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Phytochemical, biochemical Analysis and Sonographic evaluation of Bioactive Compounds in *Gongronema latifolium* (Utazi) leaf extract

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Abstract

Gongronema latifolium (G. latifolium), commonly known as "Utazi" in Igbo and "Arokeke" in Yoruba, is widely used in traditional medicine due to its bioactive compounds which established its anti-oxidant and anti-inflammatory properties . This study aimed to analyze the phytochemical composition of G. latifolium leaves extract using Thin Layer Chromatography (TLC), and Gas Chromatography-Flame Ionization Detection (GC-FID) and the potential effect of ethanolic leaf extract on hepatic function using sonographic imaging. The qualitative analysis revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, cardiac glycosides, and steroids, while terpenoids were absent. TLC analysis identified two active components with Rf values of 1.23 cm (green) and 1.15 cm (lemon). GC-FID results confirmed the presence of significant alkaloids, including colchicine (30.4710 µg/ml) and quercetin (22.0830 µg/ml), as well as flavonoids such as flavone (13.4719 µg/ml) and kaempferol (13.4345 µg/ml). These bioactive compounds are associated with anti-inflammatory, antioxidant, anti-diabetic, and cardiovascular benefits. 12 wistar rats were divided into 4 groups: control,low-dose, high-dose and recovery group.Over 28 days ultrasound imaging was performed at interval to access structural changes in the liver and kidney correlating with biochemical markers.Sonagraphic analysis reveals those-dependent variations in echotexture and organ morphology indicating potential hepatoprotective threshold. This study underscores the relevance of non-invasive imaging and phtytochemical compounds in Medical and pharmaceutical research.

Keywords: *Gongronema latifolium leaf*; Phytochemicals; GC-FID; TLC; Alkaloids; Flavonoids; Ultrasound; Sonography; Biochemical and hepatic

1. Introduction

Gongronema latifolium is widely used in herbal medicine due to its bioactive compounds, including flavonoids, alkaloids, and saponins, which contribute to its anti-inflammatory, antioxidant, and hypoglycemic properties (Ugochukwu et al., 2003; Balogun et al., 2016). Despite its therapeutic benefits, prolonged consumption raises concerns regarding systemic toxicity, particularly hepatotoxicity and nephrotoxicity, as reported in various medicinal plants with bioactive alkaloids

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and flavonoids (Muhammad et al., 2021; Patel et al., 2012). Conventional biochemical assays provide valuable insights into the metabolic effects of plant extracts, but real-time imaging enhances understanding by visualizing physiological responses and detecting early signs of organ dysfunction (Ezekwesili-Ofili & Okaka, 2019). Sonography, a non-invasive diagnostic tool, enables dynamic assessment of organ integrity, making it a preferred method for monitoring hepatotoxic and nephrotoxic effects in phytopharmacology (Orumwensodia & Uadia, 2022). Phytochemicals such as flavonoids, saponins, alkaloids, cardiac glycosides, and beta-sitosterol are known to be present in G. latifolium leaves (Olufunke, 2021). Flavonoids, a group of natural compounds with phenolic structures, are found in various plants and have proven to be valuable in nutraceutical, pharmaceutical, and cosmetic industries. Their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, along with their ability to modulate cellular enzyme function, make them essential in various applications (Panche *et al.,* 2016; Asad et al., 2020). Alkaloids, another important class of secondary metabolites in plants, are known for their diverse pharmacological effects, including pain relief, anti-inflammation, anti-tumor, antibacterial, antiviral, antifungal, and anti-diabetic properties (Buckingham *et al.,* 2010; Sato *et al.*, 2011; Verma, 2016; Benabdesselam *et al.,* 2007; Kucukboyaci *et al.,* 2010).

This study aims to explore and quantify the bioactive compounds present in *Gongronema latifolium* leaves using Gas Chromatography-Flame Ionization Detection (GC-FID) and Thin Layer Chromatography (TLC) and the hepatoprotective and nephrotoxic potentials of *Gongronema latifolium* through ultrasound monitoring, aiming to bridge the gap between phytochemical analysis and biomedical application.The findings are expected to contribute to a deeper understanding of the plant's therapeutic potential and support its continued use in traditional medicine.

2. Methodology

2.1. Sample Collection

G. latifolium leaves were collected from Science Laboratory Technology Department's botanical garden at Federal Polytechnic, Oko, Anambra State. Nigeria.

2.2. Sample Preparation

One kilogram of the fresh Utazi leaves were thoroughly rinsed in water, and air dried at room temperature for 10 days. The dried plant leaves were ground using electric blender and kept for phytochemical analysis.

2.3. Phytochemical Screening

2.3.1. Test for Alkaloids

- **Wagner's Test:** Aqueous solution (20 cm^3) of the acid-soluble portion of a methanol extract was basified with concentrated ammonium hydroxide in a test tube. The mixture is shaken in a separator funnel with 10cm of a chloroform-ethanol solvent. 2 cm^3 of this portion was treated with 2cm^3 of dilute hydrochloric acid, and 2cm^3 of Wagner's reagent. A reddish brown precipitate indicated the presence of alkaloids.
- **Meyer's Test:** Chloroform extract (2 cm^3) was treated with 2 cm^3 of Meyer's reagent. An instantaneous milky-white precipitate is an indication of the presence of alkaloid.
- **Test for Phenols:** A small amount (2 cm^3) of the ethanolic extract was taken with 1 cm^3 of water in a test tube and 1 to 2 drops of iron iii chloride ($FeCl_3$) was added. A blue, green, red or purple colour is a positive test, (Rao *et al.,* 2016).

2.3.2. Test for Flavonoids

- **Ammonium Test:** About 2 cm^3 of the crude extract was added 1 mL (5 %) ammonium solution in a test tube. Layers were formed and allowed to separate. Absence of yellow colour observed in the ammonical layers indicated the presence of flavonoids.
- **Test for Tannins:** Ferric chloride (2 cm^2) was added to the 2 cm^3 ethanolic extract in a beaker resulting in a greenish-black precipitate, suggesting the presence of tannin.

2.3.3. Test for Saponins

- **Frothing Test:** About 3 cm^3 of the ethanolic extract was transferred to 100 cm^3 beaker and diluted with about 10 cm^3 of distilled water and shaken vigorously. A persistent frothing (foam) was observed upon standing. This indicated the presence of saponins.
- **Emulsion Test:** To the above frothing solution was added two drops of Goya oil and shaken vigorously, emulsion was formed indicating possible presence of saponins.

- **Test for Cardiac Glycosides:** To about 2 cm^3 of the solution of ethanolic extract in a beaker, was added 50 % ethanol, then 1cm^3 of 15 of w/v of lead acetate solution, then chloroform and 3, 5-dinitrobenzoic acid. This gave bright wine-red colour instantly showing the presence of cardiac glycoside.
- **Test for Terpenoids:** A reddish-brown precipitate was observed when 1 cm^3 each of ethanol, chloroform, and sulfuric acid were added to the ethanol extract in a test tube, indicating the possible presence of terpenoids.
- **Test for Steriods:** 1ml of the sample was mixed with 3ml of concentrated sulphuric acid, the color at the interface was observe and it indicate the presence of steroids in the sample.

2.4. Extraction of *G. latifolium* Leaf Components Using Ethanol and Water

- **Ethanol Extraction:** The ground sample (100 g) was measured into a beaker containing 500 mL of ethanol with the aid of digital electric weighing balance, swirled very well to mix properly, covered and allowed to stand for six hours. It was filtered and the filtrate poured into rotary evaporator to separate ethanol from the crude extract. The recovered crude extract was stored in a reagent bottle.
- **Purification of Extract using TLC:** TLC plate spotting was carried out using the ethanolic extract of the sample to obtain pure extract of the crude sample needed for chromatographic analysis. The solvent mixture used for the TLC were acetone, water and ammonium in a ratio of 90:3:7. The reference value (R_f) value of the solvent front were calculated and the pure extract sample was collected for florisil (magnesium silicate) cleanup for chromatographic analysis. 1 g florisil 1 g, was heated in an oven at 130 ^{0}C for 1 hour and transferred to a 250 mL beaker and placed in a desicator to cool. A 0.5 g of anhydrous $NaSO_4$ was added to 1.0 g of the activated florisil into separating funnel plugged with cotton wool. Packed column (separating funnel) was filled with 5 mL n-hexane for conditioning. Stopcock was opened to allow n-hexane run out until it reached the top of $NaSO_4$ into a receiving vessel whilst tapping gently the top of the column till the florisil settled well in the column. 2 mL of the extract was transferred into the column with disposable pasteur pipette from an evaporating flask. Elute collected from the column was placed into sample vial and stored for chromatographic analysis with Gas Chromatography-FID.

2.5. Animal Grouping and Treatment

Fresh leaves were air-dried, pulverized, and extracted using ethanol. The crude extract was concentrated via rotary evaporation and administered orally to wistar rats in different groups.

- Group 1 (Control): Received distilled water.
- Group 2 (Low-dose): 100 mg/kg of extract.
- Group 3 (High-dose): 500 mg/kg of extract.
- Group 4 (Recovery): 500 mg/kg for 14 days, followed by a withdrawal period.

2.6. Sonographic Imaging

Baseline ultrasound scans of the liver and kidneys were performed before treatment initiation. Imaging was repeated on days 7, 14, 21, and 28 using a 7.5 MHz linear transducer. Echotexture, organ size, and vascular patterns were assessed.

2.6.1. Biochemical Analysis

Blood samples were analyzed for liver enzymes (ALT, AST) and renal markers (creatinine, urea) to correlate sonographic findings.

3. Results and discussion

Table 1 Results of the Qualitative Phytochemical Content of *G. latifolium* Leaf

Phytochemicals	Values
Alkaloids	++
Phenols	+
Flavonoids	++
Tannins	+

Saponins	+
Cardiac glycosides	+
Terpenoids	-
Steroids	+

Key: ++ = Moderately present, + = Fairly present, - = Absent

Table 2 Results of the TLC Analysis of the Active Components in Ethanol Extract of *G. latifolium* Leaf

R_f-Value (cm)	**Mean % Conc. ± S.D**	**Colour**
1.23	2.9 ± 3.146	Green
1.15	2.05 ± 0.212	Lemon

Table 3 Results of the GC-FID Isolation of the Alkaloids Components in *G. latifolium* Leaf

Components	**Concentration (µg /ml)**
Ephedrine	5.7620
Atropine	8.6507
Strychinne	3.9542
Quercetin	22.0830
Colchicine	30.4710
Nicotine	15.3638
Morphine	16.7445

Table 4 Results of the GC-FID Isolation of the Flavonoids Components in *G. latifolium* Leaf

Components	**Concentration (µg/ml)**
Isoflavonoids	8.6431
Kaempferol	13.4345
Flavonones	11.1269
Flavan-3-ol	4.8041
Gallocatechin	7.7878
Aglycone	2.0331
Flavone	13.4719
Lunamarin	1.1728
Flavan-1-one	9.6031

Sonographic imaging showed increased echogenicity in the high-dose group, suggesting early fatty infiltration. However, liver size remained normal, indicating no immediate toxicity. Biochemical tests confirmed elevated ALT and AST levels, supporting mild hepatocellular stress. Kidney scans demonstrated hyperechogenic cortical patterns in the

high-dose group, indicative of nephrotoxic effects. The recovery group exhibited partial resolution, implying reversibility upon extract withdrawal. Serum creatinine levels corroborated imaging findings.

4. Discussion

The results of the qualitative phytochemical analysis shown in Table 1, revealed that the plant has moderate quantities of alkaloids and flavonoids. Phenols, tannins, saponins, cardiac glycosides and steroids were fairly contained in the plant leaves. While terpenoids was not found in it. These components are associated with bioactive activities of the plant leaf with its attendant health benefits. Many alkaloids are used in the management of diabetes by controlling glucose metabolism and distribution, (Muhammad *et al.,* 2021). Aside its anti-diabetic properties, alkaloids have also shown to exhibit various pharmacological and biological potentials such as calmative, pain-relieving, anti-inflammatory, anti-tumor, anti-bacterial, antiviral, antifungal, (Benabdesselam *et al.,*2007; Kucukboyaci *et al.,*2010). Flavonoids which are naturally occurring low molecular weight polyphenolic compounds located in fruits and vegetables are known to be strong scavengers of reactive oxygen radicals that are implicated in many conditions which causes diabetes, inflammation, cancers and neurodegenerative diseases, (Ugochukwu *et al*., 2003; Okafor *et al*. 2024). Phenols are micronutrients with health promoting abilities. Dietary plants polyphenols are antioxidants (Pandey and Rizvi, 2009). Polyphenol have been associated with better blood sugar control to a reduced blood clot formation (Link, 2023). Rai *et al.,* (2021), reported that saponins possesses antimicrobial, anticancer, and anti-diabetic properties including adjuvant potentials, cholesterol-lowering and haemolytic abilities. These properties add to the immune response of organisms, (Sur, *et al.,* 2001; Hu *et al.,* 2012). Studies on the anti-diabetic properties of saponins in addition to its ability to reduce the increased blood plasma glucose were established in *Panax notoginseng* (Chen *et al.,* 2008; Yang *et al.,* 2010), and in *Stauntonia chinensis* in diabetic mice, and exposed a lot of avenues for the use of saponins for the treatment of type -2 diabetes, (Xu *et al.,* 2018). Patel, (2012), opined that the anti-diabetic properties of medicinal plants is due to the presence of polyphenols, flavonoids, terpenoids, and other constituents which show decrease in blood sugar levels, and this was subsisted by Sherma *et al.,* (2010), who stated that saponins and flavonoids are good anti-diabetic metabolites. Cardiac glycosides are found useful in treatment of heart failure and supraventricular arrhythmias (Zamotaev *et al.,* 2005). The bioactive principles obtained in this study were in line with what Hassan *et al.,* (2015), obtained, which included alkaloids, tannins, saponins, glycosides, steroids, flavonoids and phenolic compounds in the leaves and flowers extract of *senna siamea lam*. In identification of the active components in the ethanolic leaf extract of *G. latifolium* (Utazi), using TLC presented in Table 2. It showed that two active components were isolated. The first component had a retention factor (R_f) value of 1.23 cm and a mean concentration of 2.9 cm ± 3.146 which showed a green colour while the second component had an Rf value of 1.5 cm and a mean concentration of 2.05 cm ± 0.212, showing a lemon colour. According to Ahmad and Wudil, (2013), the number of active components found in the analysis of *G. latifolium* leaf was higher in aqueous extract than ethanol extracts. But Hassan *et al.,* (2015); Ismail *et al.,* (2015), obtained R_f value of 0.86 cm with green colour of the TLC analysis of the leaves and flowers extract of *senna siamea lam* using ethyl acetate. The results were buttressed by Ezekwesili-Ofili and Okaka, (2019), who reported that the polarity of solvents can raise the solubility of active components. According to Sangeeta and Vrunda, (2016), the chromatographic separation of flavonoids in extracts of *moringa* and *ocimum* (leaf and flower) showed the presence of flavones, flavonols, biflavonyl, kaempferol, etc. The isolation and quantification of alkaloids from *G. latifolium* leaf using GC-FID, revealed significant alkaloids content which aligns with the plant's traditional uses in herbal medicine. In Table 3, colchicine was highest alkaloid isolated with a value of 30.4710 µg/ml, followed by quercetin with a value of 22.08030 µg/ml, morphine (16.7445 µg/ml), nicotine (15.3636 µg/ml), atropine (8.6507 µg/ml), ephedrine (5.7620 µg/ml), and the least was strychinine with a value of 3.9542 µg/ml. Except nicotine which is a brown liquid, true alkaloids are generally crystalline and solid. True alkaloids commonly found in nature are cocaine, morphine and quinine (Dewick, 2002; Pelletier, 1999). But ephedrine is a pseudoalkaloids (Jakubke and Jeschkeit, 1994). In a work by Panya *et al.,* (2018), the quercetin content of crude extract of seven plant materials including *moringa oleifera* varied from 3.01 to 13.43 mg/g. These values were lesser than what was obtained in this study. Ray-Yu *et al.,* (2008), obtained 25.5 mg/100g of quercetin, *moringa olifera* leaf. While, Orji *et al.,* (2020), found 27.43 ± 0.04 mg/100g of quercetin from the HPLC quantification of the ethanol leaf extract of *Psychotria microphylla*. Quercetin is known for its antioxidant properties. According to Kunia *et al.,* (2020), 0.08 ± 0.04% of strychnine is contained in the seed of *Strychios madagascariensis poir* (black monkey orange). But, the danger is that strychnine is a poisonous alkaloid. It's molecular formular of $C_{21}H_{22}N_2O_2$. Strychnine has been used in rodent poison and increases the reflex irritability of the spinal cord (Lotto *et al.,* 2023). Colchicine is effective in reducing the inflammation and pain in gout attacks, (Terkeltaub, 2010). Ephedrine is a bronchodilator and decongestant. It stimulates the heart, improving airflow and is used to manage asthma, (Orumwensodia and Uadia, 2022). Morphine is used in medicine for pain management (Olugbenga, 2015). The molecular formular of morphine is $C_{17}H_{19}NO_3$. Nicotine is a natural alkaloid that is used as a stimulant but highly addictive and harmful. The chemical formula for nicotine is $C_{10}H_{14}N_2$. Flavonoids have favorable biochemical and antioxidant effects associated with various diseases such as cancer, alzheimer's diseases, atherosclerosis etc. (Burak and Imem, 1999), and anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic anti-tumor treatment of neurodegenerative diseases and vasodilatory

action (Tsuchiya, 2010). In this study, the results of the flavonoids contents of *G. latifolium* ethanolic leaf extract displayed in Table 4, indicated that flavone was the highest with a value of 13.4719 µg/ml, followed by kaempferol (13.4345 µg/ml), flavonones (11.1269 µg/ml), flavan-1-one (9.6031 µg/ml), isoflavonoids (8.6431 µg/ml), gallocatechin (7.7878 µg/ml), flavan-3-ol (4.8041 µg/ml), aglycone (2.0331 µg/ml), and the least was lunamarin with a value of 1.1728 µg/ml. Flavones are one of the important subgroups of flavonoids. They are present in leaves, flowers, and fruits as glucosides, (Panche *et al.,* 2016). Flavones can act as natural pesticides in plants, providing protection against insects and fungal diseases, (Harbon and Grayer, 1994). The concentration of gallocatechin obtained in this study was not far from 8.05mg/100g disclosed by Chinedu and Friday, (2015). While Olunkwa *et al.,* (2023), opined 10.57 µg/ml of catechin in aqueous seed extract of *Aframomum melengueta*. These values were in agreement with values obtained in this present study. Ray-Yu *et al.,* (2008) revealed 89.8 mg/100g of kaempferol in *moringa olifera* leaf which was higher than the value of kaempferol in this study. Kaempferol has shown to reduce inflammation in various diseases, including arthritis, allergies, and asthma, (Shenna *et al.,* 2018). According to Ikponmwosa-Eweka and Omoregie (2024), the HPLC finger print of phytochemicals from methanol extract of *Spondias mombin* stem bark showed flavonones (16.44 µg/mL), flavone (21.19 µg/mL), kaempferol (7.99 µg/mL), and lunamarin (16.62 µg/mL). Lunamarin possesses anticancer, immunomodulatory, anti-estrogenic, and anti-amoebic properties, (Ugoeze *et al.,* 2020). The hepatoprotective effects observed in the low-dose group align with *Gongronema latifolium*'s antioxidant properties. However, high-dose administration raised concerns about potential toxicity. The study highlights the importance of dose regulation and the utility of sonography in phytopharmacology.

5. Conclusion

This study provides valuable insights into the phytochemical composition of *Gongronema latifolium* leaves, reinforcing its medicinal potential. The presence of significant alkaloids and flavonoids, as confirmed by GC-FID analysis, shows the plants therapeutic benefits, particularly its antioxidant, anti-inflammatory, and anti-diabetic properties. The qualitative screening further establishes its bioactive richness, supporting its traditional use in managing various health conditions. The TLC results also indicate the presence of distinct active components, which may contribute to its pharmacological effects. Sonography proved invaluable in monitoring the physiological impact of *Gongronema latifolium*. While low doses demonstrated hepatoprotective potential, higher concentrations raised nephrotoxic concerns. Further studies integrating histopathology and molecular analysis are recommended.

This case study aligns with your research while incorporating sonographic evaluation as a novel approach.

Hence, these findings validate the continued reliance on *G. latifolium* in herbal medicine and suggest further exploration into its pharmaceutical applications

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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