

Gomphrena nitida Rothr. (Amaranthaceae): Phytochemical Evaluation and Antioxidant Activities of Roots, Stem, and Leaves

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Abstract

Gomphrena nitida Rothr. (Amaranthaceae) is traditionally used to manage oxidative stress-related ailments, infections, and inflammation. This study evaluated the phytochemical composition and antioxidant potential of root, stem, and leaf extracts obtained using hexane, ethyl acetate, and methanol. Qualitative and quantitative analyses revealed flavonoids, glycosides, steroids, and terpenoids across all extracts, with methanol and ethyl acetate fractions showing richer profiles. The highest flavonoid (36.89 mg/g quercetin equivalent) and phenolic (18.59 mg/g gallic acid equivalent) contents were found in leaf and root extracts, respectively. Root methanol extract showed the strongest DPPH activity ($IC_{50} = 168.12 \mu\text{g/mL}$), comparable to ascorbic acid ($IC_{50} = 136.97 \mu\text{g/mL}$), while root hexane extract exhibited the highest FRAP value (582.14 mg AAE/g). The strong antioxidant potential correlates with high phenolic and flavonoid contents, supporting the traditional use of *G. nitida* and highlighting its promise as a source of natural antioxidants for managing oxidative stress-related conditions.

Keywords: Antioxidant activity; *Gomphrena nitida*; Oxidative stress; Phytochemicals; Traditional medicine.

Abbreviations: DPPH: 1,1-Diphenyl-2-Picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; AAE/g: Ascorbic Acid Equivalent per gram; MPs: Medicinal plants; WHO: World Health Organisation; BHA: Butylated Hydroxyanisole; BHT: Butylated Hydroxytoluene; TBHQ: Tert Butylhydroquinone; FRIN: Forestry Research Institute of Nigeria; TSC: Total Saponin Content; TAC: Total Alkaloid Content; TPC: Total Phenolic Content; GAE/g: Gallic Acid Equivalent per gram of Extract; QUE/g: Quercetin Equivalent per gram of Extract; CE/g: Catechin Equivalent per gram of Extract; TFC: Total Flavonoid Content; TTC: Total Tannin Content; Abr: Absorbance of the Control; Aar: Absorbance of the Sample; TCA: Trichloroacetic Acid; IC50: Inhibitory Concentration at 50%; GNRH: *Gomphrena nitida* Root Hexane Extract; GNRE: *Gomphrena nitida* Root Ethyl Acetate Extract; GNRM: *Gomphrena nitida* Root Methanol Extract; GNSH: *Gomphrena nitida* Stem Hexane Extract; GNSE: *Gomphrena nitida* Stem Ethyl Acetate Extract; GNSM: *Gomphrena nitida* Stem Methanol Extract; GNLH: *Gomphrena nitida* Leaf Hexane Extract; GNLE: *Gomphrena nitida* Leaf Ethyl Acetate Extract; GNLN: *Gomphrena nitida* Leaf Methanol Extract; HAT: Hydrogen Atom Transfer; SET: Electron Transfer

INTRODUCTION

Medicinal plants (MPs) have historically been indispensable in the treatment and prevention of various human illnesses, especially in settings with limited access to formal healthcare systems. Their use involves applying whole plants or specific parts for therapeutic purposes and general well-being (Sonaye et al., 2017). Numerous herbal preparations have been developed that are reported to alleviate symptoms associated with a wide range of conditions, including infections, inflammation, respiratory ailments, and antioxidant effects that help mitigate oxidative stress-related disorders (Saggar et al., 2022). These herbal preparations commonly took the form of powders, decoctions, tinctures, infusions, macerations, poultices, and inhalations, with their formulation and usage passed

down orally through generations before eventually being documented (Orch et al., 2021). Over time, the knowledge surrounding MPs became more systematised, with contributions from disciplines such as botany, ethnobotany, ethnopharmacology, and plant ecology aiding in the identification and classification of plants with therapeutic potential (Chaachouay & Zidane, 2024). The relevance of MPs stems from their affordability, ethnomedicinal use, and the richness of their bioactive constituents. Extensive pharmacological investigations have reinforced their therapeutic roles in both indigenous medicine and contemporary clinical practices. They are natural sources of diverse secondary metabolites, including phenols, alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides, which contribute significantly to their medicinal efficacy (Falodun et al., 2025). The World Health Organisation (WHO) estimates

that up to 80% of the world's population depends on phytochemical agents for managing health conditions (Riaz et al., 2023). Secondary metabolites have demonstrated remarkable antioxidant, anticancer, cytotoxic, anti-inflammatory, cardioprotective, hepatoprotective, immunomodulatory, neuroprotective, and antidiabetic activities, positioning them as promising alternatives to synthetic compounds and potential therapeutic agents (Riaz et al., 2023). Flavonoids, phenols, and tannins are widely recognised for their antioxidant potential, primarily through their ability to scavenge free radicals, with their activity influenced by structural properties and stability. Alkaloids are also known for their potent analgesic and antimalarial effects, while saponins and tannins also exhibit notable antimicrobial and anti-inflammatory properties, highlighting the diverse therapeutic roles of plant-derived compounds (Falodun et al., 2025). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ), though widely used as food additives, have raised safety concerns due to their potential hepatotoxicity, nephrotoxicity, and neurotoxicity (Ren et al., 2025). This has prompted a global shift towards safer, plant-derived antioxidants as promising alternatives.

The Amaranthaceae family has garnered more scientific interest as scientists search for effective natural antioxidants. With 178 genera and 2,052 species, this vast family include species found in arid regions, deserts, and saline environments, with a broad geographic distribution across Africa, Asia, Europe, and the Americas (Todorović et al., 2022). The genus *Gomphrena*, widely distributed and traditionally known, is rich in phytochemicals such as flavonoids, terpenoids, phenolics, and phytosterols. Different plant parts are commonly employed in folk medicine to manage fever, inflammation, infections, and metabolic disorders (Astalakshmi et al., 2023; Tarnam et al., 2014). *Gomphrena nitida* Rothr., an underexplored herbaceous species native to tropical and subtropical regions (Hogan, 2019), has preliminary investigations indicating that the essential oils are rich in secondary metabolites with notable biological activity, although its crude extracts remain largely unexplored. Essential oils from *G. nitida* roots, stems, and leaves contain abundant compounds such as β -caryophyllene, α -humulene, and spathulenol, exhibiting cytotoxic, antioxidant, and antimicrobial properties (Sotade et al., 2025). Although its essential oil composition has been recently investigated, comprehensive studies on its qualitative and quantitative phytochemicals and antioxidant activities of crude extracts remain unexplored. This work evaluates the phytochemical constituents and antioxidant activities of *G. nitida*, contributing to the validation of its ethnomedicinal uses and underscoring its potential as a source of natural antioxidants.

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh samples of *G. nitida* comprising roots, stems, and leaves were collected from a cultivated farmland located in Orile-Igbon, Oyo State, Nigeria. This area lies within the tropical climatic zone of southwestern Nigeria and is recognised for its thriving agricultural and medicinal plant biodiversity. The plant was identified by Dr. S. A. Odewo, a plant taxonomist at the Forestry Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen with herbarium number FHI 113868 was deposited in the FRIN Herbarium for reference.

Preparation and Extraction of Plant Materials

The collected plant materials (roots, stems, and leaves) were air-dried under shade at room temperature for 14-21 days. The dried samples were separately pulverised into fine powder using a mechanical grinder to enhance extraction efficiency. A total of 200 g of powdered root, 1800 g of stem, and 1000 g of leaf were subjected to successive solvent extraction using n-hexane, ethyl acetate, and methanol, in order of increasing polarity. Each powdered sample was first soaked in n-hexane for 72 hours at room temperature (Taiwo et al., 2022). After maceration, the extract was decanted, and fresh solvent was added repeatedly until the decanted solution appeared clear, indicating that the material had been exhausted. The same process was repeated sequentially with ethyl acetate and then methanol. The resulting filtrates were passed through Whatman No. 1 filter paper, and each solvent fraction was concentrated under reduced pressure using a rotary evaporator at 35-40 °C. The concentrated extracts were further air-dried, weighed, transferred into airtight containers, and stored at 4 °C until further analysis.

Qualitative Phytochemical Analysis

Standard phytochemical methods were used to detect the presence or absence of major classes of secondary metabolites in the hexane, ethyl acetate, and methanol extracts of the roots, stems, and leaves of *G. nitida* (Yadav et al., 2016). The following phytochemicals were tested for:

Test for Saponins: 5 mL of each extract was diluted with 5 mL of distilled water, shaken vigorously and heated. Persistent frothing indicated the presence of saponin.

Test for Alkaloids: 2 mL of each extract was treated with a few drops of Hager's reagent. The appearance of a yellow precipitate confirmed the presence of an alkaloid.

Test for Flavonoids: 1 mL of each extract was treated with 1 mL of 10% lead acetate solution. The appearance of a yellow colouration indicated the presence of a flavonoid.

Test for Phenols: To 2 mL of each extract, 3-4 drops of ferric chloride solution were added. The appearance of

a bluish-black colouration indicated the presence of phenol.

Test for Tannins: 2 mL of each extract was diluted with 2 mL of distilled water, followed by the addition of 2-3 drops of 5% ferric chloride solution. The appearance of a greenish precipitate confirmed the presence of tannin.

Test for Coumarins: 2 mL of each extract was mixed with 3 mL of 10% sodium hydroxide solution. The appearance of a yellow colouration indicated the presence of coumarin.

Test for Diterpenes: 2 mL of each extract was dissolved in water, followed by the addition of 3 mL of copper acetate solution. A green colouration confirmed the presence of a diterpene.

Test for Cardiac Glycosides: 2 mL of each extract was treated with 2 mL of glacial acetic acid containing a drop of ferric chloride, followed by careful addition of concentrated sulphuric acid. Formation of a brown ring at the interphase confirmed the presence of a cardiac glycoside.

Test for Steroids: 2 mL of each extract was mixed with 2 mL of chloroform, followed by the addition of 2 mL of concentrated sulphuric acid. A reddish-brown ring at the junction indicated the presence of a steroid.

Test for Terpenoids: 2 mL of each extract was mixed with 2 mL of acetic anhydride, followed by the careful addition of 2–3 drops of concentrated sulphuric acid. Development of a deep red colouration indicated the presence of a terpenoid.

The results were recorded as either present (+) or absent (-) based on the characteristic colour changes or precipitate formation for each test.

Quantitative Phytochemical Analysis

Quantitative estimations of selected phytochemicals were carried out on the hexane, ethyl acetate, and methanol extracts of the roots, stems, and leaves of *G. nitida*. They were determined using standard procedures.

Determination of Total Saponin Content (TSC) (%)

20 g of each sample was measured into a conical flask, followed by the addition of 100 mL of 20% aqueous ethanol. The mixture was heated in a hot water bath at about 55 °C for 4 hours with continuous stirring. After heating, the mixture was filtered, and the residue was re-extracted with an additional 200 mL of 20 % aqueous ethanol. The combined filtrates were concentrated to about 40 mL over a water bath at about 90 °C. The concentrate was transferred into a 250 mL separatory funnel, and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was collected, while the ether layer was discarded, and the process was repeated to ensure purification. 60 mL of n-butanol was added, and the combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The mixture was then evaporated in a waterbath, and the residue was oven-dried to a constant weight. The TSC was

determined and expressed as a percentage, applying the method described by Obadoni and Ochuko (2002) and Akwas *et al.* (2025).

$$\% \text{ Saponin content} = \frac{(\text{Weight of filter paper} + \text{residue}) - (\text{Weight of filter paper})}{\text{Weight of sample analysed}} \times 100$$

Formula 1

Determination of Total Alkaloid Content (TAC) (%)

The TAC was determined using the gravimetric method as described by Harborne (Harborne, 1998) and later modified by Akwas (Akwas *et al.*, 2025). A 5 g portion of the sample was weighed into a 250 mL beaker, followed by the addition of 200 mL of 10% acetic acid in ethanol. The mixture was covered and left to stand for 4 hours. The resulting solution was filtered, and the filtrate was concentrated in a water bath to approximately one-quarter of the initial volume. Concentrated ammonium hydroxide solution was then added dropwise to the concentrate until complete precipitation occurred. The mixture was allowed to settle, and the precipitate obtained was washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid fraction, which was oven-dried at 40-50°C to a constant weight. The TAC was calculated and expressed as a percentage yield (%).

$$\% \text{ Alkaloid content} = \frac{(\text{Weight of filter paper} + \text{residue}) - (\text{Weight of filter paper})}{\text{Weight of sample analysed}} \times 100$$

Formula 2

Determination of Total Phenolic Content (TPC) (GAE/g)

The TPC was determined using the Folin-Ciocalteu assay (Marinova *et al.*, 2005; Tijani *et al.*, 2025). 1 mL of extract was mixed with 1 mL of Folin–Ciocalteu reagent in a 25 mL volumetric flask. After 5 minutes, 10 mL of 7% sodium carbonate was added, and diluted to volume with water. The mixture was incubated for 90 minutes at room temperature. The absorbance was then measured in triplicate at 750 nm against a blank with a Spectrumlab 752s UV/Visible spectrophotometer. The TPCs were calculated based on the calibration curve of gallic acid ($y = 0.0372x + 0.029$; $R^2 = 0.9883$ and expressed as mg gallic acid equivalent per gram of dried sample (GAE/g).

Determination of Total Flavonoid Content (TFC) (QUE/g)

The TFC was measured by the aluminium chloride colourimetric assay (Marinova *et al.*, 2005). The reaction mixture consisted of 1 mL of extract and 4 mL of distilled water in a 10 mL volumetric flask. To this, 0.30 mL of 5% sodium nitrite was added, followed after 5 minutes by 0.3 mL of 10% aluminium chloride. After another 5 minutes, 2 mL of 1 M sodium hydroxide was

added, and the mixture was diluted to 10 mL with distilled water. Reference standard solutions of quercetin (20, 40, 60, 80, and 100 µg/mL) were prepared in the same manner. Absorbance for the reaction mixtures was measured in triplicate at 510 nm against a reagent blank using a UV/Visible spectrophotometer. The TFC was expressed as mg quercetin equivalent per gram of extract (mg QUE/g).

Determination of Total Tannin Content (TTC) (CE/g)

TTC was quantified using the Folin–Ciocalteu procedure (Galvão et al., 2018). 0.1 mL of the sample solution was added to a 10 mL volumetric flask containing 7.5 mL of distilled water, 0.5 mL of Folin–Ciocalteu phenol reagent, and 1 mL of 35% Na₂CO₃ solution. The mixture was diluted to 10 mL with distilled water, shaken well, and kept at room temperature for 30 minutes. Reference standard solutions of gallic acid (20, 40, 60, 80, and 100 µg/mL) were prepared in the same manner. Absorbance for test and standard solutions was measured at 760 nm against a reagent blank using a Spectrumlab 752s UV/Visible spectrophotometer. The TTCs were calculated based on the calibration curve of catechin ($y = 0.0022x + 0.0326$; $R^2 = 0.9852$ and expressed as mg catechin equivalent per gram of extract (CE/g).

Antioxidant Assays

DPPH Radical Scavenging Assay

The antioxidant activity of the methanol extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Elusiyan et al., 2011). A methanolic solution of DPPH (0.3 mM) was prepared, and 1 mL of the sample extract was diluted with 1 mL DPPH. After 30 minutes of incubation in the dark, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition was calculated, and the IC₅₀ (concentration of extract required to inhibit 50% of the DPPH radicals) was determined. The percentage of the DPPH radical scavenging was calculated using the equation as given below:

$$\% \text{ Inhibition of DPPH} = \frac{(\text{Abr} - \text{Aar})}{\text{Abr}} \times 100 \quad \text{Formula 3}$$

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing ability of the methanol extracts was evaluated using the FRAP assay (Sotade et al., 2025). A 1 mL portion of the plant extract was combined with 2.5 mL of 20 mM phosphate buffer (7.4 pH) and 2.5 mL 1%, w/v potassium Ferricyanide. The resulting mixture was incubated at 50 °C for 30 minutes. Subsequently, 2.5 mL of 10%, w/v trichloroacetic acid (TCA) and 0.5 mL of 0.1%, w/v ferric chloride were introduced, and the mixture was allowed to stand for 10 minutes. Absorbance was then recorded at 700 nm using a spectrophotometer. Ascorbic acid was used as a positive reference standard, and all determinations were performed in triplicate.

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± SEM. Linear regression analysis was employed to determine IC₅₀ values, using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

The qualitative phytochemical screening of *G. nitida* root, stem, and leaf extracts in hexane, ethyl acetate, and methanol revealed the presence of diverse secondary metabolites (Table 1). The results indicated variations in metabolite distribution across plant parts and solvent systems. Flavonoids, cardiac glycosides, steroids, and terpenoids were consistently present across all the extracts. Notably, diterpenes were detected only in the methanol extracts of root, stem, and leaves. Saponins were absent in hexane extracts but abundant in ethyl acetate and methanol extracts. Alkaloids were widely distributed in stem and leaf extracts, but absent in root hexane and ethyl acetate and leaf ethyl acetate extracts. Phenols and tannins were detected in most extracts, except the hexane fractions of the stem and leaves. Coumarins appeared only in ethyl acetate and methanol fractions, except for the leaf ethyl acetate extract, where they were absent. Among the solvent extracts tested, methanol demonstrated the highest efficiency in extracting diverse classes of phytochemicals, followed by ethyl acetate, whereas hexane accounted for the least phytochemicals. This result agrees with previous studies that reported higher extraction efficiency of polar solvents for bioactive metabolites such as flavonoids, phenols, and saponins (Chatepa et al., 2024).

These phytochemical classes, consistently detected across all extracts, have been widely reported to exhibit diverse therapeutic activities. The prevalent occurrence of flavonoids in all the tested extracts is particularly significant, as these polyphenolic compounds demonstrate strong antioxidant capacity, alongside antiviral and anti-inflammatory properties, and have been reported to play therapeutic roles in the treatment of hypertension, diabetes, ulcers, and rheumatic fever (M. O. Taiwo & Olaoluwa, 2020). Cardiac glycosides, traditionally used in cardiovascular therapy, have also been reported to exhibit anticancer, antiviral, and immunomodulatory activities, highlighting their broader pharmacological relevance (Omole et al., 2025). Steroids have been reported to display diverse pharmacological activities, including anti-inflammatory, anticancer, antiproliferative, antihypercholesterolemic, antimicrobial, and antiviral effects, underscoring their therapeutic relevance in both pharmacology and medicine (Dembitsky, 2023). Terpenoids, a structurally diverse class of isoprene-derived metabolites, exhibit wide-ranging biological activities including antioxidant,

antimicrobial, anti-inflammatory, anticancer, and antiallergic effects, highlighting their pharmacological significance across multiple therapeutic applications (Câmara et al., 2024).

Quantitative Phytochemical Screening

The quantitative analysis of *G. nitida* revealed the presence of saponins, alkaloids, phenols, flavonoids, and tannins in varying concentrations across the root, stem, and leaf extracts (Table 2). Saponin content ranged from 0.41% in GNSE to 4.34% in GNSH. GNLH also recorded relatively high saponin content, 3.53%, while methanol extracts contained comparatively lower values, particularly leaf methanol, 1.10%. Overall, saponins were most concentrated in non-polar hexane fractions, with GNSH extract giving the highest yield. Alkaloids were detected in appreciable amounts across extracts, with the GNSH (13.52%) showing the highest concentration, followed by GNLH (12.72%). The lowest alkaloid levels were recorded in GNRH (5%) and GNSH (5%). Generally, alkaloids accumulated more in the stem and leaf extracts compared to the roots. The phenolic concentration varied across plant parts and solvent extracts. The highest value was recorded in the GNRM (18.59 GAE/g), while moderate levels were observed in the GNRE (8.96 GAE/g) and GNSM (6.50 GAE/g). Flavonoids were the most abundant class of metabolites quantified, with GNLE (36.89 QUE/g) showing the highest concentration, followed by GNRH (22.63 QUE/g) and GNRE (14.00 QUE/g). The lowest TFC were observed in GNSE (3.48 QUE/g) and GNRM (5.19 QUE/g). Overall, flavonoids were present in all solvent extracts, with higher yields generally obtained in non-polar to moderately polar solvents. The TTC showed variable distribution across the extracts. The highest TTCs were recorded in the GNRM (5.38 CE/g) and GNSE (5.33 CE/g) extracts. Moderate levels were found in the GNRE (4.03 CE/g) and GNSM (3.44 CE/g) extracts. The lowest concentrations occurred in the GNLH (1.86 CE/g) and GNSH (2.51 CE/g). These results indicate that tannins were present in all solvent extracts, with relatively higher yields in the methanol and ethyl acetate fractions of root and stem tissues.

The quantitative phytochemical screening of *G. nitida* extracts revealed significant variation in the concentration of bioactive compounds across plant parts and solvent types, highlighting the influence of both plant tissue and extraction solvent on phytochemical yield. Phenolics and tannins were generally abundant in the root extracts, suggesting that the root constitutes a primary site for their biosynthesis and accumulation, corroborating reports that roots often serve as reservoirs of polyphenols and condensed tannins, which play essential roles in plant defence, nutrient mobilisation, and soil interactions (Dehghanian et al., 2022). In this study, flavonoids were most abundant in the ethyl acetate extracts of *G. nitida*. Similarly, *Rosmarinus officinalis* showed higher flavonoid content in the ethyl acetate

fraction compared to the n-hexane fraction (Esati et al., 2024). Saponins and alkaloids were most abundant in the stem and leaf hexane extracts, indicating their solubility in nonpolar solvents. This observation is supported by findings in *Crinum zeylanicum*, where successive solvent extraction revealed high proportions of saponins and alkaloids, with significant concentration in the hexane fraction (Daben et al., 2021). Comparable studies within the genus *Gomphrena* have also reported similar phytochemical profiles, as reported in *Gomphrena globosa*, where quantitative analysis showed that phenolics, flavonoids, and flavonols constituted the major phytochemicals (Tang et al., 2022). Similarly, *Gomphrena celosioides* exhibited high concentrations of polyphenols and flavonoids, with ethanolic extracts recording the highest levels, which aligns with the predominance of these compounds observed in *G. nitida* (Thuy et al., 2020). These findings emphasise the predominance of polyphenolic compounds within the genus *Gomphrena*, while the present study provides the first detailed quantitative phytochemical profiling of *G. nitida*, thereby expanding chemotaxonomic knowledge and highlighting its potential as a source of biologically active secondary metabolites.

Antioxidant Activity Determined by DPPH and FRAP Methods

The antioxidant activity of the different solvent fractions of *G. nitida* root, stem and leaves was evaluated using two assays, which are 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays.

DPPH Radical Scavenging Assay

The DPPH radical scavenging assay is one of the most widely employed methods for assessing antioxidant activity because it is simple, rapid, cost-effective, and reliable (Barbouchi et al., 2024). It measures the capacity of extracts or compounds to donate hydrogen atoms and stabilise free radicals, where lower IC₅₀ values are indicative of stronger antioxidant potential (Falodun et al., 2025). The free radical scavenging potential of *G. nitida* extracts was evaluated using the DPPH assay. The results of the DPPH assay showed that the inhibitory percentages vary from 37.04 ± 0.06 (GNSH) to 70.46 ± 0.06 (GNSM) for the tested extracts, and from 24.38 ± 0.09 to 96.40 ± 0.10 for the Ascorbic acid (Standard). The scavenging activity of all extracts increased with concentration, with ascorbic acid exhibiting the highest percentage inhibition of DPPH radicals (96.40% at 500 µg/mL). The variation in percentage inhibition of DPPH radical across all tested concentrations (125–500 µg/mL) is graphically illustrated in Figure 1, clearly showing the concentration-dependent increase in activity for all extracts, with ascorbic acid consistently exhibiting the strongest inhibition. The methanol root extract showed the highest radical scavenging ability, with an IC₅₀ value of 168.12 µg/mL, followed by the stem ethyl acetate

extract (181.73 $\mu\text{g/mL}$) and methanol stem extract (226.34 $\mu\text{g/mL}$), when compared to the IC_{50} of Ascorbic acid (136.97 $\mu\text{g/mL}$), while the hexane stem extract had the weakest activity (535.33 $\mu\text{g/mL}$) as shown in Figure 1. Among the tested extracts, activity decreased in the order: GNRM > GNSE > GNSM > GNLM > GNRE > GN LH > GNRH > GNLE > GNSH. The corresponding IC_{50} values were 168.119, 181.726, 226.335, 315.126, 372.605, 411.188, 448.836, 495.226, and 535.331 $\mu\text{g/mL}$, respectively. The IC_{50} is represented in Table 3.

The results obtained in this study demonstrate that *G. nitida* extracts possess significant antioxidant properties, as indicated by their concentration-dependent scavenging of DPPH radicals. Extracts with lower IC_{50} values indicate stronger antioxidant activity, suggesting a high capacity for neutralising free radicals. The high radical scavenging activity observed in the methanol and ethyl acetate extracts may be attributed to the presence of phenolic and flavonoid compounds, which are well known for their ability to donate hydrogen atoms or electrons to DPPH radicals. These findings are consistent with earlier reports that highlight the contribution of phenolics to antioxidant activity in medicinal plants (Ali et al., 2014; Falodun et al., 2025). Although several studies have demonstrated a strong correlation between phenolic content and antioxidant activity in medicinal plants, the antioxidant potential of plant extracts cannot be attributed solely to phenolic compounds. The overall antioxidant activity often results from synergistic or antagonistic interactions among different phytoconstituents, including non-phenolic compounds such as terpenoids, saponins, and alkaloids (Barbouchi et al., 2024). The relatively lower scavenging activity of the hexane extract may be due to the nonpolar nature of the solvent, which limits the extraction of highly polar antioxidant constituents such as phenolics and flavonoids. This observation corroborates previous findings where non-polar extracts exhibited reduced free radical scavenging potential compared to polar fractions (Barchan et al., 2014). The strong DPPH scavenging ability observed in the methanol and ethyl acetate extracts suggests that *G. nitida* contains bioactive compounds capable of donating protons to stabilise reactive species, thereby preventing oxidative damage to biomolecules such as lipids, proteins, and nucleic acids. A related study on *G. globosa* reported potent DPPH radical scavenging activity of its ethanol extract, attributed to flavonoids, saponins, and terpenoids (Ningrum & Wijayanti, 2020). This comparable result supports the strong antioxidant activity observed in *G. nitida*, indicating that species within the *Gomphrena* genus exhibit comparable phytochemical compositions responsible for free radical scavenging.

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay evaluates the ability of antioxidants to donate electrons

by reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, thus indicating their reducing potential (Falodun et al., 2025). The FRAP of *G. nitida* extracts is presented in Table 4. The FRAP values ranged from 73.57 ± 0.20 to 582.14 ± 0.00 mg AAE/g extract across the tested concentrations (125–500 $\mu\text{g/mL}$). Ascorbic acid, used as the standard, exhibited the highest reducing power, 609.03 ± 0.17 , 449.80 ± 0.17 , and 436.81 ± 0.17 mg AAE/g at 500, 250, and 125 $\mu\text{g/mL}$, respectively. Among the extracts, the hexane root extract (GNRH) demonstrated the highest ferric reducing activity at all concentrations, with values of 582.14 ± 0.00 , 419.64 ± 0.30 , and 405.36 ± 0.30 mg AAE/g extract at 500, 250, and 125 $\mu\text{g/mL}$, respectively. This was followed by the ethyl acetate root extract (GNRE), which recorded 353.47 ± 0.45 , 287.40 ± 0.17 , and 228.08 ± 0.62 mg AAE/g extract across the same concentrations. The methanol root extract (GNRM), ethyl acetate stem extract (GNSE), and methanol stem extract (GNSM) also showed appreciable reducing power, with values ranging from 287.80 ± 0.30 to 175.10 ± 0.45 mg AAE/g extract. However, the leaf extracts exhibited comparatively lower FRAP values, with the methanol leaf extract (GNLM) showing the least activity (165.67 ± 0.25 , 126.15 ± 0.18 , and 117.78 ± 0.18 mg AAE/g extract at 500, 250, and 125 $\mu\text{g/mL}$, respectively).

In this study, *G. nitida* extracts exhibited appreciable ferric reducing activity that increased with concentration, reflecting their capacity to participate in redox reactions and suggesting the presence of abundant redox-active phytochemicals. Among the extracts, the strong activity of the root extract, particularly the hexane and ethyl acetate extracts, indicates that both non-polar and moderately polar compounds contribute substantially to the overall antioxidant potential of the plant. The strong reducing power of the ethyl acetate extract might result from the abundance of phenolic and flavonoid metabolites, while the hexane extract likely contains nonpolar antioxidants such as terpenoids and steroids, consistent with the phytochemical screening results. Numerous studies have shown that phenolic compounds, owing to their intrinsic redox properties, function as effective reducing agents and radical scavengers, thereby playing a crucial role in antioxidant defence mechanisms (Asfaw, 2023). The present findings therefore reinforce the relationship between phenolic compounds and reducing power in plant extracts.

Comparative Evaluation of Antioxidant Activities of *G. nitida* Extracts using DPPH and FRAP Assays.

The antioxidant activity of *G. nitida* extracts, as assessed by DPPH and FRAP assays, revealed distinct but complementary mechanisms of action. The DPPH assay primarily reflects the hydrogen atom transfer (HAT) ability of antioxidants, while the FRAP assay measures their single electron transfer (SET) ability (Falodun et al., 2025). Both mechanisms are crucial indicators of an

extract's overall redox behaviour and its ability to mitigate oxidative stress. In this study, variation in antioxidant activities across assays suggests that the active compounds within the extracts exhibit different propensities for hydrogen atom donation and electron transfer. Extracts with strong DPPH scavenging activity, such as the methanol root extract (GNRM) and ethyl acetate stem extract (GNSE), demonstrated efficient radical scavenging capacity through hydrogen donation. However, hexane root (GNRH) and ethyl acetate root (GNRE) extracts displayed greater ferric reducing power in the FRAP assay, signifying higher electron-donating capability. These differences could be attributed to the diverse polarity and solubility of antioxidant constituents in each solvent extract, which influence their mechanisms of redox interaction.

Generally, extracts rich in phenolic and flavonoid compounds exhibit strong antioxidant activity in both assays, given their dual HAT and SET mechanisms. The correlative relationship observed between DPPH and FRAP outcomes highlights the importance of employing multiple assays to evaluate antioxidant activities, as each targets distinct reaction mechanisms and cannot independently represent total antioxidant capacity. The DPPH and FRAP assays indicate that *G. nitida* possesses a broad spectrum of antioxidant mechanisms driven by both HAT and SET processes. This dual activity supports its ethnomedicinal use as a natural source of antioxidants capable of mitigating oxidative stress and related degenerative conditions.

Table 1. Qualitative phytochemical screening showing presence of diverse secondary metabolites in *Gomphrena nitida* extract.

S/N	PHYTOCHEMICALS	OBSERVATION	GNRH	GNRE	GNRM	GNSH	GNSE	GNSM	GNLH	GNLE	GNLM
1	Saponins	Frothing	-	+	+	-	+	+	-	+	+
2	Alkaloids	Yellow precipitate	-	-	+	+	+	+	+	-	+
3	Flavonoids	Yellow colouration/precipitate	+	+	+	+	+	+	+	+	+
4	Phenols	Bluish-black colouration	+	+	+	-	+	+	-	+	+
5	Tannins	Greenish precipitate	+	+	+	-	+	+	+	+	+
6	Coumarins	Yellow colouration	-	+	+	-	+	+	-	-	+
7	Diterpenes	Green colouration	-	-	+	-	-	+	-	-	+
8	Cardiac Glycosides	Brown ring at interphase	+	+	+	+	+	+	+	+	+
9	Steroids	Reddish-brown ring	+	+	+	+	+	+	+	+	+
10	Terpenoids	Deep red colouration	+	+	+	+	+	+	+	+	+

+ = Present; - = absent;

Table 2. Quantified Phytochemical Constituents in *Gomphrena nitida* Extracts.

Extracts	Total Saponins (%)	Total Alkaloids (%)	Total Phenolics (GAE/g)	Total Flavonoids (QUE/g)	Total Tannins (CE/g)
GNRH	ND	5.00 ± 0.02	ND	22.63 ± 0.17	3.12 ± 0.04
GNRE	2.40 ± 0.05	5.93 ± 0.04	8.96 ± 0.19	14.00 ± 0.11	4.03 ± 0.03
GNRM	2.20 ± 0.03	5.11 ± 0.03	18.59 ± 0.19	5.19 ± 0.17	5.38 ± 0.05
GNSH	4.34 ± 0.07	13.52 ± 0.04	ND	10.89 ± 0.11	2.51 ± 0.05
GNSE	0.41 ± 0.03	5.00 ± 0.02	17.14 ± 0.00	3.48 ± 0.07	5.33 ± 0.05
GNSM	2.20 ± 0.05	10.58 ± 0.02	6.50 ± 0.19	8.81 ± 0.06	3.44 ± 0.04
GNLH	4.00 ± 0.06	12.72 ± 0.03	ND	7.07 ± 0.17	1.86 ± 0.06
GNLE	3.57 ± 0.07	12.20 ± 0.02	15.46 ± 0.34	36.89 ± 0.11	2.97 ± 0.05
GNLM	2.39 ± 0.05	9.49 ± 0.02	14.45 ± 0.21	8.67 ± 0.11	2.59 ± 0.04

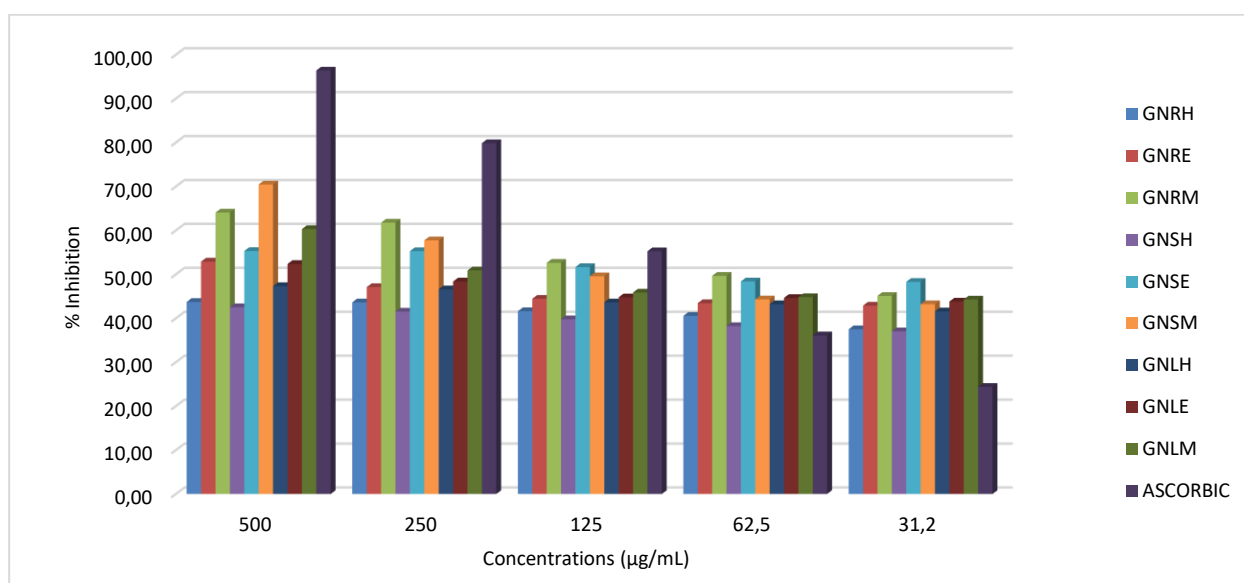
ND: Not Detected

Table 3. DPPH radical scavenging activity of different solvent extracts of *Gomphrena nitida*.

Samples	IC ₅₀ (µg/mL)
GNRH	448.836
GNRE	372.605
GNRM	168.119
GNSH	535.331
GNSE	181.726
GNSM	226.335
GNLH	411.188
GNLE	495.226
GNLM	315.126
ASC	136.968

Table 4. Ferric Reducing Antioxidant Power of different solvent extracts of *Gomphrena nitida*.

Ferric Reducing Antioxidant Power (mg AAE/g extract)			
SAMPLES	500	250	125
GNRH	582.14 ± 0.00	419.64 ± 0.30	405.36 ± 0.30
GNRE	353.47 ± 0.45	287.40 ± 0.17	228.08 ± 0.62
GNRM	287.80 ± 0.30	244.74 ± 0.45	215.18 ± 0.30
GNSH	298.12 ± 0.45	226.98 ± 0.45	165.18 ± 0.30
GNSE	325.00 ± 0.79	252.68 ± 0.60	219.74 ± 0.17
GNSM	316.07 ± 0.30	268.95 ± 0.62	175.10 ± 0.45
GNLH	200.28 ± 0.07	116.15 ± 0.30	78.10 ± 0.21
GNLE	170.24 ± 0.12	108.97 ± 0.18	73.57 ± 0.00
GNLM	165.67 ± 0.25	126.15 ± 0.18	117.78 ± 0.18
ASCORBIC	609.03 ± 0.17	449.80 ± 0.17	436.81 ± 0.17

**Figure 1.** % Inhibition at different concentrations (µg/mL) of *Gomphrena nitida* Extracts.

CONCLUSION

The consistent detection of diverse metabolite classes across plant parts and solvent extracts highlights *G. nitida* as a promising source of pharmacologically active compounds with broad therapeutic potential. Quantitative phytochemical screening revealed that non-polar hexane extracts were richer in alkaloids and saponins, whereas polar methanol and moderately-polar ethyl acetate extracts contained higher concentrations of phenolics, flavonoids, and tannins. Among plant parts, the leaf exhibited the highest flavonoid content, the stem accumulated more alkaloids and saponins, while the root methanol extract was particularly rich in phenolics and tannins. These phytochemical trends correspond closely with the antioxidant profiles observed in DPPH and FRAP assays, confirming that phenolic and flavonoid-rich fractions contributed significantly to both HAT and SET activities.

This study, therefore, represents the first comprehensive qualitative and quantitative phytochemical and antioxidant activity of *G. nitida*, providing novel insights into its chemical diversity and

redox potential. The findings provide biochemical justification for the ethnomedicinal use of *G. nitida* and corroborate previous reports on related species, validating the genus-wide predominance of polyphenolic compounds. The results establish *G. nitida* as a promising natural source of antioxidants with potential applications in mitigating oxidative stress and managing related degenerative diseases. Future research involving bioassay-guided isolation and structural elucidation of the active compounds is essential to further validate their pharmacological significance.

Conflict of interest: The authors declare that there is no conflict of interest.

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