EVALUATING THE COMPOSITION OF PHYTOEXTRACT OF *Cymbopogon citratus*: A FURTHER EVIDENCE OF ITS DIVERSITY IN ETHNOMEDICINE AND PHYTOPHARMACEUTICALS APPLICATIONS

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ABSTRACT

The medicinal value of plants lies in their bioactive constituents which usually allow them to act as remedies to several ailments. The present study determines the biochemical constituents of the aqueous extract of Cymbopogon citratus leaves. The bioactive principles and GC-MS analysis were carried out on aqueous leaves extract of *Cymbopogon citratus* using standard biochemical and analytical procedures. Spectroscopic quantitative phytochemical analysis of the aqueous extract of leaves of *Cymbopogon citratus* was found to contain 64.18 mg/g (alkaloids), 63.78 mg/g (Steroids), 58.13 mg/g (flavonoids), 48.18 mg/g (phenolics), 64.28 mg/g (saponnins), and 62.14 mg/g (tannins), while GC-MS analysis revealed 23 constituents in which they were all identified. The major constituents were three (3) compounds with percentage peak height of 23.20% (1- Nonadecane), 15.88% (1-Pentadecane) and 11.48% (Behenic alcohol), other constituents less than ten percentage peak area are 4.51% (1-Dodecanol, 3.56% (2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-d), 5.17% (Isopropyl myristate), 4.27% (n-hexadecanoic acid), 6.49% (Behenic alcohol), 4.91% (Oleic acid), 4.25% (propanoic acid, decyl ester), 2.89% (Octacosanol), 3.07% (Bis (2-ethylhexyl) phthalate while other constituents were less than 2%. It is therefore evident that the studied plant material contains various phytocomponents and is recommended as a plant of phytopharmaceutical importance possessing antimicrobial, antioxidant, anti-inflammatory, flavouring and hemolytic potentials.

KEYWORDS: extract, bioactive principles, herbal plant, gas chromatography

INTRODUCTION

Ethnomedicine provides a basic understanding of plant medicinal properties. The identification and standardization of active compounds in any medicinal plant is critical for the production of new drugs (Ojatula and Owoyemi, 2022). Herbal medicines are a group of herbs that contain a number of important compounds for medicine and pharmacy (Wink, 2015), and they are found sustaining body systemic for good health and well-being (Ojatula, 2019). Natural products of plant origin are naturally occurring compounds that are end products of secondary metabolism, often; they are unique compounds for particular organisms or classes of organisms, and they are found taking an important place in health services all over the globe (Alviano and Alviano, 2009). And they are found providing man with different kinds of healing resources, which among them include several types of plant-based medicines and chemicals known widely as potent source of alternative therapy (Ojatula and Ikuesan, 2022).

Most of the natural products isolated from medicinal plants are secondary metabolites which include alkaloids, tannins, flavonoids and phenols (Harvey, 2001). Some of the products have nutritive value, anti-diabetic, anti-malarial, anti-fungal, anti-bacterial and against many other diseases (Prasad and Bisht, 2011). Medicinal plants have been used extensively as a source for numerous active constituents for treating human diseases and they as well, have high content of therapeutic value (Fans, 2008). From ethnomedicinal accounts, it has been revealed that holistic healing is found and recorded in the use of natural substances, particularly of plant

origin, for preventive and curative potentialities. This is as a result of the phyto-compounds endowed with medicinal plants at diverse range of concentrations. One of the many medicinal plants possessing such healing potentials as indigenously claimed by the Nigerian practitioners of traditional medicine is *Cymbopogon citratus.*

Natural therapeutic substances of plant origin typically contain several different pharmacologically active compounds that may act individually or synergistically to improve health as well as play significant role in the functioning of the body and growth (Afolayan and Jimoh, 2009). Traditionally, these natural substances of plant origin, are used as food as well as medicines (Ubwa *et al*., 2014). Many ethnobotanical studies suggested that medicinal plants play major role in maintenance of life, especially of the rural community as they are used as drugs and food (Verma and Kaushal, 2014).

Researcher enthusiasm lies to re-examine each plant with a new approach regarding their probable use for food and medicine. Primary metabolites such as carbohydrate, proteins, vitamins, sterols and lipids occur in plants and provide food with nutrition (Verma and Kaushal, 2014), while secondary metabolites, such as phenols, flavonoids, tannins, alkaloids, terpenoids, lignin, quinones, coumarins and amines are the best antioxidants (Zheng and Wang, 2001). Similarly, many wild or domesticated plants provide essential biochemical and energy, besides supplementary resources of vitamins and minerals that sustain the suitable physiological equilibrium of the body. However, it has been reported that sometimes nutritional potential of uncultivated plant species is superior compared to the cultivated variety (Ebert, 2014).

Cymbopogon citratus (Dc.) Stapf. is an important plant that has been in use since ancient times for their local recipes. *Cymbopogon citratus* (Lemon grass) belonging to the family *Gramineae* is an aromatic coarse perennial herb of 1.5 m high with rhizomes and densely tufted fibrous root (Adekomi *et al*., 2012). It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Omotade, 2009). It is a fast-growing aromatic grass native to South India and Sri Lanka, now widely cultivated in other tropical and subtropical countries of Africa, America and Asia (Chanthal *et al*., 2012). The studied plant had been ethnomedicinally reported to have diverse medicinal uses (Erhabor *et al*., 2015; Ojatula and Ikuesan, 2022). Freshly cut and partially dried leaves are used extensively in Ayurvedic medicine. The grass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, fever, hypertension, leprosy, malaria, ophthalmic, pneumonia, nervous, gastrointestinal and vascular disorders (Karkala and Bhushan, 2014). Studies indicated that *Cymbopogon citratus* possesses various pharmacological activities such as antioxidant (Hanisa *et al*., 2011; Garg *et al*., 2012) and antibacterial properties (Danlami *et al*., 2011). The studied

plant contains bioactive components like alkaloids, saponins, flavonoids, and tannins. The effects of the flavonoid content in *Cymbopogon citratus* leaves include anti-inflammatory, antimicrobial and antiviral properties which have great potential as phytopharmaca for ameliorating health challenges in human. Certain flavonoids contain active components to be antimicrobial and antiviral (Kaewseejan *et al*., 2015). The presence of tannins in natural compound/substances of plant origin are found exhibiting the potential as an antibacterial (Kaewseejan *et al*., 2015).

The purpose of the study was to analysed and as well clarify the bioactive phytocompounds of *Cymbopogon citratus* leaves, keeping in mind its ethnomedicinal usage. It is hoped that the study would culminate in the discovery and development of plant-based therapies to combat various diseases and disorder among humans, animal inclusive.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were procured from certified suppliers (Sigma-Aldrich GmbH, Sternheim, Germany) and were of the highest analytical standard.

Plant Material and Authentication

Fresh leavess of *Cymbopogon citratus* (Lemon grass) was collected from Igboegunrin Community in Ilaje Local Government Area of Ondo State, Nigeria. A voucher specimen number OAUSTECH/104 was assigned to the sample after authentication at the Herbarium of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria and then deposited at the Herbarium Section of Botany Unit of the University.

Sample Preparation

Fresh leavess of *Cymbopogon citratus* were cut and separated from the plant's stem, rinsed in water, and spread out on laboratory tables to dry at room temperature. The plant material was then transferred to a 40 °C oven for 10 minutes, where it was reduced to fine powder with the help of a mechanical grinder.

Extraction of Plant Material

Powdered plant material of 200 g was macerated in 1 liter of aqueous for 48 hours. The mixture was tapped with porcelain cloth before being filtered with No. 1 Whatman filter paper. Before further testing, the filtrate was concentrated using a rotary evaporator and the raw

concentrate was deposited at 4 ^{o}C in a refrigerator for further analytical use.

Spectroscopic Phytochemical Analysis

Determination of total alkaloid content

To a portion (1 cm^3) of the extract was added with 5 cm^3 of phosphate buffer (pH 4.7) and 5 cm^3 Bromo Crystal Green (BCG) solution and the mixture was shaken with 4 cm^3 of Chloroform. The extract was collected in a 10 cm^3 volumetric flask and is diluted to makeup the final volume with Chloroform. The blank was prepared as above but without the extract and the absorbance of the complex in chloroform was measured at 470 nm against the blank. Atropine was used as a standard to generate the atropine standard curve which was used to determine the atropine equivalence of the fraction.

Blank solution: A portion, 5 cm^3 of pH 4.7 phosphate buffer and 5 cm^3 of BCG solution was mixed and then extracted with 5 cm^3 of chloroform. Extract was collected in 10 cm^3 volumetric flasks and then adjusted the volume to the mark with chloroform. A standard curve of absorbance against concentration of Atropine was plotted and used for estimation of the Atropine equivalence (AE) of test sample. This was determined according to the method described by Trease and Evans (1989).

Determination of flavonoids content

The total flavonoid content of the plant extracts was determined using Aluminium Chloride colorimetric method. Quercetin was used as standard and the flavonoid content of the extracts was expressed as mg of quercetin equivalent /gm of dried extract (Kumar *et al*., 2017). To a portion (1 cm^3) of the plant extract was taken in a test tube which is added with 2 cm^3 of 5% $NaNO_2$ and 3 cm^3 of $AlCl_3$ (10%) was added to this after 5 minutes, the reaction mixture was treated with 2 cm^3 of 1 M NaOH in another 5 minutes and the reaction mixture was made up to 10 cm^3 with water and the absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was taken. For the blank, the extracts were replaced with an equal volume of distilled water. The Flavonoids content in extracts was expressed in terms of Quercetin equivalents. A standard curve of absorbance against quercetin concentration was plotted, and used for estimation of the quercetin equivalence (QE) of test sample.

Estimation of total phenolic content

Total phenolic content of samples was determined employing the method involving the

use of Folin-Ciocalteu reagent (FCR) as oxidizing agent and with Gallic acid as standard. Preparation of blank solution: In a 20 cm^3 volumetric flask 1.5 cm^3 Folin Ciocalteau reagent, 1 cm^3 distilled water and 4 ml 20 % sodium carbonate was mixed in a 20 cm^3 volumetric flask. A portion (1 cm^3) of test sample solution and the various concentrations of the gallic acid standards were placed in different test tubes. To each of the test tubes, 1 ml of distilled water and 1.5 cm^3 Folin Ciocalteu's reagent was added, the mixture was covered with aluminium foil and allowed to incubate at room temperature for 5 minutes. Afterwards, 4 cm^3 of 20 % (w/w) Na^2CO^3 was added to each of the test tube, the mixtures were agitated and placed in a water bath at a temperature of 40 ^oC for 30 minutes. The test tubes were placed in ice water to quench the reaction. The absorbance of the test samples and standards at 765 nm using UV/VIS spectrophotometer against blank was measured.

Determination of total steroids content

A portion (20 mg) of AD-E was suspended in chloroform, covered and heated at 60 ^oC for 30 minutes in water bath with shaking. The suspension was filtered. The resultant marc was thereafter, extracted with 20 cm3 of chloroform and filtered. The volume of the combined filtrate was adjusted to 50 cm^3 with same solvent (chloroform). To 10 cm^3 volumetric flasks, 5 cm^3 of combined filtrate was transferred and 2 cm^3 of Liebermann-Burchard (LB) reagent was added. The volume was adjusted with chloroform. The absorbance was measured using a UV/VIS spectrophotometer 5 min after the addition of the reagent LB at 625 nm wavelength. Preparation of blank: In 10 cm^3 volumetric flask 5 cm^3 of chloroform, 2 cm^3 of LB was added and adjusted the volume to the 10 cm^3 mark with chloroform. It was allowed to stand for 5 minutes and the absorbance was measured at 625 nm.

Determination of saponin content

The total flavonoid content of the plant extracts was determined using Aluminium Chloride colorimetric method. Quercetin was used as standard and the flavonoid content of the extracts was expressed as mg of quercetin equivalent /gm of dried extract (Kumar *et al*., 2017). To a portion (1 cm^3) of the plant extract was taken in a test tube which is added with 2 cm^3 of 5 % $NaNO_2$ and 3 cm^3 of $AlCl_3$ (10 %) was added to this after 5 minutes, the reaction mixture was treated with 2 cm^3 of 1 M NaOH in another 5 minutes and the reaction mixture was made up to 10 cm^3 with water and the absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was taken. For the blank, the extracts were replaced with an equal volume of distilled water. The saponins content in extract was expressed in terms of Quercetin equivalents. A standard curve of absorbance

against quercetin concentration was plotted, and used for estimation of the quercetin equivalence (QE) of test sample.

Estimation of tannins content

The use of Folin-Ciocalteu reagent (FCR) as oxidizing agent and with Gallic acid as standard was employed for the determination.

Preparation of blank solution: In a 20 cm^3 volumetric flask 1.5 cm^3 Folin Ciocalteau reagent, 1 cm^3 distilled water and 4 ml 20 % sodium carbonate was mixed in a 20 cm^3 volumetric flask. A portion (1 cm^3) of test sample solution and the various concentrations of the gallic acid standards were placed in different test tubes. To each of the test tubes, 1 ml of distilled water and 1.5 cm^3 Folin Ciocalteu's reagent was added, the mixture was covered with aluminium foil and allowed to incubate at room temperature for 5 minutes. Afterwards, 4 cm^3 of 20 % (w/w) Na_2CO_3 was added to each of the test tube, the mixtures were agitated and placed in a water bath at a temperature of 40 ^OC for 30 minutes. The test tubes were placed in ice water to quench the reaction. The absorbance of the test sample and standard at 765 nm using UV/VIS spectrophotometer against blank was measured.

Gas Chromatography-Mass Spectroscopy Analysis

The aqueous extract of the leaves was analyzed using Gas Chromatography Mass Spectroscopy for the identification of the phytochemical compounds present. A solvent blank analysis was first conducted using 1 µl of ethyl lactate. Then 1 µl of the reconstituted aqueous extract solution was employed for GC-MS analysis as previously described with modifications by (Paranthaman *et al.*, 2012). GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100 % Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999 %) as carrier gas at a constant flow of 1ml/ minute and a sample injection volume of 1 µl which was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 minutes), with an increase of 10 °C/minute, to 200 °C, then 5 °C/minute to 280 °C, ending with a 9 minutes isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550 Da. Total run time was 30 min.

Identification of phytocompounds

The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV

and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library using NISP Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak height and data processing were carried out by Turbo-Mass - OCPTVS-Demo SPL software.

RESULTS AND DISCUSSION

Spectroscopic Quantitative Phytochemicals Detected in Extract

Results on **Table 1** reveal the total amount of phytochemicals detected in the extract of *Cymbopogon citratus*. The analysis revealed the presence of a variety of plant secondary metabolites such as alkaloids, saponins, tannins, steroids, flavonoids, and phenols in varying concentrations. The extract of the studied plant material showed higher concentrations of secondary metabolites The presence of various secondary class metabolites identified which are bioactive constituents puts these results in line with GC-MS analysis that was carried out, the phytochemical analysis showed that it contains secondary metabolites compounds group: alkaloids (64.18 mg/g), flavonoids (58.13 mg/g), phenols (48.18 mg/g), saponins (68.28 mg/g), tannins (62.14 mg/g) and steroids (63.78 mg/g). They are noted to be defense chemical compounds of plants produced in the plant tissue (Komansilan *et al*., 2012). The studied plant could thus be used for the management of various healthy conditions associated with the metabolites screened.

Table 1. Total amount of phytochemical in aqueous leaves extract of *Cymbopogon citratus*

Plant fraction	Alkaloids mg/g	Steroids mg/g	Flavonoids mg/g	Phenols mg/g	Saponins mg/g	Tannins mg/g
C. citratus aqueous leaves extract	64.18	63.78	58.13	48.18	68.28	62.14

The concentrations of phenols and flavonoids contributed the anti-oxidant activities of the plant extract as reported by Mehmood *et al*. (2022), and this may be ascribed to the presence of phenolic plant secondary metabolites, thereby suggesting that polyphenol compounds remain the primary contributors to the antioxidant ability possessed by *Cymbopogon citratus* leaves. Bach and Rohmer (2012) reported that saponins, which was reportedly found in the

extract of the studied plant material, inhibit Na^+ efflux by blockage of the influx of concentration in the cells, activating a Na+ - Ca_2^+ antiporter in cardiac muscles and further said the increase in Ca_2^+ influx through this antiporter strengthens the contraction of heart muscles. The valuable pharmaceutical properties of *Cymbopogon citratus* may also be attributed to the presence of alkaloids which Heinrich *et al*. (2021) reported to possess a stimulating effect, act as topical anesthetic in ophthalmology, efficient pain reliever, antipiuretic and other medicinal functions. High flavonoids content as reported in this research study, with other antioxidant vitamins and enzyme may help provide protection against oxidative stress induced diseases thereby contributing to the total antioxidative defense system of the human body. This assertion is based on the reported studies of Ullah *et al*. (2020) who attributed antioxidant properties of medicinal plants to presence of flavonoids and may account for the high lipid peroxidation inhibition. The medicinal potentials of the herbal plant may be attributed to the present metabolites in appreciable quantum concentrations.

Gas Chromatography-Mass Spectrometry Analysis

The use of herbs and other medicinal plants as remedies for diseases in both human and animals is as old as man himself. Through the nominal elucidation of active components of medicinal plants; novel bioactive compounds have been discovered and subsequently modified structurally to improve activity or reduce adverse effects (Cragg *et al*., 2013; Ezealisiji and Nwoka, 2020). Using GC-MS analysis (**Figure 1**), 23 phytcompounds possessing therapeutics potentials have been elucidated (**Table 2**). The major constituents were three (3) compounds with percentage peak height of 23.20 % (1- Nonadecane), 15.88 % (1-Pentadecane) and 11.48 % (Behenic alcohol), other constituents less than ten percentage peak area are 4.51% (1-Dodecanol, 3.56 % (2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-d), 5.17 % (Isopropyl myristate), 4.27 % (n-hexadecanoic acid), 6.49 % (Behenic alcohol), 4.91 % (Oleic acid), 4.25 % (propanoic acid, decyl ester), 2.89 % (Octacosanol), 3.07 % (Bis(2-ethylhexyl) phthalate while other constituents were less than 2% as in **Table 2**.

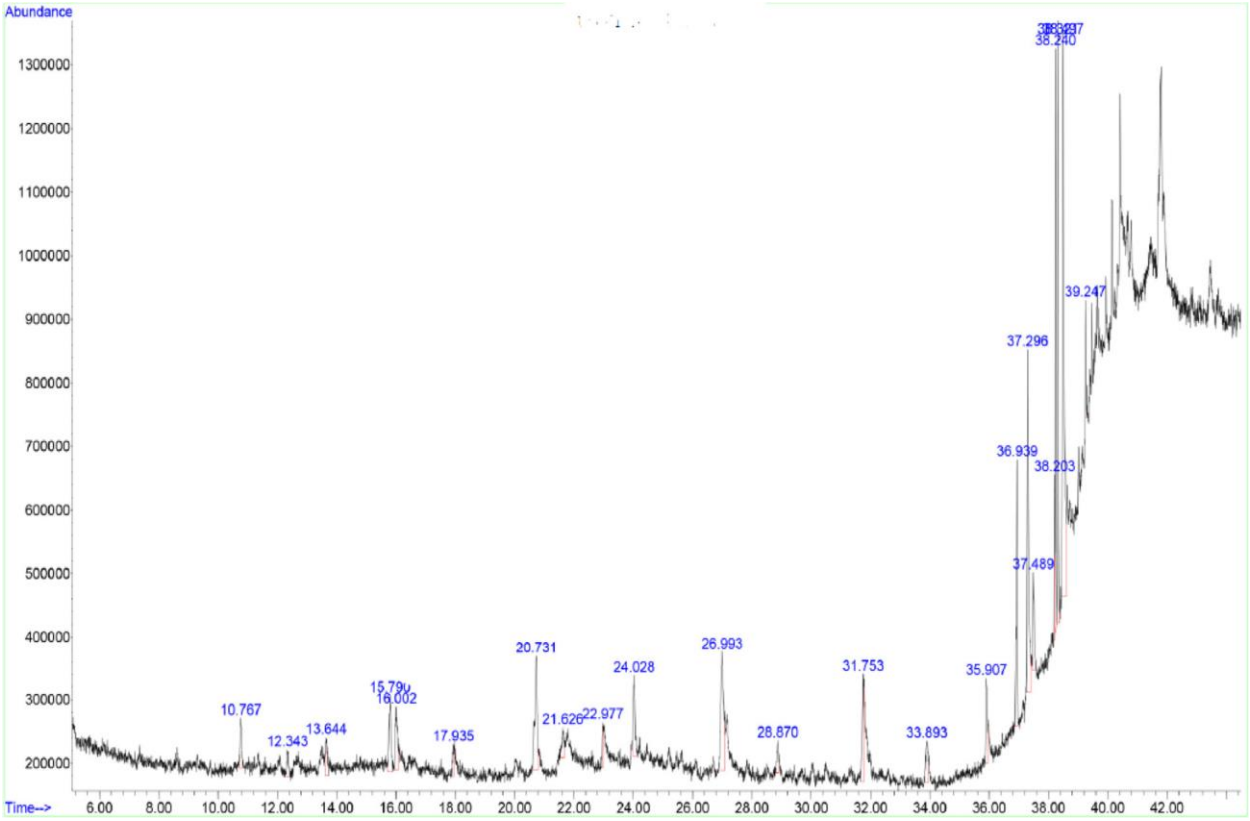

Figure 1: Total ion chromatogram (TIC) of aqueous leaves extract of *Cymbopogon citratus*

The bioactive constituents identified include unsaturated hydrocarbon, acids, alcohols and esters which are bioactive compounds; of-which medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body (Ojatula, 2021). For example, Squalene, phytol and some other compounds identified in the plant material of the studied plant, has been reported as viable antioxidant, antitumor, cancer-preventive, cardio-protective, and immunostimulant (Ezealisiji and Nwoka, 2020; Ojatula and Nwanja, 2023). Also, 1-Dodecanol is a biologically active compound, as it serves as a membrane stabilizer, energy source, energy storage and nutrient (Ezealisiji and Nwoka, 2020); and it has been shown to possess antimicrobial property (Ezealisiji and Nwoka, 2020).

Table 2: Peak report of the total ion chromatogram (TIC) of aqueous leaves extract of *Cymbopogon citratus* by GC-MS.

Peak #	R. Time	A/H Name	Height	Height %	Area	Area %
1	4.030	1-Decene	304305	0.80 1.41	429770	0.60
2	8.069	1-Dodecanol	1717607	4.51 2.95	5062684	7.05
3	10.105	Tridecane, 6-methyl-	406068	1.07 2.13	863685	1.20
4	11.581	1-Pentadecene	6043116	15.88 1.85	11151287	15.53
5	11.700	Tetradecane	299888	0.79 1.79	537510	0.75

6	12.640	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-d	1354662	3.56 1.63	2202321	3.07
7	13.249	2,4-Di-tert-butylphenol	429091	1.13 1.62	693251	0.97
8	14.310	1-Nonadecene	8827926	23.20 1.53	13472983	18.77
9	17.047	Isopropyl myristate	1967375	5.17 1.81	3562988	4.96
10	18.251	(2,3,5,6-Tetrafluorophenyl) methyl 3-(2,2-dic	386472	1.02 1.97	760399	1.06
11	19.027	Dibutyl phthalate	311290	0.82 1.90	590938	0.82
12	19.100	n-Hexadecanoic acid	1624151	4.27 2.30	3734095	5.20
13	19.564	Behenic alcohol	4369082	11.48 2.05	8940135	12.45
14	20.152	9-Tricosene, (Z)-	321208	0.84 2.18	701542	0.98
15	20.739	Behenic alcohol	2471531	6.49 2.07	5120445	7.13
16	21.371	Oleic Acid	1595334	4.19 2.49	3965076	5.52
17	22.138	Acetic acid n-octadecyl ester	264707	0.70 2.25	596178	0.83
18	22.191	Phytol	251992	0.66 1.64	413338	0.58
19	23.097	Propanoic acid, decyl ester	1615448	4.25 1.65	2670923	3.72
20	24.007	Octacosanol	1101210	2.89 1.70	1870716	2.61
21	25.215	Bis(2-ethylhexyl) phthalate	1169938	3.07 2.04	2386584	3.32
22	25.734	Hexacosanol	508993	1.34 1.80 1-	916012	1.28
23	27.414	Squalene	712458	1.87 1.62	1151987	1.60

Different phytochemicals have been found to possess a wide range of bioactivities, which may help in protection against chronic diseases. For example, Alkaloids protects against degenerative/chronic diseases such as arthritis, stroke and lots more, while saponins protect against hypercholesterolemia, and found exhibiting antibiotic properties (Akindele and Adeyemi, 2007). Steroids and triterpenoids were reported exhibiting analgesic properties. The Steroids and saponins exhibits central nervous system activities (Aiver and Kolammal, 1962). Flavonoids have been referred to as nature's biological response modifiers, because of its inherent ability to modify the body's reaction to allergies and virus, and as well possessing anti-inflammatory, anti-microbial and anti-cancer activities (Argal and Pathak, 2006).

CONCLUSION

The present study revealed presence of quantum concentrations of biochemical compounds in leaves of the studied herbal plants, which may therefore, justify both its nutritional and ethnomedicinal application to health of mankind; of which medicinally valued bioactive components like, flavonoids, steroids, alkaloids and phenolic compounds are liable being precursors in the synthesis of useful drugs. The GC-MS analysis revealed 23 compounds in which all were identified with three major peaks height of 15.88 % (1-pentadecene), 23.20 % (1-nonadecene) and 11.48 % (behenic alcohol) while the others were less than 10 %. Thus signifies, therapeutically diversified bioactive constituents in *Cymbopogon citratus* leaves, where their quantum presence in natural compound of plant origin will be very resourceful for the manufacturing of new drugs for treatment/management of various diseases in humans, animal inclusive.

COMPETING INTERESTS

Authors declared none competing interest

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