Journal of Food and Nutrition Sciences
2021; 9(5): 124-130
http://www.sciencepublishinggroup.com/j/jfns
doi: 10.11648/j.jfns.20210905.13
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Nutritional Evaluation of the Leaf Meal of *Gongronema latifolia*

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To cite this article:
Bassey Ukorebi. Nutritional Evaluation of the Leaf Meal of *Gongronema latifolia*. *Journal of Food and Nutrition Sciences*. Vol. 9, No. 5, 2021, pp. 124-130. doi: 10.11648/j.jfns.20210905.13

Received: July 24, 2021; **Accepted**: August 16, 2021; **Published**: October 12, 2021

Abstract: *Background:* The leaves of *G. latifolia* have long been employed in ethnomedical practices, and as food additive among the local populations in Nigeria. This study was carried out to evaluate the nutritive value of *G. latifolia* through the proximate, mineral and phytochemical analysis of *G. latifolia* leaf meal (GLLM), as well as analysis of amino acid profile of GLLM protein. *Methods:* Standard analytical procedures were employed for proximate, mineral and phytochemical analysis of GLLM, and amino acid analysis of GLLM protein. *Results:* Values obtained for proximate fractions on dry matter basis were: crude protein, 14.25%; Ether extract, 2.84%; and Ash, 6.26%. Others included Crude Fiber, 2.84%; Nitrogen free extractive, 60.39%, and Metabolizable energy, 2903.41Kcal/kg. The concentrations (mg/100g) of the five minerals investigated were Calcium, 10.80; Magnesium, 45.0; Potassium, 486.0; Sodium, 3.86, and Phosphorus, 395.0. Amino acid analysis of the leaf meal protein of the plant revealed that it contains nutritionally important essential and non-essential amino acids in concentrations that compare favorably with standard provisional requirement pattern: The total amino acids (TAA) content was 66.38g/100g protein; Total Essential amino acid (TEAA) with histidine was 32.53g/100g protein; Total Non-essential Amino acid (TNEAA) was 33.85g/100g protein; Percentage Total Essential Amino acid with Histidine was 49.01 protein; Percentage Essential Amino acid without Histidine recorded 45.71g/100g protein; Percentage Total Non-essential Amino acid was 50.99g/100g protein. The amino acid scores, vis-avis standard provisional scoring pattern were: Leucine, 95.7%; Isoleucine, 85.0%; Lysine, 72.7%; Methionine + cysteine, 48.6%; Phenylalanine + Tyrosine, 115.0%; Threonine, 75.0%; Valine, 80.0% The amino acids which were in abundance relative to others were: Glutamic acid, Aspartic acid, Leucine, Arginine, Valine, Phenylalanine, Lysine, Glycine and Isoleucine with the values of 9.27, 7.75, 6.70, 4.42, 4.09, 4.01, 3.97, 3.60 and 3.39 (g/100g protein), respectively. However, the first limiting amino acid(s) recorded in its amino acid profile were the two Sulphur-containing amino acids, methionine + cystein, the sum of which scored 48.60% in the amino acid scoring system. The phytochemical factors studied and their percentage compositions wer: alkaloids, 1.03%; flavonoids, 0.37%; saponins, 0.47% and tannins 0.55%. Other phytochemicals measured (mg/100g) included Phenols, 0.17; Phytates, 0.12, and Cyanogenic glycosides, 7.07. C*onclusion:* The result of this study suggests that Gongronema latifolia could contribute significant nutritional benefits to human and livestock nutritional needs. However, further research is needed to ascertain specific administration regimen of GLLM or the phyto-nutrients of *G. latifolia* leaf extracts.

Keywords: Nutritional Evaluation, Leaf Meal, *Gongronema latifolia*, *Utasi,* Phytochemicals

1. Introduction

Gongronema latifolia is a wild dicotyledonous flowering creeping plant of Tropical origin. It is characterized by broad deep green leaves with leaf stock of about 15cm, borne on the nodes of its creeping stem, which is also of deep green coloration. Its natural habitat includes wet tropical upland areas, inland valleys and swampy locations where it grows luxuriantly. In the later location, the vegetation of the plant is perennial, but often tends to be deciduous in dry upland areas [1]. *G. latifolia* can be found abundantly in Cross River State, Nigeria, where it is readily available in the Southern part, up to areas beyond the central region of the State.

Other States of Nigeria where the plant could be found include Akwa Ibom, Abia, Imo, Anambra, Enugu, and Ebonyi. In Cross River and Akwa Ibom, the plant is called

utasi by the Efik and Ibibio tribes.

Among the local communities, *G. latifolia* is used as condiment in traditional soups. It is also used in the preparation of such meals as plantain, yam, and cocoyam porridge. Sometimes the fresh leaves of utasi are eaten raw with palm oil for medicinal purposes. Indeed, the plant plays a significant role in folk medicine among the local populations, where its leaf-meal extract is used as a traditional remedy for malaria, stomach-ache, diarrhea and typhoid fever. This information was corroborated by the work of [2], and the reports of [3]. It is also commonly reported among the natives that *G. latifolia* leaf meal extract is a blood booster, particularly in post-natal blood replenishment and in convalescence. Reported the use of *G. latifolium* in some West African communities to treat cough, intestinal worms, dysentery [4], dyspepsia and malaria. According to [5], the phytonutrients of *G. latifolium* leaf extract exhibits strong inhibitory activity against human lung carcinoma, and human breast adenocarcinoma *in vitro*. Indeed, it has been asserted that the *G. latifolium* plant is a reservoir of many natural antioxidants [6]. Accordingly, Iweala et al [5] maintained that the phytonutrients of G. latifolium leaf extract exhibited scavenging activity against 1, 1-Diphenyl-2-picnylhydrazyl (DPPH) *in vitro*. Earlier reports by [7-9] also opined that phytochemicals could prevent cancer by their antioxidant activity as free radical scavengers.

Reported [10] the antioxidant activity of tannin extract from *G. latifolium* leaves on partially purified lipoxygenase from *Cucumeropsis manii* seeds, and suggested that the extract could be included in food processing to hinder the deteriorative effect of lipoxygenase. Reports of [11] showed that sorghum beer brewed with extracts of *G. latifolium* to impart bitter taste and flavor as substitute to hops used for beer production was adjudged better flavored than beer samples produced with *V. amygdalina*, and *Garcinia kola*, and compared favorably with hopped beer in term of flavor and taste.

Morebise [12] suggested that Researchers should look into the possibility of using *G. latifolium* leaf extracts as approved medicinal formulations or supplements. He was also of the opinion that commercial use of the extracts as food supplements or for food preservation is an interesting area which Researchers can focus on. The foregoing suggests that utasi might be endowed with phytonutrients, the additive and synergistic activities of which could be beneficial in animal nutrition and health.

There is paucity of information on the nutritive value of *G. latifolia*. This study therefore investigated the fundamental nutritive profile of the plant, as might be relevant in livestock as well as human nutrition.

2. Materials and Methods

2.1. Preparation of G. latifolia (utasi) Leaf Meal (GLLM)

Fresh leaves of *G. latifolia* were harvested from the forest area of Ovonum village in Obubra Local Government Area, Cross River State, Nigeria. Harvested leaves were dried under shade to prevent inactivation of its chemical constituent by direct insolation. The air-drying was carried out for 5 – 7 days, until the leaves became crispy to the touch. The dried leaves were then milled to particle sizes that would pass through a 1 mm sieve, using a hammer mill. The *G. latifolia* leaf meal (GLLM) so prepared was stored in air-tight plastic containers prior to its use for the various laboratory analyses described below.

2.2. Chemical Analysis

Samples of GLLM were subjected to different types of chemical analysis including proximate analysis, Mineral analysis, Amino acid analysis and phytochemical screening.

2.2.1. Proximate Analysis

Samples of GLLM were subjected to proximate analyses using the methods of [13], to determine its moisture and dry matter contents. Other proximate fraction, including Crude protein (Cp), Crude fibre (CF), Ether extract (EE), Ash, and Nitrogen free Extractives (NFE) were determined as percentages of the Dry matter. Metabolizable energy was determined by calculation, using proximate fractions, by the formula of [14].

2.2.2. Mineral Analysis

The method described by [13] was used for mineral analyses. Accordingly, one gram of GLLM sample was placed in a Pyrex crucible and 10 ml of pure HNO_3 was added. This was incinerated in GallenKamp microwave oven at 250°C for 18 hours, thereafter it was diluted with distilled water to the volume of 25 ml and filtered through a filter paper. The mineral content was then determined using Atomic Absorption Spectrophotometer (Perkin-Elmer Model 403, Norwalk CT, USA). However, Phosphorus determination was done by titration method.

In all analysis, triplicate determinations were carried out.

2.2.3. Amino Acid Determination

Fresh samples of GLLM were subjected to acid hydrolysis using 6N HCl. Thereafter the hydrolysate was recovered by removing the acid through evaporation at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0), stored in plastic bottles and kept in a freezer. Between 5 – 10 micro-liters of the hydrolysate was loaded into a TSM amino acid analyzer (Technicon Sequential Multi-sample Amino acid analyzer (TSM), Technicon Instruments Corporation, New York). The TSM analyzer separated and analyzed free acidic, neutral and basic amino acids of the hydrolysate, at the end of which it produced a chromagram which represented corresponding values of the different amino acids.

2.2.4. Phytochemical Screening

Quantitative phytochemical analysis was carried out to determine the content of alkaloids, Flavonoids, Tannins, Saponins, Cyanogenic glycosides and Phenols. Alkaloids were determined by the alkaline precipitation method described by

[15]; Ethyl acetate method elucidated by [16] was used to determine Flavonoids; determination of Tannins was carried out by methods described by [17]; the double solvent extraction method [15] was used in the determination of Cyanogenic glycosides; whilst Phenols were determined by the methods of [17]. All analyses were carried out in triplicates.

3. Results

3.1. Proximate/Mineral Composition

The proximate/mineral composition of GLLM is presented in Table 1. The analysis shows that the leaves of *Gongronema latifolia* is sufficiently endowed with nutrients that are important in livestock and human nutrition.

Table 1. *Proximate/Mineral Composition of GLLM.*

Nutrient	Concentration
Moisture (%)	8.04
Dry Matter (%)	91.96
Crude Protein (% DM)	14.25
Ether Extract (% DM)	2.84
Ash (% DM)	6.26
Crude Fibre (% DM)	2.84
Nitrogen Free Extractives	60.39
Metabolizable Energy (Kcal/Kg)	2903.41
Calcium (mg/100g)	10.8
Magnesium (mg/100g)	45.0
Potassium (mg/100g)	486.0
Sodium (mg/100g)	3.86
Phosphorus (mg/100g)	395.3

Values are means of triplicate determinations.

3.2. Phytochemical Composition of GLLM

Results of the quantitative phytochemical analysis of GLLM (Table 2), shows that Alkaloids had the highest concentration (1.03%), followed by Tannins, Saponins and Flavonoids, respectively. Among the compounds measured (in mg/100g), Cyanogenic glycosides recorded the highest value (7.07mg/100g), whilst Phenols and Phytates were 0.17 and 0.12mg/100g, respectively.

Table 2. *Phytochemical Composition of GLLM.*

Compound	Concentration
Alkaloids (%)	1.03
Flavonoids (%)	0.37
Saponins (%)	0.47
Tannin (%)	0.55
Phenols (mg/100g)	0.17
Phytates (mg/100g)	0.12
Cyanogenic glycosides (mg/100g)	7.07

Values are means of triplicate determinations.

3.3. Amino Acid Composition of GLLM

Table 3 shows the amino acid profile of GLLM protein (g/100g protein) The amino acids that were in abundance relative to others were Glutamic acid (Glu), 9.27; Aspatic acid (Asp), 7.75; Leucine (Leu), 6.70; Arginine (Arg), 4.42; Alanine (Ala), 4.09; Valine (Val), 4.01; Phenylalanine (Phe), 3.97; Lysine (Lys), 3.97; Glysine (Gly), 3.60; and Isoleucine (Ile), 3.39g/100g protein, respectively.

This result indicates that GLLM protein is endowed with ten essential amino acids including Glutamine and seven non-essential amino acids. The analytical procedure used could not detect other amino acids.

Table 3. *Amino acid profile of GLLM protein (g/100g) of protein.*

Amino acid	Concentration
Lysine	3.97
Histidine	2.19
Arginine	4.42
Aspartic acid	7.75
Threonine	3.02
Serine	2.14
Glutamic acid	9.27
Proline	2.97
Glycine	3.60
Alanine	4.09
Cysteine	0.86
Valine	4.01
Methionine	0.86
Isoleucine	3.39
Leucine	6.70
Tyrosine	2.90
Tryptophan	ND
Phenylalanine	3.97

ND: Not Detected.

4. Discussion

4.1. Significance of Proximate Composition of GLLM

The proximate composition of the leaf meal of *G. latifolia* refers. Values of its Crude protein (Cp) and Nitrogen free extractives (NFE) on Dry matter basis (14.25 and 60.39%, respectively) are quite reasonable for a leaf meal. The Cp value compares favorably with those of some multipurpose tree leaves like *Enterolobiam cyclocarpum*, which contains 14.45% cp; and *Pterocarpus santalonoides* (15.32% cp). However, when compared to *Gliricidia sepium* (19.26%) and *Leucaena leucocephala* (26.27%) reported by [18], it tends to rank low. The Crude fibre content (CF) of 2.84% is amazingly low for a leaf meal, far less than those of other plants leaves investigated and compares favourably with those of cereal grains used in livestock feeding [19]. This properly recommends GLLM above its counterparts for monogastric feeding programmes. Its Metabolizable Energy (ME) content of 2903.41 Kcal/Kg competes with those of conventional energy concentrates such as maize, Guinea corn, millet, wheat, and others; whilst its general ash and mineral concentrations are comparable with those of most plant leaves investigated.

Of the five minerals assayed (mg/100g), Sodium (3.86) and Calcium (10.8) were the least abundant, while Phosphorus (395.3) and Potassium (486.0) were the highest in concentration. The value of Potassium in *G. latifolia*, tends to agree with the reports of [20] which noted that Phosphorus and Calcium are always found together in the body (within the blood, teeth and bones), as well as in animal products like milk and in poultry products like egg, as well as egg shell.

The ratio of Calcium to Phosphorus in the body is of significant importance for certain physiological processes. In this study, the Ca/P ratio of 0.027 in GLLM is less than the recommended dietary ratio of 0.05, this implies that GLLM Calcium concentration may not be sufficient for normal Calcium/Phosphorus metabolism in the body unless its use in feeding programmes is carried out with Calcium supplementation. According to [21], low Ca/P ratio in the diet facilitates calcinations in the small intestine. Indeed, apart from its role in the skeletal and associated structures, Calcium is also important in blood clotting, muscle contraction and in certain enzymatic processes.

The ratio of Sodium to Potassium (Na/K ratio) measured in mg/100g in GLLM was found to be 0.008. The ratio of Sodium to Potassium (Na/K ratio) in the body is of human health care concern because it is a factor in the control of high blood pressure. Accordingly in this respect, a ratio of less than one (1.00) has been recommended [21]. It would therefore seem that the consumption of *G. latifolia* could be beneficial to humans in alleviating the malaise in question.

4.2. Phytochemical Significance of GLLM

Out of the several thousands of alkaloids known, two forms (aristolochic acid and pyrrolizidine), are of particular nutritional concern because of their toxicity. Aristolochic acids are nephrotoxic, carcinogenic and mutagenic [22], whereas pyrrolizidine is often implicated in hepatotoxicity [23]. These compounds also cause damage to lungs and kidney [24, 25]. Within the limits of this study, it would be difficult to predict whether the toxic forms of alkaloids are present in the 1.03% level of the compound detected in GLLM or not. Further investigations are required to ascertain such.

Tannins are reputed for their capacity to bind dietary proteins thereby reducing the nutritive value of feeds in that regard. The 0.55% of tannins in GLLM appears to be too low to elicit such deleterious effects when the leaf meal is incorporated in a diet. This could however be verified in a feeding trial.

Phytates usually form insoluble salts with calcium, this effect is likely to adversely affect the absorption of dietary calcium, and/or utilization of the element in the animal body.

The concentration of saponins in GLLM was 0.47%. Saponins are steroids or tri-terpenoid glycosides which are characterized by their bitter or astringent taste, foaming properties, and hemolytic effects on red blood cells. Saponins have been shown to possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic and permeabilization of the intestine) effects [26, 27].

Another important phytochemical detected in GLLM was cyanogenic glycoside, with a value of 7.07mg/100g. This concentration is higher than those recorded for some legume seeds, as reported by [28]. The toxicity of cycanogenic glycosides and their derivatives is dependent on the release of hydrogen cyanide (HCN) on hydrolysis. Cyanide toxicity can occur in animals including humans at a dose between 0.5 and 3.5mg HCN per Kilogram body weight [29]. The deleterious effects exhibited by a lethal dose of HCN is characterized by its rapid reaction with serum metal ions of iron and copper, leading to a series of reactions to form cyanohaemoglobin which is not an oxygen carrier. Large doses of cyanogenic glycosides would therefore cause death, by inhibition of cell respiration.

Reported that up to 30% dietary inclusion of GLLM in the diets of weaner rabbits elicited no deleterious effects on hematological and serum biochemical characteristics of the animals [30]. This suggests that the phytochemical factors in GLLM are not such as could cause health hazards in humans and livestock. This however may not be the case with higher dietary levels of the leaf meal than those used in the report, more investigations are needed to arrive at more specific findings.

4.3. Appraisal of Amino Acid Composition of GLLM

Amino acids are a group of organic molecules consisting of a basic amino group (-NH_2), an acidic carboxyl group (-COOH), and an organic R-group (side chain) that is unique to each amino acid. Amino acid are the building blocks of proteins, a macro molecule which catalyze the vast majority of chemical reactions that occur in the cell and also provide many of the structural elements in the cell, and bind cells together into tissues. Amino acids are needed for growth, reproduction, productive activities and maintenance of the organism. Some amino acids like glycine and glutamic acid are involved in the transmission of impulses in the nervous system. Some compounds of biological importance arise from amino acids. In addition, the metabolism of amino acids give rise to many important products in the body, and the products in turn take part in some important functions in the body [31].

The values of Leucine (6.70g/100g protein), and Phenylalanine (3.97g/100g protein) in GLLM protein are higher than the reference values of 4.20g/100g protein and 2.80g/100g protein, respectively [32]; lysine value (3.97g/100g protein), and the value of valine (4.01g/100g protein) compare favourably to the reference values of 4.20g/100g protein and 4.20g/100g protein, respectively [32]. However, GLLM methionine and threonine values of 0.86g/100g protein and 3.02g/100g protein, respectively are far below the reference standards of 2.2g/100g protein and 17.5g/100g protein, respectively [21]. This indicates that GLLM protein cannot be used as a sole protein source in diets. For adequate supply of amino acids in diets with a GLLM based protein concentrate, there would therefore be need for the supplementation for methione and threonine, since they are essential amino acids.

Table 4. *FAO Reference Values for some Essential amino acids compared with their GLLM counterparts.*

AMINO ACID	VALUE (g/100 protein)[a]	GLLM VALUE (g/100g protein)
Leucine	4.20	6.70
Phenylalanine	2.80	3.97
Lysine	4.20	3.97
Methionine	2.20	0.86
Valine	4.20	4.01
Threonine	17.50	3.02

[a] Adapted from FAO (1970).

Sundry parameters are presented in Table 5. The total amino acid (TAA) content of 66.38g/100g protein indicates that GLLM is rich in amino acids, and will contribute significantly to the supply of the same in diets.

Table 5. *Essential, Non-essential, Acidic, Neutral and Basic amino acids of GLLM.*

Amino acid classification	Concentration (g/100g)
Total Amino Acids (TAA)	66.38
Total Non-essential amino acids (TNEAA)	33.85
Percentage TNEAA	50.99
Total Essential Amino Acids (TEAA): with Histidine	32.53
Total Essential Amino Acids (TEAA): without Histidine	30.34
Percentage TEAA with Histidine	49.01
Percentage TEAA without Histidine	45.71
Essential Aliphatic Amino Acids (EAAA)	14.10
Total Neutral Amino Acids (TNAA)	52.90
Percentage Total Neutral Amino Acids (% TNAA)	79.69
Total Basic Amino Acids (TBAA)	10.58
Percentage Total Basic Amino Acids (% TBAA)	15.94
Total Sulphur Amino Acids	01.72
Percentage Total Sulphur Amino Acids	02.30

When compared to some plants protein sources, TAA content of GLLM is higher than those of pumkin, gourds and melon seeds (38.3; 53.6; and 53.4g/100g protein, respectively) reported by [33] in [34]; soybean, 44.4g/100g protein [35]; pigeon pea, 45.2g/100g protein, reported by Nwokolo [36]. This implies that though GLLM is lower in proximate protein content than afore mentioned plant protein sources, it is nutritionally a better protein source than them. The TAA value of GLLM is however lower than that reported for cashew nuts (75.8g/100g protein) by [37].

The total essential amino acid (TEAA) value of GLLM plus histidine was 32.53g/100g protein. This is higher than those reported for some tropical leaf meals including *L. leucocephala,* 11.94g/100g protein [37]; *G. sepium,* 11.59g/100g protein [38]; *S. sesban*, 9.04g/100g [39]; *P. chilensis*, 6.67g/100g [40]; *S. grandiflura*, 7.53g/100g [41]; and *M. esculenta,* 8.13g/100g protein [42]. This seemingly gives GLLM protein an edge over these sources with regards to essential amino acid profile. Additionally, its percentage total essential amino acid (% TEAA) of 49.01 with histidine indicates that GLLM protein contains almost as much essential amino acids as the non-essential amino acids.

Amino Acid scoring pattern of GLLM is shown in Table 6. Amino acid scoring is usually done by comparing the amino acid content of a material or diet with that of a reference protein containing all the essential amino acids in amounts sufficient to meet requirements without any excess. Through this comparison, it is possible to determine the nutritional quality of a protein or mixture of proteins, by calculating the deficit of each essential amino acid below the amount in the reference protein needed to meet the essential amino acid and nitrogen requirements of animal or human subjects. The quality of dietary protein can be measured in various ways [43]. For purposes of protein requirement, the amino acid score can be calculated in terms percentage of adequacy described by [31] as follows:

$$^{*}\text{AAs} = AAs = \frac{Xa}{Xb} x100$$

[*] Adapted from [31].

Where AAs = Amino acid score; Xa = g amino acid per 100g test protein; Xb = g amino acid in requirement pattern.

The GLLM amino acids scoring table (Table 6) shows that apart from tryptophan which was not detected in GLLM amino acid analysis, the amino acids score of GLLM were generally good. However, the scoring table also reveals that the first and second limiting amino acids of GLLM would be the sulfur containing amino acids (methionine + cysteine) and lysine (48.6% and 72.7%), respectively. Considering this result, it would be necessary supplement GLLM with methionine and lysine (the limiting amino acids), as well as tryptophan which was not detected, if GLLM is to be used as a sole protein source in the diet.

Table 6. *Amino Acid scores of GLLM.*

Amino acid	Provisional amino acid scoring pattern[a] (g/100g protein)	Amino acid in GLLM (g/100g protein)	Amino acid scores of GLLM protein (%)
Leu	7.0	6.7	95.7
Ile	4.0	3.4	85.0
Lys	5.5	4.0	72.7
Met + Cys	3.5	1.7	48.6
Phe + Tyr	6.0	6.9	115.0
Thre	4.0	3.0	75.0
Try	1.0	Not detected	-
Val	5.0	4.0	80.0

[a]Adapted from Ekeanyanwu and Ononogbu (2009).

5. Conclusion

The findings of this investigation suggests that *Gongronema latifolia* could contribute significant nutritional benefits to human and livestock nutritional needs.

The proximate composition of GLLM shows that its NFE fraction is sufficiently high to support the energy needs of man and livestock. Its low crude fibre content would be advantageous in the food and nutrition of monogastrics.

Apart from its calcium/phosphorus content, the mineral concentration levels/ratios of GLLM are nutritionally beneficial. Its Sodium/potassium ratio in particular could facilitate the

control of high blood pressure as corroborated by [12].

The levels of phytonutrients assayed are likely not deleterious, but would rather seem beneficial as corroborated by [30].

Its amino acid profile is quite fascinating, and could be of great nutritional benefits, especially if used with supplementation with the sulfur-containing amino acids which are limiting, as well as threonine.

With the foregoing, it would seem important to recommend that more investigations are needed to determine the toxic levels of the phytochemicals and to ascertain specific administration regimen of GLLM or the phytonutrients of *G. latifolia* leaf extracts.

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