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Phytochemical Screening and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the Fermented Maize Aqueous Extract of *Enantia Chlorantha* Stem Bark

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ABSTRACT

Enantia chlorantha (EC) is sought after by the rural communities in South Western Nigeria and some other African countries for the treatment of many ailments. Despite its widespread use, the phytochemical and the Gas Chromatography-Mass Spectrometry (GC-MS) screening of the fermented maize aqueous extract remain undetermined. This study was carried out with the aim to evaluate the phytochemical and GC-MS analysis of the plant stem bark. The phytochemical screening was carried out using standard methods while the identification and the quantification of the bioactive components were determined by GC-MS single-phase ion mode and the spectra from the detected compounds were matched with known compounds of the National Institute of Standards and Technology (NIST). The qualitative phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids and steroids in the extracts. Thirty-seven (37) bioactive chemical constituents were identified by GC-MS. Out of these, thirty-one (31) were fatty acids and its derivatives. These bioactive

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constituents include Oleic acid (29.98%), n-Hexadecanoic acid (18.59 %), Eicosanoic acid (7.34%), 2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen–1-yl)-2-butenal (6.03%) and 17-(acetyloxy)-, (4. beta.)- 18-Oxokauran-17-yl acetate Kauran-18-al (3.19%) as the predominant. The study revealed that the fermented maize aqueous extract of *E. chlorantha* stem bark have appreciable amount of pharmacologically important phytochemicals and the bioactive chemical constituents present in the extract may also serve as potential drug target for drug discovery and development. Fermented maize water may also be used as solvent of choice apart from water and alcohol.

Keywords: *Enantia chlorantha*; phytochemical screening; fermented maize water; eicosanoic acid.

INTRODUCTION

Enantia chlorantha (EC) is an ornamental tree that may grow up to 30 m high, with dense foliage and spreading crown. The plant extracts (especially aqueous and ethanolic extracts) have been widely used in folk medicine for the treatment of a large number of human ailments especially in rural communities in South Western Nigeria. The stem bark is mostly preferred (even though the roots and the leaves may also be used), and decoctions, tinctures or infusions may be prepared.

In Cameroon, the stem bark extract of *E. chlorantha* is used to treat jaundice and urinary tract infections [1]. This is a typical example of the "doctrine of signatures" commonly found in African Traditional Medicine where the colour of a plant part is linked to its ethnomedicinal use [2]. The yellow to orange colour of the plant extracts is due to the presence of the three major water-soluble alkaloids, i.e. palmitine, jathorrizine and columbamine. The herbal preparations of *E. chlorantha* are usually kept in some households and used often, even without obvious symptoms of malaria, but rather as malaria prophylactics [3].

The knowledge of the phytochemistry of this epoch-making plant species has been greatly utilized in modern medicine and herbal preparations. A number of medicinally useful phytochemicals have been isolated, purified and employed in the prophylaxis and treatment of a myriad of health conditions.

Medicinal plants play an important role in the human health care system [4]. Plants are good sources of biologically active compounds known as phytochemicals. Phytochemicals such as alkaloids, flavonoids, phenolic acids, tannins, terpenoids, lignin, quinones, coumarins, amines, and others found in plants are biologically active and contribute to the antioxidant capacity of the plants in which they are found [5]. In this present study, the qualitative phytochemical screening (Table 1) revealed the presence of alkaloids, cardiac glycosides, flavonoids and steroids. [6] – [9] also reported the presence of these phytochemicals in the stem bark extracts of *E. chlorantha*.

Phytochemicals are secondary plant metabolites found in various parts of plants; they have diverse roles in plants. These include the provision of vigour to plant; attraction of insects for pollination and feeding, defence against predators and provision of colour while some are simply waste products. A whole range of pharmacological activities have been ascribed to alkaloids which include antimalarial, anticancer [10], antibacterial [11], antitussive, antipyretic, hallucinogenic and antihypertensive [12], anti-hyperglycaemic [13], antiarrhythmic, vasodilatory [14] or as a template for drug discovery [15].

Despite *E. chlorantha* widespread use, the phytochemical constituents and the bioactive components of the fermented maize aqueous extract remain undetermined to the best of our knowledge. This study investigated the phytochemical and chemical constituents of the fermented maize aqueous extract of *Enantia chlorantha* stem bark.

MATERIALS AND METHODS

Collection of Plant Materials

The stem bark of *Enantia chlorantha* used in this study was sourced locally from herb seller and it was immediately labeled with the local name (Awopa or Osupupa or Dokitaigbo in Yoruba, Osomolu in Ikale, Kakerim in Boki) with which it was purchased. The material was identified by a Botanist (UBH- E485) in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

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Preparation and Extraction of Plant Materials

The fresh stem bark of *E. chlorantha* were washed under clean tap water to remove contaminants, debris and dust particles and were cut into pieces after which they were air-dried under shade at room temperature. Thereafter, the dried plant materials were pulverized using an electric blender. One thousand grams (1000 g) of the pulverized stem bark were macerated with 5L fermented maize water under constant stirring for 72 hours [16]. The resulting extract was filtered using Whatman No.42 filter paper (125 mm) into clean containers. The filtrate was concentrated using a freeze dryer at the National Centre for Energy and Environment, University of Benin, Benin City and the extract was thereafter stored in sterile containers and kept at 2 – 8 ^{0}C till when needed.

Laboratory Preparation of Fermented Maize Water

Three hundred grams (300 g) of yellow corn variety purchased was sorted and pre-washed severally with clean water to remove debris and dust particles. Thereafter, the washed grains were subjected to 48 hr fermentation under aseptic measures according to the modified method of [17]. The grains were steeped in sterile stainless steel container with cover. At every 12 hr, the steeped water was decanted and the fermenting grains were re-washed with warm water (20 - 25 ^{o}C) and fermented for 48 hr [18]. Thereafter, the grains were drained, ground with hand mill and sieved with muslin cloth. The recovered waste was further subjected to 24 hr fermentation, after which it was milled and sieved. The resulting filtrate was utilized as fermented maize water.

Phytochemical Screening of the Fermented Maize Aqueous Extract of *Echlorantha* Stem Bark

The preliminary phytochemical screening to detect the presence of secondary metabolites such as alkaloids, tannins, steroids, flavonoids, anthraquinones was carried out using standard procedures as described by Harborne [19], [20].

GC-MS Analysis of the Fermented Maize Aqueous Extract of *E. chlorantha* Stem Bark

The analysis of the fermented maize aqueous extract was carried out using Agilent Technologies 7890A coupled with Agilent Technologies 5975CVL MSD, the carrier gas (mobile phase) was helium with linear velocity of 1 ml/min while the stationary phase was the column Agilent technology HP5 MS with length 30m, internal diameter of 0.320 mm and thickness of 0.25 microns. The volume of the sample injected was 1 µL, oven initial temperature was 80 °C to hold for 2 minutes and the final temperature of 240 ^{0}C to hold for 10 minutes. The data was processed with Shimadzu's LabSolutions software V4.3. Compounds identified were compared with the compounds in the National Institute of Standard and Technology database. The retention time, name, molecular weight, molecular formula, and percent peak area were determined for each compound. The identification of the components in the extract was based on the comparison of their mass spectra and the retention time with the literature data and by computer matching with the NIST and WILEY Mass Spectra libraries as well as by comparison of the fragmentation pattern of the mass spectra data with those that are reported in the literature.

RESULTS AND DISCUSSION

The results obtained from the phytochemical composition of the fermented maize aqueous extracts of *E. chlorantha* stem bark were presented in Table 1. The extract contained alkaloids, cardiac glycosides, flavonoids and steroids while phenols, reducing sugars, saponins, tannins and anthraquinone were not detected.

Table 1: Phytochemical composition of the fermented maize aqueous extracts of *E. chlorantha* stem bark.

Phytochemicals	**Plant Extract**
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+

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Phenols	-
Reducing sugars	-
Saponins	-
Tannins	-
Steroids	+
Anthraquinone	-

+ = detected; - = not detected.

The results of the GC – MS screening of the fermented maize aqueous extract of *E. chlorantha* stem bark identified thirty-seven (37) bioactive chemical constituents. Out of these, thirty-one (31) were fatty acids and its derivatives. These bioactive compounds have been reported to possess various biological and pharmaceutical activities (Table 2).

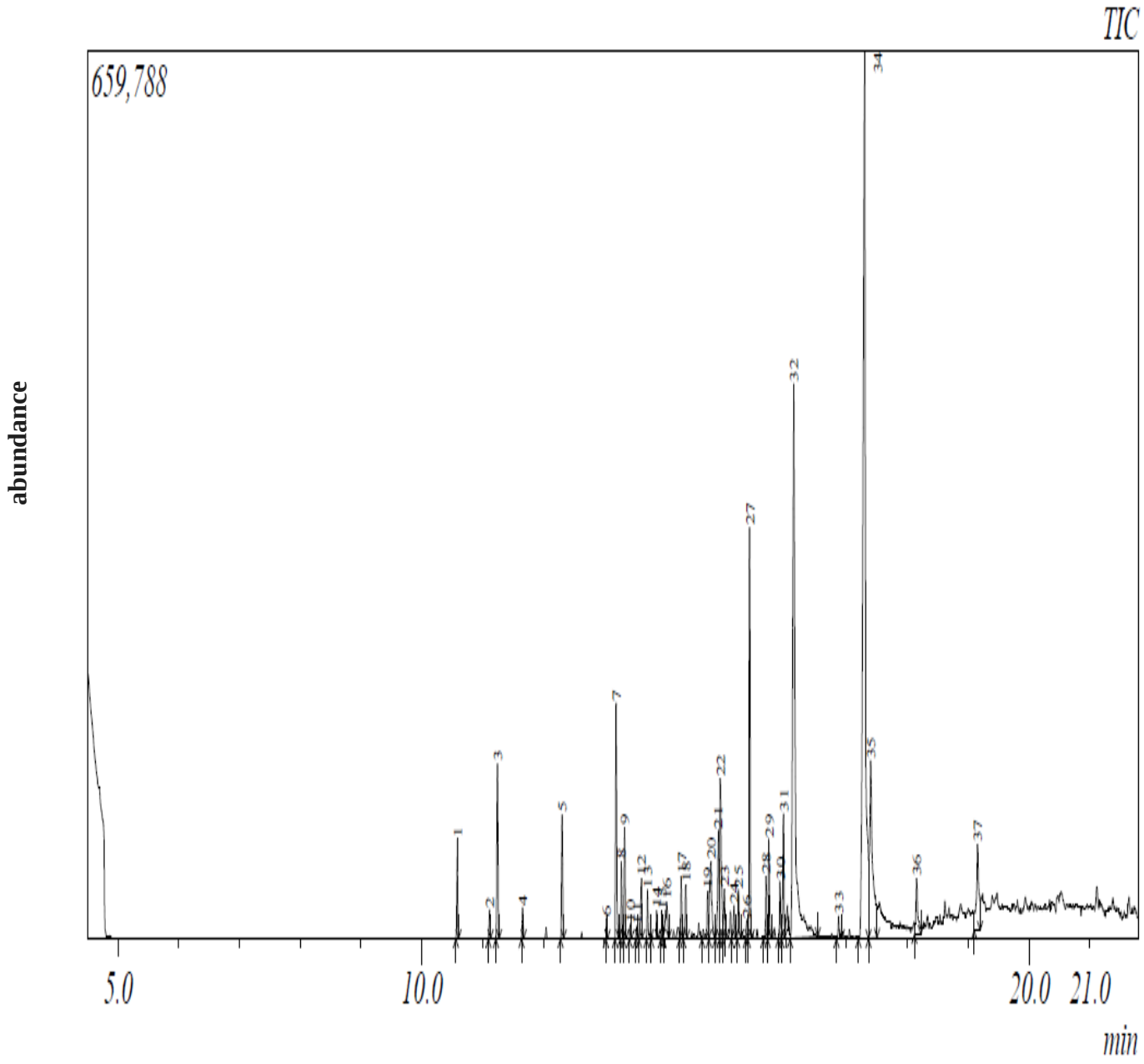

Fig. 1. GC chromatogram of the fermented maize aqueous extract of *E. chlorantha* stem bark.

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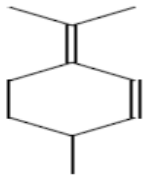
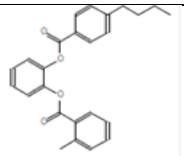
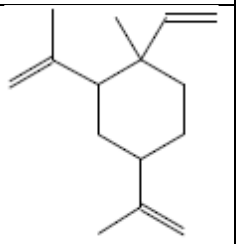
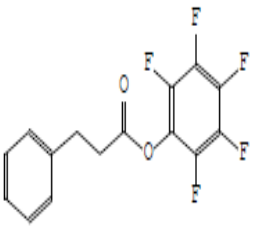
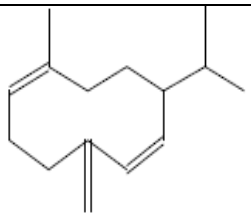
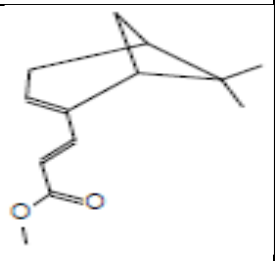
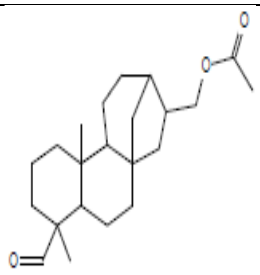
Table 2. Compounds isolated from the fermented maize aqueous extract of *E. chlorantha* stem bark.

RT	Area (%)	Name of Compound	MW	MF	Structures
10.584	1.43	3-methyl-6-(1-methylethylidene) Cyclohexene	136.23	$C_{10}H_{16}$	
11.117	0.35	(4-butylbenzoyl)-o'-(2-methylbenzoyl) 1,2-Benzenediol	388.50	$C_{25}H_{24}O_4$	
11.249	2.52	1-ethenyl-1-methyl-2,4-bis (1-methylethenyl) Cyclohexane	204.35	$C_{15}H_{24}$	
11.659	0.38	pentafluoro phenyl 3-Phenylpropionic acid	316.05	$C_{15}H_9F_5O_2$	
12.308	1.65	1-methyl-5-methylene-8-(1-methylethyl) 1,6-Cyclodecadiene	204.35	$C_{15}H_{24}$	
13.042	0.16	3-(6,6 dimethylbicyclo [3.1.1] hept-2-en-2-yl)-, methyl ester 2-Propenoic acid	206.28	$C_{13}H_{18}O_2$	
13.201	3.19	17-(acetyloxy)-, (4. beta.)- 18-Oxokauran-17-yl acetate Kauran-18-al	346.51	$C_{22}H_{34}O_3$	

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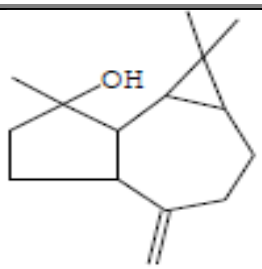
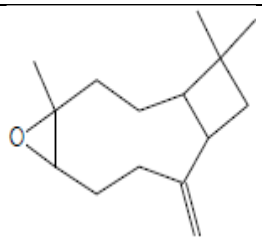
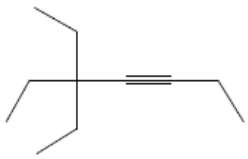
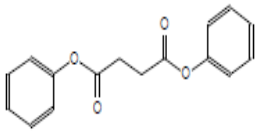
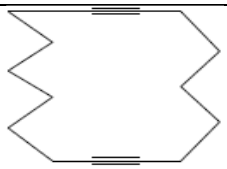
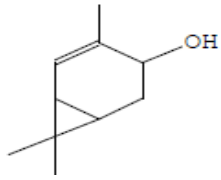
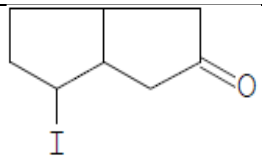
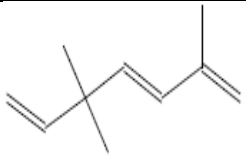
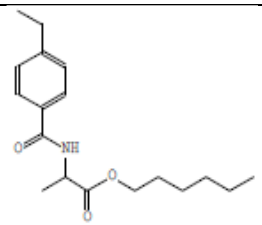
13.284	0.92	decahydro-1,1,7-trimethyl-4-methylene 1H-Cycloprop[e]azulen-7-ol	220.35	$C_{15}H_{24}O$	
13.343	1.45	Caryophyllene oxide	220.19	$C_{15}H_{24}O$	
13.432	0.21	5,5-diethyl-3-heptyne	152.28	$C_{11}H_{20}$	
13.541	0.14	diphenyl ester Succinic acid	270.28	$C_{16}H_{14}O_4$	
13.613	1.02	1,8-Cyclopentadecadiyne	202.34	$C_{15}H_{22}$	
13.717	0.59	trans-2-Caren-4-ol (essential oil)	152.24	$C_{10}H_{16}O$	
13.869	0.33	hexahydro-4-iodo-4-Iodohexahydro-2(*1 H*)-pentalenone	250.08	$C_8H_{11}IO$	
13.952	0.33	2,5,5-trimethyl- 1,3,6-Heptatriene	136.23	$C_{10}H_{16}$	
14.035	1.04	N-(4-methylbenzoyl)-, hexyl ester D-Alanine	305.41	$C_{18}H_{27}NO_3$	

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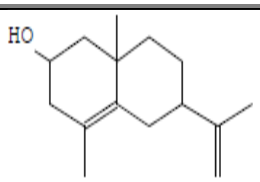
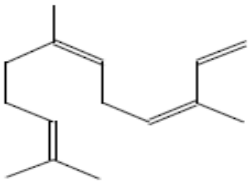
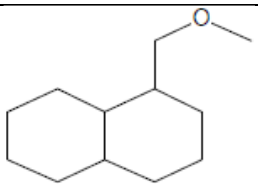
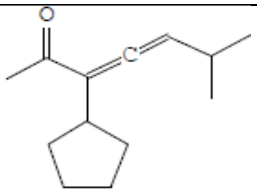
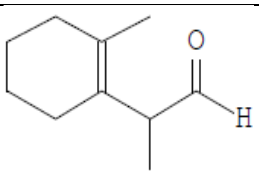
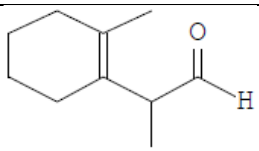
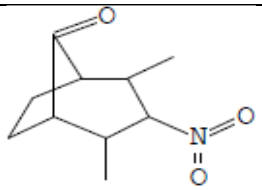
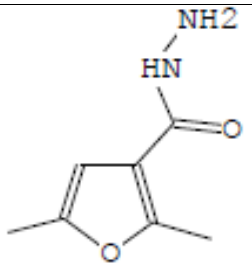
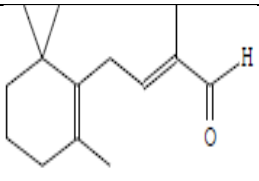
14.276	0.80	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	220.40	$C_{15}H_{24}O$	
14.347	0.80	(Z, Z)-. alpha. –Farnesene	204.35	$C_{15}H_{24}$	
14.706	0.85	1-methoxymethyl-1-(Methoxymethyl)decahydronaphthalene Decalin	182.16	$C_{12}H_{22}O$	
14.882	2.00	3-cyclopentyl-6-methyl 3,4-Heptadien-2-one	192.29	$C_{13}H_{20}O$	
14.983	0.77	1-Cyclohexene-1-acetaldehyde, alpha.,2-dimethyl- 2-(2-Methyl-1-cyclohexen-1-yl) propanal	152.24	$C_{10}H_{16}O$	
15.141	0.55	1-Cyclohexene-1-acetaldehyde, alpha.,2-dimethyl- 2-(2-Methyl-1-cyclohexen-1-yl) propanal	152.24	$C_{10}H_{16}O$	
15.211	0.72	2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one	197.23	$C_{10}H_{15}NO_3$	
15.351	0.30	3-Furancarboxylic acid, 2,5-dimethyl-, hydrazide	154.17	$C_7H_{10}N_2O_2$	
15.401	6.03	2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl) -(2E)-2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal	206.32	$C_{14}H_{22}O$	

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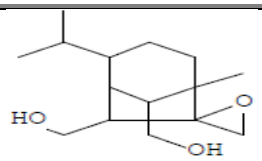
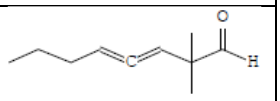
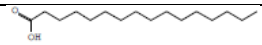
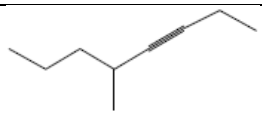
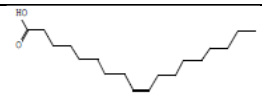
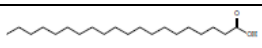
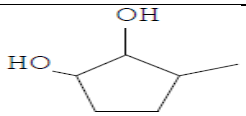
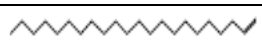
15.719	1.55	Spiro-6-(bicyclo [3.2.1] octane)-2'-(oxirane)	254.37	$C_{15}H_{26}O_3$	
15.902	0.85	2,2-Dimethylocta-3,4-dienal	152.24	$C_{10}H_{16}O$	
16.125	18.59	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	
16.867	0.39	5-methyl-3-octyne	124.22	C_9H_{16}	
17.288	29.98	Oleic Acid	282.50	$C_{18}H_{34}O_2$	
17.393	7.34	Eicosanoic acid	312.53	$C_{20}H_{40}O_2$	
18.152	1.54	3-methyl-3-1,2-Cyclopentanediol	116.16	$C_6H_{12}O_2$	
19.153	2.69	1-Docosene	308.58	$C_{22}H_{44}$	

RT = retention time; MW = molecular weight; MF = molecular formula; BHT = butylated hydroxytoluene; SDS-PAGE = Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis.

The presence of alkaloids in the plant extracts as shown in Table 1 is in agreement with the previous study carried out by [21] on phytochemical analysis of *E. chlorantha* stem bark. The presence of phytochemicals in medicinal plants is responsible for their activities in the treatment of diseases [22]. The high content of alkaloids as observed in this present study contributed to the bitter taste of the extracts because alkaloids are very alkalic.

Cardiac glycosides are majorly used in the treatment of cardiac arrhythmia and congestive heart failure, whereby they inhibit Na^+/K^+-ATPase pump that usually causes positive ionotropic effects and electrophysiological changes [23], [24] while the presence of flavonoids in the stem bark might be responsible for the use of this plant for the treatment of malaria and cough.

Gas chromatography-mass spectrometry (GC–MS) analysis of the fermented maize aqueous extract of *E. chlorantha* stem bark revealed the presence of thirty-seven (37) bioactive compounds. The relative abundant bioactive compounds in the fermented maize aqueous extract of *E. chlorantha* was in the order Oleic acid (29.98%) > n-Hexadecanoic acid (18.59 %) > Eicosanoic acid (7.34%) > 2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen–1-yl)-2-butenal (6.03%) > 17-(acetyloxy)-, (4. beta.)- 18-Oxokauran-17-yl acetate Kauran-18-al (3.19%).

From the literature, these bioactive compounds play crucial roles in homeostasis and the general metabolism of the human body. Oleic acid was reported to have anaemiagenic, anti-androgenic, anti-inflammatory, choleretic, anti-cancer, dermatitigenic and antibacterial properties and prevent insect pests [25], [26]. n-Hexadecanoic acid has anti-inflammatory, antioxidant, anti-androgenic, hypocholesterolaemic properties, acts as an haemolytic inhibitor [27] and potent mosquito larvicide [28] while Eicosanoids are potent lipid mediators that are involved in many central physiological processes such as parturition, haemostasis and renal function [29].

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CONCLUSION

The results of the phytochemical screening revealed that the fermented maize aqueous extract of *E. chlorantha* stem bark have appreciable amount of pharmacologically important phytochemicals and the bioactive chemical constituents present in the extract may also serve as potential drug target for drug discovery and development. Fermented maize water may also be used as solvent of choice apart from water and alcohol.

Further studies should be carried out at molecular level to isolate and characterize the pure compounds responsible for all these activities.

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