PROXIMATE AND VITAMIN CONTENTS ANALYSIS OF Gongronema latifolium (UTAZI) LEAF

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Abstract

This study investigated the proximate, vitamins A, B complex, C, E, D and K of Gongronema latifolium (Utazi) leaf. Standard analytical methods were used for the proximate determination. UV- visible spectrophotometer was used for vitamin A, D, E and K, and titrimetric method was used to determine the vitamin C. The results of the analysis showed that the leaf contains 1.84 ± 0.847% of moisture, 4.25 ± 0.776% of ash, 1.54 ± 0.632% of fat, 7.23 ± 1.645 of fibre, 9.80 ± 0.495% of protein and 74.68 ± 0.837% of carbohydrate. Vitamin A content was 19.604 ± 0.204. Vitamins B1, B2, B3, B5, B6, B7, B9 and B12 were 0.215 ± 0.0195 mg/kg, 0.2145 ± 0.0025 mg/kg, 0.945 ± 0.116 mg/kg, 2.119 ± 0.04 mg/kg, 0.4715 ± 0.0275 mg/kg, 6.939 ± 0.32 mg/kg, 4.4475 ± 0.725 mg/kg and 0.245 ± 0.095 mg/kg, respectively. Vitamins C, D, E and K were 641.762 ± 50.007 mg/100g, 1.819 ± 0.113 mg/kg, 10.383 ± 0.264 mg/kg and 2.225 ± 0.242 mg/kg respectively. The results indicated that Gongronema latifolium leaf contains reasonable quantities of fibre, protein and carbohydrates, vitamins A, B5, B7, B9 and E. Vitamin C content was highest. The least contained vitamin was vitamin D. The findings will contribute to the broader knowledge of the potential health benefits of G. latifolium leaf consumption and its nutrient-rich dietary source.

Key words: *Gongronema latifolium,* Proximate, Vitamins, Leaf

INTRODUCTION

In Africa, using herbaceous plants to heal illnesses has been a long-standing tradition since the dawn of time (Ezeonwu, 2011). Alternative healthcare is still accepted and used worldwide. Drugs used to treat a variety of illnesses are often derived from medicinal plants. The plants can be used by themselves or in conjunction with other plants. These plants have been linked to dietary and medical advantages (Avwioro, 2010; Oroka and Ureigho, 2015). In recent years, the prevention of cancer and cardiovascular diseases has been associated with theingestion of spices, fresh fruits, vegetables, or teas rich in natural antioxidants (Virgili *et al.,* 2001). One of the sought after vegetables is *Gongronema latifolium* (*G. latifolium*). *G. latifolium* is known as "Utazi" in the South-Eastern part of Nigeria (Adebayo *et al.,* 2022). It has soft woody stem which produces adventitious roots in soil (Osuagwu *et al.,* 2013). The plant is grown at a farm by conventional medical practitioners as a vegetable (Ahamefule *et al.,* 2006). *G. latifolium* is commonly used in soup as vegetable, or applied dried as powdery spice (Ugochukwu *et al.,* 2003; Antai *et al.*, 2009; Balogun

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et al., 2016). Edet *et al.,* (2011); Offor and Uchenwoke, (2015) noted that the leaf extract of *G. latifolium* was used to treat diabetes, malaria, hypertension, and constipation. The extract of *G. latifolium* is consumed across Nigeria for the maintenance of blood glucose level (diabetes), treatment of viral hepatitis, bilharzia, and other microbial infections, and as a cleansing purge by Muslims during Ramadan (Schneieder and Brietmeiere, 2003; Owu *et al.,* 2012; Chioma *et al.*, 2014; Mosango, 2022). Additionally, fresh leaves are chewed by asthmatic patients to relieve wheezing while oral cold macerated preparation of roots of *G. latifolium* is prescribed for the treatment of asthma (Essien *et al.,* 2007; Adegbenro *et al.,* 2021). One of the famous polyherbal preparations for hepatitis and malaria is a decoction of *G. latifolium, Mormodica charantia or Vernonia amygdalina* and *Ocimum gratissimum* given to help cleanse the liver (Ihesie, 2022). According to Abeer, (2021), *G. latifolium* increases breastfeeding mother's return, increases postpartum contraction reduction, and boost hunger. The leaf of *G. latifolium* is rich in fats, proteins, vitamins, minerals and many essential amino acids collectively contributing to its high nutritional value (Egbung *et al.,* 2011; Adebajo *et al.,* 2012), and also a source of iron (Joe, 2021). Vitamins are the building blocks of a healthy body (Adegbenro *et al.,* 2021; Paulin *et al.,* 2021). Vitamins are required in small quantities, but their deficiency can lead to various health problems. Proper nutrition and a balanced diet are crucial for maintaining optimal vitamin levels (Xiu and Price, 2009; Wendt, 2015; Price 2015; Gratzer 2016; Suzuki, and Shimamura, 2018; Kögl and Tönnis, 2018; Andrew, 2018; Trumbo and Poos, 2019; Acob, 2020; Gregory and Salmon 2020). *G. latifolium* contains essential oils, alkaloids, saponins and pregnanes (Ye *et al.,* 2013; Analike *et al.,* 2022). Over the past decade, there has been a resurgence of interest in the investigation of natural materials as a source of potential drugsubstance (Paulin *et al.,* 2021). Therefore, the need to determining the quantities of proximate and vitamins contents of the leaf of *G. latifolium*.

MATERIALS

Sample Collection: Fresh utazi leaves (about 10 kg) was purchased from local farmers at Amoakpala and Oko in Orumba North Local Government Area, Anambra State, Nigeria.

METHODS

Preparation of the Sample

Gongronema latifolium (Utazi) leaves were thoroughly rinsed using clean water and then ground using grinding machine into fine powder; the ground sample was weighed and kept for analysis.

Proximate Analysis

Ash Content: Empty platinum crucible was washed, dried and the weight was noted. Two gram of wet sample was weighed into the platinum crucible and heated in a muffle furnace at 500℃ for 3hours. The sample was cooled in a desiccator after burning and weighed.

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% Ash content = $\frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$

Where W_1= weight of empty platinum crucible, W_2 = weight of platinum crucible and sample before burning, W_3 = weight of platinum and ash.

Moisture Content: A petri dish was washed and dried in the oven. Two gram of the sample was weighed into petri-dish, and the weight of the petri dish and sample was noted before drying. The petri-dish and sample were put in oven, heated at 100℃ for 1hour and allowed to cool. The weight was noted. It was further heated for another 1 hour until a steady weight was obtained and the weight noted. The drying procedure was repeated until a constant weight was obtained.

% Moisture content = $\frac{W_1 - W_2}{Weight\ of\ sample} \times \frac{100}{1}$

Where W_1= weight of petri-dish and sample before drying, W_2= weight of petri-dish and sample after drying.

Crude Fat Content: Five gram of the sample was dried in a flask in an oven at 105-110℃ for about 30 minutes and transferred into a dessicator and allowed to cool. The flask was filled with 300 ml petroleum ether (boiling point 40-60℃). The extraction thimble was lightly plugged with cotton wool and Soxhlet apparatus was assembled and allowed to reflux for about 6 hours. The thimble was carefully removed and petroleum ether at the top of the set up was allowed to drain into a container for re-use. When the flask was almost free of petroleum ether, the sample was removed and dried at 105-110℃ for 1 hour. It was transferred from the oven into a dessicator and allowed to cool, and then weighed.

Crude Fibre: Two gram of the defatted sample from crude fat analysis was boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25g of H_2SO_4 per 100 ml of solution. The solution was filtered through linen on a fluted funnel and further washed with boiling water in a beaker until the washings are no longer acidic which was confirmed by testing with litmus paper. The residue was transferred to another beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25g of carbonate free NaOH per 100 ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, and further dried in an electric oven and weighed. It was finally incinerated, cooled and weighed.

The loss in weight after incineration × 100 is the percentage of crude fibre.

%Crude fibre = $\frac{Weight\ of\ fibre}{Weight\ of\ Sample}$ X 100.

Crude Protein: This was done by Kjeldahl method described by Chang (2003). The total nitrogen was determined and multiplied with factor 6.25 to obtain protein content.

Carbohydrate: The carbohydrate content was determined by subtraction method (%Total Carbohydrate = [100 - %(Moisture + Protein + Fat + Ash + Fibre)].

Vitamin Analysis: The analysis was carried out using Official Methods of Analysis by Association of Analytical Chemists (AOAC, 2005).

Vitamin A (Retinol): One gram of the sample and the standard were mixed with 30mL of absolute alcohol and 3mL of 50mL KOH solution was added to it and boiled gently for 30 minutes under reflux. After washing with distilled water, vitamin A was extracted with 3 X 50mL of diethyl ether.

The extract was evaporated to dryness at low temperature and was then dissolved in 10mL of isopropyl alcohol. One millilitre of standard vitamin A solution prepared and that of the dissolved extract were transferred to separate curvettes and their respective absorbances were read in a Thermo Fisher Scientific spectrophotometer at 325nm with a reagent blank at zero.

Calculation: $= \frac{Absorbance\ of\ Sample \times Concentration\ of\ Standard}{Absorbance\ of\ Standard}$

Vitamin B_1 (Thiamine) and B_2 (Riboflavine): One gram of sample was weighed into a conical flask, the sample was dissolved with 100mL of deionized water, and it was shaken thoroughly and heated for 5 minutes and allowed to cool and filtered. The filtrate was poured into a curvette and their respective wavelengths for the vitamins set to read the absorbance using Thermo Fisher Scientific spectrophotometer.

Vitamin B1 = 261nm, Vitamin B2 = 242nm.

Concentration (mg %) = $\frac{A \times DF \times Volume\ of\ curvette}{5}$. Where A = Absorbance, E = extinction coefficient = 25 for B_1 and B_2, DF = dilution factor

Vitamin B_3 (Niacin): Five gram of the sample was dissolved in 20mL of anhydrous glacial acetic acid and warmed slightly. Five mililitres of acetic anhydride was added and mixed. 2–3 drops of crystal violet solution was added as indicator and 0.1M perchloric acid was added to titrate to a greenish blue colour. Vitamin $B_3 = \frac{Titre\ value \times 0.0122}{0.1}$

Vitamin B_5 (Pantothiamine): Standard Preparation: The working standard (0.25mL) of vitamin B_5 was taken into 25mL volumetric flask with a solution mixture (chloroform and methanol in ratio of 1:9). It was gently shaken to mix thoroughly and was made up to the mark.

Sample Preparation: The sample (0.25mL) was measured into a 25ml volumetric flask containing a solution mixture (chloroform and methanol in a ratio of 1:9). It was gently shaken to mix thoroughly and absorbances recorded at 246nm against the blank.

Vitamin B_6: Five gram of the sample was dissolved in a mixture of 5mL of anhydrous glacial acetic acid and 6mL of mercury (II) acetate solution. Two drops of crystal violet was added as indicator and 0.1M perchloric acid was used to titrate to green colour end point. Calculation: each mL of 0.1M perchloric acid is equivalent to 0.02056g of $C_8H_{11}NO_3$ HCl.

Vitamin B_7 (Biotin): Sample Preparation: The sample (0.1mL) was taken into a separator. In the separator, 5mL of water was added, mixed well and added 5mL chloroform. The chloroform layer was discarded and the water layer was taken into 50mL volumetric flask by passing it through anhydrous sodium sulphate and made up to 50mL with water. Sample and blank solutions (2ml) were taken into test tubes. In each test tube, 2mL of 0.2% solution of phenylhydrazine (in hydrochloric acid and alcohol in ratio of 1.5v/v) was added and mixed well. After, it was heated on a water bath until it almost dried and cooled at room temperature. Two mililitres of the solution mixture (ammonia and alcohol in ratio of 1:1) was added in each test tube and 1mL pyridine was added. Absorbance was recorded at 548nm against the blank. Standard cobalamine was also treated and analyzed same as the sample.

Vitamin B_9 (Folic acid): The sample (0.2 milliliters) was weighed and taken into separator. Five milliliters of water was added and mixed well and 5ml of chloroform was used to extract the sample. The water was discarded and chloroform was taken in dry 50ml volumetric flask by passing it through anhydrous sodium sulphate and was made up to 50mL with chloroform. The sample and the blank solutions were each put into separate test tubes. Two milliliters of 0.2% solution of phenylhydrazine (in hydrochloric acid and alcohol in ratio of 1.5v/v) was added in each of the test tubes and were well mixed. They were then each heated on water bath until almost dryness and cooled at room temperature. Fifteen milliliters solution mixture (ammonia and alcohol in ratio of 1:1) each was added in each of the test tubes. Its absorbances was recorded at 635nm against blank.

Vitamin B_{12} (Cobalamine): Twenty five milligram of sample was dissolved in 250mL of deionized water. The absorbance was read at 361nm.

Concentration (mg%) = $\frac{A \times DF \times Volume\ of\ curvette}{E}$

Where A =Absorbance, E = Extinction coefficient =25, DF = dilution factor =5

Vitamin C (Ascorbic Acid): A weighed sample was homogenized in 6% EDTA/TCA solution. The homogenate was filtered and used for analysis. 20mL of 30% KI solution was added to the homogenate followed by 100mL of distilled water. 1mL of 1% starch solution was added to it and it was titrated against 0.1M $CUSO_4$ solution. The end point was marked by a black coloration. A reagent blank was also titrated. Vitamin content was calculated based on the relationship below. 1mL of 0.1 mole CUSO4 = 0.88mg Vitamin C. Vitamin C mg/100 = $\frac{100\ \text{x}0.88\ \text{x Titre} - \text{blank}}{Weight}$

Vitamins D and E: Total vitamin D and E were estimated by the method described by AOAC, (2023).

Vitamin K: Total vitamin K was estimated by the method described by Dutta, (2011).

RESULTS AND DISCUSSION

Proximate Analysis

The results of the proximate contents of *Gongronema latifolium* (Utazi) leaf are presented in Tables 1.

Table 1: The Proximate Composition of *Gongronema latifolium* leaf.

Parameters (%)	Values
Moisture	1.84 ± 0.847
Ash	4.25 ± 0.776
Fat	1.54 ± 0.632

Fibre	7.23 ± 1.645
Protein	9.80 ± 0.495
Carbohydrate	74.68 ± 0.837

G. latifolium is believed to have rich nutritional and medicinal values. The results of proximate composition (Table 1) showed that the moisture content of the sample leaf was 1.84 ± 0.487%. Balogun *et al.,* (2016), reported a moisture composition of 15%, which is high when compared with the result of this study. The variation could be traced to the extent of drying, which implied that the plant leaves used may have not been properly air-dried. The moisture content has great value in the preservation of food materials and high moisture content is an index of spoilage and short shelf life. The leaves indicates moderate moisture content which could minimize degradation by microorganisms during storage. The value for ash content shown in Table 1 was 4.25 ± 0.776%; the result is in agreement with the findings of Nwachoko *et al.,* (2017) that reported a mean score of 4.96% for ash content of *G. latifolium*. Adeyeye and Olaleye, (2020) reported ash content of 8.50%, which was higher than what was obtained in the present analysis. *G. latifolium* leaf contains reasonable amount of inorganic noncombustible materials. The fat content obtained in the analysis as presented in Table 1 was 1.54 ± 0.632%. The value was not in line with 4.60%; obtained by Adeyeye and Olaleye, (2020). This may be caused by the environmental conditions, especially, the soil composition of the area from where the plant leaves were harvested. Some dietary fats are needed in the body to facilitate absorption of fat soluble vitamins and carotenoids. Studies have suggested that total dietary fat intake is linked to an increased risk of obesity and diabetes (Astrup, 2005). The crude fibre content was determined to be 7.23 ± 1.645%. Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer (Mayo Clinic, 2024). The protein content was 9.80 ± 0.495% as shown in Table 1. A report by Etukudo *et al.,* (2015) indicated 18.29 ± 0.30 as the protein content of the *G. latifolium* leaf. Protein helps to repair and build body's tissues. It drives metabolic reactions, maintains pH and fluid balance, and keeps the immune system strong (Offor and Uchenwoke, 2015). The carbohydrate content according to Table 1 was 74.68 ± 0.837%. The result was higher than what was reported by Adeyeye and Olaleye, (2020) which was 39.0%. Carbohydrate serves as carbon sources for the synthesis of energy and other bi-molecules. It is produced by plants during photosynthesis.

Excessive intake of carbohydrate usually results in hyperglycaemia and leads to storage of large amount of fats in the adipose tissues, resulting in obesity (Gupta, 2022). The variation in composition could be as a result in environmental factor, geographical location, soil nutrient, method of cultivation, seasonal variation.

Vitamin Analysis

The results of the vitamin content analysis of *G. latifolium* are indicated in Table 2.

Table 2: **Vitamins Content of *Gongronema latifolium* (Utazi) leaves**

Vitamins (mg/kg)	Values
A (mg/g)	19.604 ± 0.204
B1	0.2150 ± 0.0195
B2	0.2145 ± 0.0025
B3	0.9450 ± 0.116
B5	2.1190 ± 0.040
B6	0.4715 ± 0.0275
B7	6.9390 ± 0.320
B9	4.4475 ± 0.725
B12	0.2450 ± 0.095
C (mg/100g)	641.762 ± 50.007
D	1.819 ± 0.113
E	10.383 ± 0.264
K	2.225 ± 0.242

The determination of vitamin contents of *G. latifolium* (Utazi) leaves is crucial for understanding the nutritional composition of this traditional leafy vegetable. The vitamin A content in *G. latifolium* leaf shown in Table 2 was found to be 19.604 ± 0.204 mg/g. This fat-soluble vitamin is essential for vision, immune function, and overall skin health (Acob, 2020). The detected amount indicated a substantial presence of vitamin A, contributing to the nutritional value of *G. latifolium*

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leaf and this partly aligns with 360µ/100g obtained by Balogun *et al*. (2016). Also, in a study by Offor *et al*. (2015), it was observed that the retinol content of *G. latifolium* was 37mg/100g, and this was higher than what was obtained in this study. Also, in Table 2, it is shown that the content of vitamin B1 is 0.215 ± 0.0195 mg/kg) which aligned it with findings in plant-based foods of a study carried out by Adah and Philips, (2010), on green leafy vegetables. They proposed that thiamine in *G. latifoluim* is an essential nutrient playing a crucial role in energy metabolism. However, variations in thiamine content may be influenced by factors such as soil conditions, climate, and plant species according to a research carried out by Rucker and Moyer (2011). The concentration of vitamin B2 (0.2145 ± 0.0025 mg/kg) is within the range observed in various vegetables in the studies undertaken by Tian (2020). Tian (2020) revealed that riboflavin is essential for redox reactions in cellular energy production. Adah and Philips (2010) claimed that variability in riboflavin content is attributed to plant genetics, maturity, and post-harvest handling. The concentration of (niacin) vitamin B3 (0.945 ± 0.116 mg/kg), presented in Table 2, is consistent with levels found in leafy greens studied by Abdulla and Al-Marzoqi, (2016). Wu and Yuan, (2011) proposed that niacin is crucial for DNA repair and metabolic processes. Environmental factors and genetic variations contribute to niacin fluctuations in plants (Krueger and Klotz, 2020). The vitamin B5 concentration (2.119 ± 0.04 mg/kg) falls within the range reported for vegetables by Makanjuola (2020). Vitamin B5 is vital for fatty acid synthesis. Plant growth stage, climate, and soil nutrients affect vitamin B5 levels (Lipschitz, 2006). The obtained value of vitamin B6 (0.4715 ± 0.0275 mg/kg), in Table 2 is comparable to levels found in other green leafy vegetables according to reports by Adah and Philips, (2010). Vitamin B6 is essential for amino acid metabolism. The concentration of vitamins B7 and B9 (6.939 ± 0.32 mg/kg), and (4.4475 ± 0.725 mg/kg) are consistent with values reported for edible plants by Wu and Yuan (2011). Vitamin B7 (Biotin) plays a role in fatty acid synthesis, while vitamin B9 (folate) is crucial for DNA synthesis (Andrew, 2018). The vitamin B12 content shown in Table 2 is 0.245 ± 0.095 mg/kg. Vitamin B12 is primarily found in animal products, some plants can accumulate it due to soil contamination (Price, 2015). Levels in plants are generally lower compared to animal sources (Tian, 2020). Vitamin C (ascorbic acid), a water-soluble antioxidant, was found to be 641.762 ± 50.007 mg/100g as shown in Table 2. This value highlighted the significant amount of vitamin C content in *G. latifolium* leaf, and showcasing its potential as a rich source of this essential nutrient known for its role in collagen synthesis. Vitamin C plays an important role in immune function (Gregory and

Salmon, 2020). The high vitamin C content in *G. latifolium* leaf showed that this particular species contains more vitamin C than 290µ/100g and 28mg/100g obtained by Balogun *et al.* (2016) and Offor *et al*. (2015) respectively. Vitamin D concentration presented in Table 2, was 1.819 ± 0.113mg/kg. Vitamin D is essential for calcium absorption and bone health, while the levels detected are relatively minimal. The presence of vitamin D in *G. latifolium* leaf suggests a supplementary dietary source of this vital nutrient. Vitamin D is an essential vitamin that helps regulate calcium and phosphorus in the body. Taking vitamin D by mouth is effective for preventing and treating rickets (Kogl and Tonnis, 2018). Also in Table 2, the *G. latifolium* leaf contained 10.383 ± 0.264 mg/kg of vitamin E. Vitamin E is a potent antioxidant that plays a crucial role in protecting cells from oxidative damage (Wendt, 2015). The levels observed in *G. latifolium* leaf emphasize their potential contribution to antioxidant activities in human body. Balogun *et al.* (2016) obtained 45µ/100g of vitamin E content in *G. latifolium*. Mgbeje *et al*. (2019) obtained between 0.67-0.9mg/100g of vitamin E in four selected tropical vegetable plants. Vitamin E is seen to be the most important lipid soluble antioxidant protecting membranes from lipid peroxidation by acting as a chain-breaking antioxidant (Mensah *et al.,* 2008). The Vitamin K content in *G. latifolium* leaf was found to be 2.225 ± 0.242 mg/kg as indicated in Table 2. Vitamin K is crucial for blood clotting and bone metabolism (Trumbo and Poos, 2019). The levels found in *G. latifolium* leaf indicate a notable contribution to the dietary intake of this essential vitamin. The vitamin K content in *G. latifolium* leaf aligns with the finding of studies by Balogun *et al*., (2016) emphasizing the role of green leafy vegetables as significant sources of vitamin K.

RECOMMENDATIONS

Gongronema latifolium leaf has shown to be an important source of nutrients in terms of its proximate and vitamins contents. However, we recommend that:

1. Its elemental composition of the plant leaf should also be determined.

2. It is important that other nutritional parameters such as phytochemicals, anti-nutrients components etc be also assessed.

3. More advanced analytical techniques and methods such HPLC-MS, UV-GC etc are suggested to be employed for further analysis.

CONCLUSION

From the results of the analysis, it is evident that *G. latifolium* (Utazi) leaf contains reasonable amount of ash, fibre, protein and carbohydrates, and vitamins A, B7, C, and E, which suggest that *G. latifolium* leaf is a valuable dietary source of these food nutrients and vitamins, therefore, contributing to the overall health and wellbeing of users.

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1. **Reduce your Abstract to 250 words.**
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3. **Remove Italics from Abstract.**
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16. Include Authors and Dates where “AUTHORS” were indicated in text.

COMMENT

Paper can be published if the recommended are corrections thoroughly made.

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