

Phytochemical screening and antioxidant activity of *Gardenia gummifera* fruits

Rani Runam Kumari¹, Rajkumari Supriya Devi², Sanjeet Kumar^{2*}

¹ Department of Botany, Bhupendra Narayan Mandal University, Madhepura, Bihar, India

² Ambika Prasad Research Foundation, Odisha, India

Corresponding Author: Sanjeet Kumar

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Abstract

The present study evaluated the phytochemical composition and antioxidant activity of fruit extracts of *Gardenia gummifera* using aqueous, ethanol and n-hexane as extraction solvents. Qualitative phytochemical screening was carried out to detect primary and secondary metabolites, while quantitative analysis was performed for selected secondary metabolites. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay at concentrations ranging from 0.0625 to 1.0 mg/mL. Qualitative analysis revealed the presence of proteins, carbohydrates and starch in the aqueous and ethanol extracts, whereas sucrose, lipids and amino acids were not detected in any of the tested extracts. Among the secondary metabolites, tannins, flavonoids, phenolic groups and reducing sugars were detected in the aqueous and ethanol extracts, while saponins were detected only in the aqueous extract. Terpenoids, steroids and alkaloids were not detected in the tested extracts. Quantitative analysis showed tannin content of 68.97 mg/100 mL, saponin content of 4.6%, total phenol content of 6.36 mg/100 mL and flavonoid content of 0.57 mg/mL. In the DPPH assay, antioxidant activity increased progressively with increasing extract concentration. At 1.0 mg/mL, inhibition was 63.72% for the n-hexane extract, 61.27% for the ethanol extract and 63.55% for the aqueous extract. The findings indicate that *G. gummifera* fruits contain several biologically relevant phytochemical groups and exhibit appreciable free-radical-scavenging activity. The results provide a preliminary basis for further investigation of the antioxidant potential and phytochemical constituents of this species.

Keywords: DPPH assay, Free radicals, Phenolic compounds, Phytochemical constituents, Radical-scavenging activity

Introduction

Plants constitute an important source of structurally diverse bioactive compounds (Hossain *et al.*, 2026) and have traditionally been used for food (Dash *et al.*, 2026; Kumar, 2026) ^[1, 5], medicine (Devi *et al.*, 2026a; Devi *et al.*, 2026b) ^[1] and other ethnobotanical purposes (Majhi *et al.*, 2026) ^[4]. Phytochemicals produced by plants include primary metabolites such as carbohydrates, proteins and lipids and secondary metabolites such as phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids and steroids (Hossain *et al.*, 2025; Das *et al.*, 2025) ^[3, 11]. These compounds contribute to the biological properties of plants and have attracted considerable attention in pharmacognostic, pharmaceutical and nutritional research (Rani *et al.*, 2025 ^[23]; Das *et al.*, 2024). Antioxidants are compounds capable of inhibiting or delaying oxidative processes by interacting with free radicals and other reactive species (Gulcin, 2025). Excessive formation of reactive oxygen species can result in oxidative damage to cellular macromolecules (Juan *et al.*, 2021) ^[12]. Consequently, the identification of plant-derived antioxidant compounds has become an important area of natural-product research (Maury *et al.*, 2020) ^[20]. Phenolic compounds and flavonoids are particularly important because of their ability to participate in radical-scavenging and other antioxidant mechanisms (Mutha *et al.*, 2021) ^[21]. The genus *Gardenia* belongs to the family Rubiaceae and comprises species of considerable botanical and ethnomedicinal interest (Ouédraogo *et al.*, 2019). *Gardenia gummifera* is a plant of the Indian subcontinent that has received attention because of its traditional uses and phytochemical constituents (Reddy *et al.*, 1977; Kunert *et al.*, 2009 ^[15, 24]; Vinaykumar

et al., 2020; Maind *et al.*, 2025) ^[16, 26]. The present study was undertaken to investigate the primary and secondary phytochemical constituents and antioxidant activity of *G. gummifera* fruits.

Materials and methods

Fruits of *G. gummifera* (Fig 1) were collected from Keonjhar Forest Division, Odisha, India. Plant is identified by the authors using flora books. The fruit material was subjected to Soxhlet extraction using aqueous, ethanol and n-hexane solvents. The resulting extracts were used for qualitative phytochemical screening and antioxidant analysis (Jena *et al.*, 2025) ^[11].

Preparation of extracts: Fruit material was extracted separately using distilled water, ethanol and n-hexane solvents. The respective extracts were subsequently used for phytochemical investigation. The use of solvents with different polarities enabled preliminary assessment of the distribution of phytochemical constituents among polar and non-polar extracts (Das *et al.*, 2026) ^[4].

Qualitative phytochemical analysis: Qualitative phytochemical screening was performed to determine the presence or absence of selected primary and secondary metabolites. The primary metabolites investigated included proteins, carbohydrates, starch, sucrose, lipids and amino acids. Secondary metabolites examined included tannins, saponins, flavonoids, terpenoids, phenolic groups, reducing sugars, steroids and alkaloids. The results were recorded as positive (+) when the corresponding phytochemical was

detected and negative (–) when it was not detected (Marndi *et al.*, 2024) ^[19].



Fig 1: Fruit of *G. gummifera*

Quantitative phytochemical analysis: Selected secondary metabolites were quantitatively estimated. The phytochemicals quantified were tannins, saponins, total phenols and flavonoids. The results were expressed according to the units provided in the experimental dataset (Soni *et al.*, 2026; Acharya *et al.*, 2026) ^[1, 25].

DPPH radical-scavenging assay: The antioxidant activity of aqueous, ethanol and n-hexane fruit extracts was evaluated using the DPPH radical-scavenging assay. Extract concentrations of 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/mL were tested. The percentage inhibition of DPPH radicals was recorded for each concentration. The percentage inhibition was used as an indicator of the free-radical-scavenging capacity of the extracts (Majhi *et al.*, 2026; Kumari *et al.*, 2026) ^[4, 14].

Results and discussion

Qualitative analysis of primary metabolites: The qualitative phytochemical analysis demonstrated that proteins, carbohydrates and starch were present in both aqueous and ethanol extracts but were absent from the n-hexane extract. Sucrose, lipids and amino acids were not detected in any of the three extracts. The presence of carbohydrates and starch in the aqueous and ethanol extracts indicates that these primary metabolites were preferentially extracted by the relatively polar solvents. Their absence from the n-hexane extract is consistent with the lower affinity of non-polar solvents for such polar constituents (Table 1).

Table 1: Qualitative phytochemical analysis of primary metabolites of *G. gummifera* fruits

Primary metabolites	Aqueous extract	Ethanol extract	n-Hexane extract
Protein	+	+	-
Carbohydrates	+	+	-
Starch	+	+	-
Sucrose	-	-	-
Lipid	-	-	-
Amino acid	-	-	-

(+: Detected; -: Not detected)

Qualitative analysis of secondary metabolites: The secondary metabolite screening showed the presence of tannins, flavonoids, phenolic groups and reducing sugars in both aqueous and ethanol extracts. Saponins were detected in the aqueous extract but were not detected in the ethanol or n-hexane extracts. Terpenoids, steroids and alkaloids were not detected in any of the extracts. The n-hexane extract showed negative results for all of the tested secondary metabolites (Table 2).

Table 2: Qualitative phytochemical analysis of secondary metabolites of *G. gummifera* fruits

Secondary metabolites	Aqueous extract	Ethanol extract	n-Hexane extract
Tannin	+	+	-
Saponin	+	-	-
Flavonoid	+	+	-
Terpenoid	-	-	-
Phenolic Group	+	+	-
Reducing sugar	+	+	-
Steroids	-	-	-
Alkaloids	-	-	-

(+: Detected; -: Not detected)

Quantitative phytochemical composition: Quantitative analysis demonstrated the presence of several important secondary metabolites in *G. gummifera* fruits. The tannin content was 68.97 mg/100 mL, while saponin content was 4.6%. The total phenol content was 6.36 mg/100 mL and the flavonoid content was 0.57 mg/mL (Table 3).

Table 3: Quantitative phytochemical analysis of *G. gummifera* fruits

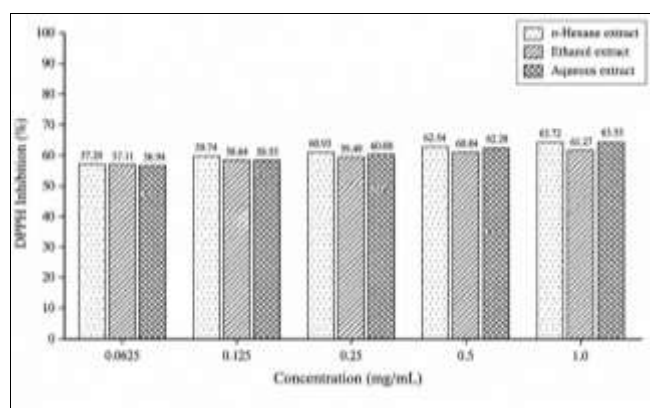
Secondary metabolites	Quantity
Tannin	68.97 mg/100 mL
Saponin	4.6 %
Total phenol	6.36 mg/ 100 mL
Flavonoids	0.57 mg/mL

The quantitative findings support the qualitative detection of tannins, phenolic compounds, flavonoids and saponins. The comparatively notable tannin value suggests that tannin-type compounds constitute an important component of the phytochemical profile of the investigated fruit material.

DPPH radical-scavenging activity: All three extracts demonstrated DPPH radical-scavenging activity, and inhibition increased with increasing concentration. The n-hexane extract showed inhibition values of 57.20, 59.74, 60.93, 62.54 and 63.72% at concentrations of 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/mL, respectively. The ethanol extract showed 57.11, 58.64, 59.49, 60.84 and 61.27% inhibition at the same concentrations. The aqueous extract demonstrated 56.94, 58.55, 60.08, 62.28 and 63.55% inhibition, respectively (Table 4). At the lowest concentration tested, the inhibition ranged from 56.94% to 57.20%, while at the highest concentration it ranged from 61.27% to 63.72%. The highest recorded inhibition was observed for the n-hexane extract at 1.0 mg/mL (63.72%), closely followed by the aqueous extract (63.55%). The ethanol extract showed comparatively lower inhibition at the highest concentration (61.27%).

Table 4: Antioxidant assay (DPPH) of *G. gummifera* fruits

Extract	Concentration (mg/mL)	Inhibition percentage (%)
n-Hexane	0.0625	57.20
	0.125	59.74
	0.25	60.93
	0.5	62.54
	1.0	63.72
Ethanol	0.0625	57.11
	0.125	58.64
	0.25	59.49
	0.5	60.84
	1.0	61.27
Aqueous	0.0625	56.94
	0.125	58.55
	0.25	60.08
	0.5	62.28
	1.0	63.55

**Fig 2:** Antioxidant activity of *G. gummifera*

The phytochemical screening demonstrated that *G. gummifera* fruits contain a range of primary and secondary metabolites. The occurrence of proteins, carbohydrates and starch in the aqueous and ethanol extracts but not in the n-hexane extract indicates that solvent polarity influenced the extraction of these compounds. This observation highlights the importance of selecting suitable extraction solvents when investigating plant phytochemistry. Among the secondary metabolites, tannins, flavonoids and phenolic groups were consistently detected in the aqueous and ethanol extracts. These phytochemical groups are of particular interest in antioxidant investigations because phenolic compounds and flavonoids possess chemical structures capable of interacting with free radicals. The presence of these compounds therefore provides a plausible phytochemical basis for the antioxidant activity observed in the present study. However, the present dataset does not establish a direct quantitative correlation between individual phytochemicals and DPPH inhibition. Saponins were detected only in the aqueous extract. This difference between extracts indicates that the distribution or extractability of individual phytochemical classes varied according to solvent. The absence of terpenoids, steroids and alkaloids in the tested extracts should be interpreted as a result of the qualitative screening procedures employed rather than definitive evidence that these compound classes are completely absent from the fruits. The quantitative analysis showed a relatively high measured tannin content compared with the other reported phytochemical parameters. Phenols and flavonoids were also detected quantitatively, while saponins accounted for 4.6% according

to the reported measurement. Because the units used for the different phytochemicals are not identical, direct numerical comparison among all four compounds should be avoided. The DPPH assay demonstrated measurable antioxidant activity in all three extracts (Fig 2). A progressive increase in inhibition was observed with increasing concentration, indicating a concentration-dependent response within the tested range. The n-hexane extract showed the highest activity at 1.0 mg/mL, with 63.72% inhibition, while the aqueous extract showed a very similar value of 63.55%. The ethanol extract showed 61.27% inhibition at the same concentration. An interesting observation is that the n-hexane extract showed antioxidant activity despite the qualitative screening reporting negative results for the tested secondary metabolites in this extract. This suggests that the measured DPPH activity cannot be attributed solely to the phytochemical classes detected by the qualitative tests. Other constituents, compounds below the detection threshold, or methodological factors may contribute to the observed radical-scavenging activity. Therefore, further chemical characterization is required before attributing the antioxidant response to particular compounds. The relatively close antioxidant responses of the aqueous and n-hexane extracts at 1.0 mg/mL are also noteworthy. The aqueous extract increased from 56.94% inhibition at 0.0625 mg/mL to 63.55% at 1.0 mg/mL, whereas the n-hexane extract increased from 57.20% to 63.72%. The ethanol extract exhibited a comparatively smaller increase over the tested concentration range.

Conclusion

The present investigation demonstrates that *Gardenia gummifera* fruits contain several primary and secondary phytochemical constituents and exhibit measurable DPPH radical-scavenging activity. Proteins, carbohydrates and starch were detected in aqueous and ethanol extracts, whereas tannins, flavonoids, phenolic groups and reducing sugars were detected among the major secondary metabolites. Saponins were detected only in the aqueous extract. Quantitative analysis recorded 68.97 mg/100 mL tannins, 4.6% saponins, 6.36 mg/100 mL phenols and 0.57 mg/mL flavonoids. All three extracts demonstrated concentration-dependent DPPH inhibition. The maximum inhibition recorded was 63.72% for the n-hexane extract, followed closely by 63.55% for the aqueous extract and 61.27% for the ethanol extract at 1.0 mg/mL. These findings suggest that the fruits of *G. gummifera* possess antioxidant potential and warrant further investigation. Future studies should include detailed chromatographic and spectroscopic characterization of the extracts, determination of total phenolic and flavonoid contents using standardized methods, evaluation of additional antioxidant assays, inclusion of appropriate positive controls, replicate measurements and statistical analysis. Such studies would help establish the compounds responsible for the antioxidant activity and clarify the potential pharmaceutical, nutraceutical and functional-food applications of *G. gummifera* fruits.

References

- Acharya S, Chouhan S, Devi RS, Kumar S. *Phytochemical profile* and pharmacological activities of *Globba racemosa* Sm. International Journal of Agriculture and Plant Science. 2026;8(3):408-412.

2. Das A, Das K, Mishra S, Kumar S. Medicinal Poaceae of India, Volume 1. Odisha: Ambika Prasad Research Foundation; 2026.
3. Das A, Gupta S, Mishra S, Kumar S. Diversity, ethnopharmacological potential and ecological significance of *Drosera* species: a carnivorous genus of India. *Indian Forester*. 2025;151(9):847-850.
4. Das K, Manjula S, Kumar NA, Sharma BP, Agrahari D, Majhi S, *et al.* Experimental methodology for antimicrobial screening of medicinal plants: a critical review of extraction, phytochemical characterisation and susceptibility testing approaches. *Asian Journal of Microbiology and Biotechnology*. 2026;11(2):18-131.
5. Dash SS, Kumar S, Mahalik G. Ethnobotanical insights: phytochemical richness, nutritional potential, and bioactivity evaluation of *Bambusa arundinacea* and *Bambusa nutans*. *International Journal of Advancement in Life Sciences Research*. 2026;9(2):110-133.
6. Devi RS, Jethy S, Kumar S. Phytochemical profiling and antibacterial assessment of *Mappia nimmoniana* (J. Graham) Byng & Stull. *e-planet*. 2026;24(1):63-71.
7. Devi RS, Satapathy KB, Kumar S. *Wetland flora* of Loktak Lake, Manipur. India: Ambika Prasad Research Foundation; 2026.
8. G. Antioxidants: a comprehensive review. *Archives of Toxicology*. 2025;99(5):1893-1997. doi: 10.1007/s00204-025-03997-2
9. Hossain E, Marndi S, Kumar R, Kumar S. Banyan family-medicinal, pharmacological and economical aspects. India: Ambika Prasad Research Foundation; 2026.
10. Hossain E, Sharma BP, Kumar S. *Ethnobotany of India, Volume 3*. India: Ambika Prasad Research Foundation; 2026.
11. Jena N, Vimala VC, Singh B, Patra A, Sharma BP, Hossain E, *et al.* Methods for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for pharmacological evaluation of medicinal plants. *Journal of Biodiversity and Conservation*. 2025;9(2):87-107.
12. Juan CA, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences*. 2021;22(9):4642. doi: 10.3390/ijms22094642
13. Kumar S. *Biological resources of North-East India, Volume 1*. India: Ambika Prasad Research Foundation; 2026.
14. Kumari S, Chouhan S, Tripathi G, Devi RS, Tudu S, Kumar S, *et al.* Ethnopharmacological evaluation of *Dendrocalamus strictus* (Roxb.) Nees. *International Journal of Geography, Geology and Environment*. 2026;8(9):35-41.
15. Kunert O, Sreekanth G, Babu GS, Rao BV, Radhakishan M, Kumar BR, *et al.* Cycloartane triterpenes from dikamali, the gum resin of *Gardenia gummifera* and *Gardenia lucida*. *Chemistry & Biodiversity*. 2009;6(8):1185-1192.
16. Maind R, Halder S, Bhat AR, Bhattacharya D, Mujahid MH, Abu-Rayyan A, *et al.* Biomimetic green synthesis of ZnO nanoparticles using *Cheilocostus speciosus* and *Gardenia gummifera* with comprehensive characterization and bioactivity assessment. *Scientific Reports*. 2025;15(1):44323. doi: 10.1038/s41598-025-30720-z
17. Majhi S, Pradhan MK, Marndi S, Rout S, Jethy S. *Gliricidia sepium* (Jacq.) Kunth (Fabaceae): antioxidant potential of the flowers of a shade tree. *Journal of Biodiversity and Conservation*. 2026;10(1):117-124.
18. Majhi S, Samantray PK, Kumar S. Seed oil bearing plants of Odisha state, their ecological, medicinal and economic values. *Indian Forester*. 2026;152(3):268-274.
19. Marndi S, Barik M, Kumar S. Phytochemical and cytotoxicity analysis of *Eulophia explanata* Lindl. (Tuber extracts). *African Journal of Biological Sciences*. 2024;6(Si4):2756-2760.
20. Maury GL, Rodríguez DM, Hendrix S, Arranz JCE, Boix YF, Pacheco AO, *et al.* Antioxidants in plants: a valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants*. 2020;9(11):1048. doi: 10.3390/antiox9111048
21. Mutha RE, Tatiya AU, Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future Journal of Pharmaceutical Sciences*. 2021;7(1):25. doi: 10.1186/s43094-020-00161-8
22. Ouédraogo K, Dimobe K, Zerbo I, Etongo D, Zare A, Thiombiano A. *Traditional knowledge* and cultural importance of *Gardenia erubescens* Stapf & Hutch. in *Sudanian savanna* of Burkina Faso. *Journal of Ethnobiology and Ethnomedicine*. 2019;15(1):28. doi: 10.1186/s13002-019-0305-4
23. Rani JJ, Das K, Sahu JK, Thakur S, Sharma S, Kumar S. Pharmacological evaluation and prediction of pharmaceutical potential of *Lasiococca comberi* Haines: a threatened medicinal tree. *Journal of Biodiversity and Conservation*. 2025;28(4):394-404.
24. Reddy GC, Rangaswami S, Sunder R. Triterpenoids of the stem bark of *Gardenia gummifera*. *Planta Medica*. 1977;32(3):206-211.
25. Soni PK, Devi RS, Kumar S, Behera B, Kumar S. Evaluation of phytochemical constituents, antioxidant and antimicrobial properties of *Gnetum edule*. *Plant Archives*. 2026;26(Suppl 2):3316-3321.
26. Vinaykumar N, Mahmood R, Krishna VE. doi: 10.1186/s40816-020-00188-7