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# Chemical and Phytochemical Components of *Gongronema Latifolium (Asclepiadaceae).*

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**ABSTRACT**

An investigation into the chemical composition of leaf samples of *Gongronema latifolium* was carried out. The ethanol extract of pulverized, air-dried leaves of *Gongronema latifolium* was subjected to serial extraction on silica gel using solvents of increasing polarity, namely, n-hexane, chloroform, ethylacetate and ethanol respectively. Column fractionation of the n-hexane extract using graded solvent mixtures of n-hexane and ethylacetate followed. The fractions were pooled and dried samples yielded four fractions: F1 – a waxy white substance, F2 – a yellowish brown oil, F3 – white pin-like crystals and F4 – roundish white crystals. GC/MS analysis of F1 and F2 revealed the presence of hydrocarbons in F1 and six fatty acylesters in F2 of which 3 are essential fatty acids, 2 saturated fatty acylesters and an aromatic dicarboxylic acyl ester. Mass spectral analysis of F3 and F4 revealed that F3 is a composite of unidentified fatty acids while F4 is β-sitosterol. Phytochemical evaluation of the crude ethanol extract and sub-fractions indicated the presence of resins, steroids, terpenoids, flavonoids, alkaloids, proteins, glycosides, carbohydrates, fats and oils.

**Keywords:** *Gongronema latifolium*, chemical components, essential fatty acyl esters, saturated fatty acyl esters, aromatic compound, β-sitosterol.

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## INTRODUCTION

The plant kingdom has long served as source of useful drugs, foods, additives, flavouring agents, colourants, binders and lubricants and it is estimated that 25% of all prescribed medicines are substances derived from plants (Gamaniel, 2000, Ogunwande *et al.,* 2007). Medicinal plants are those plants which have been claimed and confirmed to posses medicinal properties (Kunle, 2000). The World Health organization (WHO) considers a medicinal herb "as any vegetable containing in one or more of its organs any substance that can be used with therapeutical aims or as raw material for chemical-pharmaceutical synthesis. It is estimated that 80% of the population of developing countries rely on traditional medicines for their primary health care needs (WHO 2002 – 2005). Approximately 89% of the rural African population uses traditional medicines (Soef *et al*., 2002) and these traditional plants are usually found useful in those countries where modern medicines are not readily available (Bailey and Day, 1989). Plants help to provide a disease free state when properly used as herbal medicine (Nwangwu *et al.*, 2011) as they are relatively safer to utilize with little side-effect than synthetic drugs (WHO, 2002 – 2005). In addition their natural presentation mimics more closely the natural constitution of the human somatic system which follows the biochemical "lock and key principle" (Gamaniel, 2000).

In vegetables, the active components have been combined with many other substances that appear to be inactive which give the plant as a whole safety and efficacy, much superior to its isolated and pure active components (Pamplona – Roger, 2001). These components include tea phenols and flavonoids which are naturally occurring low molecular weight compounds abundantly located in fruits, vegetables and red wines associated with inhibiting oxidation of LDL-cholesterol, thereby preventing formation of plaques and streaks in arteries (Aviram *et al*., 2002, Esterbauer and Puhl, 1991). They are strong scavengers against superoxide ions, hydrogen peroxide and hydroxyl radicals produced by various chemicals (Vaya and Aviram, 2001). Some traditional anti-diabetic plants such as *Gymnema sylvestre, Evening primrose* and *Medicago sativa*, rich in micrountrients, dietary fibre, folate and $\alpha$-linoleic acid are associated with lower risk of diabetes, nerve disorder and cardio-vascular diseases (Williams *et al*.,2007; Day, 1998; Gray and Flatt, 1997, Snowdon and Philip, 1985). Inhibitory activities against microbes (Dongmu *et al*., 2007), insects and pests (Tatsadjieu *et al*., 2007), are also exhibited by alkaloids, saponins and fibres components of plants and vegetables.

*Gongronema latifolium* (*Benth*) (*Asclepiadaceae*) is a tropical plant attributed with folkoric properties. It is listed among the twenty-eight medicinally important vegetables of South West Nigeria (Ayodele, 1996) and also as one of the aromatic plants of medicinal importance from Nigeria (Ogunwade *et al.,* 2007). Several pharmacological investigations carried out on this vegetable reveal an efficacy on many ailments, such as diabetes, hypertension, ulcer, microbes, inflammation and viral activities (Ugochukwu&Babbady, 2003; Ezekwe, 2005; Ogunwande *et al*., 2007 and Eleyinmi, 2007). Akinuga *et al*., 2001 however, reported some detrimental haematological conditions associated with administration of extracts of the vegetables. A careful documentation of the chemical composition of these extracts and their possible mechanisms of action is lacking. Hence the intent of this study, to identify the chemical compositions of these bioactive compounds and provides an insight into the operation of the chemical compounds.

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## METHODOLOGY

Fresh leaves of *Gongronema latifolium* were purchased from the local markets in Nsukka, Enugu State, Nigeria. The leaves were dried and pulverized.

### Extraction

One kilogramme of the pulverised dried leaves was macerated in 5 litres of 96% ethanol for 48h. The Whatman No.1 filtrate of the macerate was dried at 40$^{O}$C to obtain the crude ethanol extract (CEE). The resulting extract (CEE) was stored in brown bottles at –10$^{O}$C for the next step.

### Fractionation Step

A known weight of the ethanol extract (75g) adsorbed unto silica gel (1:2w/w) was eluted successively with volume solvents of increasing polarity namely n-hexane, chloroform, ethylacetate and ethanol respectively to obtain n-hexane (n-HE), chloroform (CLE), ethylacetate (EAE) and residual ethanol extracts (REE). They were dried at 40$^{O}$C and used for subsequent determination.

### Graded solvent fractionation

Using a 4 x 60cm of silica gel (70 – 230 mesh), 20g of the n-hexane fraction was subjected to column fractionation using different volumes of each of solvents of graded polarity viz. n-hexane, n-hexane - ethylacetate 19:1, 9:1, 4:1 and ethylacetate. Aliquots of 50ml each were collected, dried and similar fractions (based on tlc) behaviour pooled together to give F1, F2, F3, F4. Subsequently, the resultant extracts were subjected to thin layer chromatography on silica with 0.25mm precoated silica gel,60$GF_{254}$plates (Merck) developed with hexane- ethylacetate. Salts among the fractions were recrystalised from acetone.

### Phytochemical Analysis

Conventional phytochemical methods for identification of carbohydrates, alkaloids, saponins, flavonoids, reducing sugars, tannins, resins, proteins, steroids, acidic compounds, fats and oil were employed for the identification of components according to the methods of Harbone, (1998) and Trease and Evans (2002).

### Gas Chromatography/Mass Spectrometer Analysis of F1, F2

F1 and F2 were analysed in Agilent 5973N mass selective detector coupled to Agilent 6890N gas chromatography equipped with a cross-linked 5% PH-ME siloxane, HP5 – MS capillary column 30m x 0.2mm, film thickness of 0.25μm. The carrier gas helium had a flow rate of 2ml/min, the column temperature 60 – 275$^{O}$C at 4$^{O}$C/min, injector and detector temperature of 280$^{O}$C, injector volume 2μ/j and split ratio 1.5.

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The MS operating parameters were ionization potential 70eV, ionization current 1A, ion source temperature 200$^{O}$C and resolution of 1000. Identification of components of F1 and F2 was based on comparison of the retention times and computer matching of MS fragments with NISTO2.L library.

**Nuclear Magnetic Resonance (NMR) Spectral Analysis of F3, F4**

$^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker AV400 spectrophotometer. Chemical shifts were recorded in ppm.

**Electron Impact Mass Spectral Analysis (EIMS)**

The electron impact mass spectral analysis of F4 was determined. The accurate, mass of the compound was confirmed using relative abundance of the fragments and comparing them with the mass abundance table. The mass spectrometer accurately measured the mass to charge (m/z) ratio of the ions in the gas phase. The spectral analysis was carried out in the Department of Pharmacognosy and Phytochemistry, University of Mississippi, United States of America.

## RESULTS

**Table 1: Phytochemical composition of crude ethanol extract, fractions of the crude and the n-hexane fractions of *Gongronema latifolium***

| Group | % yield | Phytochemical Compound+ |
|---|---|---|
| Crude Ethanol extract | 8.36+ | Resins, terpenoids, steroids, fats, oils, alkaloids, flavonoids, saponins, carbohydrate, reducing sugars, proteins, glycosides |
| n-Hexane extract (n-HE) | 32.7++ | Resins, terpenoid, steroids, fats and oils |
| Chloroform extract (CLE) | 25.6++ | Resins, terpenoids, steroids, fats and oils, flavonoids, alkaloids |
| Ethylacetate extract (EAE) | 14.4++ | Resins, terpenoids, fats, oils, flavonoids, glycosides |
| Residual ethanol extract (REE) | 29.1++ | Resins, terpenoids, steroid, flavonoids, saponins, alkaloid, carbohydrate, glycosides proteins, reducing sugars |
| n-Hexane Fraction (F1) | 0.04+++ | Waxy substance (white) |
| n-Hexane Fraction (F2) | 0.012++ | Yellowish brown oil |
| n-Hexane Fraction (F3) | 0.33+++ | Unidentified fatty acids |
| n-Hexane Fraction (F4) | 1.04+++ | Sterol (pin-like) |

(+) yield calculated = % of 1000g leaf sample
(++) yield calculated = % of 75g CEE
(+++) yield calculated = % of 20g n-Hexane

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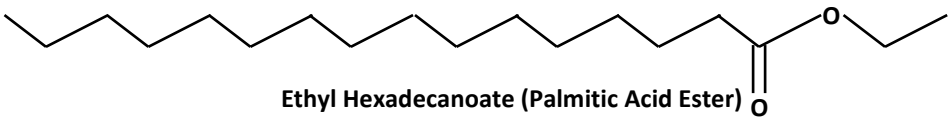
**Table 2: The Chemical Composition of F1**

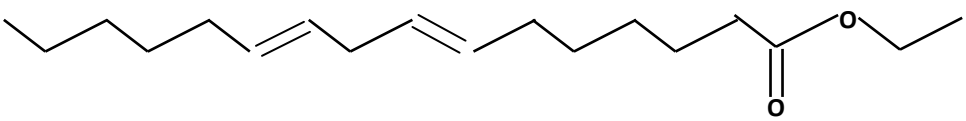
| No. | Compound | RT (mins) | Molecular Weight | Molecular Formular | MS Fragment ions |
|---|---|---|---|---|---|
| 1 | 1 – Hexadecene | 10.48 | 224 | $C_{16}H_{32}$ | 224,196, 154, 139, 125, 111, 97, 83, 69, 55 |
| 2 | (Z) – 7 – Hexadecene | 10.72 | 224 | $C_{16}H_{32}$ | 224, 140, 125, 111, 97, 83, 71, 55 |
| 3 | (Z) – 3 – Hexadecene | 10.93 | 224 | $C_{16}H_{32}$ | 224, 154, 140, 125, 111, 97, 83, 69, 57 |
| 4 | E – 15 – Heptadecene | 14.56 | 252 | $C_{17}H_{32}O$ | 252, 182, 166, 153, 140, 125, 111, 97, 83, 69, 55 |
| 5 | 1 – Nonadecene | 21.90 | 266 | $C_{19}H_{38}$ | 266, 223, 167, 153, 139, 125, 111, 97, 83, 69, 57 |
| 6 | Octacosane | 23.69 | 394 | $C_{28}H_{58}$ | 394, 324, 225, 211, 197, 183, 168, 153, 140, 127, 113, 99, 85, 71, 57 |
| 7 | Hexacosane | 26.81 | 366 | $C_{26}H_{54}$ | 366, 352, 281, 211, 169, 141, 113, 99, 85, 71, 57 |
| 8 | Tetradecyl-Oxirane | 29.74 | 240 | $C_{16}H_{32}O$ | 240, 175, 151, 123, 111, 97, 83, 69, 57 |
| 9 | Squalene | 31.11 | 410 | $C_{30}H_{50}$ | 410, 341, 217, 191, 161, 137, 95, 81, 69 |
| 10 | Tetratetracontane | 32.48 | 616 | $C_{44}H_{90}$ | 618, 408, 337, 309, 267, 210, 169, 141, 127, 113, 99, 85, 71, 57 |
| 11 | 11 – DecylTetracosane | 33.90 | 478 | $C_{34}H_{70}$ | 478, 362, 337, 253, 197, 175, 141, 111, 97, 85, 71, 57 |
| 12 | 3,7,11, 15 – Tetramethyl-2-hexadecane – 1 – 0l | 36.58 | 296 | $C_{20}H_{40}O$ | 296, 278, 256, 213, 165, 137, 123, 109, 95, 83, 71, 57 |

1.



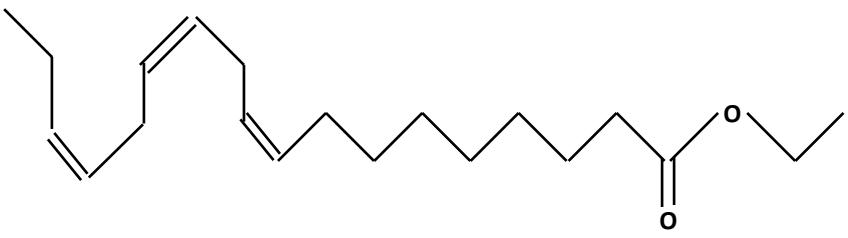

**Ethyl Hexadecanoate (Palmitic Acid Ester)**

2.




**Ethyl- 9, 12 – Octadecadienoate (Linoleic Acid Ester)**

3.



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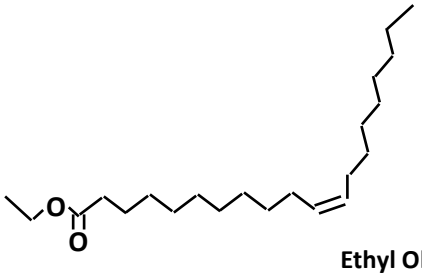
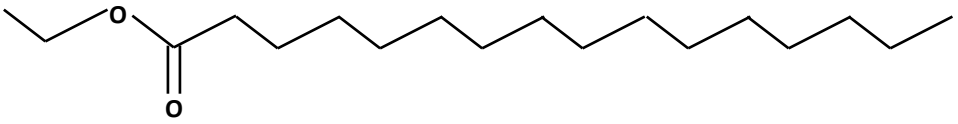
(Z,Z,Z) Ethyl -9, 12, 15 – Octadecatrienoate (Linolenic ester)

4.



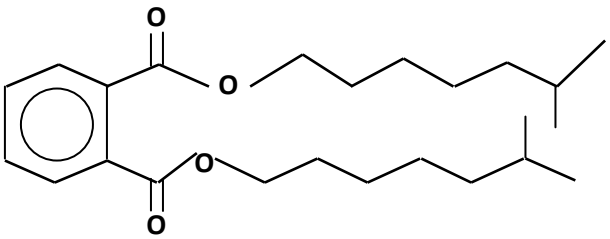
Ethyl Oleate (Oleic acid ester)

5.



Ethyl Octadecanoate (Stearic acid ester)

6.



Disoctyl 1, 2 –Benzenedicarboxylate

**Fig. 1: The Chemical Structures of F2**

**Table 3: The Chemical Composition of Sample F4**

| No. | Compound | RT (mins) | Molecular Weight | Molecular Formular | MS Fragment ions |
|---|---|---|---|---|---|
| 1 | β-Sitosterol | 3.59 | 414.71 | $C_{29}H_{50}O$ | 453, 451, 439, 438, 437, 435, 424, 423 |

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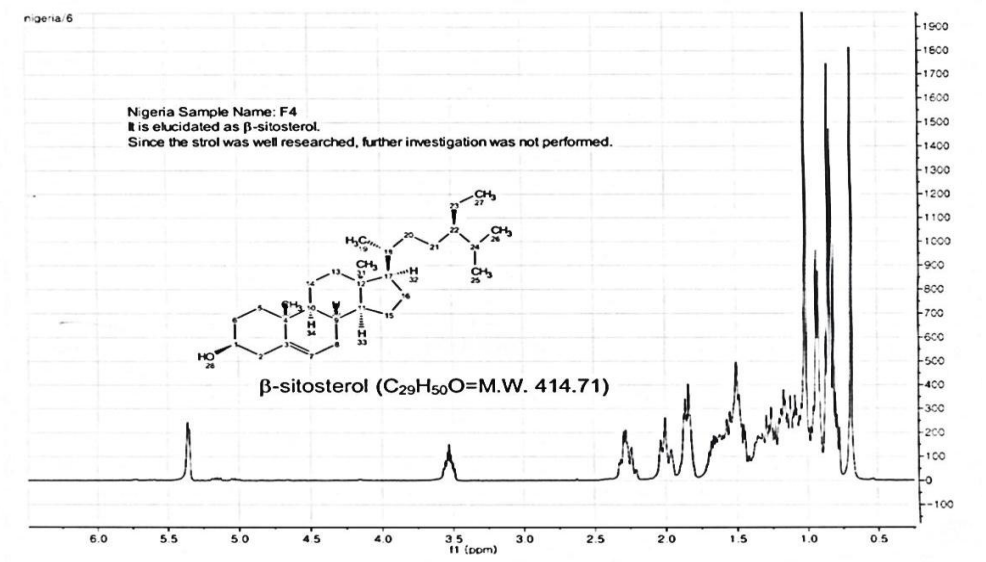


**Fig.2: $^{1}$H – NMR Spectrum of F4**

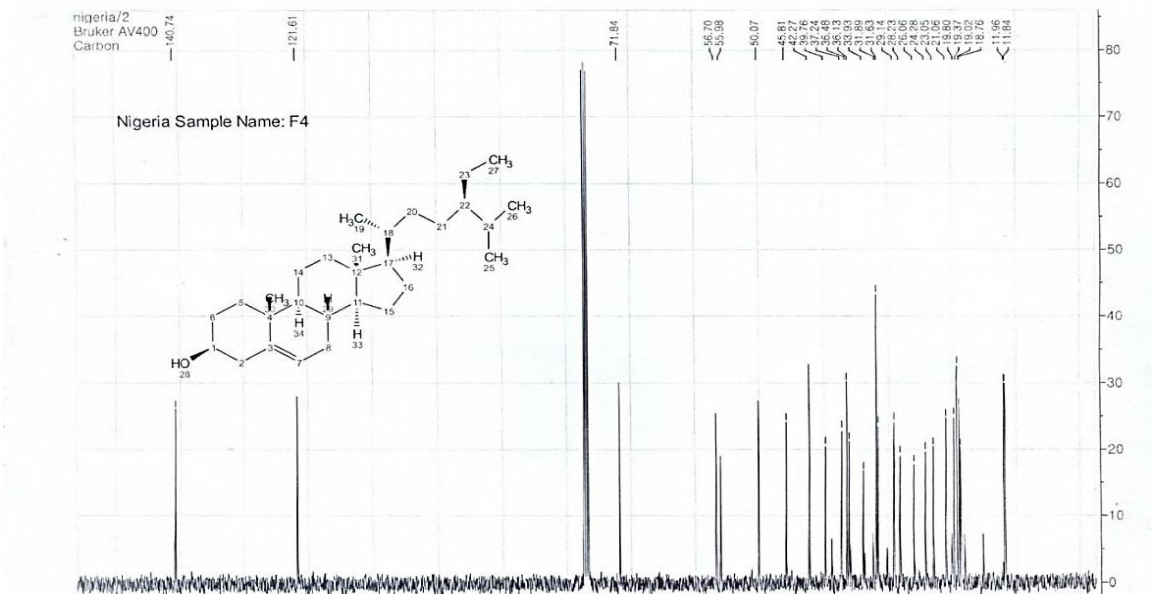


**Fig. 3:$^{12}$C-NMR Spectrucm of F4**

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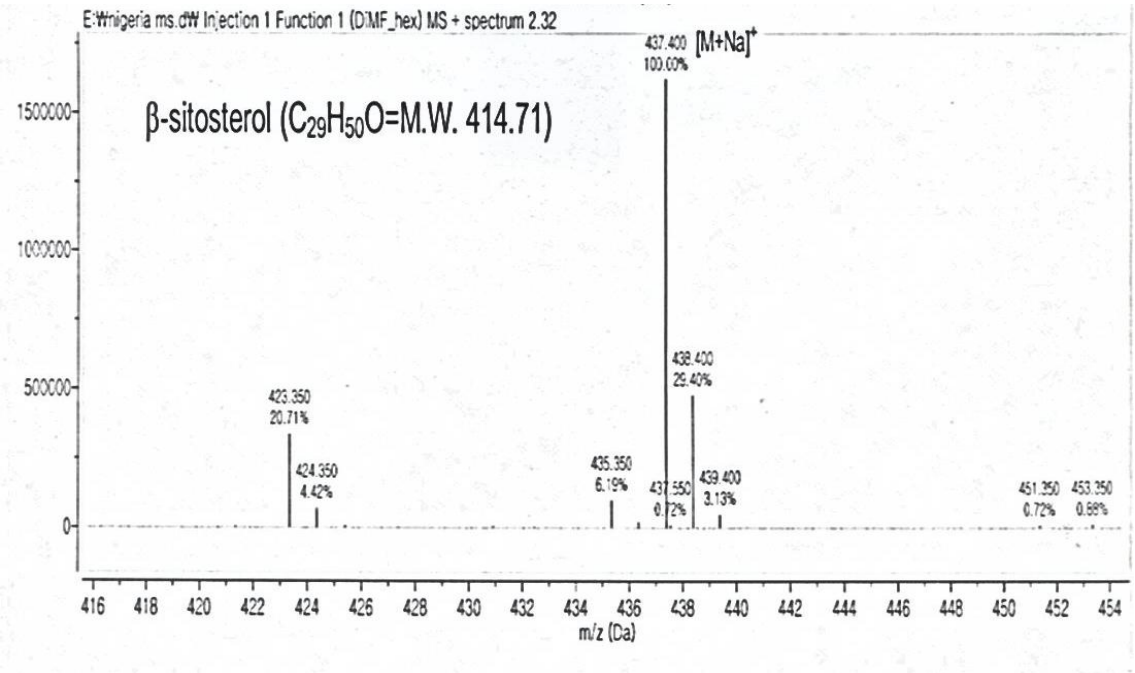


**Fig. 4 – EIMS Spectrucm of F4**

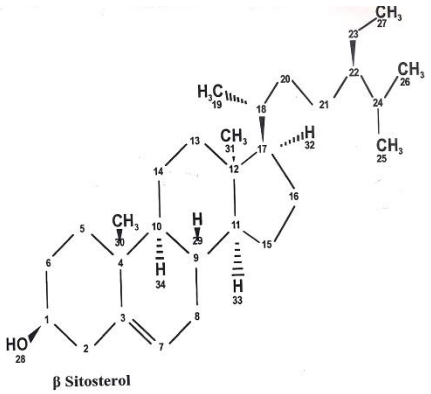


**Fig. 5: The Chemical Structure of ß-Sitosterol**

## DISCUSSION

The data from this study revealed that this vegetable extract has a rich content of phytochemicals, namely, saponins, alkaloids, flavonoids, resins, terpenoids, steroids, glycosides carbohydrates, proteins, fats and oils. This agrees with some of the works done previously on the extract by Eleyinmi (2008) and Ogunwande *et al*., (2007).

These components are associated with bioactivities having health impacts. Flavonoids which are naturally occurring low molecular weight polyphenolic compounds located in fruits and vegetables are known to inhibit formation of plaques and streaks in arteries and so hinder hypertension, and other cardiovascular diseases (Vaya and Aviram, 2001, Estabauer and Puhl, 1991). They also are strong scavengers of reactive oxygen radicals known to be involved in many conditions that cause diabetes, inflammatory diseases, cancers and neurodegenerative diseases (Ugochukwu *et al.,* 2003 and Asgary *et al.,* 1998,).

Alkaloids are implicated with antimicrobial activity and lowering glycaemic indices of experimental animal (Constantino *et al.,* 2003, Punitha *et al.,* 2005, Garcia Lopez *et al.,* 2001). Saponins are involved in ulcer protection and certain antimicrobial activity, Ukwe *et al.,* (2010) while resins and essential oils have been associated with antimicrobial, Amvam Zollo *et* al., (1998) anti-inflammation and antioxidant properties, (Lemos *et al.,* 2006, Olivieria *et al.,* 2004). Structural elucidation of the isolated compounds revealed that F1 is composed of 12 hydrocarbons including squalene, a precursor of cholesterol, some alcohols, ethers and alkenes. F2 is made up of both saturated and unsaturated esters of fatty acids. This agrees with work done by Eleyinmi *et al.,* (2008) and Ogunwande *et al.,* (2007). The unsaturated fatty acids are associated with some health benefits. Linoleic acid is a precursor of arachidonic acid which is essential in the body for the synthesis of prostaglandins. These prostaglandins enhance ulcer protection by the bicarbonate defence system of the gastric mucosa (Voet and Voet, 2011).

Unsaturated fatty acids also block lipid deposition in the arteries which is an important mechanism for hindering atherosclerosis and hypertension (Voet and Voet, 2011). Another component, β-sitosterol, is a well-known antilipidemic substance. It inhibits the absorption of cholesterol from the gut thereby promoting excretion of cholesterol in the faeces. It is now being projected or proposed for use as the next generation drugs for blood cholesterol lowering purposes (Garrette and Grisham, 2005). It may be the explanation for the antilipidemic property observed in the study on effect or *Gongronema latifolium* on diabetes mellitus by Ezekwe, (2005) and Ugochukwu *et al.,* (2003).

This, therefore, suggests that this vegetable, *Gongronema latifolium* would be a beneficial medicinal plant.

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