PHYTOCHEMICAL STUDIES AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)ANALYSIS OF THE CARBONATED DRINK EXTRACT OF *Enantia chlorantha*STEM BARK.

ABSTRACT

Enantia chlorantha (EC) is a dense forest tree found in the Eastern and Southern forests of Cameroon, Southern part of Nigeria, Gabon, Guinea, Ivory Coast, Liberia, Angola (Cabinda) and DR Congo (Province Bas-Congo) and it is mostly used for the treatment of malaria and typhoid fever. Despite its widespread use, the phytochemical and the Gas Chromatography-Mass Spectrometry (GC-MS) analysis remains largely undetermined. This study was carried out with the aim to evaluate the phytochemical composition of EC. The proximate and phytochemical tests were 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 proximate analysis reveals that the plant is richest in crude fibre (47.30 ± 0.54% by dry mass), followed by total carbohydrate (28.13 ± 0.43%), crude protein (9.67 ± 0.32%), moisture content (8.30 ± 0.54%), ash content (5.00 ± 0.24%) and lastly lipids (1.60 ± 0.04%). The qualitative phytochemical composition reveals the presence of alkaloids, cardiac glycosides, flavonoids, steroids and trace amount of tannins. Forty-seven (47) bioactive chemical constituents and forty (40) fatty acid compounds were identified by GC-MS. These include Oleic acid (17.70 %), n-Hexadecanoic acid (9.31%), 1-Hexyl-2-nitrocyclohexane (3.71%), Kauran-18-al, 17-(acetyloxy)-, (4. beta.)- (3.05%) as the predominant. The study revealed that *Enantia chlorantha* could be a useful source of nutrients and the bioactive chemical constituents may serve as potential drug target needed for drug discovery and development.

Keywords:*Enantia chlorantha*, Proximate, Phytochemical, Oleic acid.

1.INTRODUCTION

Tropical rain forest plants are known to have higher concentrations of natural chemical defences and greater diversity. The list of medicinal plants used for the treatment of malaria are numerous and inexhaustible [1,2], but the one that caught our attention during our ethnobotanical survey is *Enantia chlorantha* (*Annonaceae*) or *Annickia chlorantha*.This plant has been used extensively in traditional medicine practice in the treatment of several ailments of non-related pathophysiology [3].

Enantia chlorantha belongs to the family Annonaceae and is locally known as Awogba, Awopa, Osu pupa (Yoruba), Osomolu (Ikale), Dokitaigbo, Erumeru (South Eastern Nigeria), Kakerim (Boki in Cross River State, Nigeria), Erenba-vbogo Bini (Benin). It is widely distributed along the coasts of West and Central Africa. It is also very common in the forest regions of Nigeria [4].

Enantia chlorantha is particularly sought after by the rural communities in Nigeria for the treatment of many ailments. 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 Nigeria, *E. chlorantha* preparations can be made in the form of a drink, called 'agbo', or in the form of a powder, referred to as 'agunmu' [5].

Despite its widespread use, the phytochemical profile and the bioactive components remain largely undetermined. This study investigated the nutritional and elemental compositions of the carbonated drink extract of *Enantia chlorantha* stem bark.

2.MATERIALS &METHODS

2.1 Collection of Plant Materials

The plant samples used in this study was sourced locally from herb seller at Lagos Street, Benin City, Edo State, Nigeria and it was identified by the Botanist Dr. H. A. Akinnibosun of the Department of Plant Biology

and Biotechnology, University of Benin, Benin City for authentication and the voucher specimen of the plant was deposited at the herbarium of the University of Benin, Benin City, with the voucher number UBH- E485.

2.2 Preparation and Extraction of Plant Materials

The fresh stem bark of *Enantia 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 (1000g) of the pulverized stem bark were macerated with 5L of carbonated drink. The resulting extracts were filtered using Whatman No.42 filter paper (125mm) into clean containers and the filtrates were 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 4^0C till when needed.

2.3 Proximate Analysis of *E. chlorantha* Stem Bark

The powdered sample of the stem bark of *Enantia chlorantha* was subjected to the proximate analysis to ascertain its nutritional composition. The analysis was carried out in accordance with the Association of Official Analytical Chemists [6].

2.4 Phytochemical Screening of the Carbonated Drink Extract of *E. chlorantha* Stem Bark

The preliminary phytochemical screening to detect the presence of secondary metabolites such as alkaloids, steroids, flavonoids, tannins, anthraquinones was carried out using standard procedures as described by [7,8].

2.5 GC-MS Analysis of the Carbonated Drink Extract of *E. chlorantha* Stem Bark

The analysis of the carbonated 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 240^0C 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 Standards and Technology database. The retention time, name, molecular weight, molecular formula, and percent peak area were determined for each compound.

2.5.1 Identification of the components

The identification of the components in the extract was based on the comparison of their mass spectra and the retention timewith 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.

3.RESULTS AND DISCUSSION

The results obtained from the proximate composition analysis of *Enantia chlorantha* stem bark are presented in Table 1. The results showed that the plant is richest in crude fibre (47.30 ± 0.54% by dry mass), followed by total carbohydrate (28.13 ± 0.43%), crude protein (9.67 ± 0.32%), moisture (8.30 ± 0.54%), ash content (5.00 ± 0.24%) and lastly lipids (1.60 ± 0.04%). Dry matter (91.77 ± 2.65%) and energy content (165.60 ± 5.53%) were also significantly ($p < 0.05$) high.

Table 1: Proximate composition of *E. chlorantha* stem bark.

Constituents	Percentage composition
Crude fibre (%)	47.30 ± 0.54
Crude protein (%)	9.67 ± 0.32

Crude fat (ether extract) (%)	1.60 ± 0.04
Moisture content (%)	8.30 ± 0.54
Ash content (%)	5.00 ± 0.24
Carbohydrate (Nitrogen free extract) (%)	28.13 ± 0.43
Energy content (kcal)	165.60 ± 5.53
Dry matter (%)	91.77 ± 2.65

Values are represented as Mean ± Standard Error of Mean (SEM) performed in triplicates.

The results of the phytochemical composition of the carbonated drink extracts of *E. chlorantha* stem bark are shown in Table 2. The extract contained alkaloids, cardiac glycosides, flavonoids, steroids and trace amount of tannins while phenols, reducing sugars, saponins and anthraquinone were not detected.

Table 2: Phytochemical composition of the carbonated drink extract of *E. chlorantha* stem bark.

Phytochemicals	**Plant Extract**
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Phenols	-
Reducing sugars	-
Saponins	-
Tannins	+
Steroids	+
Anthraquinone	-

+ = component present; - = component absent.

The results of the GC – MS screening of the carbonated drink extract of *E. chlorantha* stem bark identified forty-seven (47) bioactive chemical constituents present in the plant stem bark. Out of these, forty (40) were fatty acids and its derivatives. These bioactive compounds have been reported to possess various biological and pharmaceutical activities (Table 3). The GC-MS spectrum analysis of the carbonated drink extract is shown in Figure 1.

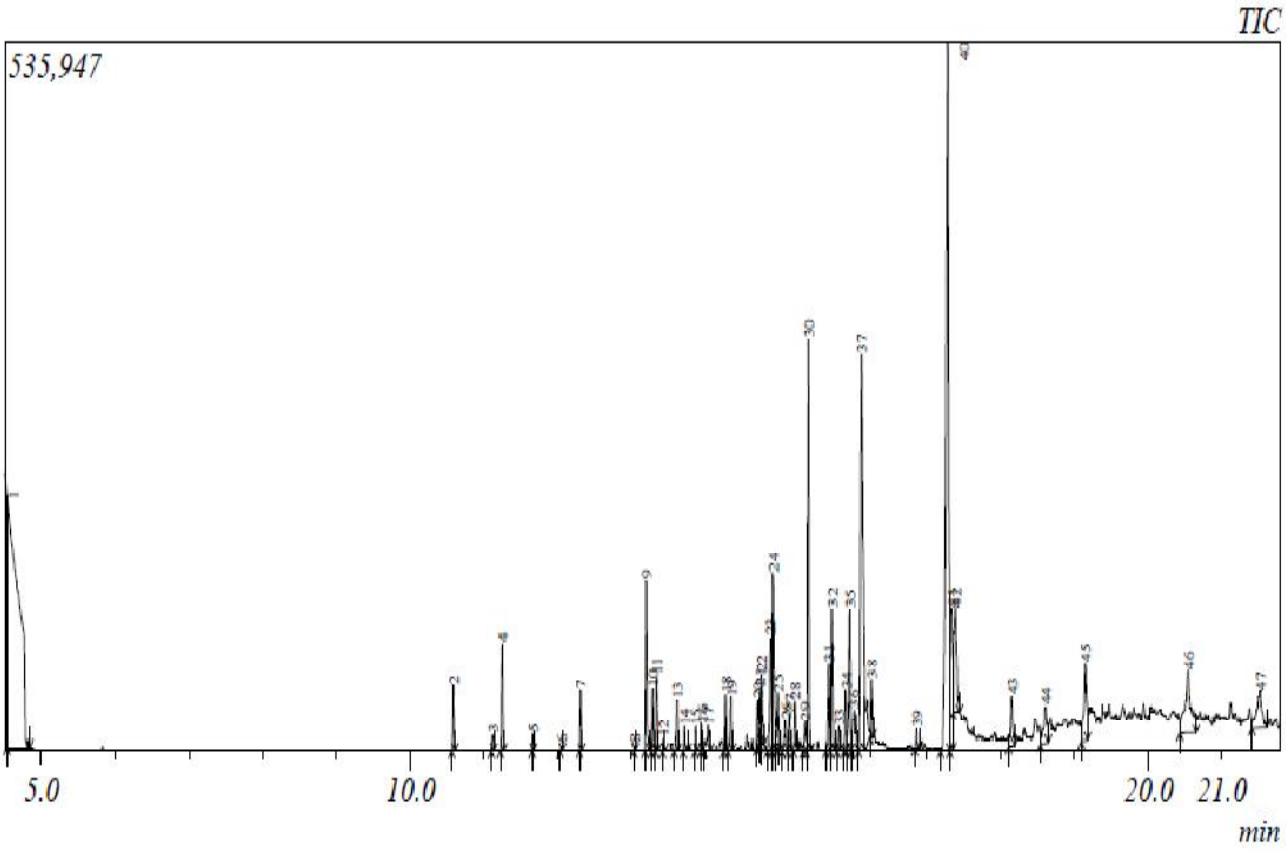
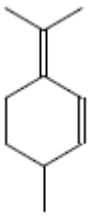
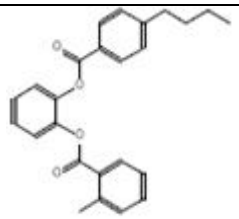
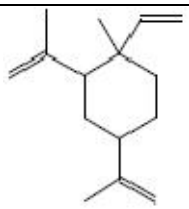
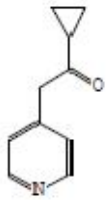
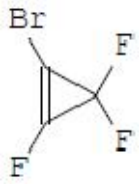
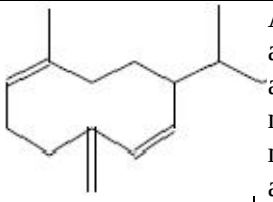
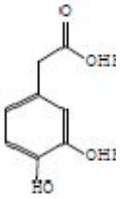
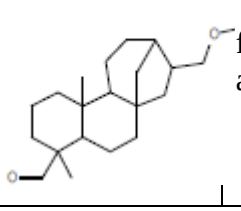
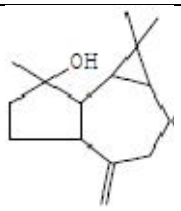
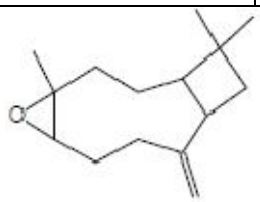
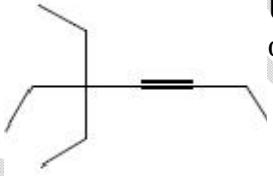
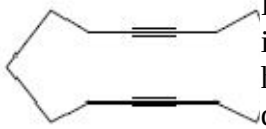
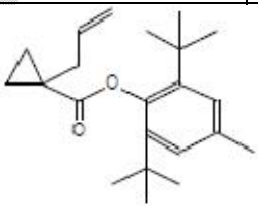
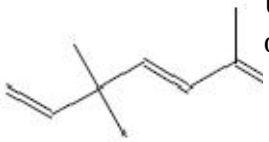
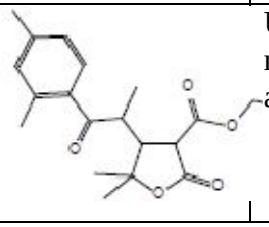
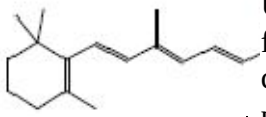


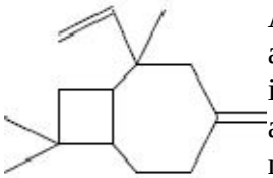
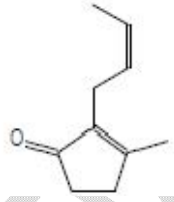
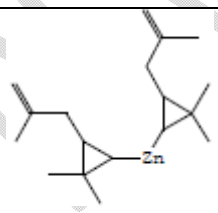
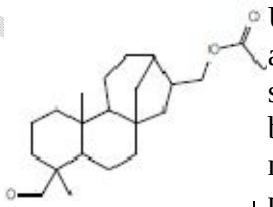
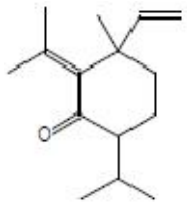
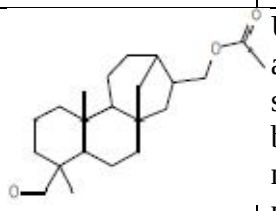
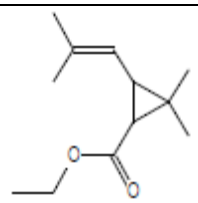
Fig. 1: GC-MS spectrum analysis of CDE of *E. chlorantha* stem bark.

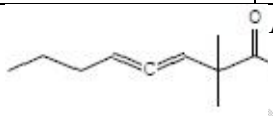
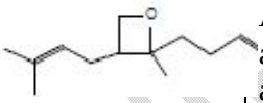
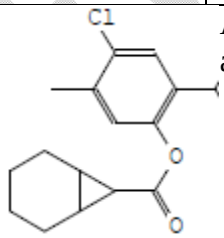
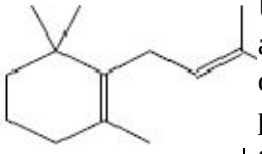
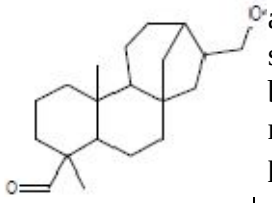
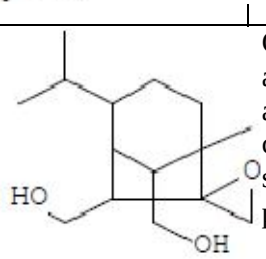
Table 3. Compounds isolated from carbonated drink stem bark extract of *E. chlorantha*

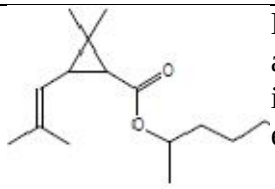
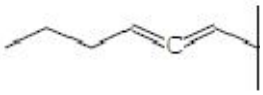
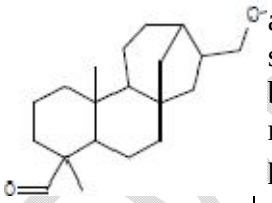
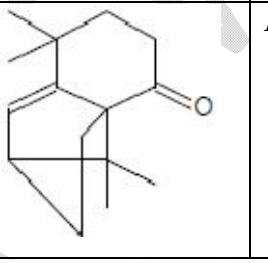
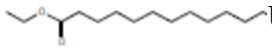
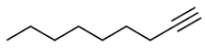
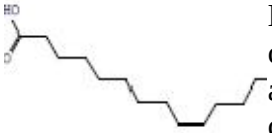
RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological importance
4.542	25.00	n-Hexane	86.18	C_6H_{14}		Used in the extraction of edible oils from seed and vegetable crops (soybeans, peanuts, corn) and used as a solvent for glues, varnishes, and inks.
10.584	0.78	Cyclohexene, 3-methyl-6-(1-methylethylidene)	136.23	$C_{10}H_{16}$		Used for the manufacture of synthetic camphor.

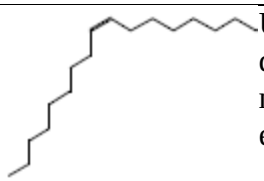
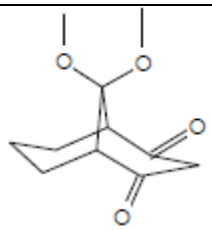
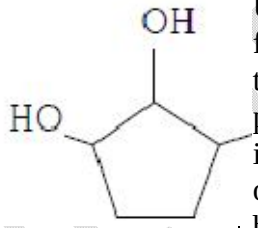
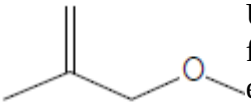
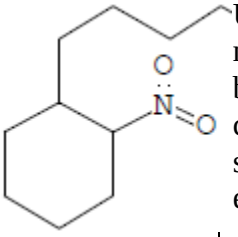
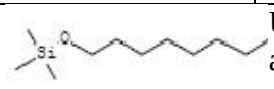
11.117	0.18	1,2-Benzenediol, o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)	388.46	$C_{25}H_{24}O_4$		An important therapeutic target for the treatment of several ailments.
11.248	1.22	Cyclohexane	204.35	$C_{15}H_{24}$		Antibacterial, antimicrobial, anti-inflammatory, food additives, anti-cancer, anti-malaria, anti tumor, anti-migraine, analgesic, sedative activities etc
11.658	0.08	Ethanone, 1-cyclopropyl-2-(4-pyridinyl)	161.20	$C_{10}H_{11}NO$		Used as a precursor in the synthesis of tryptamines like DMT and the drug indoramin along with TNF-alpha modulators etc.
12.050	0.06	Cyclopropene	172.93	C_3BrF_3		Used to label biomolecules in living systems etc.
12.308	0.59	1,6-Cyclodecadiene, 1-methyl-5-methylene	204.35	$C_{15}H_{24}$		Antibacterial, antimicrobial, anti-inflammatory, food additives, anti-cancer, anti-malaria, anti tumor, anti-migraine, analgesic, sedative activities etc
13.042	0.03	Benzene acetic acid, 3,4-dihydroxy	168.15	$C_8H_8O_4$		A fungicide and bactericide, it is used primarily in processed fruit and vegetables etc.
13.200	1.88	Kauran-18-al, 17-(acetyloxy)-, (4. beta.)18-Oxokauran-17-yl acetate	346.25	$C_{22}H_{34}O_3$		It has a role as a fungicide, an antibacterial agent and a plasticizer etc.

13.283	0.57	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1	220.35	$C_{15}H_{24}O$		People also use it as medicine. BHT is used to treat genital herpes and acquired immunodeficiency syndrome (AIDS) etc.
13.342	0.80	Caryophyllene oxide	220.35	$C_{15}H_{24}O$		People also use it as medicine. BHT is used to treat genital herpes and acquired immunodeficiency syndrome (AIDS) etc.
13.431	0.12	3-Heptyne, 5,5-diethyl	152.28	$C_{11}H_{20}$		Used as a reagent in organic synthesis etc.
13.613	0.71	1,8-Cyclotetradecadiyne	188.31	$C_{14}H_{20}$		Precursor to develop laser-induced, high-pressure and high-temperature diamonds etc.
13.717	0.24	1-Allyl-cyclopropanecarboxylic acid	328.50	$C_{22}H_{32}O_2$		Essential for the growth and functional development of the brain in infants etc.
13.868	0.17	-	-	-	-	-
13.951	0.25	1,3,6-Heptatriene, 2,5,5-trimethyl	136.23	$C_{10}H_{16}$		Used for the manufacture of synthetic camphor etc.
14.036	0.53	3-Furancarboxylic acid, 4 - [2-(2,4-dimethyl)]	346.17	$C_{20}H_{26}O_5$		Used in European folk medicine to treat numerous ailments etc.
14.276	0.59	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	258.40	$C_{18}H_{26}O$		Use as a musk fragrance for perfuming soap and cosmetics, biocides, polishes and waxes, and washing and cleaning

						products etc.
14.346	0.73	Bicyclo [5.2.0] nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-2,8,8-Trimethyl-4-methylene-2-vinylbicyclo [5.2.0] nonane	204.35	$C_{15}H_{24}$		Antibacterial, antimicrobial, anti-inflammatory, food additives, anti-cancer, anti-malaria, anti tumor, anti-migraine, analgesic, sedative activities etc.
14.706	0.54	2-Cyclopenten-1-one, 2-(2-butenyl)-3-methyl-2-[(2Z)-2-Butenyl]-3-methyl-2-cyclopenten-1-one	150.22	$C_{10}H_{14}O$		Used to flavor chewing gum and mint candies and to provide aromas in personal-care products, air fresheners, and aromatherapy oils etc.
14.741	0.74	Zinc, bis [2,2-dimethyl-3-(2-methyl-2-propenyl) cyclopropyl]	310.00	$C_{18}H_{30}Zn$		Anti-inflammatory, food additives, anti-cancer etc.
14.762	1.05	Kauran-18-al, 17-(acetyloxy)-, (4. beta.)-18-Oxokauran-17-yl acetate	346.50	$C_{22}H_{34}O_3$		Used as memory enhancer and as a dietary supplement with proven benefit in several neurological and psychiatric conditions etc.
14.882	1.68	Isoshyobunone	220.35	$C_{15}H_{24}O$		People also use it as medicine. BHT is used to treat genital herpes and acquired immunodeficiency syndrome (AIDS) etc.
14.920	3.05	Kauran-18-al, 17-(acetyloxy)-, (4. beta.)-18-Oxokauran-17-yl acetate	346.50	$C_{22}H_{34}O_3$		Used as memory enhancer and as a dietary supplement with proven benefit in several neurological and psychiatric conditions etc.
14.982	0.75	Ethyl chrysanthemate	196.29	$C_{12}H_{20}O_2$		Used primarily as a component of perfumes for creams, soaps and as a flavouring ingredient. used particularly in rose, lavender and geranium

						formulations where a sweet fruity or citrus aroma is desired etc.
15.084	0.47	1,9-Decadiyne	134.23	$C_{10}H_{14}$		Antifungal activity etc.
15.139	0.48	2,2-Dimethylocta-3,4-dienal 2,2-Dimethyl-3,4-octadienal	152.24	$C_{10}H_{16}O$		Anti-microbial activity etc.
15.211	0.63	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane 2-Methyl-3-(3-methyl-2-butenyl)-2-(4-methyl-3-pentenyl) oxetane	222.37	$C_{15}H_{26}O$		Antischistomona, antioxidant, antifungal, anticancer, antimicrobial activities etc.
15.350	0.35	4-Chloro-2-isopropyl-5-methylphenyl bicyclo [4.1.0] heptane-7-carboxylate	306.88	$C_{18}H_{23}ClO_2$		Anti-inflammatory, food additives, anti-cancer etc.
15.400	4.96	2-Butenal, 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	$C_{14}H_{22}O$		Used to study its alkylation reaction with cinnamyl alcohol in the presence of Al-substituted disordered hexagonal molecular silica catalysts. It is a raw material for phosphate anti-oxidants etc.
15.668	1.11	Kauran-18-al, 17-(acetyloxy)-, (4. beta.)-18-Oxokauran-17-yl acetate	346.50	$C_{22}H_{34}O_3$		Used as memory enhancer and as a dietary supplement with proven benefit in several neurological and psychiatric conditions etc.
15.718	1.93	Spiro-6-(bicyclo [3.2.1] octane)-2'-(oxirane),7,8-di(hydroxymethyl)-5-methyl-2-isopropyl	254.36	$C_{15}H_{26}O_3$		Can improve the polyphenol and anthocyanin content and antioxidant activity of some crop plants. Also shows a suppressive effect on the plant growth etc.

15.808	0.24	1-Methylheptyl trans-2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropane carboxylate	280.47	$C_{18}H_{32}O_2$		Hypocholesterolemic, anticancer, anti-inflammatory, insecticidal etc.
15.902	0.68	2,2-Dimethylocta-3,4-dienal	152.24	$C_{10}H_{16}O$		Anti-microbial activity etc.
15.955	2.06	Kauran-18-al, 17-(acetyloxy)-, (4. beta.)-18-Oxokauran-17-yl acetate	346.50	$C_{22}H_{34}O_3$		Used as memory enhancer and as a dietary supplement with proven benefit in several neurological and psychiatric conditions etc.
16.015	1.33	Neoisolongifolene	218.33	$C_{15}H_{22}O$		Antimicrobial activity etc.
16.117	9.31	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$		Antioxidant, antiandrogenic, lubricant, nematicidal, haemolytic inhibitor etc.
16.252	0.69	Octadecanoic acid, ethyl ester	313.50	$C_{20}H_{40}O_2$		Used for the production of detergents, photographic materials and lubricants etc.
16.866	0.17	1-Nonyne	124.22	C_9H_{16}		Used in enantioselective synthesis of cladospolide B, C and (ent)-cladospolide D. etc.
17.281	17.70	Oleic Acid	282.50	$C_{18}H_{34}O_2$		Hypochoesteroemic, anti-cancer, anaemiagenic, anti-inflammatory, choleretic, dermatitigenic, antibacterial, antiandrogenic etc.

17.338	3.02	Ethanol, 2-(9 octadecenyloxy)-, (Z)-2-cis-9 Octadecenyloxyethanol	312.54	$C_{20}H_{40}O_2$		Used for the production of detergents, photographic materials and lubricants etc.
17.389	2.57	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	212.24	$C_{11}H_{16}O_4$		Used to treat methemoglobinaemia by converting the ferric iron in haemoglobin to ferrous iron etc.
18.152	0.90	1,2-Cyclopentanediol, 3-methyl-cyclopentan-1,2-diol	116.15	$C_6H_{12}O_2$		Uses including as a food flavouring, as a solvent in the process of lacquer production, in polishes, inks, adhesives and occasionally as a high-boiling solvent etc.
18.614	1.27	1-Propene, 2-methyl-3-(1-methylethoxy)-3-Isopropoxy-2-methyl-1-propene	114.19	$C_7H_{14}O$		Used as a synthetic flavouring and in perfumes etc.
19.153	2.05	1-Octadecyne	250.46	$C_{18}H_{34}$		Used as a solvent for the synthesis of iron oxide nanospheres etc.
20.547	3.71	1-Hexyl-2-nitrocyclohexane	213.17	$C_{12}H_{23}NO_2$		Used as a volatile binding medium, a temporary binder for sealing and conservation of friable and structurally weak materials etc.
21.521	2.00	Silane, [(11 fluoroundecyl) oxy] trimethyl	262.48	$C_{14}H_{31}FOSi$		Used in some fiberglass and composites to improve mechanical strength, and electrical properties etc.

The proximate composition of the extract expressed in percentage is shown in Table 1 with a high content of crude fibre (47.30 ± 0.54 %) and the ash content the least (5.00 ± 0.24 %), suggesting that the plant extract could be used for animal feed formation and also administered to individuals with known high cholesterol levels. The crude fibres react with the cholesterol and passed out of the system as waste [9]. In the gut, dietary fibres aid in the absorption of trace elements and also increase intestinal bowel movement, help in lowering blood cholesterol, triglycerides and protect against digestive disorders and cancer [10]. Also, plants with high amounts of crude fibre are requested for the treatment of obesity, gastrointestinal disorders, cancer, coronary heart disease, hypertension and constipation [11]. Fibre also cleanses the digestive tract by removing potential carcinogens from the body, prevents the absorption of excess cholesterol, adds bulk to the diet, prevents the intake of excess starchy food [12] and may therefore guard against metabolic conditions such as diabetes mellitus and hypercholesterolemia [13]. It has also been reported that a high intake of dietary fibre is associated with enhanced insulin sensitivity and therefore may have a role in the prevention and control of Type 2 diabetes [14]. The substantial amount of fibre in *E. chlorantha* stem bark shows that they can help in keeping the digestive system healthy and functioning properly.

[15] reported that protein is essential in the human diet and is vital for various body functions such as nutrient transport, muscle contraction, body temperature, maintenance of fluid balance, formation of hormones and enzymes, sustaining strong immune function, supply of energy and adequate amount of required amino acids. Also, they are building block units and the food protein is needed to make vital hormones, antibodies, important brain chemicals, digestive enzymes and necessary elements for deoxyribonucleic acid synthesis. Some are involved in structural support, while others are involved in bodily movement or defense against germs [16]. From our results (Table 1), we observed that the crude protein (9.67 ± 0.32 %) was in accordance with the result reported by [17] who reported 10.78±0.19 %. The high crude protein content of *E. chlorantha* stem bark makes it a good source of plant protein and it could also provide the body with fuel and energy that is required for daily activities since it contains higher value in terms of carbohydrate and energy content as shown in Table 1.

The availability of energy in food and its ability to regulate blood pressure can be estimated by its fat contents. Fats insulate and protect body organs and also transport fat-soluble vitamins. Our result revealed fat content to be 1.60 ± 0.04 %. This result is lower than that reported by [17] who reported 3.78 ± 0.16 %.
Lipid provides very good sources of energy and also aids in the transport of fat-soluble vitamins, insulates and protects internal tissues and contributes to important cell processes [18]. More so, it is good to add lipids (fat) to most of our diets, because many body functions depend on lipids [19].

The amount of moisture in plant material determines its absorption and assimilation rate within an organism. Thus, the plant moisture content determines storability and plant quality since high moisture content is associated with lower storage stability [20]. The reasonable amount of moisture in most vegetables is 6 to 15 % [21]. This contributes to slowing the growth and development of microorganisms and inhibiting hydrolysis of components present in plant material, so that the material can be preserved for a long period or no risk of microbial attack [22].
Our results showed that *E. chlorantha* stem bark moisture content value was relatively low 8.30 ± 0.54 %. The low moisture content would therefore hinder the growth of spoilage microorganisms and enhance shelf life that is it has a high shelf and will not be prone to microbial attack. Our result is slightly higher than the result reported by [17] who reported 3.85 ± 0.35 %.

We recorded ash content to be 5.00 ± 0.24 % higher than that reported by [17] for the stem bark of *E. chlorantha* (2.48 ± 0.1 %) but lower than that reported by [23] and [24] who reported 16.65 ± 0.09 % and 7 % respectively. *E. chlorantha* had an ash value of 5.00 ± 0.24 % which is a reflection of the mineral contents preserved in the plant's stem bark.

Carbohydrates are the most abundant biological molecules and play important roles in the body as sources of energy as well as provision of structural materials and are required in the human body mainly for the production of energy for the body's activities [18]. The results obtained (Table 1) revealed appreciable amounts of carbohydrate content in the stem bark (28.13 ± 0.43 %). Thus, the high carbohydrate content may have contributed to the high energy value (165.60 ± 5.53 kcal) in *E. chlorantha* stem bark. Carbohydrates produced by plants are one of the three main energy sources in food, along with protein and fat. When animals eat plants, energy stored in carbohydrates is then released by the process of respiration (a chemical reaction between glucose and oxygen to produce energy, carbon dioxide, and water).
Medicinal plants play an important role in the human health care system [25]. 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 have been known to be biologically active and contribute to the antioxidant capacity of the plants in which they are found [26].

In this present study, the qualitative phytochemical screening (Table 2) of the carbonated drink extract revealed the presence of alkaloids, cardiac glycosides, flavonoids, tannins and steroids. [27-30] also reported the presence of these phytochemicals in the stem bark extracts of *E. chlorantha*.
Phytochemicals are secondary plant metabolites that occur in various parts of plants, they have diverse roles in plants which 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 [31], antibacterial [32], antitussive, antipyretic, hallucinogenic and antihypertensive [33], anti-hyperglycaemic [34], antiarrhythmic, vasodilatory [35] or as a template for drug discovery [36].
Alkaloids are a diverse group of heterogeneous compounds that contain a ring structure with a nitrogen atom located in the heterocyclic ring. It has a marked physiological action on animals and humans when used in small quantities. Examples of alkaloids with pharmacological properties reported in the literature include quinine, ephedrine, homoharringtonine, morphine, galantamine and piperine with significant antimalarial, antiasthma, anticancer, analgesic, cholinomimetic and hypoglycaemic activities respectively [36]. Also, they are among the most therapeutically efficient plant secondary metabolites and are usually used as the major backbone in some drugs like morphine, quinine, colchicine and vincristine among others [37]. An alkaloid (9-methoxycanthin-6-one) has also been identified to be a more potent antimalarial agent than chloroquine when tested against *Plasmodium falciparum* Gombak A isolate [38]. The high content of alkaloids contributed to the bitter taste of the extracts because alkaloids are very alkalic.

Cardiac glycosides are majorly used in the treatment of congestive heart failure and cardiac arrhythmia, whereby they inhibit Na^+/K^+-ATPase pump that causes positive ionotropic effects and electrophysiological changes [39,40]. The presence of flavonoids suggests the ability of this by-product to play an important role in preventing disorders associated with oxidative stress. The appreciable level of total flavonoid contents in the extract of the stem bark might be responsible for the use of this plant for the treatment of radical-related problems such as malaria and cough. Flavonoids are a group of polyphenolic potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong cardiovascular [41] anti-hepatotoxic [42], anti-microbial, anti-viral and anti-ulcer [43] activities and protection against different levels of carcinogenesis [44], anti-inflammatory [45] and anti-allergic [46].
Tannins are used for the treatment of inflammation, leucorrhoea, gonorrhoea, burn piles and diarrhoea [47] and also useful in the treatment of inflamed or ulcerated tissues and cancer [48]. These potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation [49].
Gas chromatography-mass spectrometry (GC–MS) analysis of carbonated drink extract of *E. chlorantha* stem bark revealed the presence of forty-seven bioactive compounds. The relative abundant bioactive compounds in

the carbonated drink extract was in the order Oleic acid (17.70%) >n-Hexadecanoic acid (9.31%) > 1-Hexyl-2-nitrocyclohexane (3.71%) >Kauran-18-al, 17-(acetyloxy)-, (4. beta.)- (3.05%)>Kauran-18-al, 17-(acetyloxy)-, (4. beta.) - (2.06 %).

Literature has reported that these compounds play crucial roles in the general metabolism and homeostasis of the human body. Palmitic acid and oleic acid prevent insect pests and have anaemiagenic, anti-inflammatory, choleretic, antibacterial and antiandrogenic properties [50]. Octadecanoic acid methyl ester has antifungal and antimicrobial properties [51,52]. n-Hexadecanoic acid has anti-inflammatory, antioxidant, antiandrogenic, hypocholesterolaemic, nematicide, pesticide and haemolytic inhibitor [53], potent mosquito larvicide [51]. Oleic acid has been reported to have antibacterial, anti-cancer, anaemiagenic, anti-inflammatory, choleretic, dermatitigenic and anti-androgenic properties [54].

4. CONCLUSION

This study revealed that carbonated drink extractof *E. chlorantha* stem bark contains appreciable amount of pharmacologically important phytochemicals compounds with reported medicinal potential. It could also be a useful source of nutrients and the bioactive chemical constituents may serve as potential drug target needed for drug discovery and development.Thus may be considered as a potential drug, fertilizer or feed target. Further research should be done at molecular level in isolating and characterization of the pure compounds that are responsible for all these activities.

REFERENCES

1. Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4: 191 – 198.

2.Adebayo JO, Kreltti AU.Potential antimalarials from Nigerian plants a review. The Journal of Ethnopharmacology. 2011;133(2): 269 - 302.

3.Sotade GA, Onyeneke EC, Ofoha PC, Anionye JC, Anyanwu GO. *Enantia chlorantha*: A review. Journal of Pharmacognosy and Phytochemistry.2022;11(3): 34 - 38.

4.Adesokan AA, Yakubu MT, Owoyele BV, Akanji MA, Soladoye AO, Lawal OK. Effect of administration of aqueous and ethanolic extracts of *Enantiachloranthas*tem bark on brewer's yeast induced pyresis in rats. African Journal of Biochemistry Research. 2008;2(7): 165 - 169.

5.Ishola NO, Oreagba IA, Adeneye AA, Adirije C, Oshikoya KA, Ogunleye OO. Ethnopharmacological survey of herbal treatment of malaria in Lagos, Southwest Nigeria. Journal of Herbal Medicine. 2014;4: 224 – 234.

6. AOAC. Official Methods of Analysis of AOAC International. 18th ed.; Association of Official Analytical Chemists, Washinton, DC. 2000.

7.Harborne JB.Phytochemical methods: A Guide to modern technique of plant analysis. 3rdedn, Springer, London. 1998; 88 - 98.

8. Trease GE, Evans WC. Pharmacognosy. 15th edition. London: Saunders Publishers. 2002; 585.

9. Agbaje EO, Onabanjo AO. Analgesic and antipyretic actions of *Enantiachlorantha* in some Laboratory animals. Nigerian Journal of Natural Products and Medicine. 1998;2: 24 – 25.

10.Abolaji OA, Adebayo AH, Odesanmi OS. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica, Blighia sapida* and *Parinaripolyandra*) commonly used by Pregnant Women in the Western Part of Nigeria. Pakistan Journal of Nutrition. 2007;6(6): 665 - 668.

11. Ibironke SA, Adeniyi MA, Fashakin JB. Nutritional evaluation of complementary food formulated from fermented maize, pigeon pea and soybeans. Nutrition and Food Science.2014;44(5): 464 – 470.

12. Mensah JK, Okoli RI, Ohaju-Obodo JO. Eifediyi K.Phytochemical, nutrional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African Journal of

Biotechnology.2008;7(14): 2304 – 2309.
13. Henry S.Fiber: An all natural "medicine" for Type 2 Diabetes? *Consumer Health Interactive*. 2004; http://www.ahealthyme.com.
14. Ylonen K, Saloranta C, Kronberg C, Leif G, Antti A, Suvi M, Virtanean M. Associations of dietary fiber with glucose metabolism in non-diabetic relatives of subjects with Type 2 Diabetes. Diabetes care.2003;26: 1979 - 1985.
15. Igile GO, Iwara A, Mgbeje BA, Uboh FE, Ebong PE. Phytochemical, proximate and nutrient composition of vernonia calvaona hook (*Asterecea*): A green-leafy vegetable in Nigeria. Journal of Food Research. 2013;2(6). http://dx.doi.org/10.5539/jfr.v2n6p1.
16. Bailey R.The role of proteins in the body. About.com. Guide to Biology. 2008.
17. Dawodu AO, Moses UD, Apena A, Adetoro A, Dairo JO. The proximate evaluation and phytochemistry of *Enantiachlorantha* stem bark in aqueous and ethanolic extract. Middle-East Journal of Scientific Research.2014;21(11): 2145 - 2148.
18. Adjatin A, Dansi A, Badoussi E, Loko YL, Dansi M, Azokpota P, Gbaguidi F, Ahissou A, Akoègninou A, Akpagana K, Sanni A. Phytochemical screening and toxicity studies of *Crassocephalumrubens* (Juss. ex Jacq.) *S. Moore* and *Crassocephalumcrepidioides* (Benth.) *S. Moore* consumed as vegetable in Benin. International Journal Current Microbiology Applied Sciences. 2013;2(8): 1 – 13.
19. Lehninger AL, Nelson DL, Cox MM. Lehninger Principle of Biochemistry, Worth Publishers, New York, NY, USA, 3rd edition.2000.
20. Kwenin WKJ, Wolli M, Dzomeku BM. Assessing the nutritional value of some African indigenous green leafy vegetables in Ghana. Journal of Animal and Plant Sciences.2011;10(2): 1300 -1305.
21. Rishi P, Rishi E, Kuniyal L, Mathur G. Short-term results of intravitreal dexamethasone implant (OZURDEX®) in treatment of recalcitrant diabetic macular edema: A case series. Oman Journal of Opthalmology.2012;5(2): 79.
22.Egga ES, Adeyanju O, Agyeno OE. Preliminary phytochemical, antimicrobial and proximate analysis of tender leaves of *Psidium guajava* L in Jos, Plateau State, Nigeria. Asian Review of Environmental and Earth Sciences.2014;1(2): 35 – 38.
23. Usunobun U, Okolie PN. Phytochemical analysis and proximate composition of *Vernonia amygdalina*. International Journal of Scientific World. 2016;4(1): 11 – 14.
24. Ugwu OPC, Onyeneke EC, Igwenyi IO, Aja PM, Ugwuoke KC0, Okon MB, Onyeke, SC. Proximate, vitamin, mineral and phytochemical analysis of ethanol root extract and fractions of *Sphenocentrumjollyanum*.IAA Journal of Scientific Research. 2018;4(1): 63 - 74.
25. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, Mastinu A. *Cannabis sativa*:A comprehensive ethanopharmacological review of a medicinal plant with a long history. Journal of Ethnopharmacology. 2018;227: 300 – 315.
26. Gulcin I, Topal F, Ozturk Sarikaya SB, Bursal E, Birsel G, Goren AC. Polyphenol contents and antioxidant properties of Medlar (*Mespilus germanica* L). Records of Natural Products Journal. 2011;5: 158 – 175.
27. Bassey AL, Nwafor PA, Udobang JA, Ettebong EO. Subchronic evaluation of the ethanol stem bark extract of *Enantia chlorantha* in rodents biochemical, haematological evaluation and effect on body weight. World Journal of Pharmaceutical Research. 2017;6(9): 73 – 83.
28. Nurain IO, Bewaji CO, Abubakar AA, Mustapha A, Ajani EO, Sabiu S, Usman LA, Abdulhameed HA, Ahmed SB. Antimalarial and reno-protective potentials of combined stem bark extracts of *Khaya grandifoliola* and *Enantiachlorantha* in *Plasmodium* infected mice. Iranian Journal of Toxicology. 2018;12(3): 29 – 37.
29. Abubakar A, Ahmad NS, Akanya HO, Abdulkadir A, Abubakar AN. Antiplasmodial activity of total alkaloids and flavonoids of stem bark extracts of *Enantia chlorantha* in mice. Comparative Clinical Pathology. 2020;29: 873 – 881.

30. Ibrahim LB, Idowu PF, Moses OA, Alabi MA, Ajani EO. Antidiabetic potential of stem bark extract of *Enantiachloranthaand* lack of modulation of its therapeutic efficacy in diabetic rats co-administered with lisinopril. Acta ChimicaSlovenica. 2021;68: 118 – 127.
31. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation. Current Topics in Medicinal Chemistry.2014;14(2): 239 – 252.
32. Cushnie TP, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic enhancing and antivirulence activities. International Journal of Antimicrobial Agents.2014;44(5): 377 – 386.
33.Hesse M, Thylstrup B, Skogen JC. Drug use, self-harm, suicide, and use of registry data epidemiological research. Addiction.2022;117: 1950 – 1951.
34. Qui S, Sun H, Zhang A, Xu H, Wand X. Natural product alkaloids: Basic aspects, biological roles and future perspective. Chinese Journal of Natural Medicines. 2014;12(6): 401 – 406.
35.Sinatra RS, Jahr JS, Watkins-Pitchford JM. The essence of analgesia and analgesics. Cambridge: Cambridge University Press. 2010;82 – 90.
36. Pérez-Amador MC, Muñoz Ocotero V, García JM, Castañeda AR, González E. Alkaloids in *Solanum torvum* (Solanaceae). International Journal of Experimental Botany. 2007;76: 39 – 45.
37. Jigam AA, Akanya HO, Ogbadoyi EO, Dauda BEN.*In vivo*antiplasmodial, analgesic and anti-inflammatory effects of the root extracts of Acacia nilotica del (Leguminosae). Asian Journal of Experimental Biological Sciences.2010;1(2): 315 – 320.
38. Chan KL, Choo CY, Abdullah NR, Ismail Z. Antiplasmodial studies of *Eurycoma longifolia* Jack using the lactate dehydrogenase assay of *Plasmodium falciparum*. Journal of Ethnopharmacology.2004;9: 223 – 227.
39. Ngoci SN, Matasyoh JC, Mwaniki CG, Mwendia CM. Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. Journal of Animal and Plant Sciences. 2011;11(1): 1364 –1373.
40. Ifemeje JC, Egbuna CS, Eziokwudiano JO, Ezebao FC. Determination of the anti-nutrient of composition of *Ozimumgrassinum*. International Journal of Innovation and Science Research. 2014;3(2): 127 – 133.
41. Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. Current Opinion in Lipidology. 2005;16(1): 77 – 84.
42. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. Journal of Sustainable Agriculture and the Environment.2004;6: 30 – 34.
43. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013;1(6): 168 – 182.
44. da Cruz LS, Lima RZ, de Abreu CMP, Correa AD, Pinto LMA. Caracterizaçãofisica e química das frações do fruto atemoya (gefner). Cienc Rural.2013;43: 2280 – 2284.
45. Ravishankar D, Rajora AK, Greco F, Osborn HMI. Flavonoids as prospective compounds for anti-cancer therapy. The International Journal of Biochemistry and Cell Biology. 2013;45(12): 2821 – 2831.
46. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-kB pathway in the treatment of inflammation and cancer. Journal of Clinical Investigation. 2001;107(2): 135 – 142.
47. Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, Ieri F, Romani A. Antimicrobial and antiviral activity of hydrolysable tannins. Mini-Reviews in Medicinal Chemistry. 2008;8: 1179 - 1187.
48. Sun Y, Zhang T, Wang B, Li H, Li P. Tannic acid, an inhibitor of poly (ADP-ribose) glycohydrolase, sensitizes ovarian carcinoma cells to cisplatin. Anticancer Drugs.2012;23(9): 979 – 990.
49. Ajali U.Chemistry of biocompounds, Is ted. Rhyce Kerex Publishers. Enugu, Nigeria. 2004; 102 – 112.
50.Jang JS, Yang YC, Choi DS, Ahn YJ. Vapor phase toxicity of marjoram oil compounds and their related monoterpenoids to Blattella germanica (Orthoptera: *Blattellidae*), Journal of Agricultural and Food Chemistry. 2005;53(20): 7892 – 7898, doi: 10.1021/jf051127g.

51. Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effects of *Feronia limonia* on mosquito larvae. Fitoterapia. 2000;71: 553 – 555.
52. Gehan MA, Hanan AE, Hassan AHI, Okbah MA. Marine natural products and their potential applications as antiinfective agents. World Science Journal. 2009;7(7): 872 – 880.
53. Kumar SPJ, Prada SR, Banerjee R, Thammineni C. Seed birth to death: dual functions of reactive oxygen species in seed physiology. Annals of Botany. 2015;116(4): 663 – 668.
54. Awa EP, Ibrahim S, Ameh DA. GC-MS analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of *Annona senegalensis* Pers. International Journal of Pharmaceutical Sciences and Research. 2012;3: 4213 – 4218.