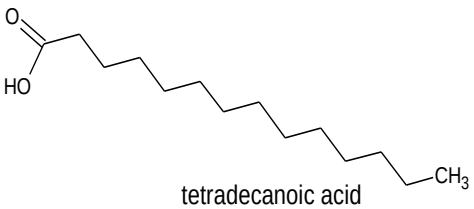
S/N	Retention Time (RT)	Molecular Weight	Name of Compound
1.	14.242	206	3, 5-bis 1, 1 dimethylethyl (Phenol)
2.	17.808	228	Tetradecanoic acid
3.	18.417	240	1, 2-epoxyhexadecane(Oxirane)
4.	19.442	270	Methylhexadecanoate (Palmitic acid)
5.	20.142	242	Hexadecanoic acid (Eicosanoic acid)
6.	21.800	294	9, 12-octadecadienoic acid (Linoleic acid)
7.	22.133	296	3, 7-dimethyldodecan-1-ol (Phytol)
8.	22.850	282	6-octadecenoic acid(Oleic acid)
9.	23.217	284	octadecanoic acid(Stearic acid)
10.	28.117	386	Cholest-5, 3-ol, 5-acetate (Cholestane)
11.	28.350	390	1,2-Benzenedicarboxylic acid (Di-n-octyl phthalate)



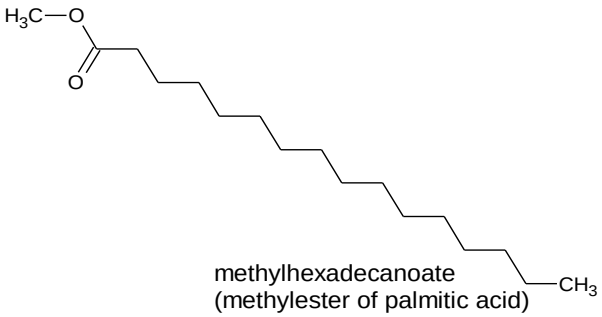
phenol - 3,5 - bis (1,1-dimethylethyl



(oxiran-2-yl)hepta-2,4,6-trien-1-ylium



tetradecanoic acid



methylhexadecanoate
(methylester of palmitic acid)

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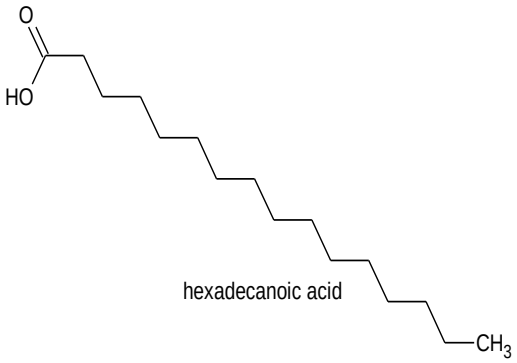
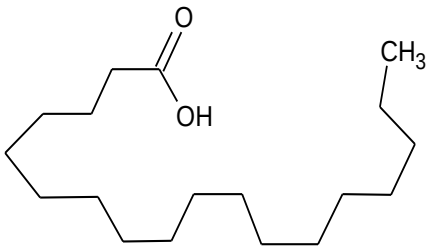
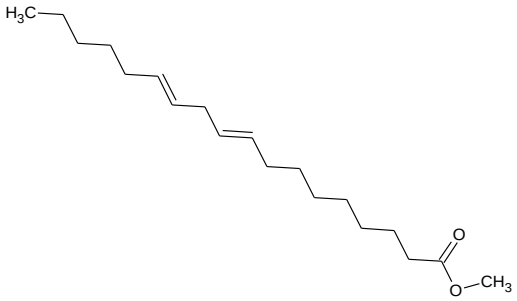


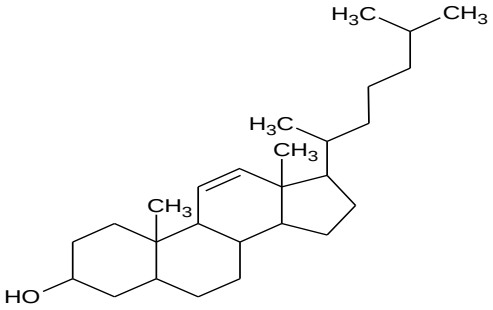
hexadecanoic acid



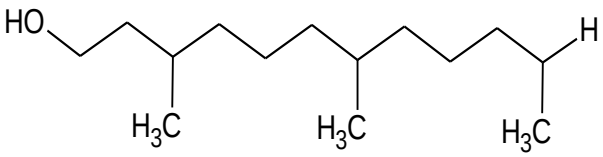
Octadecanoic acid (stearic acid)



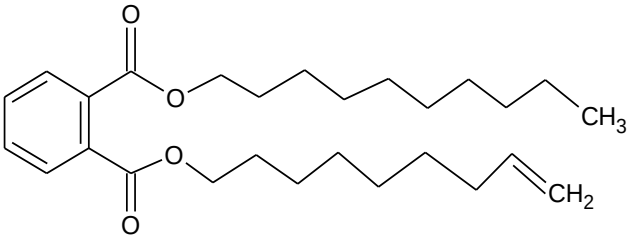
9,12 -octadecanoic acid (linoleric acid)



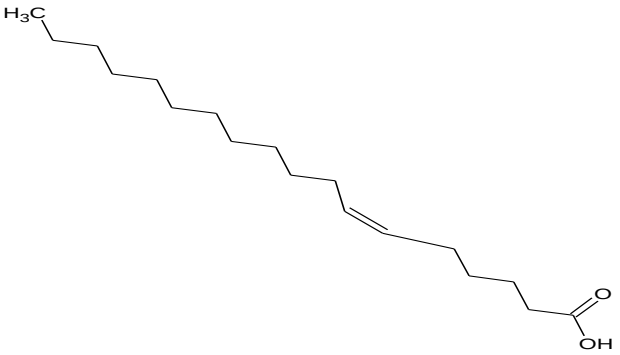
Cholest-5-en-3-ol (cholesterol base)



3,7-dimethyldodecan-1-ol



1,2-Benzenediisooctyl ester



6-octadecenoic acid

Figure-3. Shows the structure of compounds identified in contractile fraction of Vernonia amygdalina by GC-MS analysis

Table -2 Shows Activities of Phyto-components Identified in F5 Fraction of Vernonia amygdalina by GC-MS analysis

RT	Name of Compound	Compound Nature	Activity
14.242	Phenol 3, 5-bis (1, 1 dimethylethyl)	an organic compound in which an –OH group is attached to a carbon atom as part of aromatic carbon ring system	* antiseptic and disinfectant properties * phenolic antioxidant food additives BHA (butylated hydroxyl anisole) and BHT (butylated hydroxyl tuolene) * flavorant and antibacterial properties * ingredient in mouth wash formulation * Phenol ring bind ER_α and ER_β receptor sites
17.808	Tetra decanoic acid	Oxygenated terpenoid	
18.417	Oxirane (1,2-epoxyhexadecane)		* well known as preservative in food, drugs and cosmetics; * antifungal against dermatophytes; anti-tumor, analgesic, antibacterial, anti-inflammatory; anticoagulant properties; reduces liver damage; effective in killing cancer cells and treating rheumatoid arthritis
19.442	Palmitic acid (Hexadecanoic acid)	a saturated fatty acid that is the major fat in meat and dairy products	* Lubricant, Antiandrogenic, Flavor, Hemolytic, Antioxidant, Hypocholestrolemic Nematicide, Pesticide, 5-Alpha reductase inhibitor.
20.142	Pentadecanoic acid and Eicosanoic acid	the saturated fatty acid with a 20-carbon chain	* it is as a minor constituent of peanut oil (1.1%-1.7%) and corn oil (3%) * used for the production of detergents, Photographic materials and lubricants.
21.800	9,12-octadecadienoic acid (Linoleic acid)		
22.133	Phytol	acyclic diterpene alcohol	* can be used as a precursor for the manufacture of synthetic forms of Vitamin E and vitamin K_1. * Antimicrobial, anticancer, anti inflamatory,

			diuretic.
22.850	Oleic acid	fatty acid ester formed (9-octadecenoic acid)	* used as a solvent for pharmaceutical drug by the condensation of preparations involving lipophilic substances oleic acid and ethanol such as steroids. * used as a lubricant and a plasticizer * has been identified as a primer pheromone in Honeybees. * it is used by compounding pharmacies as a vehicle for intramuscular drug delivery, in some cases to prepare the daily doses of progesterone in support of pregnancy.
23.217	Stearic acid (octadecanoic acid)	C_{18} Molecule fatty acid	* vehicle for fat soluble vitamins as – A D E and K
28.117	Cholestane-3,5-diol,5-acetate (3beta,5alpha)	C_{17} molecule lipid which on hydrolysis produces fatty acid and glycerol	* Precursor for various classes of steroid hormones in plants. * Binds estrogen$_{\alpha}$ (ER_{α}) and Estrogen$_{\beta}$ (ER_{β}) receptor sites.
28.350	1,2-Benzenedicarboxylic acid: Acid-Plasticizer	an aromatic dicarboxlic	* Antimicrobial, Antifouling
28.350	Di-n-octyl phthalate: Acid-Plasticizer compound	an aromatic dicarboxlic	* Antimicrobial, Antifouling

Source: Duke's phytochemical and ethno- botanical database

3.2 DISCUSSION

Plants have been an important source of medicine. They are source of many potential drugs mainly on traditional remedies such as herbs used as popular folk medicines 10. It has been shown that in vitro screening methods could provide preliminary observations necessary to elect crude plants extracts with potentially useful properties for further chemical and pharmacological investigation [11]. There is an increasing interest in the phytochemicals compounds which could be relevant to nutrition and their role in health and

decrease [7, 12]. The combination of ideal separation techniques, Gas Chromatography (GC) with the best identification technique, Mass Spectrometry (MS) has made GC-MS an ideal technique for quantitative and qualitative analysis for volatile and semi-volatile compounds [13]. The focus of the present research is to determine the organic compounds in Vernonia amygdalina and to confirm the phytochemicals present in the plant extract that contracted the uterus. Phytochemicals have been found to have a wide range of activity which helps in protection against disease like cancer and other chronic disease [14]. In this research the GC-MS analysis revealed the presence of eleven compounds from the ethanol leaf extract fraction of Vernonia amygdalina. These phytocomponents may have synergistically caused contraction in the uterus.

4.CONCLUSION

The result confirmed the presence of contractile active compounds in the leaf extract of Vernonia amygdalina. These biologically active phytochemicals were present in the ethanol extract of the plant. The presence of various bioactive compounds in F5 fraction of Vernonia amygdalina justifies the use of the plant fraction for uterine contraction during labour.

From the result it is concluded that F5 fraction of Vernonia amygdalina contain various active compounds, which may have synergistically caused the myoepithelial cells of the uterine tissue to contract.

The plant could be recommended for use during labour to assist in contraction of uterus for delivery. It could also be used to evacuate the placenta after delivery.

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