COMPARATIVE PHYTOCHEMICAL, GC-MS, PROXIMATE, MINERALS AND VITAMINS COMPOSITION OF *GONGRONEMA LATIFOLIUM* AND *OCIMUM GRATISSIMUM* LEAF EXTRACTS.

ABSTRACT

Gongronemalatifolium and *Ocimumgratissmum*have been reported to contain phytochemicals, minerals and vitamins and these offer a great opportunity for the development of new types of therapeutics.The comparative qualitative phytochemical, gas chromatography-mass spectroscopy (GC-MS), proximate, vitamin and mineral elementcompositions of ethanol leafextracts of *Gongronemalatifolium*(GL) and *Ocimumgratissimum* (OG) was studied. The leaves of GL and OG were collected, identified, washed, sliced, air-dried and pulverized into powder.The powdered sampleswereweighed and macerated in 95% absolute ethanol for 72 hours. They were filtered and the filtrate was concentrated to dryness using a water bath at 40°C. The samples were analyzed, for phytochemical, GC-MS, proximate, vitamin and mineral compositions using standard methods. The qualitativeresults of the leaves of GLandOGshowed thatthe plant leaves contain alkaloids, saponins, tannins, flavonoids, phenols and cardiac glycosides. The GC-MS analysis of ethanol leaves of GL and OG revealed that the plantscontain 38 and 37 bioactive compounds respectively.Comparatively, the moisture content of OG (19.30 ± 0.20) was significantly higher ($P<0.05$) than GL(16.97 ± 0.52).The protein composition of OG was significantly higher ($P<0.05$) (17.18 ± 0.22)than that of*GL* (2.43 ± 0.14).Similarly, the lipid composition of GL was significant higher ($P<0.05$)(5.96 ± 0.11)than that of OG (2.34 ± 0.17). Ash composition of OGwas significantly higher($P< 0.05$)(14.87 ± 0.47) than that of GL (9.65 ± 0.20).Also, fibre content was significantly higher ($P<0.05$) in OG (14.87 ± 0.47)compared to GL(9.91 ± 0.24|). This result revealed also revealed higher percentage composition of carbohydrates (53.05 ± 1.27) and energy(343.77 ± 1.50) in OGcompare to GL(40.33 ± 0.95) and (284.51 ± 2.91).Higher mineral compositionssuch as calcium, phosphorus, magnesium was seen in OG compared to GLand a significantly higher in sodium in GLcompared to OG. Iron and Lead was found to be present only in GL. The vitamins of GLand OGwas also significantly high($P<0.05$).From the results of this study, it can be concluded that GL and OG have rich bioactive constituents which can be employed in the formulation of novelty drugs.

Keywords:*Gonglonemalatifolium, Ocimumgratissimum*, Proximate composition, Phytochemicals, Vitamins, GC-MS

INTRODUCTION

Medical plants are useful in the treatment of ailments as it containsactive metabolites. Most studies report the ability of medicinal plants to fight against diseases is due to their bioactive metabolite such as alkaloids, flavonoids, tannins, saponins and terpenes which exert biological effects on the body(16). The plants can be used singly or combined to treat ailments. Most plants have both nutritional and therapeutic benefits. *Gongronemalatifolium* and *Ocimumgratissimum* have these properties.

Gongronemalatifolium and *Ocimumgratissmum* have been reported to be used for several diseases as the contains some of these phytochemicals. Both plants are herbaceous shrub. They are commonly grown in gardens in Calabar, Cross River State, and Akwa Ibom State, Nigeria.

Gongronemalatifolium is plant that belongs to the family Asclepiadae and is locally called Utazi by the Efiks, Ibibio and Quas by Igbos. It is commonly used as spice and vegetable in the traditional folk practice (33).Traditionally, *Gongronemalatifolium* is used in the treatment of malaria, diabetes, constipation and hypertension as well as laxative in the Southern part of Nigeria (16).

Ocimumgratissimum is commonly known as clove basil, sweet basil, tea bush, scent leaf or fever plant and it is a native to Africa, Madgascar and Southern Asia.Locally, *Ocimumgratissimum*is called Ntoñ by the Efiks and Ibibios, Daidoya by the Hausas, Nchwunwu by the Igbos and Efirinby the Yorubas.*Ocimumgratissimum* is used nutritionally in spicing food in most part of Nigeria (16) and it is also used as folk medicine for the treatment of many disease conditions such as respiratory disorders, cough, fever, sore throat, kidney stones, epilepsy, dermatitis, headache, stress and mental diseases. Most studies reports that *Ocimumgratissimum* contain important bioactive constituents such as alkaloids,tannins, phytas, oligosaccharides and flavonoidsand accounts for its high medicinal use.

There are no sufficient studies on the comparative chemical composition of these leaves extracts reported. Therefore, this study investigated the comparative qualitative phytochemical, GC-MS, proximate, minerals and vitamins,analysis of 95% ethanolleaf

extracts of *Gongronemalatifolium* and *Ocimumgratissimum* in a view to obtain information on their usage in human and disease treatment.

MATERIALS AND METHODS

Materials

Chemical

A 95% Ethanol (Sigma Alrich Chemicals) was purchased from a pharmaceutical shop in Uyo Metropolis.

Equipment

Weighing balance (Kerro, Model BL-3002), water bath, chopping board, knife, electric blender, What-Man No.1 filter paper

Collection and preparation of Plant materials

Fresh leaves of *Gongronemalatifolium*and *Ocimumgratissimum*were purchased from Itam Market in Itu Local Government Area. The purchased plant leaves were wrapped and taken to the taxonomist at herbarium unit of Botany and Ecological Science Department, Faculty of Biological Science, University of Uyo,Uyo, for identification.

After identification, the fresh leaves were washed to get rid of debris, sliced and air dried for two weeks. 200g of the dried leaves each were then pulverized into powder using an electronic blender. The powdered samples were weighed and macerated in 95% absolute ethanol (Sigma Aldrich) which was obtained from at a chemical shop in for 72 hours. The solvents were then filtered off to obtain the extracts. The extracts obtained were concentrated to dryness using a water bath at a temperature of 45^0C. The dried extracts were then stored in the refrigerator.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Determination of Alkaloids

Alkaloids was determined by adding few drops of Wagners reagent into2ml of the ethanol extracts. A reddish brown precipitate formed, indicating the presence of alkaloids.

Determination of Flavonoids:

Twenty percent (20%) NaOH was added in few drops to 2ml of each extract.A yellow color was observed. Then, 70% dilute HCl was added in few drops and the yellow color disappeared. The flavonoids presence was determined by the formation and disappearance of colors.

Determination of Saponins

Each extract (2ml) was mixed with 6ml of distilled water and shaken thoroughly. Bubbles or foams formed, indicating the presence of saponins.

Determination of Tannins

10% alcoholicferric chloride was added to 1ml of each extract. A blue/blackcolor was formed, indicating the presence of tannins.

Determination of Phenols

1ml of 5% aqueous ferric chloride was added to 1ml of each extract. A blue color formed, indicating the presence of phenols.

Determination of Cardiac Glycosides

0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride was added to 1ml of each extract. A brown ringformedat the edge, indicating the presence of cardiac glycosides.

Determination of Terpenoids

0.5ml of chloroform and a few drops of concentrated sulphuric acid were added to 1ml of each extract. A reddish-brown precipitate appeared. This indicate the presence of terpenoids.

Determination of steroids

About 0.5g of each extract was dissolved in 2ml of chloroform and was filtered. 2ml of concentrated sulphuric acid was added to the filtrate. A reddish-brown color was formed at the interphase,indicating the presence of steroidal ring.

Determination of Anthraquinones

Into 0.5g of each extract was added 10ml benzene and was shaken. Then 5ml of 10% ammonia solution was added and thoroughly shaken. A pink-red or violent color was formedat the ammoniacal (lower) phase, indicating the presence of anthraquinones.

GAS CHROMATOGRAPHY- MASS SPECTROSCOPY

The samples were analyzed using agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC-MS system which are as follows: Hp 5-MS capillary standard non-polar column, dimension: 30M, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programmed (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1µl. Samples dissolved in methanol were run fully scan at a range of 40-650 m/z and the results were compared by using Nist mass Spectral library search programmed.

PROXIMATE COMPOSITION DETERMINATION

Determination of Crude Moisture

Two grams of the samples were added to the empty moisture dish and placed in an air oven. The two samples were dried in the hot air-drying oven at 1100C for 24hours. The samples were then kept in a desiccator and allowed to cool after which the crucible with the dry samples were then weighed and returned to the oven for further 24hours to make sure that the drying was completed. The weights were taken again, for each sample.

The moisture was determined using the following formula:

$$\% = \text{Moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where;

W0= weight of empty dish

W1= weight of fresh sample

W2= weight of dry sample

Crude Protein Determination

To estimate crude protein, it involves the determination of total nitrogen. The amount of crude protein was obtained by multiplying the nitrogen content by a factor of 6.25. A gram of each extract was digested by boiling in 10ml concentratedtetraoxosulphate (VI) acid, with the use of selenium as a catalyst. Boiling was done under a fume cupboard until a clear solution was obtained. The digested extracts were diluted to 100ml with distilled water. A portion of each digest (10ml) were mixed with equal volume of 45% NaOH solution in the Macham distillation apparatus. On distillation, the distillates were collected into 10ml each of basic acid solution, containing three drops of mixed indicators (methyl red and bromocresol green). A total of 50ml distillates each were collected and titrated against 0.02N H_2SO_4 solution. Titrations were done from green to deep blue point. A reagent blank was also run and titrated.

The moisture was determined using the following formula:

$$\% \text{ crude protein} = N_2 \times 6.25$$

$$\%N_2 = \frac{100}{W} \times \frac{14 \times N}{1000} \times \frac{V_f}{V_a} \times T\text{-}B$$

Where,

W= weight of sample analyzed

N= normality of titrant (H_2SO_4)

V_f= Total volume of dissolved extracts

Va= Volume of dissolved extracts distilled

T = Titre value of samples

B = Titre value of reagent blank.

Crude Lipid Determination

"A cleaned and dried round bottom flask was weighed W_1. Each of the dried samples (2g) were placed into a thimble and finally placed into a soxhlet extractor. A quantity petroleum spirit was added into the soxhlet extractor. The extraction went on for 6hours, then the apparatus set up was disconnected and the round bottom flask was dried for 10minutes and cooled in a desiccator and reweighed W_2. The difference in weight was used to calculate the percentage of lipid" (24).

Determination of Crude Fibre

Two grams of the extracts were weighed and put into 1litre control flask. Then 200ml of $H_2SO_4$was added to each extract, boiled gently for 30minutes, filtered through a poplin cloth, stretched over 9cm Buchner funnel and mixed well with hot distilled water. The two samples were taken back into the flask with spatula and 100ml of boiling 1.25% NaOH were added, boiled gently to maintain a constant volume and filtered through a poplin cloth. The residues were washed thoroughly with hot distilled water and rinsed once with 10% HCl and twice with industrial methylated ether (BP40-600C) and allowed to dry. The residues were kept overnight at 105^oC in the oven, and cooled in a desiccator. The two samples were weighed again and ashes at 550°C for 90 minutes in a muffle furnace and weighed again.

The crude fibre was determined using the following formula:

% Fibre = Dry weight –Ash weight x 100

Determination of Carbohydrates

The carbohydrate contents were determined as a difference between crude protein, sum of crude ash, lipid and crude fibre (6).

Formula:

NFE = 100% (%Ash + %crude lipid + Crude fibre + %Crude protein)

MINERAL ANALYSIS

Mg, Fe, Cu, Pb, Ca, andP wereanalyzed using atomic absorption spectrophotometer (AAS)while Na and K were analyzed using flame photometer. Using AAS, the ash solutions of the plant samples were prepared by weighing 5g each of the powdered plant samples andashed at 550°C in muffle furnace for 5hrs, and the residues were dissolved in 100 ml of deionized water. Suitable salts of the metals were used to make their standards, lamps were fixed. The standard mineral solutions were injected to calibrate the AAS using acetylene gas. An aliquot of ash solutions was injected and the concentrations obtained from the AAS. Using the flame photometer, the diluents of sample was aspirated into the Jenway Digital flame photometer using the filter corresponding to each mineral element.

VITAMINS ANALYSIS

Determination of Vitamin B1 (Thiamin)

5g of the sample extracts were homogenized with ethanol mixture (50 ml) and filtered into a 100 ml flask. The filtrates (10 ml)were pipetted and 10 ml of potassium dichromate was added. The absorbance was read at 360 nm in a spectrophotometer. A blank sample was prepared and the color also developed and read at the same wavelength.

Determination of Niacin

"5 g of each samples were treated with 50 ml of 1 N sulphuric acid and shaken for 30 minutes. Ammonia solution (three drops) was added to each sample and then filtered. Afterwards, 10 ml of each filtrate were added into different volumetric flask (50 ml) and 5 ml of 0.02ML H_2SO_4absorbance were measured in the spectrophotometer at 470nm" (23).

Determination of Riboflavin

"5 g of each sample were extracted with 100ml of 95% ethanol solution and shaken for 1 hour. These mixtures were filtered into a 100 ml flask, while 10 ml of the extracts were poured into 50 ml volumetric flask. 10 ml of the 5% potassium permanganate and 10ml of the 30% H_2O_2 were added and allowed to stand over a hot water bath for about 30 min. Furthermore, 2 ml of the 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance was measured at 510 nm in a UV/visible spectrophotometer (UV-1601 SHIMADZU)" (23).

Determination of Vitamin E (Tocopherol)

Spectrophotometrically, vitamin E contentswere determined using a modified standard method of AOAC (3) .1.5 ml of plant extract, 1.5 ml of the standard and 1.5 ml of water were pipetted out separately into 3 stoppered centrifuge tubes. 1.5 ml of ethanol and 1.5 ml of xylene were added to all the tubes. The mixtures were stirred properly and centrifuged at 300 rpm. Xylene (1.0 ml) layer was transferred into another stoppered tube. 1.0 ml of dipyridyl reagent was added to each tube andshaken properly. The mixtures (1.5 ml) were pipetted out into a cuvette and the extinction was read at 460 nm (A460). Ferric chloride solution (0.33 ml) was added to all the tubes and mixed well. The red color developed was read exactly after 15 minutes at 520 nm (A520) in a visible spectrophotometer.

Determination of Vitamin C (AscorbicAcid)

A method described by Baraleef*et al*. (7) and tritrimetric method described by (23) were used in vitamin C determination. A gram of the test samples was extracted using 50ml of 6% EDTA/TCA extractant solution. The mixtures were thoroughly shaken and allowed to stand for 20 minutes at room temperature. Then were filtered and filtrates were treated with 20ml of 30% KI solution. 10ml of distilled water was added to each mixture. 1ml of 1% starch solution was added and titrated against dilute Cu SO_4 solution.Calculation of the vitamin C content was based on the relationship that 1ml of 0.008M $CuSO_4$ is equivalent to 0.088mg Vitamin C, hence, the formula:

$$\text{Vitamin C (mg/100g)} = 100 \times 0.088 \times \frac{\text{Titre}}{W}$$

Where W= is weight of the sample

Determination of Vitamin A

Vitamin A was determined spectrophotometrically using a method described by AOAC (3). The extracts (0.5g) were homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide in a water bath at 60°C for 30 minutes. The saponified extracts were transferred to a separating funnel containing 10-15ml of petroleum ether and mixed properly. The lower aqueous layers were transferred to another separating funnel while the upper petroleum ether layer which contains carotenoids were collected. The extractions were repeated until the aqueous layers became colorless. Anhydrous sodium sulphate was added into petroleum ether extracts in small quantity to remove excess moisture. The volume of the final petroleum ether extractswas taken note. The absorbance of the yellow color was read in a visible spectrophotometer at 450nm using petroleum ether blank

RESULTS

The results of the phytochemical, GC-MS, proximate, minerals and vitamins compositions of 95% ethanol leaf extracts of *G.latifolium and O.gratissium*obtained in this study are presented in tables 1-5. The qualitativeresults of the leaves of *Gongronemalatifolium and Ocimumgratissium*showed thatthe plant leaves contain alkaloid, saponin, tannin, flavonoids, phenol and cardiac glycosides.Different phytochemical/bioactive compounds of the ethanolic extracts of *Gongronemalatifolium*and*Ocimumgratissium*were analysis using GC-MS. The chromatograms in Figure 1 and 2 and summarized in Table 2.a and.2.b.

Table 2.a and Figure 1 reveals that ethanol leaf extract *Gongronemalatifolium*contain 38 bioactive compounds. This result revealed that the percentage of bioactive compounds present in this leaf extracts are Butylated Hydroxytoluene (39.86%), Octadecanoic acid, propylester(35.479 %), 2-Amino-4-(4-cyclohexyl-phenyl)-nicotinonitril(17.85%), Hexadecanoic acid, methyl ester(4.26%), n-Propyl 11-octadecenoate (3.96%), Dodecanoic acid, propyl ester(3.01%), Phytol(2.65%), 9-Octadecenoic acid (Z)-, methyl e ster (2.33%), 9,12,15-Octadecatrien-1-)-(2.20%), 1-Docosen (1.71%), Dibutyl phthalate (1.68%), 2-Pentadecanol (1.66%), Tetradecanoic acid, propyl ester (1.58%), Oleic Acid(1.59%) , 1-Docosene(1.54%), 9-Eicosene, (E)-(1.27%), trans,trans-9,12-Octadecadienoic acid, propyl ester (1.24%), Hexadecanoic acid, methyl ester (1.18%), Hexadecanoic acid, ethyl ester (1.10%), 2-Methyl-Z,Z-3,13-octadecadienol (0.98%), 9-Eicosene, (E)-(0.83%), 17-Pentatriacontene(0.69%), Cyclooctane, methyl-(0.66%), D-Mannitol, 1,2:5,6-bis-O-(1-methyl(0.65%), 9-Eicosene, (E)-(0.61%), D-Limonene (0.59%), Acetic acid, chloro-, hexadecyl ester(0.49%), Cyclotetracosane(0.47%), Pentadecafluorooctanoic acid, octadecyl ester(0.47%), Methyl 8,10-dimethyl-hexadecanoate (0.45%), Carbonic acid, eicosyl prop-1-en-yl ester(0.32%), Cyclotetradecane, 1,7,11-trimethyl -4-(1methyl(0.22%),Cyclopentadecane(0.20%), Toluene(0.21%), Tetradecane (0.21%), Toluene(0.19%), 3-Eicosene, (E)- (0.15%), 1-Octadecene(0.10%),

Table 2.b and Figure 2 reveals that ethanolleaf extract of *Ocimumgratissium*contain 37 bioactive compounds. This result revealed that the percentage of bioactive compounds present in this leaf extracts are Butylated Hydroxytoluene(63.53%), Dodecanoic acid, propyl ester(9.27%) D-Mannitol, 1,2:5,6-bis-O-(1-methylethylidene)-(4.93%), 9-Octadecene, (E)-(1.91%), Tetradecanoic acid,

propyl ester (1.69%), 1-Octadecene (1.61%), n-Propyl 11-octadecenoate (1.30%), Dibutyl phthalate(1.29%), 14-Octadecenoic acid, methyl ester (1.20%), Hexadecanoic acid, propyl ester (1.16%)Hexadecanoic acid, methyl ester(1.14%), Hexadecane(0.99%), 9,12-Octadecadienoic acid, methyl ester, (E,E)-(0.84%), 2-Piperidinone, N-[4-bromo-n-butyl]-(0.82%), 1-Octadecanesulphonyl chloride (0.45%)Diethyl 2-(2-cyanoethyl)-malonate(0.46%), Carbonic acid, eicosyl prop-1-en-yl ester (0.43%), Heptadecanoic acid, 16-methyl-, methyl ester (0.42%), Oxirane, [(hexadecyloxy)methyl]-(0.38%), Isopropyl linoleate(0.36%), 1-Docosene (0.33%), 2-Piperidinone, N-[4-bromo-n-butyl]-(0.32%), 1-Hexadecanol, 2-methyl(0.32%), Cyclodocosane,ethyl-Butanoic acid, 4-methoxy-, methyl ester (0.31%), Ethyl tetradecyl ether (0.30%), Butylated Hydroxytoluene (0.29%), Cyclotetradecane 1-(0.29%), Octadecene(2.40%), Cyclododecane(0.23%), D-Limonene(0.21%), Octadecanoic acid, propyl ester(0.18%), 13-Tetradecen-1-ol acetate (0.15%), 1-Decanol, 2-hexyl-(0.15%), Tetracosanal(0.02%), 1-Docosene(0.01%), and 2-Piperidinone, N-[4- bromo-n-butyl]-(0.01%) .

The results of the proximate composition of *G.latifolium* and *O.gratissium*is presented in table 3.0 Moisture (%) inGL is 16.97 ± 0.52 compared to OGwhich is 19.30 ± 0.20. Hence, there is an increase ($P<0.05$) in moisture content in OGcompare to GL.The percentage protein and lipid composition of GLare 17.18 ± 0.22 and5.96 ± 0.11 and that of OGare 2.43 ± 0.14 and 2.34 ± 0.17. This shows an increase in % protein composition in GLcompare to OGand an increase in lipid composition in GLcompared to OG. Ash and fibre (%) in GL are 9.65 ± 0.20 and 9.91 ± 0.24 compared to OGwhich are 14.87 ± 0.47 and 14.87 ± 0.47. There is an increase in ash composition of OGcompared to GLand an increase in fibre content of GL compare to OG. The Carbohydrates and Energy (%) in GLare 40.33 ± 0.95and 284.51 ± 2.91compare to OGwhich are 53.05 ± 1.27and 343.77 ± 1.50. This result revealed that there is an increase in Carbohydrates and Energy content in OGcompare to GL.

Table 4.shows the mineral compositionsGL and OG. There is a significant increase($P<0.05$) in sodium, calcium, phosphorus, magnesium in OGcompared to GL and a significant increase($P<0.05$) in sodium in GLcompared to OG. Iron and Lead was found to be present in GL.

Table 5. shows the vitamins of GLand OG. There is a significantincrease (P<0.05) in riboflavin, niacin, tocopherol, vitamin C in GLcompared to OGand an increase (P<0.05) in thiamine, vitamin A in OGcompared to GL.

Table 1.:Qualitative phytochemical compositions of 90% ethanol extracts of *Gongronemalatifolium and Ocimumgratissium*

Compound	GL	OG
Alkaloid	+++	+++
Tannin	+++	+
Flavonoids	+++	+
Phenol	+++	++
Cardiac glycosides	+	++
Saponins	+++	+++

GL= *Gongronemalatifolium*, OG = *Ocimiumgratissium*

Key

+ = Present

+++ = Present in Excess
--- = Absent

Table 2.a: Gas chromatography- mass spectroscopy (GC-MS) analysis of *Gongronemalatifolium*

S/N	NAME OF COMPOUND	RETENTION TIME(MINS)	PEAK AREA%	MOLECULAR FORMULA	MOLECULAR WEIGHT(Kg/mol)	PROBALITY
1	Toluene	5.588	0.19	$C_6H_5CH_3$	92.141	53
2	Toluene	5.781	0.21	$C_6H_5CH_3$	92.141	45
3	D-Limonene	6.302	0.59	$C_{10}H_{16}$	136.238	96
4	Cyclooctane, methyl-	16.203	0.66	C_8H_{16}	112.21	70
5	Butylated Hydroxytoluene	19.426	39.86	$C_{15}H_{24}O$	220.356	98
6	9-Eicosene, (E)-	21.227	0.61	$C_{20}H_4O$	280.5	95
7	Tetradecane	21.394	0.21	$C_{14}H_{30}$	198.39	80
8	D-Mannitol, 1,2:5,6-bis-O-(1 methyl	22.499	0.65	$C_6H_{14}O_6$	182.172	64
9	Dodecanoic acid, propyl ester	23.579	3.01	$C_{17}H_{34}O_2$	270.45	99
10	9-Eicosene, (E)-	25.779	0.83	C20H4O	280.5	94
11	Carbonic acid, eicosyl prop-1-en-yl ester	27.566	0.32	H_2CO_3	62.03	35
12	Tetradecanoic acid, propyl ester	27.895	1.58	$C_{14}H_{28}O_2$	228.376	99
13	Hexadecanoic acid, methyl ester	28.653	1.18	$C_{16}H_{32}O_2$	62.9	97
14	Dibutyl phthalate	29.478	1.68	$C_{16}H_{22}O_4$	278.348	95
15	1-Docosen	29.910	1.71	$C_{22}H_{44}$	308.6	93
16	Hexadecanoic acid, ethyl ester	30.002	1.10	$C_{16}H_{32}O_2$	62.9	52
17	Acetic acid, chloro-, hexadecyl ester	31.327	0.49	CH_3COOH	60.052	64
18	Hexadecanoic acid, methyl ester	31.848	4.26	$C_{16}H_{32}O_2$	62.9	99
19	9-Octadecenoic acid (Z)-, methyl e ster	32.041	2.33	$C_{18}H_{36}$	252.5	98
20	Phytol	32.329	2.65	$C_{20}H_{40}O$	296.5	91
21	Methyl 8,10-dimethyl-hexadecanoate	32.544	0.45	$C_{17}H_{34}O_2$	270.5	87
22	2-Methyl-Z,Z-3,13-	33.169	0.98	$C_{19}H_{36}O$	280.5	87

	octadecadienol					
23	9,12,15-Octadecatrien-1-)-	33.292	2.20	$C_{18}H_{32}O_2$	280.4	93
24	1-Docosene	33.697	1.54	$C_{22}H_{44}$	308.6	94
25	trans,trans-9,12-Octadecadienoic acid, propyl ester	34.897	1.24	$C_{18}H_{32}O_2$	280.4	99
26	n-Propyl 11-octadecenoate	34.992	3.96	$C_{21}H_{40}O_2$	324.5	95
27	Octadecanoic acid, propylester	35.479	35.479	$C_{18}H_{36}O_2$	284.48	99
28	1-Octadecene	35.676	0.10	$C_{18}H_{36}$	252.486	93
29	17-Pentatriacontene	37.174	0.69	$C_{35}H_{70}$	490.9	83
30	2-Amino-4-(4-cyclohexyl-phenyl)-nicotinonitril	37.865	17.85	$C_8H_9N_3$	147.18	45
31	Cyclotetracosane	38.630	0.47	$C_{24}H_{48}$	336.6	55
32	Pentadecafluorooctanoic acid, octadecyl ester-	38.938	0.47	$C_8HF_{15}O_2$	414.1	93
33	Oleic Acid	39.474	1.59	$CH_3(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH	282.468	55
34	9-Eicosene, (E)-	39.776	1.27	$C_{20}H_4O$	280.5	83
35	Cyclopentadecane	40.622	0.20	$C_{15}H_{30}$	210.40	90
36	3-Eicosene, (E)-	40.622	0.15	$C_{20}H_{40}$	280.5	90
38	Cyclotetradecane, 1,7,11-trimethyl -4- (1-methyl	41.667	0.22	$C_{14}H_{28}$	196.372	90

Table 2.b: Gas chromatography- mass spectroscopy (GC-MS) analysis of *Ocimumgratissium*

S/N	NAME OF COMPOUND	RETENTION TIME(Mins)	PEAK AREA%	MOLECULAR FORMULA	MOLECULAR WEIGHT(Kg/mol)	PROBABILITY
1	D-Limonene	6.308	0.21	$C_{10}H_{16}$	136.238	94
2	Cyclododecane	16.208	0.23	$C_{12}H_{24}$	168.324	93
3	Butylated Hydroxytoluene	19.440	63.53	$C_{15}H_{24}O$	220.356	98
4	Butylated Hydroxytoluene	20.123	0.29	$C_{15}H_{24}O$	220.356	60
5	9-Octadecene, (E)-	21.234	1.91	$C_{18}H_{36}$	252.5	95
6	Hexadecane	21.391	0.99	$C_{16}H_{34}$	226.448	93
7	Diethyl 2-(2-cyanoethyl)-malonate	21.690	0.46	$C_7H_{12}O_4$	160.17	44
8	Butanoic acid, 4-methoxy-, methyl ester	22.240	0.31	$C_4H_8O_2$	88.11	47
9	D-Mannitol, 1,2:5,6-bis-O-(1-methy lethylidene)-	22.514	4.93	$C_6H_{14}O_6$	182.172	78
10	Ethyl tetradecyl ether	22.905	0.30	$C_{19}H_{40}O_2$	300.52	43
11	Carbonic acid, eicosyl prop-1-en-yl ester	23.178	0.43	H_2CO_3	62.03	46
12	Dodecanoic acid, propyl ester	23.579	9.27	$C_{12}H_{24}O_2$	43.2	98
13	Heptadecanoic acid, 16-methyl-, methyl ester	24.377	0.42	$C_{17}H_{34}O_2$	270.45	83
14	1-Octadecene	25.780	2.40	$C_{18}H_{36}$	252.486	95
15	1-Octadecanesulphonyl chloride	25.906	0.45	$C_{18}H_{37}ClO_2S$	353.00	83
16	Oxirane, [(hexadecyloxy)methyl]-	27.569	0.38	C_2H_4O	44.05	49
17	Tetradecanoic acid, propyl ester	27.895	1.69	$C_{14}H_{28}O_2$	228.376	99
18	Hexadecanoic acid, methyl ester	28.650	1.14	$C_{16}H_{32}O_2$	62.9	97
19	Dibutyl phthalate	29.478	1.29	$C_{16}H_{22}O_4$	278.348	95
20	1-Octadecene	29.910	1.61	$C_{18}H_{36}$	252.486	98
21	Hexadecanoic acid, propyl ester	1.850	1.16	$C_{16}H_{32}O_2$	62.9	99
22	9,12-Octadecadienoic acid,	31.923	0.84	$C_{18}H_{32}O_2$	280.452	99

	methyl ester, (E,E)-					
23	14-Octadecenoic acid, methyl ester	32.040	1.20	$C_{18}H_{32}O^{2}$	280.4	98
24	Cyclotetradecane	32.331	0.29	$C_{14}H_{28}$	196.372	74
25	1-Docosene	33.698	0.33	$C_{22}H_{44}$	308.6	94
26	Isopropyl linoleate	34.899	0.36	$C_{21}H_{38}O_2$	322.5	99
27	n-Propyl 11-octadecenoate	34.992	1.30	$C_{21}H_{40}O_2$	324.5	99
28	Octadecanoic acid, propyl ester	35.476	0.18	$C_{18}H_{36}O_2$	284.48	50
29	2-Piperidinone, N-[4-bromo-n-butyl]-	39.427	0.32	$C_9H_{16}BrNO$	234.13	86
30	2-Piperidinone, N-[4-bromo-n-butyl]-	39.771	0.82	$C_9H_{16}BrNO$	234.13	94
31	Tetracosanal	40.033	0.02	$C_{24}H_{48}O$	352.647	91
32	1-Hexadecanol, 2-methyl-	40.431	0.32	$C_{16}H_{34}O$	242.44	90
33	13-Tetradecen-1-ol acetate	40.799	0.15	$C_{16}H_{30}O_2$	254.41	89
34	1-Docosene	40.844	0.01	$C_{22}H_{44}$	308.6	89
35	1-Decanol, 2-hexyl-	40.965	0.15	$C_{10}H_{22}O$	158.28	87
36	2-Piperidinone, N-[4- bromo-n-butyl]-	41.086	0.01	$C_9H_{16}BrNO$	234.13	91
37	Cyclodocosane,ethyl-	41.32	0.32	$C_{12}H_{24}$	168.324	97

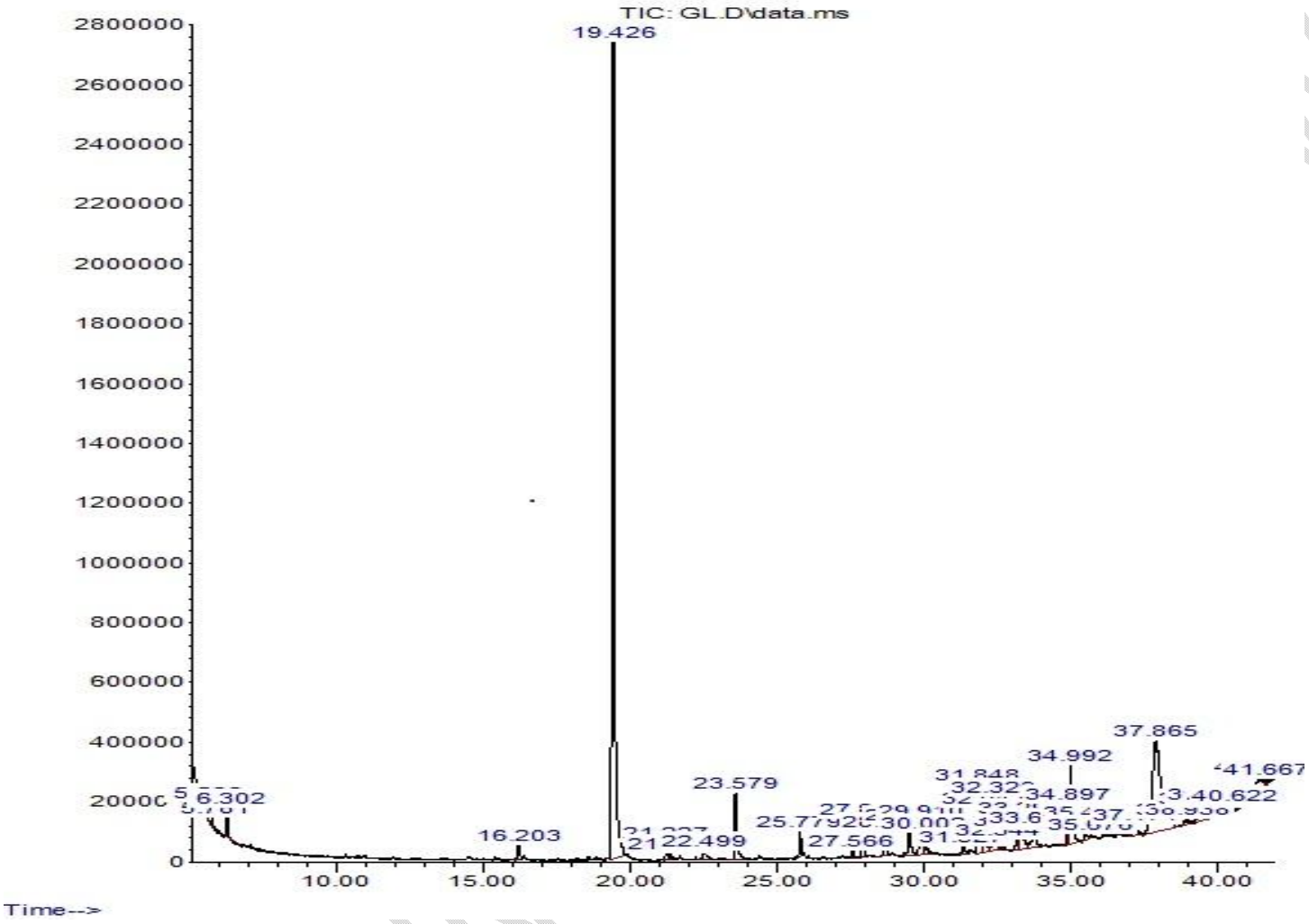

Figure 1.: GC-MS spectra of all the compounds detected from the ethanolleafextract of *Gongronemalatifolium.*

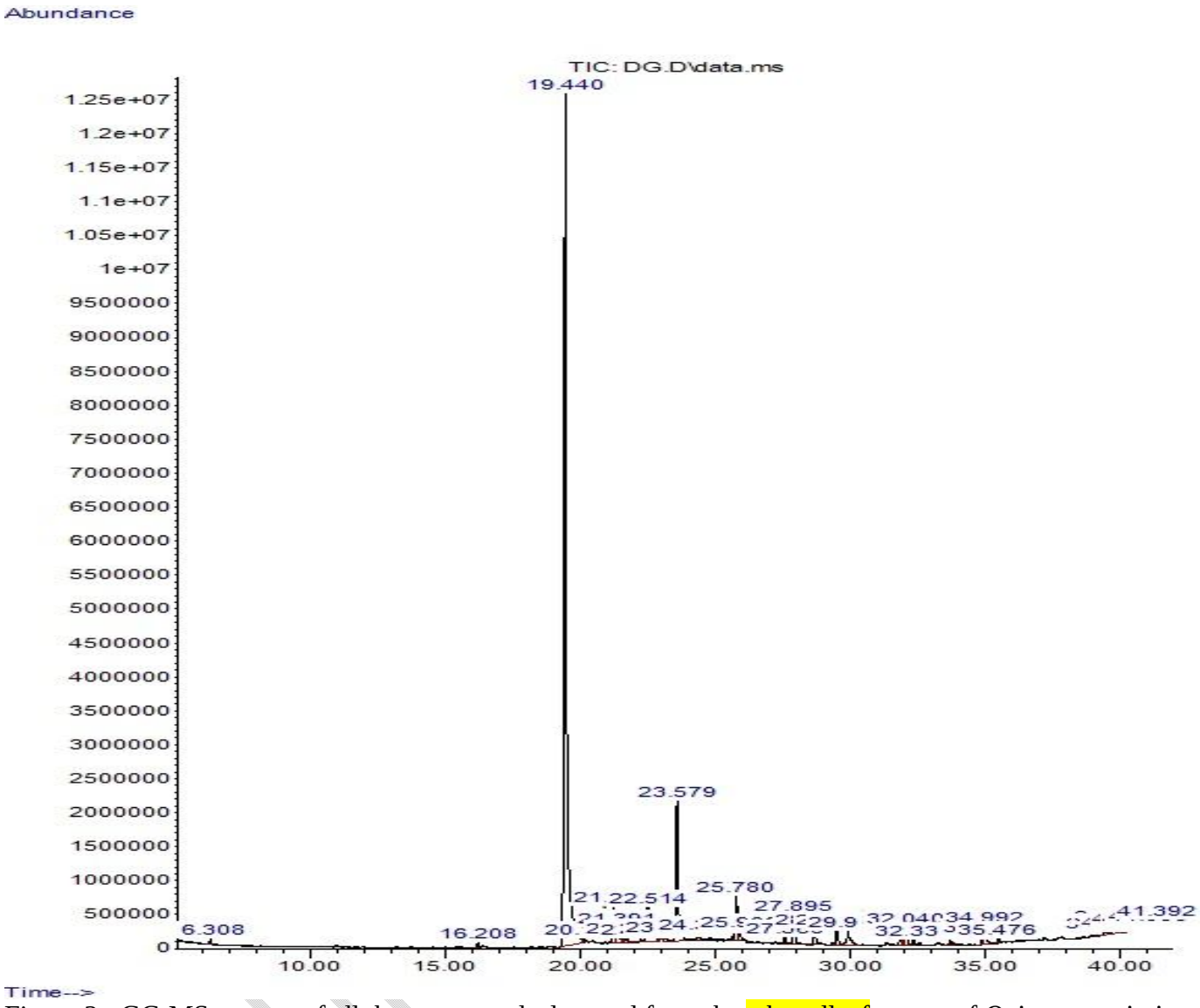

Figure 2.: GC-MS spectra of all the compounds detected from the ethanolleafextract of *Ocimumgratissium*

Table 3:Proximate compositions (mg/100g) of ethanol leaf extracts of *Gongronemalatifolium* and *Occimumgratissium*

Proximate	***Gongronemalatifolium***	***Ocimumgratissimum***
Moisture	16.97 ± 0.52	19.30 ± 0.20*
Protein	17.18 ± 0.22	2.43 ± 0.14*
Lipids	5.96 ± 0.11	2.34 ± 0.17*
Ash	9.65 ± 0.20	14.87 ± 0.47*
Fibre	9.91 ± 0.24	8.01 ± 0.57
Carbohydrates	40.33 ± 0.95	53.05 ± 1.27*
Energy	284.51 ± 2.91	343.77 ± 1.50*

Data presented as Mean ± Standard Error of Mean (SEM). Mean value for *Gongronemalatifolim* and *Ocimumgratissimum* were compared using 'Independent Student t Test' and they were considered significantly different at $p < 0.05$. Asterisk (*) indicates that the mean value for *Ocimumgratissimum* is significantly different from mean value for *Gongronemalatifolium.*

Table 4.:Mineral compositions (mg/100g) of ethanol leaf extracts of *Gongronemalatifolium* and *Ocimumgratissium*

Mineral	***Gongronemalatifolium***	***Ocimumgratissimum***
Sodium	107.78 ± 0.32	199.05 ± 1.32*
Potassium	333.74 ± 0.71	321.24 ± 0.83*
Calcium	113.99 ± 0.55	247.04 ± 0.61*
Magnesium	51.19 ± 0.44	339.66 ± 0.85*
Phosphorus	124.38 ± 0.45	459.32 ± 1.60*
Iron	21.32 ± 0.60	
Lead	41.31 ± 0.81	

Data presented as Mean ± Standard Error of Mean (SEM). Mean value for *Gongronemalatifolium and Ocimumgratissimum* were compared using 'Independent Student t Test' and they were considered significantly different at $p < 0.05$. Asterisk (*) indicates that the mean value for *Ocimumgratissimum* is significantly different from mean value for *Gongronemalatifolium*.

Table 5.:Vitamins compositions (mg/100g) of ethanol leaf extracts of *Gongronemalatifolium* and *Ocimumgratissium*

Vitamins	***Gongronemalatifolium***	***Ocimumgratissimum***
Thiamine	0.14 ± 0.02	0.49 ± 0.05*
Riboflavin	2.16 ± 0.02	0.46 ± 0.02*
Niacin	1.03 ± 0.02	0.24 ± 0.02*
Tocopherol	4.75 ± 0.34	1.90 ± 0.13*
Vitamin C	18.04 ± 0.07	11.32 ± 0.65*
Vitamin A	352.88 ± 1.75	901.66 ± 0.40*

Data presented as Mean ± Standard Error of Mean (SEM). Mean value for *Gongronemalatifolium* and *Ocimumgratissimum* were compared using 'Independent Student t Test' and they were considered significantly different at $p < 0.05$. Asterisk (*) indicates that the mean value for *Ocimumgratissimum*is significantly different from mean value for *Gongronemalatifolium.*

DISCUSSION

Phytochemicals are compounds which are produced by plants and these chemicals helps to reduce the risk of diseases caused by fungi, bacteria and viruses. On qualitative analysis of the leaf extracts of *Gongronemalatifolium and Ocimumgratissium*, it was seen that these leaf extracts contain phytochemicals such as alkaloids, flavonoid, saponins, cardiac glycosides, tannins and phenolic compounds. The presence of these phytochemicals in the leaves of *Gongronemalatifolium and Ocimumgratissium*revealed that these plants maybe of medicinalimportant(16).

Most experimental studies show that some of these phytochemical components elicit numerous biological activities against diabetes, anemia and many other disease conditions. Studies revealed that saponins which is present in both plants can reduce the level of plasma cholesterol and therefore is a potential remedy for atherosclerosis, diabetes, obesity and other health conditions(5). Serum cholesterol reductive capacity of saponins is by initiating resin-like action, reducing enterohepatic reabsorptionof bile acids(31).The liver enhances the conversion of cholesterol to bile acid leading to concomitant hypocholesterolemia (20, 25). Na^+ efflux is inhibited by saponins resulting in an increased Na^+ concentration in cells, thereby activating a Na^+- $Ca2^+$ antiport (27). Increased cytosolic Ca^{2+} is produces by this effect and strengthens heart muscle contraction, causing a reduction in congestive heart failure (27).Proteins leakage and degradation of cell wall enzymes from the cell can also be caused by saponins (36).

Hypoglycemic and hypolipidemic effects of alkaloids, flavonoids and tannins has been reported by many studies (34). Most metabolic activities in the body can be regulated by alkaloids. Because of the antioxidant property of flavonoid, it can be very effective on endothelial function, thereby reducing LDL oxidation (9). Flavonoids can also be used in targeting cancer tumor as it can inhibit the promotion of growth and progression of tumors (29). Plant growth regulation, development and disease resistance can be enhanced by phenols and in combined state with flavonoids can initiate series of activities such as antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory among others(21,4). The leaves of *Gongronemalatifolium* and *Ocimiumgratissimium* is seen to

contain tannin and this phytochemical has an antiviral activity.Inhibition of pathogenic fungi, prophylactic and therapeutic effect against cancer cells by tannins through different mechanism (22).

Gas chromatography-mass spectrometry is an analytical method that make use of gas chromatographyand mass spectroscopy features. It is used in the identification of different substances such as drugs, plant chemicals, food and flavor and unknown samples. This technique is used in quantifying an amount of substances by comparing the relative concentration among the atomic masses in the generated spectrum. The present study reveals that the ethanol leaf extracts of *Gongronemalatifolium and Occimiumgratissimium*contains D-limonene, D-mannitol, 1,2,5,6-bis-o-(1- methyl), carbonic acid,eicosyl prop-1-en-yl-ester, tetradecanoic acid, hexadecanoic acid, debutyl phthalate, 1-1docosene, 9-octadecanoic acid, n-propyl 11-octadecenoate, octadecanoic acid, 1-octadecene,cyclooctane hydroxytoluene, 9-eicosene, acetic acid, chlorohexadecyl ester, methyl 8,10- dimethyl-hexadecanoate, 2-methyl-Z,Z,Z-3,13- octadecanienol, 9,12,15-octadecatrien-1, tran,trans-9-12-octadecadienoic acid, propyl ester, 17-pentriacontene,, 2-amino-4-(4-cyclohexyl –phenyl) nicotinonitril, cyclotetracosane, pentadecafluorooectanoic acid, oleic acid, phytol, cyclopentadecane, 3-eicosene and toluene.

D-limonene possess pharmacological activities. Limonene possess bioactivities like the antiviral, anti-inflammatory, anti-tumor, and antibacterial agents(35). Reports shows that limonene can act as prodrug due to the therapeutic potency of its metabolites such as effective agents (13). The antimicrobial activity of limonene is due to its lipophilic and lipid layer changing the properties and functions of the cell wall and leading to the loss of intracellular components and cell death(14). Also, most experimental studies report the antidiabetic potential of limonene. Limonene antidiabetic potential is due to its ability to decrease blood glucose levels, enhances insulin levels, decreased MDA levels and improved activities of CAT, SOD, GR and GSH levels. Studies reports limonene great influence in plasma lipoprotein levels. Limonene is also reported to have anti-tumor effect, due to its ability to cause G_0/G_1 cancer cell cycle arrest through the inhibition of post- translational modification of signal transduction of proteins involved in the Ras/ MAPK pathway by depleting farnesylatedRas level (35). Equally, literatures report the antioxidant properties of D-mannitol. D-mannitol has free radical scavenging properties (10), protecting active substance like hyaluronic acid against radical degradation by oxygen derived

free radicals (2). Mannitol can act through the up-regulation of catalase level which is decreased by oxidative stress (18). Also, the hypoglycemic effect of mannitol is due to its partial absorption by the body. Hexadecanoic acid possess some biological activities such as antioxidant, hypocholesterolemia, nematicide and pesticide(28). Studies also reports that aside from the flavoring role of 9,12-octadecadienoic acid, it is also responsible for important pharmacological actions such as antioxidant, anti-cancer, anti-fungal, anti-anociceptive, hepatoprotective, depressant and wound healing activities(15). 9,12,15- octadecatrienoic acid (Z,Z,Z) exhibits antiarthritic, anti-androgenic, hepato-protective, anti-inflammatory, antieczemic, 5-alpha reductase, hypocholesterolemic, antiacne and antihistamine effects(17). Study report that 9,12,15- octadecantrienic and phytol may play a significant role in the treatment of cancer (19).

Proximate and mineral composition is important in human and animal nutrition and understanding the modes of action of these medicinal plants is important (16). These compositions in plants makes medicinal plants more important than chemotherapy in complex diseases such as diabetes (30). The present study revealed the leaves of *Gongronemalatifolium and Ocimumgratissimum*contain moisture, protein, lipids, ash, fibre, carbohydrates and energy.For one to have a good health,nutrient is needed. Nutrient supple energy, build the body structures, regulate and control of body processes. (16). Medicinal plants provide dietary supplements and some may promote bowel regularity and enhance frequent waste elimination including bile acid. The proximate analysis revealed that the studied *Gongronemalatifolium and Ocimumgratissimum*are good sources of carbohydrate and protein. The carbohydrates and proteins present in*Gongronemalatifolium and Ocimumgratissimum*may be a mixture of bioactive sugars, glycoproteins or proteins which give most of the vegetables their medicinal potency against certain diseases (16). Reports shows diseases like obesity, diabetes, breast cancer, hypertension and gastrointestinal disorder can be reduced by dietary fibers(26). Fibre has a physiologic effect in the gastrointestinal tract (GIT) function, promoting the reduction of extracolonic pressure which is beneficial in diverticular diseases (16). Blood cholesterol and blood sugar can be lowered by dietary fibre. It absorbes fats and cholesterol. Diabetes and postprandial hyperglycemia management have been implicated by dietaryfibres(16).

This study also revealed the presence of Na,K, P, Fe, Ca, Mg and Pb in the leaves extracts of *Gongronemalatifolium and Occimumgratissimum.* Most of these mineral acts as cofactor in enzymatic activities. The presences of this nutritional elements makeplants a source of nourishment for the body. Hemoglobin contain an essential trace element-iron and hemoglobin function in the transportation of oxygen from the lungs to tissues and metabolic waste like CO_2 from the tissues back to the lungs for excretion. Iron also plays an important role in the normal functioning of the central nervous system (1). Hemoglobin also serves as buffer to regulate changes in blood ph.

In this study, the presence of vitamins such as A, C, thiamine, riboflavin, niacin and tocopherol were also seen in the ethanol leaf extracts of these plants. The micronutrients- antioxidant vitamins and minerals roles have been reported in many studies (8). Most antioxidant enzymes or defense system of the body processes involved in lipid metabolism in general make use of mineral elements (12). Nutritional disorders such as serum increase in cholesterol and triacylglycerol can occur due to absence or imbalance in nutritional content of diet (34). Micronutrients present in these plants exert antihyperglycemic action and ameliorate macro vascular complication (34).

CONCLUSION

The wide distribution of nutrients in the leaf extracts of *Gongronemalatifolium and Occimumgratissimum*studied gives vital information as a rationale for its possible use by human and animal for nutritional purposes and as a tonic appetizer in folk medicine. Because of the phytochemical compositions, these plants therapeutic traces promote good health and proper functional mechanism in the body.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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