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Bioactive and antioxidant properties of ethanol extract, dichloromethane and butanol fractions of *Gongronema latifolium* leaf

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Abstract

Gongronema latifolium Benth. is a tropical medicinal plant of significant ethnomedicinal importance in West Africa. This study evaluated the bioactive and antioxidant properties of the ethanol extract, dichloromethane (DCM) and butanol fractions of *G. latifolium* leaf. Following maceration and fractionation by Vacuum Liquid Chromatography (VLC), samples were subjected to qualitative phytochemical screening, Gas Chromatography–Mass Spectrometry (GC-MS) analysis, and in vitro antioxidant evaluation comprising DPPH radical scavenging, ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC). Phytochemical screening confirmed alkaloids, flavonoids, saponins, tannins, and cardiac glycosides in the ethanol extract. GC-MS identified 83 compounds in the crude extract dominated by Undecane, 2,6-dimethyl- (7.79%), Tridecane (4.38%), and γ-Terpinene (4.02%); 53 compounds in the DCM fraction, with 14-Octadecenoic acid methyl ester (6.81%), γ-Terpinene (6.12%), and Phytane (3.23%) as major constituents; and 86 compounds in the butanol fraction. At 100 μg/mL, DPPH inhibition was 72% for both the crude extract and DCM fraction, and 23% for the butanol fraction, versus 92% for ascorbic acid. FRAP absorbance values at 100 μg/mL were 1.98, 2.01, and 2.06 for crude, DCM, and butanol fractions, respectively, approaching the ascorbic acid standard (2.08). TPC was highest in the crude extract (39.8 mgGAE/g), while TFC was highest in the DCM fraction (87.16 mgRE/g). These findings establish a rich phytochemical profile and significant antioxidant capacity in *G. latifolium* leaf, with the crude extract and DCM fraction showing the most promising activity, supporting further investigation as sources of bioactive pharmaceutical compounds.

Keywords: *Gongronema latifolium*; Antioxidant activity; Phytochemical screening; GC-MS analysis; Total phenolic content; Total flavonoid content

1. Introduction

Reactive oxygen species (ROS) and other free radicals are continuously generated as by-products of normal cellular metabolism. Under physiological conditions, endogenous antioxidant defence systems maintain redox homeostasis; however, when ROS generation overwhelms these defences, a state of oxidative stress ensues [1]. Oxidative stress has been implicated in the pathogenesis of numerous chronic and degenerative diseases including type 2 diabetes mellitus, cardiovascular disorders, cancer, neurodegenerative conditions, and inflammatory diseases [1,2]. The global burden of these conditions has intensified interest in the identification of safe, effective, and affordable antioxidant agents, particularly from natural plant sources, as alternatives to synthetic antioxidants associated with carcinogenic risk and metabolic side effects [2].

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Medicinal plants have served as the cornerstone of healthcare in many parts of the world, particularly across Africa, Asia, and Latin America. In sub-Saharan Africa, the vast majority of the population depends on herbal medicine for primary healthcare needs, owing to cultural tradition, geographical accessibility, and persistent challenges of access to orthodox medicine [3]. These plants are rich repositories of structurally diverse secondary metabolites including alkaloids, terpenoids, flavonoids, phenolic acids, saponins, and glycosides, which underpin their observed pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic effects [1,3].

Gongronema latifolium Benth. (family: Apocynaceae, formerly Asclepiadaceae), commonly known as "utazi" in south-eastern Nigeria and "arokeke" in south-western Nigeria, is a tropical perennial climbing plant native to the rainforests of West and Central Africa [1,2]. The plant is widely used as a spice, vegetable, and medicinal herb in Nigeria, Cameroon, Ghana, and other sub-Saharan African countries. Ethnomedically, various parts of the plant, particularly the leaves, are used in the management of diabetes mellitus, malaria, hypertension, pain, and liver diseases [1,2]. In Senegal and Ghana, the boiled leaf extract is also used as a laxative [2].

Scientific investigation has progressively validated several of these traditional claims. Balogun *et al.* [1] provided a comprehensive pharmacological review of the plant, establishing that its extracts exhibit significant antioxidant, hypoglycaemic, hypolipidaemic, hepatoprotective, and antimicrobial activities, attributing these effects to its phytochemical composition which includes flavonoids, saponins, alkaloids, oleanolic acid derivatives, antioxidant fatty acids, and tannins. Ojo *et al.* [3] demonstrated that leaf extract of *G. latifolium* significantly improved antioxidant defence, attenuated neuroinflammation, and restored oxidative stress markers in streptozotocin-induced diabetic rats. Ugochukwu and Babady [4] further established that both aqueous and ethanolic leaf extracts significantly enhanced superoxide dismutase activity and reduced glutathione levels in diabetic hepatocytes, suggesting antioxidant mechanisms are central to the plant's therapeutic action. Okochi *et al.* [5] confirmed significant concentrations of phenols, flavonoids, and tannins alongside strong in vitro antioxidant activity in *G. latifolium* leaf preparations. Nneoyi-Egbe *et al.* [6] further demonstrated that the plant's phytochemical richness including alkaloids, flavonoids, and tannins is consistent across both fresh and dried leaf samples.

Gas Chromatography–Mass Spectrometry (GC-MS) is a validated and highly sensitive analytical tool for identifying volatile and semi-volatile bioactive compounds in medicinal plant extracts [1,5]. Fractionation of crude plant extracts using polarity-based solvent systems enables the selective enrichment of compounds with specific physicochemical properties, facilitating more precise attribution of biological activities to particular chemical classes. Despite the expanding body of knowledge on *G. latifolium*, comparative GC-MS profiling of its fractionated extracts alongside systematic antioxidant characterisation remains limited in the literature. This study therefore aimed to identify the bioactive compounds in the ethanol extract, dichloromethane (DCM) and butanol fractions of *G. latifolium* leaf by GC-MS analysis, and to evaluate their antioxidant properties using DPPH radical scavenging activity, ferric reducing antioxidant power, total phenolic content, and total flavonoid content assays.

2. Materials and methods

2.1. Collection and Identification of Plant Material

Fresh leaves of *G. latifolium were* purchased from Itam Market, Uyo, Akwa Ibom State in March 2025, and identified by Prof. Margaret Esshiet, Department of Botany and Ecological Science, Faculty of Biological Sciences, University of Uyo. Herbarium specimen was prepared and a voucher number (UUPH 9(a) issued and was deposited at the Department of Pharmacognosy and Natural Medicine herbarium, Faculty of Pharmacy, University of Uyo, Nigeria.

2.2. Extraction and Fractionation of Plant Material

The leaves were washed thoroughly with clean water to remove dirt and contaminants, manually chopped, and pounded using a mortar and pestle to increase surface area for extraction. Nine kilograms (9 kg) of *G. latifolium* leaf was macerated for 72 h in 24 litres of solvent (80% ethanol), with intermittent shaking to enhance the extraction of bioactive compounds. This was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in a water bath at 45°C.

The extract of *G. latifolium* (230 g) was dissolved in methanol (100 mL) and adsorbed onto a silica gel G and packed in a Buckner funnel connected to a vacuum pump. The sample was partitioned successively and exhaustively with n – hexane, DCM, ethyl acetate and methanol using the Vacuum Liquid Chromatographic (VLC) techniques to obtain their

respective fractions. The fractions were concentrated to dryness in vacuo at 40°C and the dried fractions were stored in a deep freezer (-4 °C) for further studies.

2.3. Phytochemical Screening

The qualitative phytochemical screening was conducted on the ethanol extract of *G. latifolium* leaves in accordance with standard methods to identify the various classes of bioactive compounds present [7,8].

2.3.1. Test for Saponins

Froth Test: The extract (0.2 g) of the plant was shaken vigorously with 5 mL of distilled water in a test tube. The formation of froth that persisted for 15 minutes after standing indicated the presence of saponins [7].

2.3.2. Test for Tannins

Ferric Chloride Test: The extract (0.1 g) was dissolved with 5 mL of distilled water and filtered followed by the addition of 2 drops of 5% ferric chloride to the filtrate. The presence of tannins was indicated by the appearance of a blue-black precipitate [8].

2.3.3. Test for Flavonoids

Shinoda's Reduction Test: To 2 mL of the extract solution prepared by dissolving 0.1 g of extract in 5 mL of distilled water, 1 mL of concentrated hydrochloric acid was added followed by a few pieces of magnesium metal. The appearance of orange colour indicated the presence of flavonoids [8].

Alkaline Reagent Test: The extract (0.1 g) was dissolved in 5 mL of distilled water and 10% NaOH solution was added and shaken. An intense yellow colour appeared in the test tube. The solutions became colourless with the addition of a few drops of 10% HCL indicating the presence of flavonoids [9].

Ammonia Test: The plant extract (0.2 g) was dissolved in 4 mL of ethyl acetate, warmed and filtered. Dilute ammonia solution (10%; 2 mL) was added and the solution was shaken. A few drops of 5% potassium hydroxide solution was added, and a few drops of dilute hydrochloric acid was added. A yellow colouration in the lower ammonia layer indicated the presence of flavonoids [8].

2.3.4. Test for Cardiac Glycosides

- **Salkowski's Test:** To 0.1g of the crude extract dissolved in 5 mL of chloroform, 1 mL of concentrated tetraoxosulphate (IV) acid was gently added by running it down the side of the test tube to form a distinct lower layer. A reddish brown ring at the interphase indicated the presence of a steroidal ring of cardiac glycoside [7].
- **Keller-Kiliani Test:** To 0.1g of the extract dissolved in 5 ml of glacial acetic acid, one drop of 5 % ferric chloride solution was added. The resultant solution was underlaid with 1 mL of concentrated tetraoxosulphate (IV) acid by running it down the side of the test tube to form a lower layer. The presence of the deoxy sugar of the cardiac glycosides was indicated by the appearance of a brown ring at the interphase [7].
- **Liebermann's Test:** To 0.1 g of the extract dissolved in 4 mL acetic anhydride and cooled well in ice, concentrated tetraoxosulphate (IV) acid (1 mL) was carefully added to form a lower layer. A colour change from violet to blue to green at the interphase indicated the presence of a steroidal nucleus of the cardiac glycoside [8].

2.3.5. Test for Alkaloids

The crude extract (0.2 g) of the plant material was boiled with 5 mL of 5% HCL in a test tube in a boiling water bath. The mixture was filtered after cooling. To the filtrate, two drops of Dragendorff's precipitating reagent were added and observed. An orange precipitate was considered as an indication of the presence of alkaloids [8].

2.4. Gas Chromatographic–Mass Spectrometry (GCMS) Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) was employed to identify and characterize volatile and semi-volatile bioactive compounds in the crude extract and fractions of *G. latifolium* leaf. Bioactive compounds of crude and fractions were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

2.5. Antioxidant Evaluation of Crude Extract, Dichloromethane and Butanol Fractions of *G. latifolium*

DPPH (1,1 -diphenyl-2-picryl hydrazyl) radical scavenging activity, reducing antioxidant power assay, estimation of total phenolic content, as well as estimation of total flavonoid content, were conducted as an evaluation of the antioxidant potentials of the extracts and fractions of *G. latifolium*.

2.5.1. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The method of Bloise [10] was employed in determining the free radical scavenging activity of the samples. According to the method, 3 mL of different concentrations (20- 100 μg/mL) of the extract, fractions of leaf and standard drug (ascorbic acid) in different test tubes was added to 1.0 mL of 0.1 % DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured at the wavelength of 517 nm using UV/ visible spectrophotometer (Hewlett Packard, England) against a DPPH control containing only 3mL of methanol in place of plant samples (that is containing reagents except test samples). The experiment was carried out in triplicate. Percentage scavenging activity was calculated using the equation.

$$\% \text{ DPPH scavenging} = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control}\ X\ \frac{100}{1}$$

2.5.2. Determination of Reducing Antioxidant Power

The reducing antioxidant power activity of the samples was determined using the method of Oyaizu [11]. Various concentrations (20-100 μg / mL) of the plant extract, fractions and ascorbic acid (2 mL) were mixed individually with the mixture containing 2 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2mL of 1% potassium ferric-cyanide (K3 Fe (CN), the mixture obtained was incubated at 500 c for 20 minutes then 2 mL of trichloacetic acid (10 %) was added, the mixture was centrifuged at 650rpm for 10 minutes, the upper layer of the solution 5 mL was mixed with 5 mL of distilled water and 1.0 mL of ferric chloride (0.1%). The absorbance was measured at the wavelength of 700 nm against a blank sample. The assays were carried out in triplicates and the results were expressed as mean absorbance + standard error of mean.

2.5.3. Estimation of Total Phenolic Content

The total phenolic content of the samples was determined spectrophotometrically with Folin-ciocalteau reagent using the modified method of Wolfe and Liu [12]. An aliquot of the extract and fractions (0.5 mL; 1 mg/ mL) was mixed with 2.5 mL of 10% Folin-ciocalteau reagent and 2 mL of 7% NA2CO3. The resulting mixture was vortexed for 15 seconds and incubated at 400 c for 30 minutes for colour development. The same procedure was used to prepare gallic acid at various concentrations (20-100 μg/ mL) for the standard calibration curve and the procedure was carried out in triplicate. The absorbance of the samples was measured at 765 nm using a UV/ visible spectrometer. Total phenolic content was expressed as mg gallic acid equivalent per gram of the sample from the calibration curve using the equation Y= mx + c; where Y is the absorbance and x is the gallic acid equivalent of the sample (mgGAE/g) (i.e. total phenolic content).

2.5.4. Estimation of Total Flavonoids Content

The total flavonoid content was estimated using the procedure described by Zhishen *et al.* [13]. A total of 1 mL of plant extract and fractions (1 mg/ mL) were diluted with 200 μL of distilled water separately followed by the addition of 150 μL of sodium nitrite (5%) solution. This mixture was incubated for 5 minutes, then 150 μL of aluminium chloride (10%) solution was added and allowed to stand for 6 minutes, then 2 mL of sodium hydroxide (4%) solution was added and the mixture made up to 5 mL with distilled water. The mixture was shaken well and left for 15 minutes at room temperature. The same procedure was used to prepare rutin at different concentrations (20-100μg/ mL) for the standard calibration curve. The absorbance was measured at the wavelength of 510 nm using a UV/ visible spectrometer. The procedure was carried out in triplicate. The total flavonoid content was expressed as milligram rutin equivalent per gram (mgRE/ g) of extract and fractions using the standard curve.

2.6. Statistical Analysis

The data obtained were analyzed using a statistical package for social sciences (SPSS) version 25. Analysis of variance (ANOVA) was conducted to determine the significant differences among the groups. Multiple comparison tests using the least significant difference (LSD) method were performed to identify which specific groups differ significantly. The means of the group were compared, with differences compared statistically significant at $P < 0.05$. Results are presented as Mean ± Standard Error of the Mean (SEM).

3. Results

3.1. Qualitative Phytochemical Screening of Ethanol Extract of *G. latifolium*

Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, and steroids in the ethanol extract of *G. latifolium* (Table 1). These results are consistent with prior documentation of this plant's phytochemical profile and collectively provide the pharmacological basis for the reproductive and antioxidant effects observed in this study.

Table 1 Qualitative phytochemical screening of crude ethanol extract of *G. latifolium*

Secondary Metabolite	Test	Inference
Tanins	Ferric chloride test	+
Alkaloids	Dragendorff' s reagent test	+
Saponins	Froth tests	+
Flavonoids	Shinoda's reduction test Alkaline reagent test Ammonia test	+ + +
Cardiac glycosides	Salkowski's test Keller Kiliani test Liebermann's test	+ + +

Key: – absent, + = Present

3.2. GC-MS screening of *G. latifolium* crude extract, dichloromethane and butanol fractions

GC-MS screening of *G. latifolium* crude extract, dichloromethane and butanol fractions chromatogram are represented in Figure 1, 2 and 3.

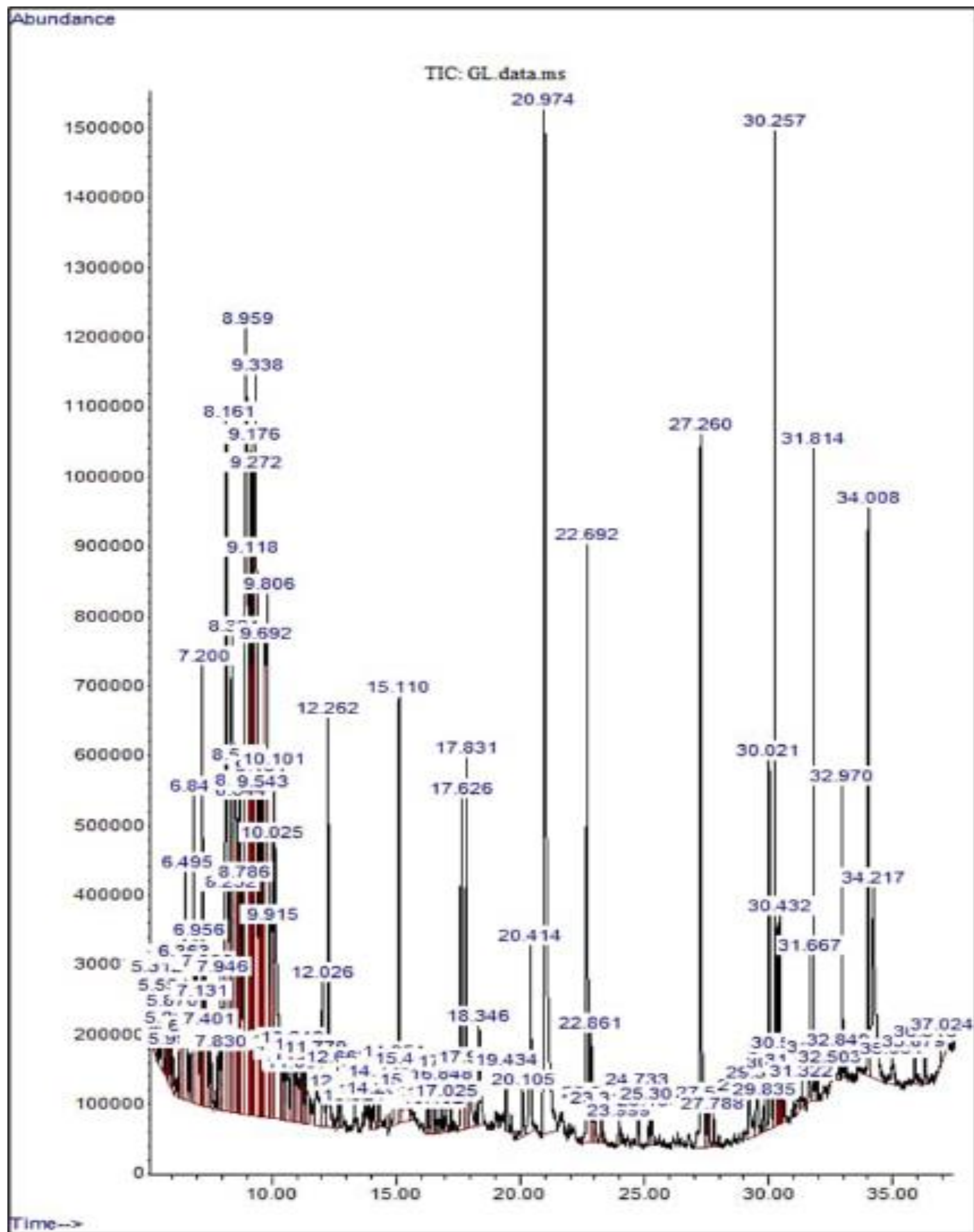

Figure 1 Chromatogram of *G. latifolium* crude extract

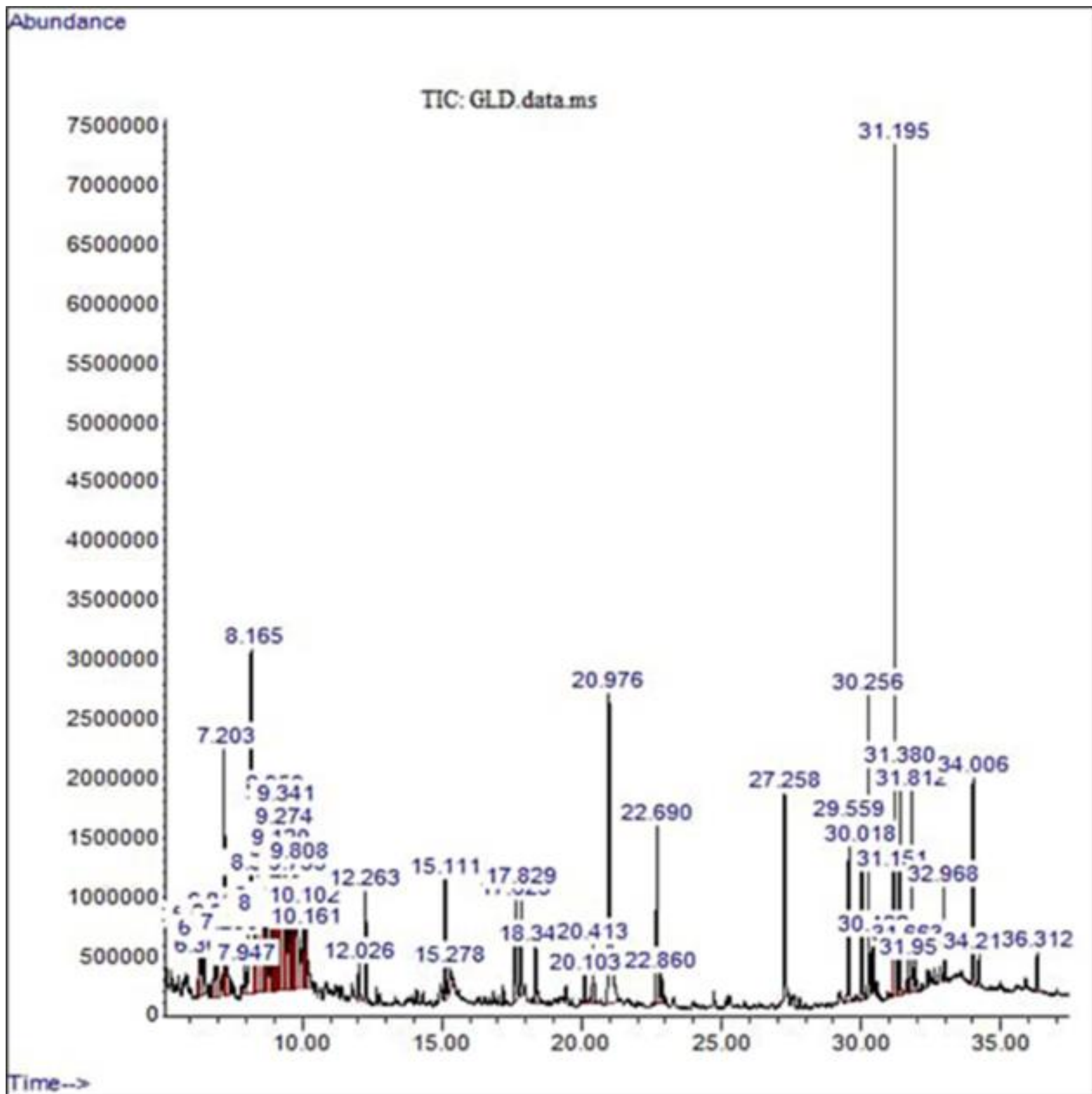

Figure 2 Chromatogram of dichlomethane fraction

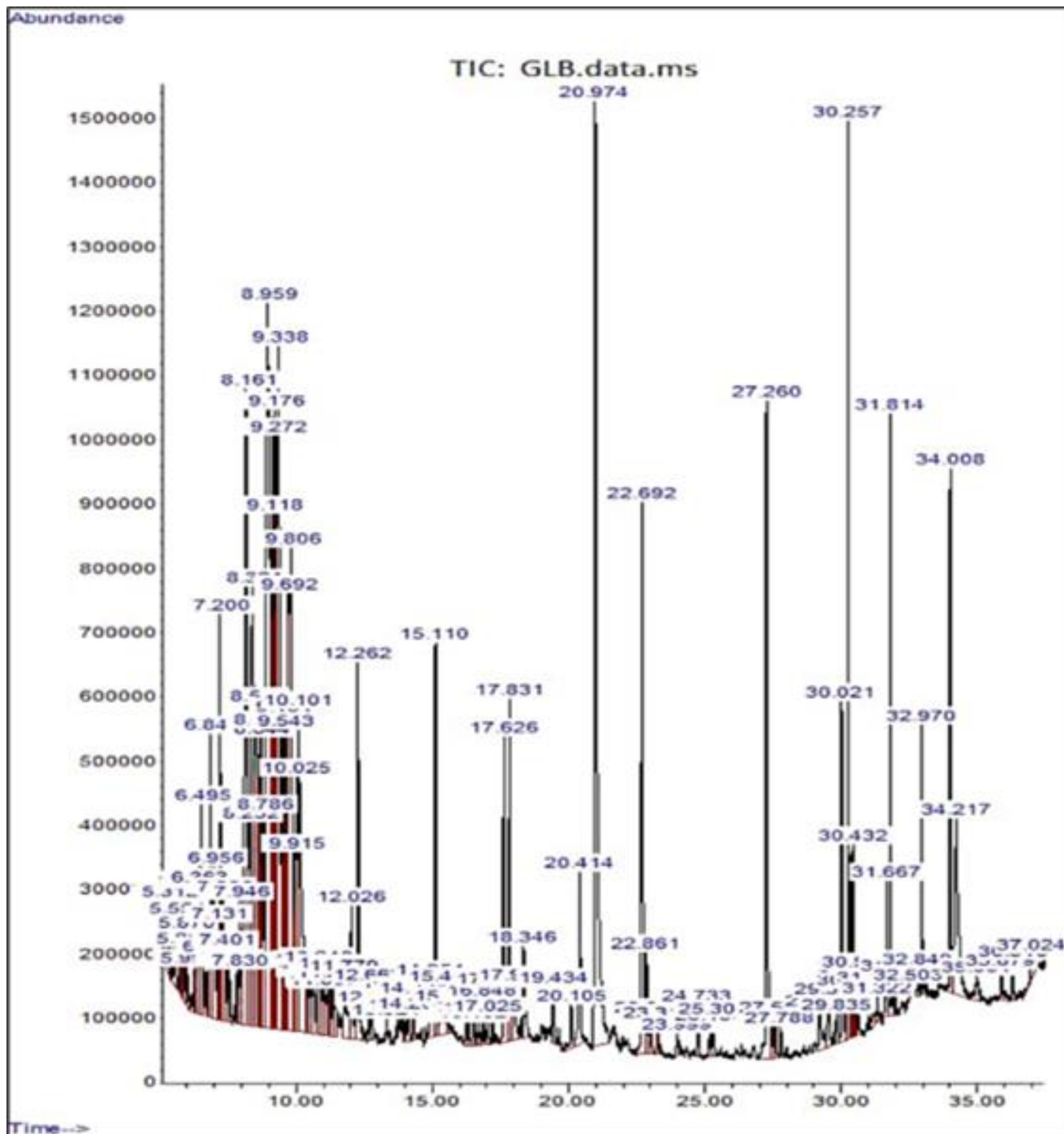

Figure 3 Chromatogram of butanol fraction

3.3. Antioxidant Studies

DPPH radical scavenging activity showed concentration-dependent inhibition for all samples. At 100 µg/mL, crude extract and DCM fraction both achieved 72% inhibition, while butanol fraction showed only 23% inhibition. FRAP showed DCM fraction as the strongest reducing agent (absorbance 2.01), followed by butanol fraction (2.06) and crude extract (1.98). Total flavonoid content was highest in DCM fraction (87.16 mg RE/g). Total phenolic content was highest in crude extract (39.8 mg GAE/g) as shown in Figure 4, 5, 6 and 7 respectively.

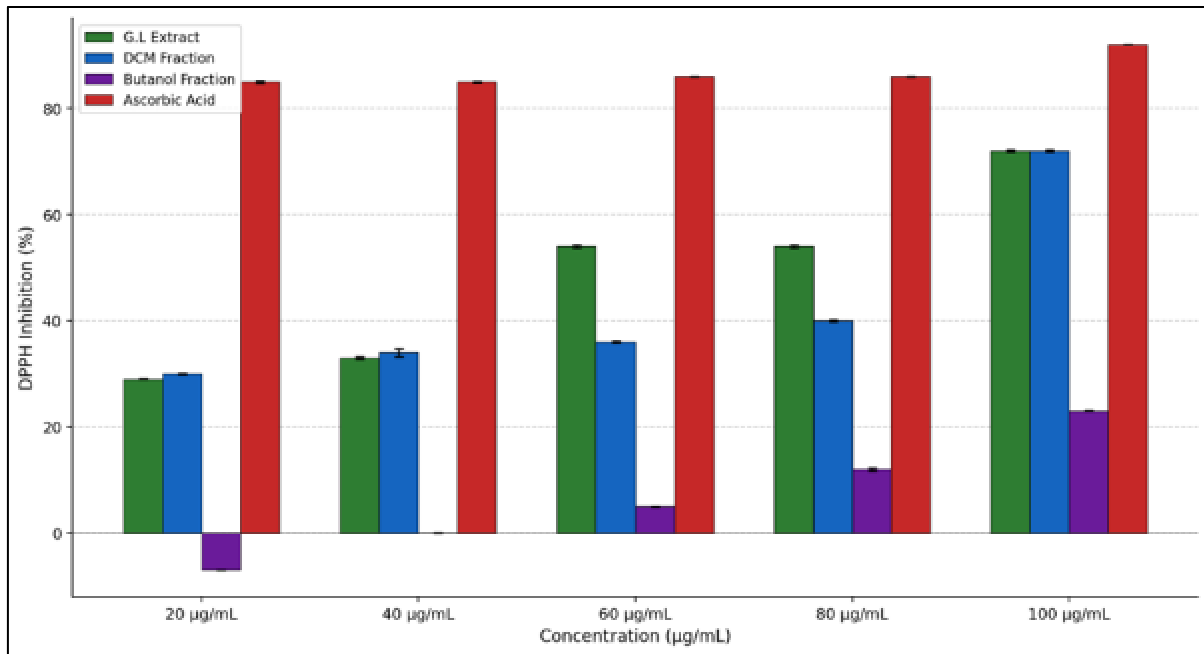

Figure 4 DPPH inhibition of *G. latifolium* crude extract, dichlomethane fraction and butanol fraction across various concentrations (20–100 µg/mL)

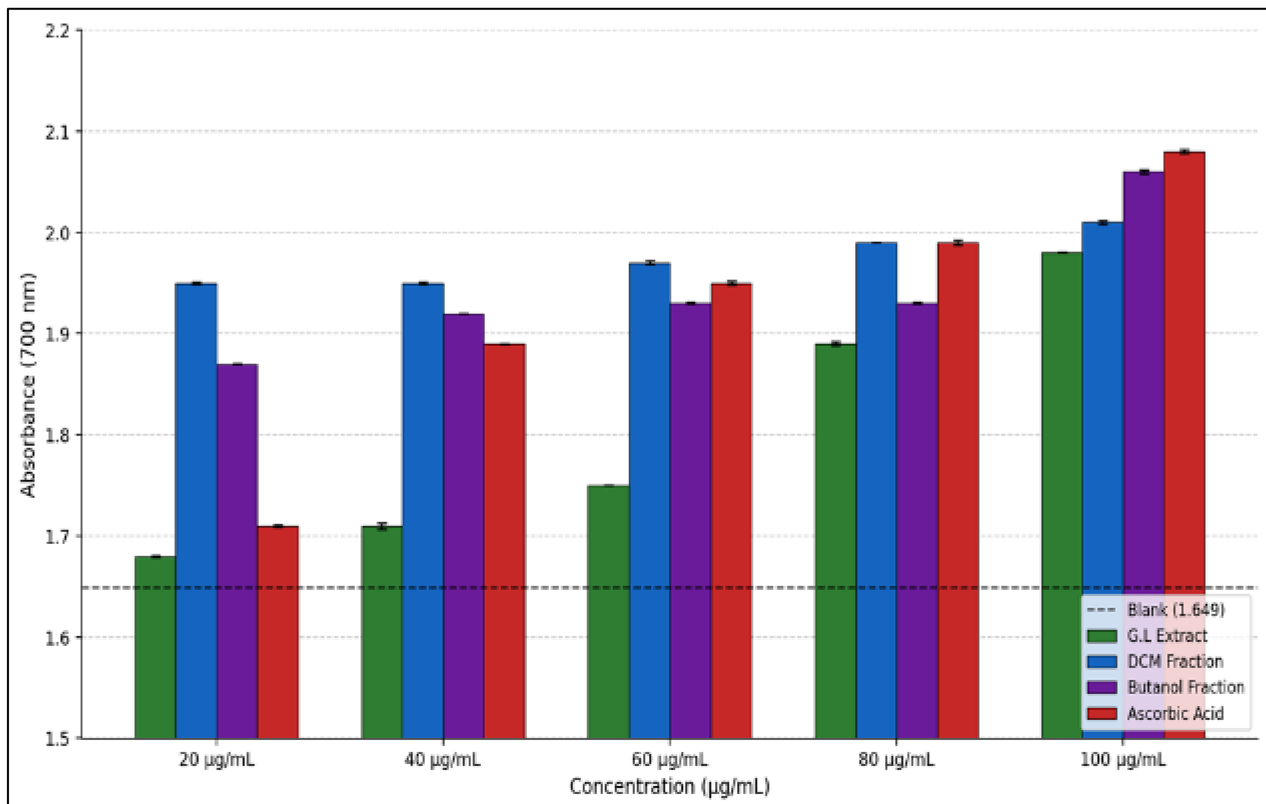

Figure 5 Ferric reducing power activity of *G. latifolium* crude extract, dichlomethane fraction and butanol fraction across various concentrations

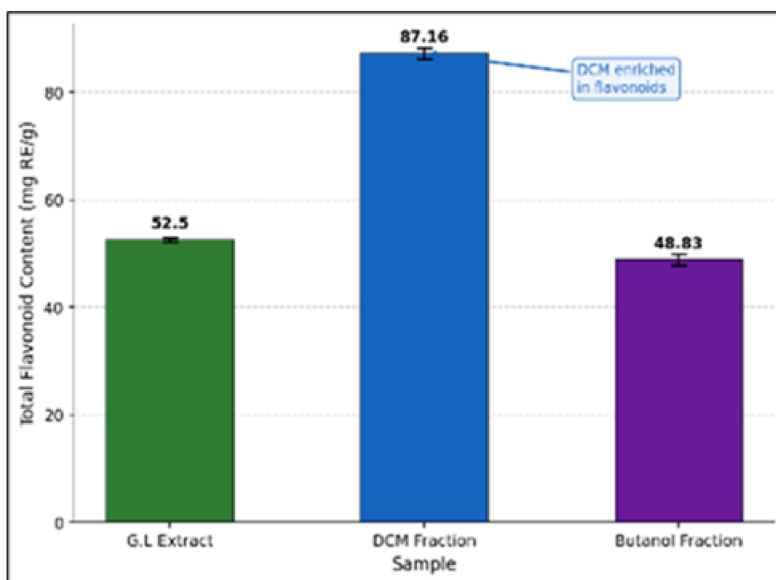

Figure 6 Total flavonoid content of *G. latifolium* crude extract, dichlomethane fraction and butanol fraction expressed as mg rutin equivalent per gram (mgRE/g)

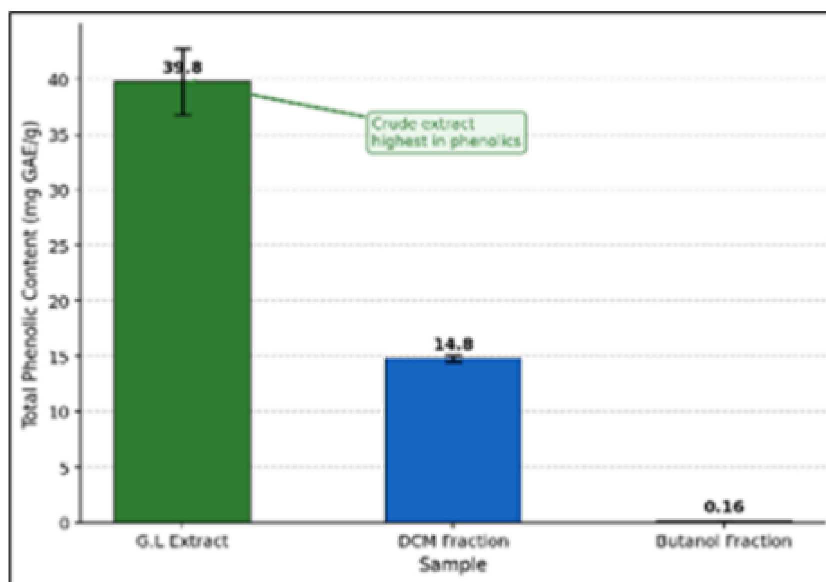

Figure 7 Total phenolic content of *G. latifolium* crude extract, dichlomethane fraction and butanol fraction expressed as mg gallic acid equivalent per gram (mgGAE/g)

4. Discussion

The qualitative phytochemical screening of the ethanol extract of *G. latifolium* leaf confirmed the presence of alkaloids, flavonoids, saponins, tannins, and cardiac glycosides. These findings are consistent with prior documentation of the phytochemical composition of this plant. Balogun *et al.* [1] reported the presence of flavonoids, saponins, alkaloids, steroids, oleanolic acid derivatives, and glycosides in *G. latifolium* extracts, attributing them to its broad pharmacological activities. Similarly, Okochi *et al.* [5] confirmed significant concentrations of phenols (53.105 mg/g), flavonoids (9.635 mg/g), saponins (5.480 mg/g), and tannins (1.175 mg/g) in *G. latifolium* leaves. The presence of alkaloids is pharmacologically significant given their well-established roles in analgesic, antimicrobial, antispasmodic, and cytotoxic activities. Saponins are associated with hypocholesterolaemic and immune-modulating effects, while tannins exhibit potent astringent, antimicrobial, and antioxidant properties [1,2]. The detection of cardiac glycosides is consistent with

the plant's reported use in the management of heart-related conditions in ethnomedicine [2]. Collectively, the phytochemical richness of *G. latifolium* leaf provides a strong biochemical basis for its diverse medicinal applications.

GC-MS profiling of the crude ethanol extract identified 83 compounds, dominated by alkane hydrocarbons, terpenoids, and long-chain lipid derivatives. The most abundant compound in both the crude extract and butanol fraction was Undecane, 2,6-dimethyl- (7.79%), a branched-chain alkane documented in several essential oils and aromatic plant extracts. The detection of long-chain alkanes and their derivatives across the crude and butanol fractions reflects the broad extraction capacity of ethanol as a solvent, extracting both polar and non-polar constituents.

Among the biologically most significant compounds identified across all three samples was γ-Terpinene, a monocyclic monoterpene hydrocarbon present in the crude extract (4.02%), DCM fraction (6.12%), and butanol fraction (4.02%). γ-Terpinene has been extensively characterised for its pharmacological properties. Li and Liu [14] demonstrated its remarkable antioxidant behaviour, establishing that it efficiently scavenges DPPH and ABTS free radicals and protects methyl linoleate, DNA, and human erythrocytes from oxidative damage. Ramalho *et al.* [15] confirmed that γ-Terpinene effectively modulates acute inflammatory responses in experimental mouse models, significantly suppressing carrageenan-induced oedema Nooshadokht *et al.* [16]. further confirmed the antioxidant activities of γ-Terpinene, alongside significant antiprotozoal effects, and demonstrated its ability to increase intracellular reactive oxygen species in treated parasites while simultaneously providing antioxidant protection in the host microenvironment The notably higher proportion of γ-Terpinene in the DCM fraction (6.12%) compared to the crude and butanol fractions provides a plausible chemical explanation for the consistently superior DPPH radical scavenging and FRAP performance of the DCM fraction observed in this study. p-Cymene (1.46%) was identified in both the crude extract and butanol fraction. This aromatic monoterpene has been documented for its antibacterial, antifungal, antiviral, and anti-inflammatory activities, and is known to act synergistically with other monoterpenes such as carvacrol and γ-Terpinene to amplify biological effects [16]. Thymol (0.46%), detected exclusively in the DCM fraction, is a well-characterised phenolic monoterpenoid and one of the most potent natural antimicrobial and antioxidant agents in plant secondary metabolites [16]. Though present at low concentration, thymol's high intrinsic potency may meaningfully contribute to the DCM fraction's biological activity. E-15-Heptadecenal (3.09%), identified in the crude and butanol fractions, is a long-chain fatty aldehyde class compound with reported antimicrobial relevance in aromatic plant preparations.

The DCM fraction exhibited a distinctly different and pharmacologically notable compound profile. Its most abundant compound, 14-Octadecenoic acid methyl ester (methyl myristoleate, 6.81%), belongs to the fatty acid methyl ester class. Methyl palmitate (hexadecanoic acid methyl ester, 1.80%), also detected in the DCM fraction, is a well-studied endogenous fatty acid methyl ester with documented anti-inflammatory and anti-fibrotic properties. It has been demonstrated that methyl palmitate inhibits phagocytic activity in macrophages while differentially modulating cytokine expression, nitric oxide, and COX-2, and has shown efficacy in preclinical models of pulmonary and liver fibrosis [17]. Additionally, Squalene (0.53%) was identified in the DCM fraction, a finding of particular pharmacological interest. Squalene, as a natural unsaturated triterpene, is a highly effective antioxidant; its multiple double bonds enable it to quench singlet oxygen efficiently and prevent lipid peroxidation at the cellular membrane surface [18]. Ibrahim and Mohamed demonstrated that squalene exerts its antioxidant effect through activation of the Keap1-Nrf2-ARE signalling pathway, stimulating the transcription of antioxidant and detoxifying enzymes, while simultaneously downregulating NF-κB-mediated pro-inflammatory signalling [18]. Du *et al.* [19] further established that squalene scavenges free radicals, inhibits ROS generation, and enhances antioxidant enzyme activities including SOD, catalase, and glutathione peroxidase The presence of squalene in the DCM fraction may therefore contribute to its strong antioxidant and potential anti-inflammatory activity. Phytane (Heptadecane, 2,6,10,14-tetramethyl-, 3.23%), a saturated isoprenoid compound, was identified in the DCM fraction alongside Aromandendrene (0.99%), a bicyclic sesquiterpene previously reported for its antimicrobial and antioxidant properties in essential oils of various medicinal plant species.

In the DPPH radical scavenging assay, the crude extract and DCM fraction demonstrated equivalent and potent activity at 100 μg/mL (72% inhibition), while the butanol fraction showed markedly lower activity (23% inhibition). All samples exhibited concentration-dependent inhibition, consistent with established free radical scavenging behaviour of plant-derived antioxidants and consistent with findings by Okochi *et al.* who similarly observed concentration-dependent DPPH scavenging in *G. latifolium* preparations [5]. The substantially weaker activity of the butanol fraction may be attributed to the selective partitioning during VLC fractionation, wherein more polar and less antioxidant-active compounds are preferentially eluted into the butanol phase, simultaneously depleting the fraction of lipophilic terpenoids such as γ-Terpinene that are principal contributors to DPPH scavenging activity.

The FRAP assay demonstrated that all three samples possessed significant ferric ion reducing capacity, with absorbance values at 100 μg/mL of 1.98 (crude), 2.01 (DCM), and 2.06 (butanol), all closely approaching the ascorbic acid standard

(2.08). Notably, the butanol fraction showed a relatively stronger FRAP performance (2.06) compared to its modest DPPH inhibition (23%). FRAP measures electron-donating capacity, whereas DPPH primarily measures hydrogen atom transfer and electron donation; the discrepancy between these two assay results for the butanol fraction suggests that its compounds may preferentially act as reductants rather than free radical chain-breakers [5,6].

Total phenolic content (TPC) was highest in the crude ethanol extract (39.8 mgGAE/g), substantially exceeding the DCM fraction (14.8 mgGAE/g) and butanol fraction (0.16 mgGAE/g). This is consistent with the established capacity of aqueous-alcoholic solvents like 80% ethanol to extract a broad spectrum of phenolic compounds of varying polarities [1,6]. Phenolic compounds contribute to antioxidant activity through multiple mechanisms including free radical scavenging, metal chelation, and singlet oxygen quenching, and their high concentration in the crude extract correlates with its strong DPPH inhibition performance [1,5]. The marked decline in TPC across fractions, particularly in the butanol fraction, reflects the redistribution and selective exclusion of phenolics during solvent partitioning.

Total flavonoid content (TFC) was highest in the DCM fraction (87.16 mgRE/g), substantially exceeding both the crude extract (52.5 mgRE/g) and butanol fraction (48.83 mgRE/g). This enrichment of flavonoids in the DCM fraction during VLC fractionation indicates that DCM preferentially extracts lipophilic to semi-polar flavonoid aglycones, which are generally more potent antioxidants than their water-soluble glycosylated counterparts due to higher intrinsic radical scavenging capacity and greater membrane permeability [1,5]. The elevated TFC in the DCM fraction provides a consistent explanation for its equivalent DPPH scavenging performance to the crude extract despite its lower TPC, and for its superior FRAP absorbance. Nneoyi-Egbe *et al.* confirmed that flavonoid concentrations in *G. latifolium* leaves are measurable and consistent across different processing conditions, reinforcing the significance of flavonoids as a core bioactive class in this plant [6].

Collectively, the findings confirm that *G. latifolium* leaf possesses a rich and diverse array of bioactive compounds with considerable antioxidant capacity. The DCM fraction and crude ethanol extract emerged as the most pharmacologically promising, exhibiting strong and complementary antioxidant profiles. The identification of γ-Terpinene, squalene, methyl palmitate, p-Cymene, thymol, and fatty acid methyl esters across the extract and fractions provides a robust phytochemical basis for the observed biological activities and the plant's established ethnomedicinal uses. These findings lay the groundwork for further bioactivity-guided isolation of individual active compounds and in vivo validation studies aimed at translating the antioxidant potential of *G. latifolium* into evidence-based therapeutic applications.

5. Conclusion

This study has demonstrated that the ethanol extract, dichloromethane (DCM) and butanol fractions of *Gongronema latifolium* leaf are rich in diverse bioactive phytochemicals, including alkaloids, flavonoids, saponins, tannins, and cardiac glycosides. GC-MS profiling revealed a complex array of pharmacologically relevant compounds across all three preparations, with γ-Terpinene, squalene, methyl palmitate, p-Cymene, and fatty acid methyl esters among the most significant identified constituents. The crude extract and DCM fraction demonstrated the strongest DPPH radical scavenging activity (72% at 100 μg/mL), with all fractions exhibiting substantial ferric reducing antioxidant power comparable to the ascorbic acid standard. The crude extract recorded the highest total phenolic content (39.8 mgGAE/g), while the DCM fraction recorded the highest total flavonoid content (87.16 mgRE/g), collectively accounting for the superior antioxidant profiles of these two preparations. These findings provide scientific validation for the traditional medicinal use of *G. latifolium* and highlight its considerable potential as a natural source of antioxidant and bioactive compounds. Further studies involving bioactivity-guided fractionation, isolation of individual active compounds, in vivo antioxidant validation, and toxicity profiling are recommended to fully exploit the therapeutic potential of this plant.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest, whether financial or non-financial, regarding the publication of this work.

Statement of ethical approval

All animal experiments were carried out in accordance with the guidelines of the University of Uyo, Uyo Animal Ethics Committee with approval number UU_FBMSREC_2026_004

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