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Phytochemical profiling and Antioxidant activity of *Gloriosa superba*

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Abstract

Gloriosa superba, an important medicinal climber of the family Colchicaceae, and commonly known as flame lily is valued for its diverse therapeutic potential. The plant has records in the Ayurvedic treatments in India. However, habitat exploitation and unsustainable way of collection have somehow decreased in its population. Mahanadi River in Odisha harbours important habitats for such medicinal plants. The plant has been traditionally utilized in the management of gout, arthritis, inflammation, wounds and other ailments, while its pharmacological potential includes anti-inflammatory, antioxidant, antimicrobial, analgesic and cytotoxic activities. The present study was carried out to collect the plant parts, identify using photographs and with key morphological characters. The study mainly aims to document the qualitative and quantitative phytochemical profile, bioactive potential through quantitative antioxidant assay. Phytochemical investigations revealed presence of important primary and secondary metabolites, including proteins, carbohydrates, phenolic compounds, flavonoids, tannins, saponins, terpenoids, steroids and alkaloids. The quantitative phytochemical analysis revealed that it contains appreciable amounts of secondary metabolites, with tannin (56.87 mg/100 mL) being predominant, followed by saponin (10.24%), phenol (4.96 mg/100 mL) and flavonoids (0.1 mg/mL). The quantitative antioxidant assay evaluated through DPPH assay in both ethanolic and aqueous extracts showed higher inhibition (54.09-59.03%) for ethanolic extracts than the aqueous extract (45.60-57.65%), indicating stronger free-radical-scavenging potential of the ethanol extract of the study plant. However, all parts of *G. superba*, particularly the tubers, are highly poisonous and excessive exposure to colchicine and related alkaloids may cause severe toxicity. *G. superba* represents a valuable source of pharmacologically important phytochemicals, its medicinal utilization requires appropriate identification, standardized extraction, dosage control and rigorous toxicological evaluation. The findings emphasize the therapeutic significance of *G. superba* and support further investigation of its bioactive constituents for safe and controlled pharmaceutical applications.

Keywords: Phytochemical analysis; antioxidant activity; DPPH assay; medicinal climber

Introduction

Gloriosa superba is an important medicinal climber belonging to the family Colchicaceae. It is commonly known as glory lily or flame lily and distributed in tropical and subtropical regions of Africa and Asia and occurs in several parts of India. The species is generally found in tropical deciduous forests, forest margins, scrublands, grasslands, hedges, thickets, field boundaries and other relatively open habitats (Peele *et al.*, 2022; Joshi *et al.*, 2024) [15]. In India, *G. superba* has been reported from several states, including Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Maharashtra, Odisha, Uttar Pradesh, West Bengal, Jharkhand and parts of northern India. Its occurrence has been reported across diverse ecological regions of India (Kumar *et al.*, 2026; Sharma *et al.*, 2026) [16, 26]. Despite its wide distribution, wild populations have declined in many areas because of habitat destruction, indiscriminate collection, overexploitation of tubers and seeds and poor natural regeneration. It also has a long history of ethnomedicinal use in Ayurvedic and traditional African and Asian medicine (Padmapriya *et al.*, 2015) [22]. Various plant parts, especially the tubers, roots, seeds and leaves, have traditionally been employed for treating several ailments (Peele *et al.*, 2022; Kumar and Sagar, 2015; Padmapriya *et al.*, 2015) [18, 22, 23]. The tubers are

traditionally used for the treatment of gout, arthritis, rheumatism, muscular and joint pain, inflammation,



Fig 1: *Gloriosa superba* in its habitat near the bank of Mahanadi River, Cuttack, Odisha

wounds, skin disorders, intestinal worms and certain gastrointestinal complaints (Sharma *et al.*, 2026) [26]. Tubers have also been used traditionally in preparations associated with snakebite and other inflammatory disorders (Boddu *et al.*, 2024; Peele *et al.*, 2022; Joshi *et al.*, 2024; Sharma *et al.*, 2026) [5, 15, 23, 26]. In some traditional systems, preparations of the plant have been used for reproductive disorders, including as an abortifacient and to induce labour (Peele *et al.*, 2022; Padmapriya *et al.*, 2015) [22, 23]. Leaves and other aerial parts have also been used traditionally for certain skin ailments, wounds and inflammatory conditions, while the seeds have considerable medicinal and pharmaceutical importance because of their high alkaloid content (Kumar and Sagar, 2015; Jasmine *et al.*, 2020b) [11, 18]. The present work aims to collect the *G. superba* from the Mahanadi River areas of Cuttack, Odisha and evaluate the qualitative phytochemical and quantitative screening of primary and secondary metabolites in addition to access the quantitative secondary metabolites and evaluate the antioxidant activity.

Methodology

Authors have collected the plant material of the study plant from the banks of Mahanadi River basin of Cuttack, Odisha. Photographs of the plants was also taken during the collection of the study plant. Morphology of the plant was recorded and compared with the regional flora books for identification. It has a long history and literature on the traditional therapeutic uses (Jena *et al.*, 2025) [14]. The collected plant materials (whole plant) were cleaned and cut into small pieces and shade dried before course grounded. The qualitative phytochemical analysis was screened for the primary (proteins, carbohydrates, starch, sucrose, lipids and amino acids) and secondary metabolites (tannins, saponins, flavonoids, terpenoids, phenolics, reducing sugars, steroids and alkaloids). Further quantitative screening of secondary metabolites (Tannin, saponin, phenol and flavonoid) was estimated. The bioactivity for antioxidant activity was assessed quantitatively to correlate the phytochemical present in the study plant.

Qualitative phytochemical analysis

For qualitative phytochemical analysis, standard colour and precipitation tests were employed to detect primary

metabolites (proteins, carbohydrates, starch, sucrose, lipids and amino acids) and secondary metabolites (tannins, saponins, flavonoids, terpenoids, phenolics, reducing sugars, steroids and alkaloids). The presence of individual metabolites was confirmed based on characteristic colour changes, precipitate formation or froth production (Kumar *et al.*, 2013; Devi *et al.*, 2022; Marndi *et al.*, 2024; Jena *et al.*, 2024) [8, 13, 17, 20].

Quantitative phytochemical analysis

The quantitative phytochemical estimation was carried out following standard procedures:

Estimation of total phenol

Total phenolic content was estimated following Swain and Hillis (1959) [29]. 0.5 g of plant material was extracted with 60% methanol and centrifuged at 5000 rpm for 20 min. The extract was treated with 0.1 N HCl, sodium nitrite-molybdate reagent and 1 N NaOH, followed by incubation in the dark for 20 min. Absorbance was measured at 515 nm, and phenolic content was calculated from the gallic acid standard curve.

Estimation of Total Tannin

Tannin content was estimated according to Sadasivam and Manickam (2009) [25]. 5mg of plant material were boiled with distilled water for 30 min and centrifuged at 2000 rpm for 20 min. The supernatant was reacted with Folin reagent and 20% sodium carbonate and incubated in the dark. Absorbance was recorded at 720 nm, and tannin content was determined using a tannic acid standard curve.

Estimation of Total Saponin

Total saponin was estimated following Obadoni and Ochuko (2001) [21]. 5 gm of plant material were extracted with aqueous ethanol at 45°C for 4 h, filtered and concentrated. The extract was partitioned with diethyl ether and n-butanol, and the n-butanol fraction was washed with 5% sodium chloride. The fraction was evaporated and dried at 40°C to constant weight. Saponin content was expressed as a percentage of the initial sample weight.

% of Saponin = (final weight of sample/initial weight of extracts) x 100

Estimation of total flavonoid

Total flavonoid content was determined by the aluminium chloride colorimetric method (Chang *et al.*, 2002; Shirazi *et al.*, 2014; Raina and Misra, 2023) [7, 24, 28]. 100 µL of extract was mixed with sodium nitrite, followed by aluminium chloride and sodium hydroxide, and incubated in the dark for 15 min. Absorbance was measured at 510 nm, and flavonoid content was calculated from a quercetin standard curve.

Quantitative antioxidant activity through dpph assay radical-scavenging activity

For antioxidant evaluation, dried plant material was extracted by maceration using selected solvents for 48 h, followed by filtration, concentration and preparation of working extracts in 1% DMSO. DPPH radical-scavenging activity was assessed using a 0.1 mM DPPH solution. Extracts at different concentrations were mixed with DPPH in a 1:1 ratio and incubated in the dark at room temperature for 10 min. Absorbance was measured at 517 nm, and the

percentage radical-scavenging activity was calculated. Quercetin was used as the reference standard, and inhibition percentage was plotted against extract concentration to compare antioxidant activity (Abubakar and Haque 2020; Baliyan *et al.*, 2022; Majhi *et al.*, 2026) ^[1, 4, 19].

Inhibition (%) = [(Control absorbance – Sample absorbance) / Control absorbance] × 100

Results and Discussion

Morphology

Gloriosa superba is a glabrous, herbaceous, deciduous perennial climber, generally growing to about 2-3 m or more depending on environmental conditions. The plant develops from a characteristic fleshy, subterranean, tuberous rootstock, which is usually bifid or V-shaped and serves as the principal underground storage and perennating structure (Figure 1) (Kumar and Sagar, 2015; Peele *et al.*, 2022; Kumar *et al.*, 2026) ^[16, 18, 23]. The stem is slender, green, soft and climbing, and may branch depending upon the growing conditions. The leaves are simple, entire, green and lanceolate to ovate-lanceolate, with a distinctive tendril-like modification at the apex. This leaf-tip tendril enables the plant to attach itself to surrounding vegetation and climb upwards (Padmapriya *et al.*, 2015; Kumar *et al.*, 2026) ^[16, 22]. The flowers are the most conspicuous feature of the plant. They are generally solitary and borne on long pedicels. Each flower possesses six narrow tepals, which are typically yellow, orange, scarlet or crimson and become strongly reflexed at maturity. The margins are characteristically wavy or undulate, giving the flower its flame-like appearance and providing the basis for the common name "flame lily" (Peele *et al.*, 2022; Kumar *et al.*, 2026; Sharma *et al.*, 2026) ^[16, 18, 23, 26].



Fig 2: Informant on the ethnobotanical uses of *Gloriosa superba*

Ethnobotany

The local communities near the Mahanadi River bank of Cuttack informed that the tubers of *G. superba* is applied to get rid of arthritis and joint pain (Figure 2).

Qualitative analysis of primary metabolites

The qualitative phytochemical screening of the whole plant of *G. superba* revealed a differential distribution of primary metabolites among the different solvent extracts (Table 1).

Protein was detected in both aqueous and ethanol extracts. Carbohydrates showed strong presence in the aqueous extract and moderate positivity in the ethanol extract. Starch was detected only in the aqueous extract. The predominance of proteins and carbohydrates in the aqueous fraction can be attributed to the relatively polar nature of these primary metabolites. The absence of these constituents in n-hexane is consistent with the non-polar character of the solvent, which is more suitable for extracting lipophilic compounds rather than polar macromolecules. The absence of detectable lipids in all three extracts may indicate either their low concentration in the tested material or concentrations below the detection limits of the qualitative assays.

Table 1: Qualitative analysis of primary metabolites of *Gloriosa superba*

Solvent Extracts	Primary metabolites detected
Aqueous Extract	Protein, carbohydrate and starch
Ethanol Extract	Protein and carbohydrate
n-Hexane	NIL

Qualitative analysis of secondary metabolites

The screening of secondary metabolites demonstrated a more pronounced phytochemical diversity in the aqueous and ethanol extracts (Table 2). Tannins were detected in both aqueous and ethanol extracts. Saponins were detected only in the aqueous extract. Terpenoids, phenolic compounds, reducing sugars and steroids were detected in both aqueous and ethanol extracts. Alkaloids were detected only in the aqueous extract, while they were absent from ethanol and n-hexane extracts. The absence of detectable secondary metabolites in the n-hexane extract indicate that the compounds investigated by these qualitative assays were predominantly polar or moderately polar constituents.

Table 2: Quantitative analysis of secondary metabolites of *Gloriosa superba*

Solvent Extracts	Secondary metabolites detected
Aqueous Extract	Tannin, saponin, terpenoid, phenolic compound, reducing sugar, steroid and alkaloids.
Ethanol Extract	Tannin, terpenoids, phenolic compound, reducing sugar, steroid and alkaloids.
n-Hexane	NIL

Quantitative phytochemical composition

Quantitative estimation further confirmed the presence of several important secondary metabolites in the whole plant of *G. superba*. Tannin was the most abundant quantified constituent, with a concentration of 56.87 mg/100 ml, followed by phenol at 4.96 mg/100 ml. The saponin content was 10.24%, whereas the flavonoid concentration was comparatively low at 0.10 mg/ml (Table 3).

Table 3: Quantitative phytochemical analysis of *G. superba*

Secondary metabolites	Availability
Tannin	56.87 mg /100 mL
Saponin	10.24%
Phenol	4.96 mg /100 mL
Flavonoids	0.1 mg / mL

The relatively high tannin content indicates that tannin-rich phenolic compounds constitute an important component of the phytochemical profile of the investigated plant material.

The substantial tannin content may contribute to some of the biological properties including antioxidant and antimicrobial activities. Tannins are polyphenolic compounds capable of interacting with proteins and microbial cell components and may therefore contribute to antimicrobial effect (Fabbrini *et al.*, 2022) ^[10]. Likewise, the phenolic content of 4.96 mg/100 ml may contribute to the antioxidant potential of the plant through hydrogen- or electron-donating mechanisms (Camarda *et al.*, 2026; Ernawati *et al.*, 2022) ^[6, 9]. The saponin content of 10.24% was estimated. Saponins are amphiphilic compounds that possess a wide range of biological activities, including antimicrobial, antioxidant and membrane-interacting properties. Their detection exclusively in the aqueous extract in the qualitative analysis is consistent with their generally greater affinity for polar extraction systems. The comparatively low flavonoid concentration (0.10 mg/ml) agrees with the weak or non-detectable response obtained in the qualitative flavonoid assay. Previous studies have demonstrated considerable variation in the phytochemical profile and colchicine concentration of *G. superba* among different accessions and ecotypes (Jasmine *et al.*, 2020a; Jasmine *et al.*, 2020b; Sharma and Mishra, 2024) ^[11, 12, 27].

Quantitative antioxidant activity through dpph assay radical-scavenging activity

The DPPH radical-scavenging assay was evaluated for the whole-plant extracts of *Gloriosa superba* and demonstrated that percentage inhibition increased progressively with increasing extract concentration.

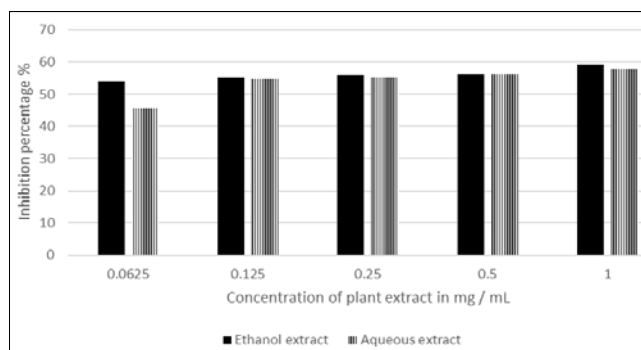


Fig 3: DPPH Assay Radical-Scavenging Activity of ethanol and aqueous extracts of *G. superba*

The ethanol extract exhibited highest inhibition ranging from 54.09% to 59.03%, whereas the aqueous extract showed comparatively lower activity, increasing from 45.60% to 57.65% over the same concentration range (Figure 3). The comparatively higher activity of the all-tested concentrations may indicate the presence of non-polar or moderately lipophilic antioxidant constituents capable of donating hydrogen atoms or electrons to neutralize DPPH radicals.

The observed antioxidant potential of *G. superba* may be associated with its diverse phytochemical composition. Phenolic compounds, flavonoids, tannins and other secondary metabolites are widely recognized for their ability to scavenge free radicals through hydrogen donation and electron-transfer mechanisms. Earlier research activities revealed the presence of diverse range of phytochemicals including alkaloids, phenolics, flavonoids, terpenoids, tannins, saponins, steroids, glycosides and other secondary

metabolites (Kumar and Sagar, 2015; Jasmine *et al.*, 2020a; Ahire, 2020 ^[2]; Sharma and Mishra, 2024; Balamurugan and Sivakumar, 2026) ^[3, 18]. The most important phytochemical group is the tropolone alkaloids, among which colchicine is the principal bioactive compound. Other reported alkaloids and colchicine-related compounds include 3-demethylcolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, colchicoside, gloriosine, lumicolchicine, N-formyl-deacetylcolchicine and superbine (Peele *et al.*, 2022; Joshi *et al.*, 2024; Sharma *et al.*, 2026) ^[15, 23, 26]. Therefore, the antioxidant activity observed in the present study may reflect the combined contribution of different phytoconstituents present in the whole-plant extracts. However, the toxicological profile of *G. superba* is dominated by colchicine and related alkaloids. (Kumar and Sagar 2015; Jasmine *et al.*, 2020a) ^[11, 12]. Its medicinal use requires strict dose control and appropriate processing, as excessive exposure to colchicine can result in serious or potentially fatal poisoning (Padmapriya *et al.*, 2015; Peele *et al.*, 2022) ^[22, 23].

Conclusion

Gloriosa superba is a medicinally important but also highly toxic plant possessing a diverse array of bioactive phytoconstituents. The phytochemical profile indicates the presence of several primary and secondary metabolites, including phenolics, flavonoids, tannins, saponins, terpenoids, steroids and alkaloids, which may contribute to its biological activities. However, the therapeutic potential of the species must be considered alongside its significant toxicity, particularly due to colchicine and related alkaloids. Therefore, *G. superba* represents a promising source of pharmacologically active compounds, but further studies on compound isolation, toxicity evaluation, dose standardization and mechanism of action are essential for its safe and effective utilization.

Conflict of interest

Authors confirm that there is no conflict of interest.

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