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Phytochemical profile and pharmacological activities of *Globba racemosa* Sm

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Abstract
Globba racemosa Sm. is an underexplored terrestrial herb belonging to the family Zingiberaceae that occurs mostly in moist and shaded habitats. Several *Globba* spp. has been reported for their therapeutic potential and associated bioactive compounds. However, studies related to *Globba racemosa* remains limited. The present study thus evaluated the qualitative and quantitative phytochemical composition and DPPH radical-scavenging activity of leaf extracts of *G. racemosa* prepared using aqueous, ethanol and n-hexane solvents. Qualitative phytochemical of primary metabolites revealed the presence of proteins and carbohydrates and secondary metabolites such as tannins, saponins, phenols and reducing sugars were detected in aqueous extracts. Quantitative analysis revealed tannin (33.73 mg/100 ml), saponin (10.40%), phenol (3.16 mg/100 ml) and flavonoid (0.61 mg/ml) contents in the leaves. The DPPH assay demonstrated highest inhibition (61.16%), followed by the aqueous extract (59.77%) at 1.0 mg/ml concentration. *G. racemosa* leaves contain several biologically important phytoconstituents and exhibit appreciable antioxidant activity, particularly in the ethanol extract. The study suggest that *G. racemosa* may have potential as a source of natural antioxidant compounds and warrant further investigation for the isolation and characterization of its bioactive constituents.

Keywords: DPPH assay, morphology, phenol, tannin, Zingiberaceae

Introduction
The genus *Globba* L. belonging to the family Zingiberaceae is a small terrestrial herb distributed mainly across South and Southeast Asia, including India, Nepal, Bhutan, Bangladesh, Sri Lanka, China and Southeast Asia. The genus shows considerable morphological diversity, particularly in floral characters, inflorescence structure, anther appendages which are important for species identification and classification (Yadav and Gowda 2024; Maity *et al*., 2025) [10, 23]. Recent taxonomic work has considerably improved the understanding of Indian *Globba*. Several *Globba* species also have considerable ethnobotanical and medicinal importance. Aslam and Ahmad (2017) [2] documented the traditional uses of *Globba* species for ailments including cough, asthma, stomach disorders, fever, pain, conjunctivitis, mouth ulcers, rheumatism and postpartum-related conditions. Among the medicinally important species, *Globba marantina* is regarded as an extra-pharmacopoeial medicinal plant and is traditionally used for conditions such as cough, cold, asthma, rheumatism, conjunctivitis and snakebite; its roots and leaves have also been documented in traditional medicine in Odisha. Phytochemical investigations further support the medicinal significance of the genus. Earlier research has reported carbohydrates, amino acids, steroids, alkaloids, flavonoids, cardiac glycosides, saponins, tannins, terpenoids, fatty acids, coumarins and phenolic compounds in tuber extracts of *G. bulbifera*, with the ethanolic extract showing a comparatively broad range of phytoconstituents (Rao and Kaladhar 2014) [18]. Phytochemical databases also report several compounds from the rhizome of *G. marantina*, including β-bisabolene, safrole, p-cymene, borneol and geranyl acetate. *Globba racemosa* is a small, perennial, rhizomatous herb that occurs in moist and shaded habitats of tropical and subtropical regions and forms part of the rich herbaceous flora of the Indian subcontinent (Mohanty *et al*., 2025; Shen *et al*., 2026) [14, 20]. However, species-specific information on the phytochemistry and biological activities of *G. racemosa* remains comparatively limited. The present study aims to document the morphology, habitat, qualitative phytochemical profiling of primary and secondary metabolites and quantitative phytochemical profiling of secondary metabolites of leaves extracts of *Globba racemosa.*

Fig 1: *Globba racemosa* a) in its habitat b) flower morphology

Methodology
During the floristic survey, the team documented the habitat and natural occurrence of *Globba racemosa* in the Rourkela

Forest Division, Odisha. Field photographs were taken, and plant specimens were collected for taxonomic identification using standard regional floras. Leaves were also collected for phytochemical profiling and antioxidant activity analysis (Gunjal *et al*., 2025; Kumar 2025) [6, 14].

Qualitative analysis for primary metabolites (Kumar *et al*., 2013; Marndi *et al*., 2024) [8, 12]

Plant extraction was carried out using the Soxhlet extraction method with different solvents of varying polarity.

Test for Protein: 1 ml of plant extract was mixed with 2–5 drops of Millon's reagent and heated in a water bath for 30 min. A pink colour indicated the presence of protein.

Test for Carbohydrate: 1 ml of extract was mixed with 2–5 drops each of Fehling's solutions A and B and heated in a water bath for 30 min. An orange-red precipitate indicated the presence of carbohydrates.

Test for Starch: 1 ml of extract was treated with 2-3 drops of concentrated HCl and heated for 1-2 min. NaOH and Benedict's reagent were then added and the mixture was heated in a water bath. A red-orange colour indicated the presence of starch.

Test for Sucrose: 1 ml of extract was mixed with 2-3 drops of iodine solution. A blue-black colour indicated the presence of sucrose.

Test for Lipid: 1 ml of extract was mixed with 25 drops of Sudan III reagent. The appearance of pink droplets indicated the presence of lipids.

Test for Amino Acid: 1 ml of extract was mixed with 5 drops of ninhydrin reagent and heated in a water bath for 30 min. Development of a purple colour (Ruhemann's purple) indicated the presence of amino acids.

Qualitative analysis for secondary metabolites (Devi *et al*. 2022; Jena *et al*. 2024 and Marndi *et al*. 2024) [5, 7]

Plant extraction was carried out using the Soxhlet extraction method with different solvents of varying polarity.

Test for Tannin: 1 ml of leaf extract was treated with 3–5 drops of 10% lead acetate. Formation of a gelatinous precipitate indicated the presence of tannins.

Test for Saponin: 1 ml of extract was mixed with 1 ml distilled water and shaken vigorously. Persistent froth indicated the presence of saponins.

Test for Flavonoids: 1 ml of extract was treated with 2 ml of 2% NaOH followed by 3–4 drops of dilute HCl. An intense yellow colour that disappeared after acidification indicated flavonoids.

Test for Terpenoids: 1 ml of extract was mixed with 5–6 drops of chloroform and heated briefly, followed by 5–6 drops of concentrated H_2SO_4. A reddish-brown interface indicated terpenoids.

Test for Phenols: 1 ml of extract was treated with a few drops of 5% ferric chloride. A dark bluish-black colour indicated phenolic compounds.

Test for Reducing Sugars: 1 ml of extract was treated with Fehling's solutions A and B and heated in a water bath. Formation of a red-orange precipitate indicated reducing sugars.

Test for Steroids: 1 ml of extract was mixed with 1 ml each of chloroform and concentrated H_2SO_4. The development of red and yellow colour with green fluorescence indicated steroids.

Test for Alkaloids: 1 ml of extract was treated with 3–4 drops of Dragendorff's reagent. Formation of a reddish-brown precipitate indicated alkaloids.

Quantitative analysis for secondary metabolites

Estimation of phenol (Swain and Hills, 1959) [22]

For phenol extraction, 0.5 g of plant material was taken in triplicate, crushed with 60% methanol using a mortar and pestle, and centrifuged at 5000 rpm for 20 min. For estimation, 0.1 and 0.2 ml of each extract were diluted to 1 ml with 60% methanol, followed by the addition of 1 ml of 0.1 N HCl and 1 ml of sodium nitrite–molybdate mixture. After incubation for a few minutes, 5 ml distilled water and 2 ml of 1 N NaOH were added, and the mixtures were kept in the dark for 20 min. Absorbance was measured at 515 nm, and the phenol content was determined from the standard calibration curve.

Estimation of tannin (Sadasivam and Manickam, 2009) [19]

For tannin estimation, a tannic acid standard solution was prepared, and different concentrations were reacted with Folin reagent and 20% sodium carbonate, followed by incubation in the dark for 40 min. Absorbance was measured at 720 nm to prepare the standard curve. For sample extraction, 5 mg of plant material was boiled with 75 ml distilled water for 30 min and centrifuged at 2000 rpm for 20 min. The supernatant was collected, diluted to 100 ml and 1 ml of the extract was treated with Folin reagent and 20% sodium carbonate. After shaking and incubation, absorbance was recorded at 720 nm, and tannin content was calculated from the standard curve.

Estimation of phenol (Sadasivam and Manickam, 2009) [19]

For phenol estimation, three replicate samples (0.5 g each) were separately ground with 60% methanol using a mortar and pestle. The extracts were centrifuged at 5000 rpm for 20 min and the process was repeated five times. Aliquots of 0.1 and 0.2 ml of each extract were taken in separate test tubes, with a blank included, and the volume was adjusted to 1 ml with 60% methanol. Then, 1 ml of 0.1 N HCl and 1 ml of sodium nitrite-molybdate reagent were added sequentially, allowing a few minutes between additions. The mixtures were diluted with 5 ml distilled water, followed by 2 ml of 1 N NaOH, and incubated in the dark for 20 min. Absorbance was measured at 515 nm, and the total phenol content was calculated using a gallic acid standard calibration curve.

Estimation of saponin

For total saponin estimation, 5 g of plant material was extracted with 100 ml of 20% aqueous ethanol by stirring for 30 min and heating at 45°C for 4 h. The mixture was filtered, and the residue was re-extracted with 100 ml of 25% aqueous ethanol. The combined extracts were concentrated and partitioned twice with 20 ml diethyl ether, discarding the ether layer. The aqueous fraction was then extracted with 30 ml n-butanol and washed twice with 10 ml of 5% sodium chloride solution. The n-butanol fraction was evaporated and dried at 40°C to constant weight and the saponin content was calculated (Shirazi *et al*., 2014) [21].

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

Estimation of Flavonoid

For flavonoid content estimation, 100 µl of aqueous plant extract was mixed with 100 µl of 5% sodium nitrite and allowed to stand for 5 min. Then, 150 µl of aluminium chloride was added and incubated for 6 min, followed by the addition of 200 µl of 1 M sodium hydroxide. The volume was made up to 3 ml with distilled water and incubated in the dark at room temperature for 15 min. Absorbance was measured at 510 nm, and the flavonoid content was calculated from the quercetin standard curve. (Chang *et al*., 2002; Shirazi *et al*., 2014; Raina and Misra, 2023) [4, 16, 21].

Antioxidant Activity

Antioxidant activity was evaluated using DPPH free radical scavenging assay. For plant extraction, 1 g of finely powdered plant material was macerated with 10 ml of distilled water or ethanol for 48 h with occasional stirring. The extracts were filtered, concentrated in a hot-air oven, and the crude extracts were dissolved in 1% DMSO to prepare working solutions. For the DPPH assay, a 0.1 mM DPPH solution was prepared in methanol and stored in the dark. Different concentrations of the extracts were mixed with DPPH solution in a 1:1 ratio, while appropriate sample blanks and controls were maintained. The reaction mixtures were incubated in the dark at room temperature for 10 min, and absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The percentage of DPPH radical-scavenging activity was calculated, and inhibition percentage was plotted against extract concentration. Quercetin was used as the standard antioxidant (Abubakar and Haque 2020 [1]; Baliyan *et al*., 2022 [3]; Majhi *et al*., 2026) [11].

% Inhibition = {Absorbance (control) – Absorbance (sample) / Absorbance (control)} x 100

Results and discussion

Morphology and habitat

It is an erect or inclined herb belonging to the family Zingiberaceae with oblong, elliptic-oblong or ovate-oblong leaves and acuminate long apex. Leaves are glabrous above and softly pubescent beneath. Panicle narrow and sometimes pubescent. Flowers are yellow or yellow-orange including stamen. Corolla tube 2-3 times the length of calyx and puberulous. Petals broadly ovate, longer than or equal to the staminodes, median hooded and shortly horned. Lip obovate, equal to or longer than the reflexed petals, bifid or 2-lobed with lanceolate lobes, the two halves reduplicate and cleft about one-fourth way down. Ovary smooth or verrucose. Capsule smooth or verrucose. Seeds tomentose (Figure 1). It is usually found in moist, shaded and humid habitats, particularly in tropical and subtropical regions. It typically grows along forest margins, understorey vegetation, stream banks, moist valleys and damp rocky slopes.

Phytochemical analysis

Primary metabolites are essential biomolecules required for the normal growth, development, and physiological functioning of plants. They include proteins, carbohydrates, lipids, amino acids, starch and sucrose, which are directly involved in fundamental metabolic processes. The qualitative phytochemical analysis of *Globba racemosa* of both the aqueous and ethanol extracts exhibited strong presence of proteins. In contrast, the n-hexane extract showed no detectable proteins, which is expected because proteins are generally insoluble in non-polar solvents. Carbohydrates were also detected in both the aqueous and ethanol extracts but were absent in the *n*-hexane extract. This finding is consistent with the polar nature of carbohydrates, which are readily extracted by water and ethanol but not by non-polar solvents such as *n*-hexane (Table 1). No detectable levels of starch, sucrose, lipids or free amino acids were observed in any of the three extracts. The absence of starch and sucrose may indicate either their negligible concentration in the leaves or their hydrolysis into simpler sugars during sample processing. Similarly, the negative results for lipids of the leaves extracts may be due to the presence of only trace amounts of extractable lipids under the conditions employed, while free amino acids were below the detectable limits of the qualitative assays. The results demonstrate that aqueous and ethanol extracts are more efficient than *n*-hexane in extracting polar primary metabolites from *Globba racemosa* leaves.

Table 1: Qualitative phytochemical analysis of leaves extract of *G. racemose*

Solvent Uses	Primary and secondary metabolites detected
Aqueous Extract	Protein, Carbohydrate, Tannin, Saponin, Phenol and Reducing sugar
Ethanol Extract	Protein, Carbohydrate, Tannin, Saponin, Phenol and Reducing sugar
h-Hexane	Not detected

Table 2: Quantitative Phytochemical Analysis of leaves extract of *G. racemosa*

Secondary metabolites	Quantity
Tannin	33.73 mg /100ml
Saponin	10.40 %
Phenol	3.16 mg /100ml
Flavonoids	0.61mg/ml

Qualitative phytochemical analysis of secondary metabolites is a fundamental step in evaluating the medicinal potential of plants. It enables the preliminary identification of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenolics, and steroids, which are known to possess diverse pharmacological activities. The qualitative phytochemical analysis of the secondary metabolites in the leaves of *G. racemosa* revealed that tannins showed strong presence of both aqueous and ethanol extracts. Saponins and reducing sugars were detected in both aqueous and ethanol extracts, while flavonoids were detected only in the aqueous extract. Terpenoids, phenolic groups, steroids, and alkaloids were absent in all three extracts. The absence of secondary metabolites in the *n*-hexane extract indicates that the bioactive constituents of *G. racemosa* leaves are predominantly polar and are more efficiently extracted using aqueous and ethanol solvents. Unlike qualitative screening, which only indicates the presence or absence of phytochemicals, quantitative estimation measures the exact amount of bioactive compounds (Table 2). The concentration of these metabolites is closely associated with the biological activities of plants, including antioxidant, antimicrobial, anti-inflammatory, anticancer and antidiabetic properties. The quantitative phytochemical analysis of *G. racemosa* leaves revealed considerable variation in the concentration

of secondary metabolites. Tannins were the most abundant phytochemical, with a concentration of 33.73 mg/100 ml, followed by saponins (10.40%), phenols (3.16 mg/100 ml) and flavonoids (0.61 mg/ml). The high tannin content may be due to the presence of rich source of polyphenolic compounds, which are known for their antioxidant, antimicrobial and astringent properties. The appreciable level of saponins indicates potential anti-inflammatory, immunomodulatory, and antimicrobial activities (Swain and Hillis 1959) [22]. Although present in comparatively lower amounts, phenols and flavonoids contribute significantly to the plant's antioxidant capacity by scavenging free radicals and protecting cells against oxidative stress (Shirazi *et al*., 2014) [21].

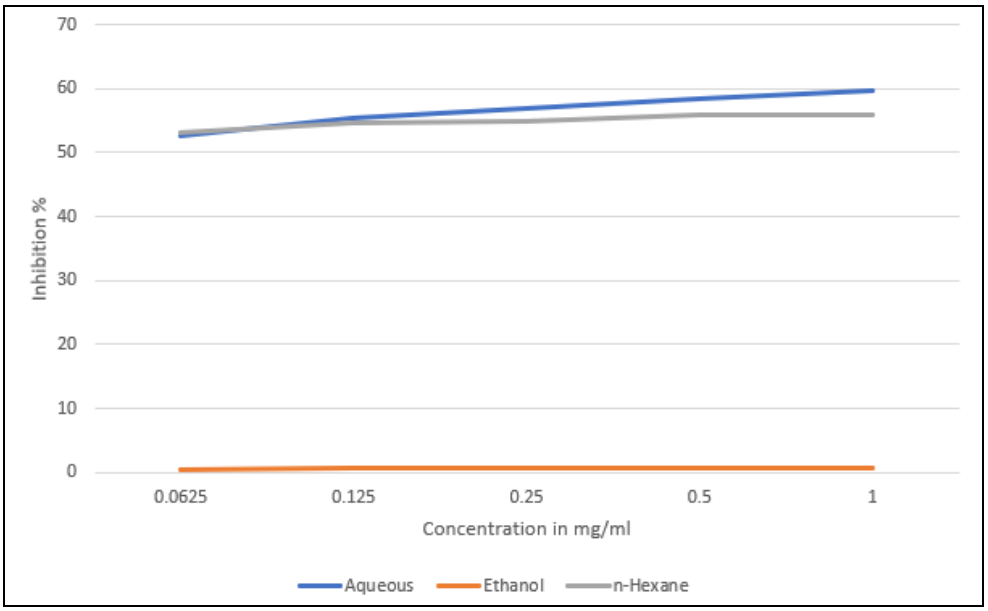

Fig 2: DPPH assay for evaluating the antioxidant activity of leaves extracts of *G. racemosa*

Antioxidant activity

The antioxidant activity of *Globba racemosa* leaf extracts was evaluated using the DPPH free radical scavenging assay. All three extracts exhibited antioxidant activity, with the percentage inhibition increasing as the extract concentration increased from 0.0625 to 1.0 mg/ml. Among the tested extracts, the ethanolic extract demonstrated the highest antioxidant activity, with DPPH inhibition increasing from 57.32% at 0.0625 mg/ml to 61.16% at 1.0 mg/ml. The aqueous extract showed moderate antioxidant activity, increasing from 52.53% to 59.77%, whereas the *n*-hexane extract exhibited the lowest scavenging activity, ranging from 53.03% to 55.98% (Figure 2). The superior antioxidant activity of the ethanolic extract may be attributed to its higher extraction efficiency of bioactive polyphenolic compounds, particularly tannins and phenolics, which are effective hydrogen or electron donors capable of neutralizing DPPH free radicals. Although the aqueous extract also showed appreciable antioxidant activity, its scavenging capacity was slightly lower than that of the ethanolic extract. Further, the relatively weaker activity of the *n*-hexane extract demonstrates that non-polar solvents extracted fewer antioxidant constituents.

Conclusion

The present study explores the pharmacological potential of *G. racemosa* that contain a range of important primary and secondary metabolites, particularly tannins, saponins, flavonoids, reducing sugars, proteins and carbohydrates. *G. racemosa* leaves possess promising phytochemical and antioxidant potential and may serve as a useful source of natural bioactive compounds. Further studies are recommended to isolate and characterize the compounds responsible for the observed antioxidant activity and to validate their pharmacological potential.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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