



Proximate, Phytochemical and Gas Chromatography-mass Spectrometry (GC-MS) Analysis of the Carbonated Drink (Soft drink) Extract of *Enantia chlorantha* Stem Bark

Gabriel Akinwale Sotade ^{a*}, Samuel Ifidon Ojeaburu ^b,
Ozi Salami Abdulrahim Onimisi ^c, Ahmed U. Otokiti ^d,
Paul Temidayo Duile ^a, Ayotunde Sherif Azees ^e,
Rotimi Elijah Abioye ^f and Victor A. C. Biodun ^g

^a Molecular Biology Department, Federal College of Veterinary and Medical Laboratory Technology (NVRI), Vom, Plateau State, Nigeria.

^b Biochemistry Department, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

^c Sports Medicine Department, Federal Ministry of Sports Development, Abuja, Nigeria.

^d Internal Medicine Department, Icahn School of Medicine, Mount Sinai, New York City, USA.

^e Department of Community Medicine and Primary Care, Federal Medical Center, Abeokuta, Ogun State, Nigeria.

^f ICT and Library Division, Planning Department, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

^g Administrative Department, Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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*Corresponding author: E-mail: walesotade@gmail.com;

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 ($47.30 \pm 0.54\%$ by dry mass), followed by total carbohydrate ($28.13 \pm 0.43\%$), crude protein ($9.67 \pm 0.32\%$), moisture content ($8.30 \pm 0.54\%$), ash content ($3.00 \pm 0.24\%$) and lastly lipids ($1.60 \pm 0.04\%$). 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 to cure a particular ailment and the brand of the carbonated drink may also be used as solvent of choice apart from water and alcohol.

Keywords: *Enantia chlorantha*; proximate; phytochemical; oleic acid; 7up; natural chemical defences, rural communities.

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, but the one that caught our attention during our ethnobotanical survey is *Enantia chlorantha* (*Annonaceae*)" [1,2]. "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" [5]. 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' [5-8].

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 AND 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 a particular brand of carbonated drink that is used in my village to treat a particular ailment. 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°C 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 [9].

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 Harborne [10,11].

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 μ L, oven initial temperature was 80°C to hold for 2 minutes and the final temperature of 240°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 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 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.

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 \pm 0.54% by dry mass), followed by total carbohydrate (28.13 \pm 0.43%), crude protein (9.67 \pm 0.32%), moisture (8.30 \pm 0.54%), ash content (3.00 \pm 0.24%) and lastly lipids (1.60 \pm 0.04%). Dry matter (91.77 \pm 2.65%) and energy content (165.60 \pm 5.53%) were also significantly ($p < 0.05$) high.

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.

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 Fig 1.

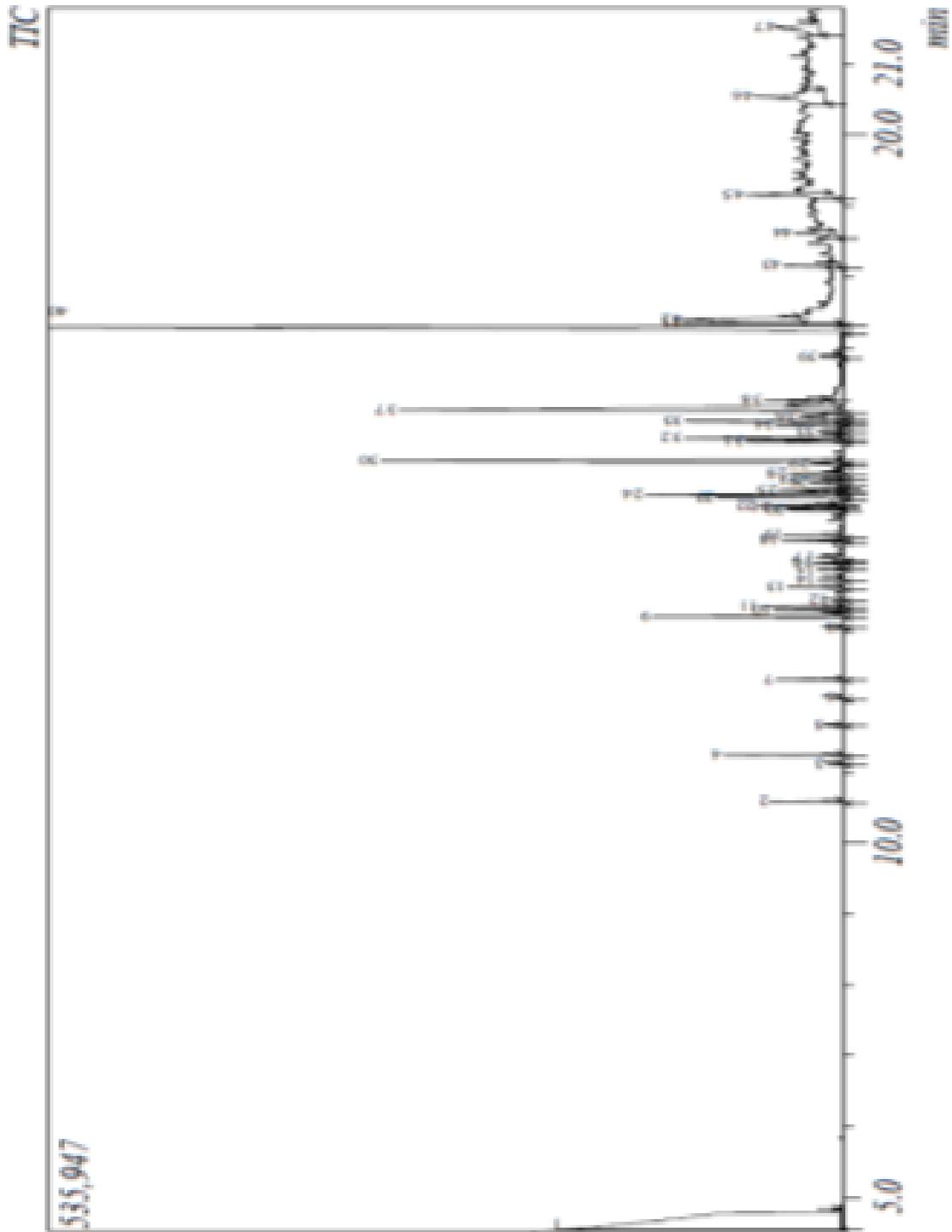


Fig. 1. GC-MS spectrum analysis of CDE of *E. chlorantha* stem bark

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 (%)	3.00 ± 0.24
Carbohydrate (Nitrogen free extract) (%)	28.13 ± 0.43
Energy content (kcal)	165.60 ± 5.53
Dry matter (%)	91.77 ± 2.65

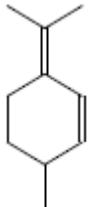
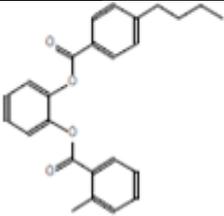
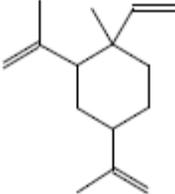
Values are represented as Mean ± Standard Error of Mean (SEM) performed in triplicates

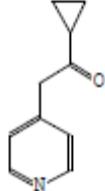
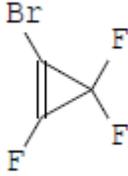
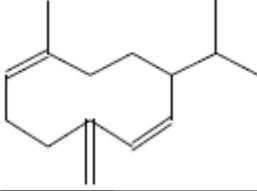
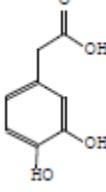
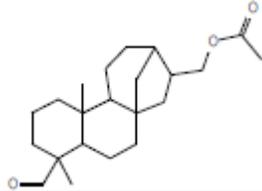
Table 2. Phytochemical composition of the carbonated drink extract of *E. chlorantha* stem bark

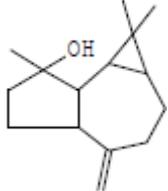
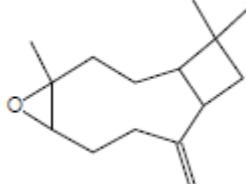
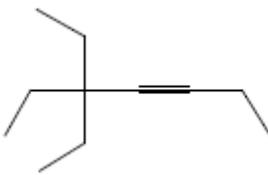
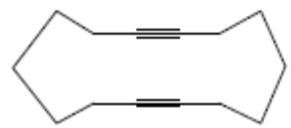
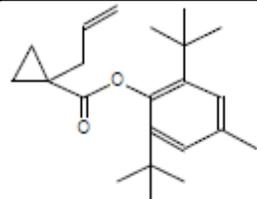
Phytochemicals	Plant Extract
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Phenols	-
Reducing sugars	-
Saponins	-
Tannins	+
Steroids	+
Anthraquinone	-

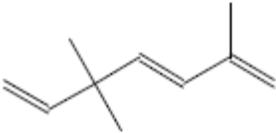
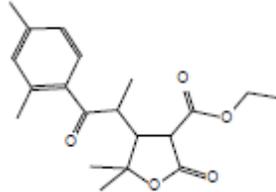
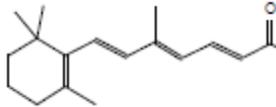
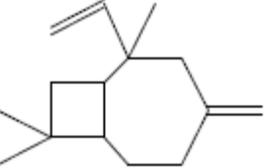
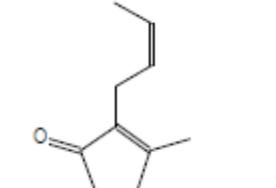
+ = component present; - = component absent

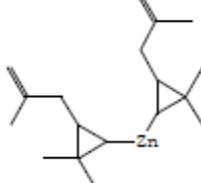
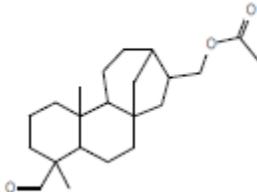
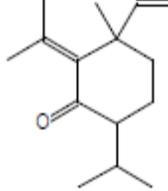
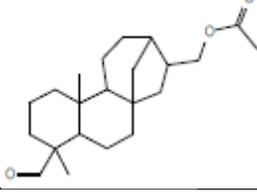
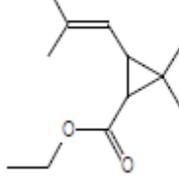
Table 3. Compounds isolated from carbonated drink stem bark extract of *E. chlorantha*

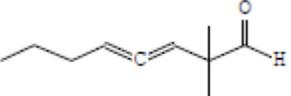
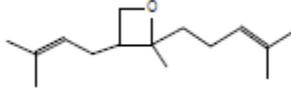
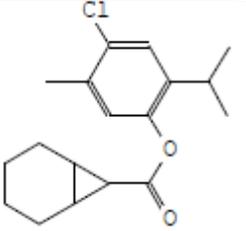
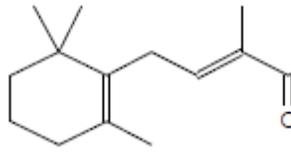
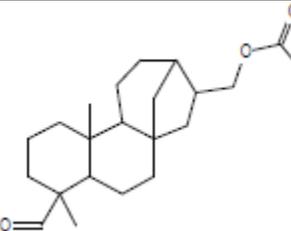
RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
4.542	25.00	n-Hexane	86.18	C ₆ H ₁₄		Used in the extraction of edible oils from seed and vegetable crops (soybeans, peanuts, corn) and used as a solvent for glues, varnishes, and inks.
10.584	0.78	Cyclohexene, 3-methyl-6-(1-methylethylidene)	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-tumour, anti-migraine, analgesic, sedative activities etc.

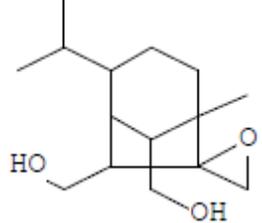
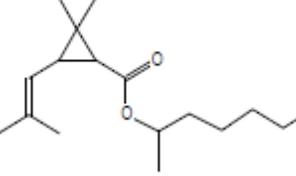
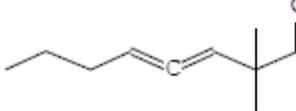
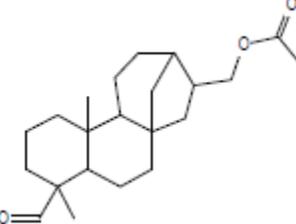
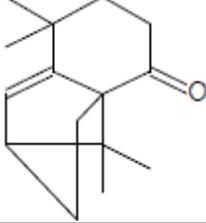
RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
11.658	0.08	Ethanone, 1-cyclopropyl-2-(4-pyridinyl)	161.20	C ₁₀ H ₁₁ NO		Used as a precursor in the synthesis of tryptamines like DMT and the drug indoramin along with TNF-alpha modulators etc.
12.050	0.06	Cyclopropene	172.93	C ₃ BrF 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 ₈ H ₈ O 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.

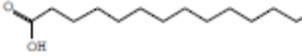
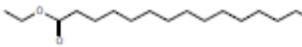
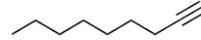
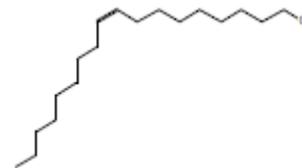
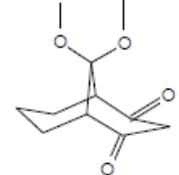
RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
13.283	0.57	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1	220.35	C ₁₅ H ₂₄ O		People also use it as medicine. BHT is used to treat genital herpes and acquired immunodeficiency syndrome (AIDS) etc.
13.342	0.80	Caryophyllene oxide	220.35	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	-	-	-	-	-

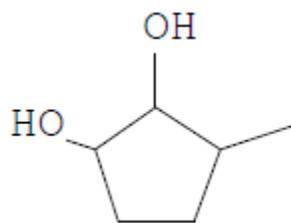
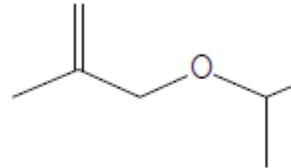
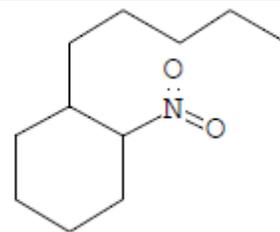
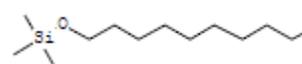
RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
13.951	0.25	1,3,6-Heptatriene, 2,5,5-trimethyl	136.23	C ₁₀ H ₁₆		Used for the manufacture of synthetic camphor etc.
14.036	0.53	3-Furancarboxylic acid, 4 - [2-(2,4-dimethyl)]	346.17	C ₂₀ H ₂₆ O ₅		Used in European folk medicine to 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.

RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
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.

RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
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.

RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
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.

RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
16.117	9.31	n-Hexadecanoic acid	256.42	C ₁₆ H ₃₂ O ₂		Antioxidant, antiandrogenic, lubricant, nematocidal, 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 ₉ H 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		Hypocholesterolemic, anti-cancer, anaemiagenic, anti-inflammatory, choleric, 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.

RT	Area %	Name of Compound	MW	MF	Structures	Pharmacological Importance
18.152	0.90	1,2-Cyclopentanediol, 3-methyl-cyclopentan-1,2-diol	116.15	C ₆ H 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 ₇ H 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 ($47.30 \pm 0.54\%$) and the ash content the least ($3.00 \pm 0.24\%$), 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 [12]. 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 [13]. Also, plants with high amounts of crude fibre are requested for the treatment of obesity, gastrointestinal disorders, cancer, coronary heart disease, hypertension and constipation [14]. 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 [15] and may therefore guard against metabolic conditions such as diabetes mellitus and hypercholesterolemia [16]. "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" [17]. The substantial amount of fibre in *E. chlorantha* stem bark shows that they can help in keeping the digestive system healthy and functioning properly.

Igile et al. [18] 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" [19]. From our results (Table 1), we observed that the crude protein ($9.67 \pm 0.32\%$) was in accordance with the result reported by [20] who reported $10.78 \pm 0.19\%$. 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 $1.60 \pm 0.04\%$. This result is lower than that reported by [20] who reported $3.78 \pm 0.16\%$.

"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" [21]. "More so, it is good to add lipids (fat) to most of our diets, because many body functions depend on lipids" [22].

"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" [23]. "The reasonable amount of moisture in most vegetables is 6 to 15 %" [24]. "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" [25].

Our results showed that *E. chlorantha* stem bark moisture content value was relatively low $8.30 \pm 0.54\%$. 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 [20] who reported $3.85 \pm 0.35\%$.

We recorded ash content to be $3.00 \pm 0.24\%$ which is slightly higher than that reported by [20] for the stem bark of *E. chlorantha* ($2.48 \pm 0.1\%$) but lower than that reported by [26] and [27] who reported $16.65 \pm 0.09\%$ and 7% respectively. *E. chlorantha* had an ash value of $3.00 \pm 0.24\%$ 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" [21]. The results obtained (Table 1) revealed appreciable amounts

of carbohydrate content in the stem bark ($28.13 \pm 0.43\%$). 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 [28]. "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" [29].

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. [30-33] 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 [34], antibacterial" [35], antitussive, antipyretic, hallucinogenic and antihypertensive [36], anti-hyperglycaemic [37], antiarrhythmic, vasodilatory [38] or as a template for drug discovery [39].

"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" [39]. "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" [40]. "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" [41]. The high content of alkaloids contributed to the bitter taste of the extracts because alkaloids are very alkaline.

"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 inotropic effects and electrophysiological changes" [42,43]. "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" [44] anti-hepatotoxic [45], anti-microbial, anti-viral and anti-ulcer [46] activities and protection against different levels of carcinogenesis [47], anti-inflammatory [48] and anti-allergic [49].

"Tannins are used for the treatment of inflammation, leucorrhoea, gonorrhoea, burn piles and diarrhoea and also useful in the treatment of inflamed or ulcerated tissues and cancer" [50,51]. "These potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation" [52].

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, choleric, antibacterial and antiandrogenic properties” [53]. “Octadecanoic acid methyl ester has antifungal and antimicrobial properties” [54,55]. n-Hexadecanoic acid has anti-inflammatory, antioxidant, antiandrogenic, hypocholesterolaemic, nematicide, pesticide and haemolytic inhibitor [56], potent mosquito larvicide [54]. “Oleic acid has been reported to have antibacterial, anti-cancer, anaemiagenic, anti-inflammatory, choleric, dermatitogenic and anti-androgenic properties” [57].

4. CONCLUSION

This study revealed that carbonated drink extract of *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 to cure a particular ailment and the brand of the carbonated drink may also be used as solvent of choice apart from water and alcohol.

Further research should be done at molecular level in isolating and characterization of the pure compounds that are responsible for all these activities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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