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Chemical composition and antibacterial activity of *Gongronema latifolium*

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Abstract: Chemical composition of *Gongronema latifolium* leaves was determined using standard methods. Aqueous and methanol *G. latifolium* extracts were tested against thirteen pathogenic bacterial isolates. Crude protein, lipid extract, ash, crude fibre and nitrogen free extractives obtained are: 27.2%, 6.07%, 11.6%, 10.8% and 44.3% dry matter respectively. Potassium, sodium, calcium, phosphorus and cobalt contents are 332, 110, 115, 125 and 116 mg/kg respectively. Dominant essential amino acids are leucine, valine and phenylalanine. Aspartic acid, glutamic acid and glycine are 13.8%, 11.9% and 10.3% respectively of total amino acid. Saturated and unsaturated fatty acids are 50.2% and 39.4% of the oil respectively. Palmitic acid makes up 36% of the total fatty acid. Extracts show no activity against *E. faecalis*, *Y. enterolytica*, *E. aerogenes*, *B. cereus* and *E. agglomerans*. Methanol extracts were active against *S. enteritidis*, *S. cholerasius* ser *typhimurium* and *P. aeruginosa* (minimum inhibitory concentration (MIC) 1 mg; zone of growth inhibition 7, 6.5 and 7 mm respectively). Aqueous extracts show activity against *E. coli* (MIC 5 mg) and *P. aeruginosa* (MIC 1 mg) while methanol extracts are active against *P. aeruginosa* and *L. monocytogenes*. *G. latifolium* has potential food and antibacterial uses.

Key words: *Gongronema latifolium*, Chemical composition, Antibacterial activity
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INTRODUCTION

Gongronema latifolium, commonly called 'utazi' and 'arokeke' in the South Western and South Eastern parts of Nigeria, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu *et al*., 2003). Reports by various authors showed that it contains essential oils, saponins and pregnanes among others (Schneider *et al*., 1993; Morebise and Fafunso, 1998; Morebise *et al*., 2002). Ugochukwu and Babady (2003), Ugochukwu *et al*.(2003) and Ogundipe *et al*.(2003) reported that aqueous and ethanolic *G. latifolium* extracts had hypoglycemic, hypolipidemic and antioxidative properties while Morebise *et al*.(2002) showed that it has anti-inflammatory properties. These reports are focused mainly on the medicinal properties of the plant with little attempts at investigating their potential nutritional and food processing/preservation values.

There is a current shift towards evaluating the chemical composition and nutritive value of tropical plants, many of which are medicinal. Aletor and Adeogun (1995) reported on the nutrient components of 17 leafy vegetables in Nigeria. Apori *et al*.(2000) reported on the chemical composition and nutritive value of *Chromolaena odorata* leaves. Corlett *et al*.(2002) reported on *Acorus gramineus*, *aff. Angelica*, *Dendranthema indicum*, *Eupatorium lindleyana*, *Sedum aff. sarmentosum*, and *Sedum aff. Spectabile*. Ahamefule *et al*.(2006) reported on the nutritive value of *Napoleona vogelii* and *Grewia pubescens*; while Eleyinmi *et al*.(2006) reported on the chemical composition of *Garcinia kola* seed and hull. The need to widen raw material base of agro-allied industry and the current shift away from the use of synthetic chemicals in food processing necessitate a further evaluation of widely available but underutilised

tropical medicinal plants like *G. latifolium*. Information on potential food uses of *G. latifolium* is scanty. It is therefore important that further studies are carried out to assess its nutritive value and assess its potential use as food/feed supplement. The nutrient information reported in this study would enhance efforts to promote wider use of the plant as part of a broader program aimed at educating local populations on the nutritional benefits of the many wild plants existing in their environment.

The objective of the research work reported here was to determine the chemical composition and evaluate the antibacterial properties of methanol and water extracts of *G. latifolium* leaves with a view to investigating their potentials for use in various systems.

MATERIALS AND METHODS

Materials and reagents

The major raw material used in this work is freshly harvested *G. latifolium* leaves obtained from a local farm in Akure, Ondo State, Nigeria. The samples were taken to the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria for identification and packed in clean sterile sample bags. All reagents used were analytical grade.

Sample preparation

Leafy parts of freshly harvested *G. latifolium* leaves were cut, rinsed, air-dried, oven dried (50 °C, 10~12 h), milled, packaged in sterile 1 mm thick high-density polyethylene sachet, labelled and stored in a refrigerator [(3±1) °C] until used.

Analytical methods

1. Proximate and mineral analysis

Dry-milled *G. latifolium* leaves were analyzed for fat (method No. 930.09), crude fiber (method No. 930.10) and total ash (method No. 930.05) as described by AOAC (1990). Crude protein was determined (N×6.25) using the Leco-N nitrogen determinator (Model FP-428, Leco Corporations, MI, USA). The nitrogen free extractive (NFE) was obtained by difference. The moisture content was determined by drying the sample to a constant weight in an air circulating oven at 70~80 °C. The mineral contents, namely: Na, K, Ca, Mg, Cu, Mn, Co, Cr, Hg, Pb, Mo and Se contents were determined as described by Whiteside and Milner (1984) using a Pye Unicam SP9 atomic absorption spectrophotometer (Pye Unicam Ltd., York Street, Britain). Total phosphorus was determined by the spectrophotometric molybdovanadate method (No. 970.39) as described by AOAC (1990). Regression equations were used to calculate the amount of metals in each sample (using their absorbance and dilutions).

2. Fatty acid (FA) analysis

Oil was extracted from dry-milled *G. latifolium* leaves using Goldfisch apparatus, saponified and then esterified using 10% (v/v) boron trifluoride-methanol (Fisher Scientific Co., Canada) via method No. 969.33 described by AOAC (1990) to fatty acid methyl esters (FAMEs). The FAMEs were analyzed on a cool on-column injection gas liquid chromatography (GLC, Varian 3400, Varian Inc., Walnut Creek, CA, USA) equipped with a flame-ionization detector and a SP2560 fused silica capillary column (100 m×0.25 mm i.d.; Supelco Inc., USA). The carrier gas was helium with a flow rate of 1.5 ml/min. The injector temperature was programmed from 50 to 230 °C at 150 °C/min and maintained for 9.4 min. Column temperature was programmed from 45 to 175 °C at 13 °C/min (maintained for 27 min), then to 215 °C at 4 °C/min (maintained for 45 min). Detector temperature was set at 270 °C. Peak area integration for fatty acids was made using a Shimadzu Ezchrom Data System (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). The fatty acids were identified by comparing their relative retention times with reference standards (Nu-Chek-Prep, Inc., Elysian, Mn 56028) of known composition.

3. Amino acid profile

Separation and quantification of amino acids was accomplished on hydrolyzed samples with a high performance liquid chromatography and a fluorichrom detector (excitation 340 nm emission 450 nm) as described by Sedgwick *et al*.(1991). Separation was achieved using Supelcosil 3 micron LC-18 reverse phase column (4.6 mm×150 mm; Supelco Inc., USA) equipped with a guard column (4.6 mm×50 mm) packed with Supelcosil LC-18 reverse phase packing (20~40 μm). Amino acid standard solution (Sigma, St Loius, Missouri, USA) was used for calibration and quantification.

Determination of antibacterial activity

1. Preparation of extracts

Ten grams of dry-milled *G. latifolium* leaves was extracted with 200 ml of 70% methanol with continuous stirring (30 min), centrifuged (10000 r/min, 15 min) and filtered (Whatman No. 1) into a 250-ml round-bottom flask. Methanol was removed under reduced pressure, dried under nitrogen atmosphere and freeze dried to obtain methanol extract (MEx) as shown in Fig.1. To obtain water extracts, 10 g of dry-milled *G. latifolium* leaves was extracted with 150 ml of distilled water, with continuous heating and stirring (30 min) on a mechanical shaker. The resulting slurry was centrifuged (10000 r/min, 15 min), filtered under vacuum (using the Buchner funnel) and freeze dried to obtain aqueous extract (AqEx) (Fig.1).

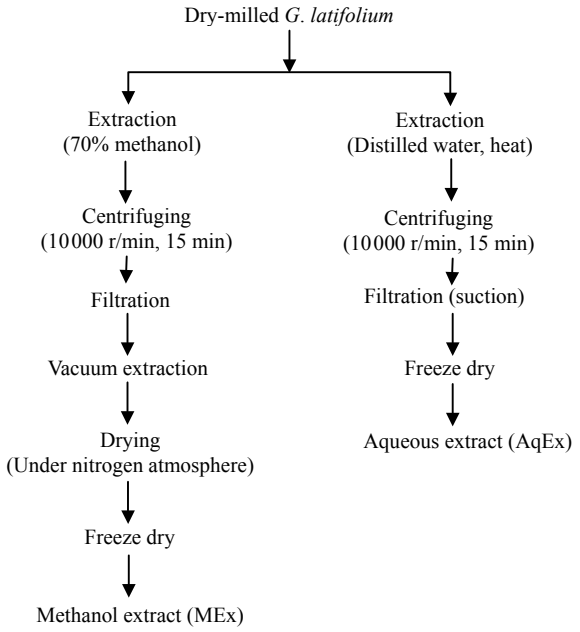

Fig.1 Preparation of methanol and aqueous extracts of *G. latifolium* leaves

2. Test organisms

The test microorganisms used for the antimicrobial activity screening namely: *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (DH5), *Enterobacter aerogenes*, *Enterobacter agglomerans* (ATCC 27155), *Salmonella enteritidis* (ATCC 13076), *Salmonella cholerasius* ser *typhimurium* (ATCC 23564), *Staphylococcus aureus* (ATCC 23235), *Pseudomonas aeruginosa* (ATCC 14207), *S. aureus* subsp *aureus* (ATCC 6538), *Enterobacter faecalis* (ATCC 7080), *Yersinia enterolytica* (ATCC 23715) and *Listeria monocytogenes* (HBP 463) were obtained from the Microbiology Unit, Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Canada. Each bacterial strain was suspended in Mueller-Hinton broth (Difco, France) and incubated at 37 °C for 18 h. Mueller-Hinton Agar (MHA, Difco, France) was used for testing antibacterial activity.

3. Paper disc agar diffusion method

Six millimetres sterile paper discs were soaked with 10~50 μl aliquot of 100 mg/ml of MEx and AqEx respectively. Ten microlitres aliquot of each extract contains about 1 mg of extract. Each paper disk was then impregnated with the equivalent of 1~5 mg of each extract and dried at 37 °C overnight. The discs were applied to the surface of MHA seeded with 5-h broth culture of the test bacteria and incubated for 18 h at 35 °C. Antibiotic susceptibility disc (ampicillin) was used as the positive control, while 70% methanol and distilled water were used as negative controls. The minimum inhibitory concentration, defined as the lowest concentration able to inhibit any visible microorganism growth, was determined by measuring cell growth after 18 h incubation at 35 °C at the different extract concentrations used. All data are presented as the mean values of triplicates readings.

4. Statistical analysis

Means of triplicate measurements and standard errors were determined for each sample using standard procedures.

RESULTS

Proximate and mineral composition

The proximate and mineral composition of dry milled *G. latifolium* leaves is presented in Table 1. Results show the following percent dry matter (%DM) composition for *G. latifolium*: crude protein 27.2, lipid extract 6.07, ash 11.6, crude fiber 10.8 and NFE 44.3. Potassium is the dominant mineral (332 mg/kg). Values obtained for sodium (110 mg/kg), calcium (115 mg/kg), phosphorus (125 mg/kg) and cobalt (116 mg/kg) were comparable. Chromium, molybdenum, nickel, selenium and lead were not detected in the leaves.

Table 1 Proximate and mineral composition of *Gongronema latifolium* leaves

	Composition
Proximate (%DM)	
Crude protein	27.2±0.02
Lipid extract	6.07±0.03
Ash	11.6±0.29
Crude fibre	10.8±0.23
NFE (by difference)	44.3
Minerals (mg/kg)	
Na	110.0±2.53
K	332.0±4.72
Ca	115.0±5.24
Mg	54.0±1.83
P	125.0±1.09
Cu	43.5±1.68
Co	116.0±2.45

Values are means of three replicate readings; Cr, Mo, Mn, Ni, Se, Pb and Hg were not detected

Amino acid composition

The amino acid composition of *G. latifolium* is presented in Table 2 and compared with the WHO (1985) ideal amino acid in Table 3. Noteworthy in the amino acid profile of *G. latifolium* is the presence of high amounts of aspartic acid, glutamic acid and glycine (13.8%, 11.9% and 10.3% total respectively). The dominant essential and non-essential amino acids in *G. latifolium* are leucine, valine, phenylalanine and aspartic acid, glutamic acid, glycine respectively. The proportion of essential to non-essential amino acid is 41.1% to 58.9%. The pattern of amino acid in *G. latifolium* leaves is comparable with the WHO (1985) standards with respect to several amino acids (Table 3).

Fatty acid composition

The fatty acid profile of *G. latifolium* leaf oil is shown in Table 4. Results showed that *G. latifolium* leaf oil is composed of 50.2% (21.2 mg/100 mg) saturated fatty acids (SFA), 10.4% (4.39 mg/100 mg) monounsaturated fatty acids (MUFA) and 39.4% (16.6 mg/100 mg) polyunsaturated fatty acids (PUFA). The predominant SFA in *G. latifolium* leaf oil is palmitic acid ($C_{16:0}$) which make up 36% of the total fatty acid (TFA) and 76.27% of SFA. Oleic acid ($C_{18:1\ n9}$), the major MUFA in *G. latifolium* leaf oil is 7.13% of TFA and 53.3% of MUFA. The PUFA:SFA ratio of *G. latifolium* leaf oil is 1.11. About 76.9% of the PUFA of *G. latifolium* leaf oil can be attributed to the presence of oleic, linoleic and α-linoleic acid.

Table 2 Amino acid composition (g/100 g DM) of *Gongronema latifolium* leaves

Amino acids (abbreviation)	Mean±*SEM*	Percent of total amino acid
Threonine (Thr)	0.94±0.05	3.75
Valine (Val)	1.94±0.04	7.73
Isoleucine (Ile)	1.18±0.11	4.70
Leucine (Leu)	2.25±0.45	8.97
Lysine (Lys)	1.43±0.40	5.70
Tyrosine (Tyr)	0.82±0.05	3.27
Methionine (Met)	0.18±0.02	0.73
Phenylalanine (Phe)	1.58±0.09	6.30
Cysteine (Cys)	ND	−
Tryptophan (Trp)	ND	−
Total essential amino acid	10.3	41.1
Arginine (Arg)	1.93±0.06	7.69
Aspartic acid (Asp)	3.46±0.50	13.78
Glutamic acid (Glu)	2.98±0.08	11.86
Serine (Ser)	1.56±0.01	6.22
Histidine (His)	0.35±0.01	1.40
Proline (Pro)	ND	−
Glycine (Gly)	2.59±0.50	10.31
Alanine (Ala)	1.91±0.20	7.61
Total non-essential amino acid	14.8	58.9
Total protein	25.1	−

Values are means of three replicate readings±*SEM*; ND: Not determined

Table 3 Amino acid composition of *G. latifolium* leaves compared with the WHO "ideal protein[1]"

Amino acids	*Gongronema latifolium*		WHO ideal protein (%)
	Total amino acid (%)[2]	Amino acid/ideal (%)	
Threonine	3.75	110	3.4
Valine	7.73	221	3.5
Isoleucine	4.70	168	2.8
Leucine	8.97	136	6.6
Lysine	5.70	98.2	5.8
Tryptophan	−	−	1.1
Phenylalanine+ Tyrosine	9.57	152	6.3

[1]WHO, 1985; [2]Total protein, 25.118 g/100 g DM

Antibacterial activity

The zone of growth inhibition and minimum inhibitory concentration of aqueous and 70% methanol extract of *G. latifolium* extracts on the test organisms is shown in Table 5. Results showed that the extracts show no activity against *E. faecalis*, *Y. enterolytica*, *E. aerogenes*, *B. cereus* and *E. agglomerans*. Methanol extracts show activity against

Table 4 Fatty acid composition (mg/100 mg) of *Gongronema latifolium* leaf oil

Fatty acid	*Gongronema latifolium* leaf oil	
	Quantity* (mg/100 mg)	Composition (%)
Saturated		
Myristic ($C_{14:0}$)	0.71±0.01	1.68
Pentadecanoic ($C_{15:0}$)	0.33±0.02	0.78
Palmitic ($C_{16:0}$)	15.2±0.05	36.00
Margaric ($C_{17:0}$)	0.29±0.04	0.70
Stearic ($C_{18:0}$)	1.92±0.01	4.55
Arachidic ($C_{20:0}$)	1.17±0.02	2.78
Behenic ($C_{22:0}$)	1.56±0.04	3.69
∑Saturates	21.20	50.20
Monounsaturated		
Myristoleic ($C_{14:1}$)	0.46±0.01	1.08
Palmitoleic trans ($C_{16:1}$)	0.50±0.02	1.18
Palmitoleic C ($C_{16:1}$)	0.13±0.01	0.31
Oleic C ($C_{18:1\ n9}$)	3.01±0.03	7.13
Oleic C ($C_{18:1\ n7}$)	0.29±0.02	0.70
∑Monoenes	4.39	10.40
Polyunsaturated		
Linoleic ($C_{18:2}$)	13.11±0.050	31.10
α-Linolenic ($C_{18:3\ n3}$)	2.98±0.032	7.07
Arachidilenic ($C_{20:2\ n6}$)	0.28±0.021	0.65
Docosahexaenoic ($C_{22:6\ n3}$)	0.24±0.010	0.57
∑Polyenes	16.61	39.40
∑Unsaturates	21.00	49.80
∑Essential FA ($C_{18:2}$+$C_{18:3}$)	16.10	38.20

*Values are means of three replicate readings

S. aureus, *S. aureus* subsp *aureus*, *L. monocytogenes*, *S. enteritidis*, *S. cholerasius* ser *typhimurium* and *P. aeruginosa*. The highest activity observed was against *S. enteritidis*, *S. cholerasius* ser *typhimurium* and *P. aeruginosa* with minimum inhibitory concentration (MIC) of 1 mg plant extract and zones of growth inhibition 7, 6.5 and 7 mm respectively. When 5 mg methanol extract was used against these organisms (data not shown), the zones of growth inhibition were 17, 8 and 22 mm respectively. At this level of methanol extract usage, activity was low compared to the control (ampicillin) which had 30 and 27 mm zones of growth inhibition against *S. enteritidis* and *S. cholerasius*. Aqueous extracts showed activity against *E. coli* (MIC 5 mg) and *P. aeruginosa* (MIC 1 mg) only with *P. aeruginosa*, the more susceptible of the two.

DISCUSSION

Chemical composition

G. latifolium is a good source of protein. Its protein content (27.2% DM, Table 1) is quite high and compares favourably with percent DM values reported for chickpea (24.0%), cowpea (24.7%), lentil (26.1%), greenpea (24.9%), fluted pumpkin leaves (22.4%), *Tamarindus indica* (24.3%), *Mucuna*

Table 5 Zone of growth inhibition and minimum inhibitory concentration of aqueous and methanol extracts of *Gongronema latifolium* leaves

Test organisms	Zone of growth inhibition (mm)			[a]MIC (mg)	
	[b]Control	AqEx	MEx	AqEx	MEx
Staphylococcus aureus (ATCC 23235)	40	[c]ND	7	ND	5
S. aureus subsp *aureus* (ATCC 6538)	40	ND	7	ND	5
E. faecalis (ATCC 7080)	26	ND	ND	ND	ND
Y. enterolytica (ATCC 23715)	14	ND	ND	ND	ND
L. monocytogenes (HBP 463)	ND	ND	8	ND	5
Escherichia coli (DH5)	25	8	ND	5	ND
Enterobacter aerogenes	10	ND	ND	ND	ND
Bacillus cereus (ATCC 14579)	8	ND	ND	ND	ND
Bacillus subtilis (ATCC 6051)	11	[d]NT	NT	NT	NT
Enterobacter agglomerans (ATCC 27155)	10	ND	ND	ND	ND
S. enteritidis (ATCC 13076)	30	ND	7	ND	1
Salmonella cholerasius ser *typhimurium* (ATCC 23564)	27	ND	6.5	ND	1
Pseudomonas aeruginosa (ATCC 14207)	ND	7	7	1	1

[a]Concentration is expressed as the amount of extract (mg) per 6 mm blank antibiotic discs; [b]Ampicillin paper discs were used as positive control; 70% methanol was used as negative control and exhibited no activity against the test organisms; [c]ND: Not detectable within the range tested; [d]NT: Not tested

flagellipes (24.9%), *Hibiscus esculentus* (23%) and *Parkia biglobosa* (20.9%) (Glew *et al*., 1997; Akwaowo *et al*., 2000; Ajayi *et al*., 2006; Iqbal *et al*., 2006). Consumption of 100 g (DM) of *G. latifolium* may be capable of providing 27 g of protein which satisfies recommended daily allowance of protein for children. Thus, *G. latifolium* leaves appear to represent a potentially rich source of some, but not all, of the essential amino acids that are essential for humans (Tables 2 and 3). The crude fat content of *G. latifolium* (6.07%) compare favourably with percent DM values reported for leafy vegetables like *Brachystegia eurycoma* (5.87%) and *Tamarindus indica* (7.20%) (Ajayi *et al*., 2006). A child consuming 100 g of *G. latifolium* would be ingesting approximately 6.07 g of fatty acid which translates into 54.6 kcal of energy or about 3%~3.5% of their daily total energy requirement. Apart from providing energy, the lipid fraction of *G. latifolium* contains modest but useful amounts of the essential fatty acid, linoleic acid (31.1%, Table 4). Linoleic acid is an important component of membrane phospholipids, a precursor to another critical fatty acid one finds in virtually all tissue membranes of humans, namely arachidonic acid (Glew *et al*., 2004). Arachidonic acid is important for another reason; it is metabolized to various prostaglandins which regulate many normal processes, including blood pressure and gastric acid secretion (Lauritzen *et al*., 2001). Prostaglandins play a critical role in inflammation and anaphylaxis (Glew *et al*., 2004). About 76.9% of the PUFA of *G. latifolium* leaf oil can be attributed to the presence of oleic, linoleic and α-linoleic acid. These fatty acids are important from the nutritional and stability point of view. Nutritionally, edible triglycerides, such as those in olive oil (which are rich in oleic acid), have cardioprotective effects, as opposed to dietary fats that are rich in saturated fatty acids and which are associated with increased risk of macrovascular diseases (e.g., stroke, heart attack) (Glew *et al*., 2004). The presence of PUFA is very important in human feeding and physiology, the most important ones being n-3 fatty acid which are predominant in cold water and deep sea fishes. With the current emphasis on increasing polyunsaturated and monounsaturated fats intake, the use of *G. latifolium* leaf oil in food processing/formulations may be acceptable. However, further tests would have to be carried out to determine the specific use of the oil for food and/or industrial purposes. The chemical composition of *G. latifolium* suggests that it may find use in food/feed formulation/supplementation operations. This would be particularly so where protein content is of prime importance.

Antibacterial activity

The activity of methanol extract of *G. latifolium* against *S. aureus* (MIC 5 mg, Table 5) was low compared with those reported for methanol extract of *Azadirachta indica* leaf (MIC 2.0 mg) and *Ginkgo biloba* (MIC 0.5 mg) against *S. aureus* (Ross, 2001). It is noteworthy that methanol extracts show activity against *P. aeruginosa* (MIC 1 mg and zone of inhibition 7 mm) and *L. monocytogenes* (MIC 5 mg and zone of inhibition 8 mm) while the control showed no activity against these two organisms. Aqueous extracts show no activity against *S. aureus*. This observation agrees with previous reports by Oshodi *et al*.(2004) that aqueous extracts of *G. latifolium* extracts show no activity against *S. aureus*. Results obtained in this work complement previous report on the usefulness of *G. latifolium* (Ugochukwu and Babady, 2002; Ugochukwu *et al*., 2003; Farombi, 2003; Morebise *et al*., 2002). Although compounds responsible for the observed bioactivity are unknown at this point, preliminary works and other reports show the presence of saponins and flavonoids which had been shown to possess antioxidant and antimicrobial properties (Morebise and Fafunso, 1998; Hernández *et al*., 2000). This notwithstanding, the isolation, purification and identification of bioactive compounds in *G. latifolium* is crucial to a fuller understanding of the observed activity and their potential for use in food and non-food systems.

CONCLUSION

This work has shown that *G. latifolium* leaves have potentials for use in food formulation operations in view of its amino acid profile and fatty acid contents. The observed antibacterial activity in the plant suggests that it may play dual role in food and non-food systems where it may also find use. Its full potential for utilisation in these systems is however dependent on the full characterisation of biologically active components in the plant.

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