

Chapter 13

Evaluation of medicinal values through TLC & phytochemical analysis of *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch., flowers

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Abstract: Present study investigates the phytochemicals of *Dendrobium aphyllum* flowers using different extracts and TLC analysis, revealing the presence of diverse bioactive compounds, providing insights into the secondary metabolites of this orchid species.

Keywords: Bioactive compounds, therapeutics, orchids

INTRODUCTION

For decades, global researchers have been searching for new therapeutic agents to develop effective formulations due to growing microbial resistance and drug failure (Kumar et al., 2013; Kumar and Jena, 2014; Jena et al., 2024). In this context, exploring traditional practices and plant uses is crucial. Orchids, featured in mythology and folk medicine, have been recognized for their potential in indigenous therapeutic formulations (Chakraborty et al., 2022) and unique eco-medico food values (Kumar and Thorat, 2018; Kumar et al., 2022; Sharma et al., 2024). Despite their unique potential, orchids remain largely unexplored (Kumar and Kumar, 2020; Kumar et al., 2021). Present study aims to document the

secondary metabolites of *Dendrobium aphyllum* flowers, an epiphytic orchid (Kumar et al., 2022; Figure 1). Phytochemical screening of n-hexane, ethanol, and aqueous extracts was conducted, along with thin-layer chromatography, providing baseline data for future bioactivity research.

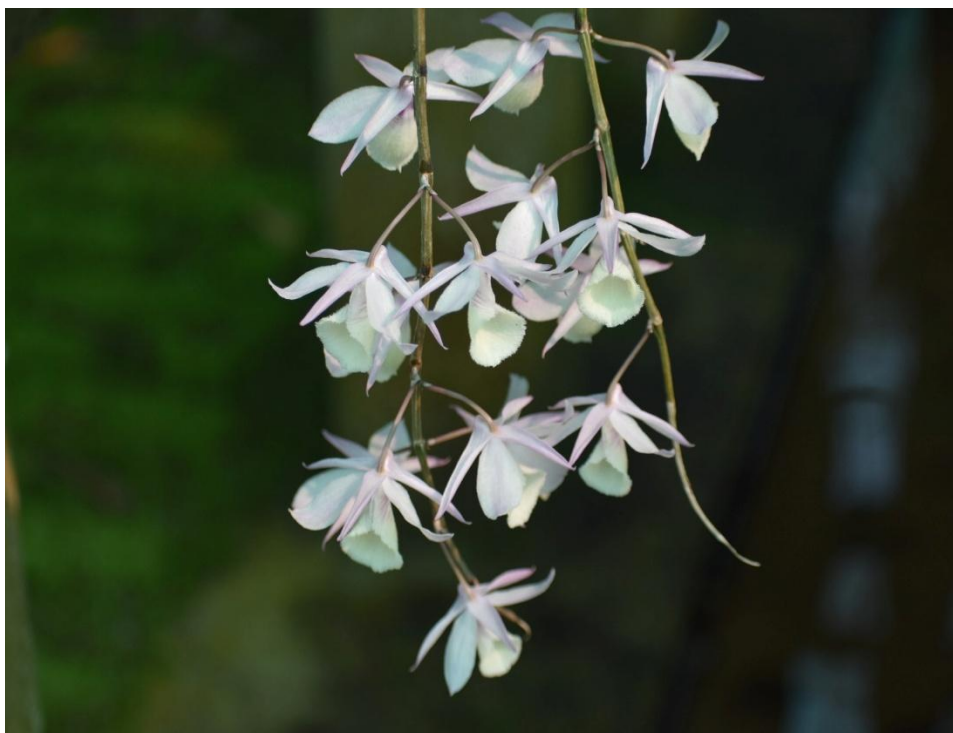


Figure 1: Flowers of *D. aphyllum*

METHODOLOGY

The flowers of *D. aphyllum* were collected from Khordha district of Odisha, India (Figure 2). The Plant species was identified by Dr. Sanjeet Kumar (Figure 1). The Soxhlet extraction method was adopted using n-hexane, ethanol, and distilled water for phytochemical analysis. Detection of selected secondary metabolites were conducted using standard methods (Devi *et al.*, 2023; Ankari *et al.*, 2024; Marndi *et al.*, 2024; Darshani *et al.*, 2025; Das *et al.*, 2025; Jayalakshmi *et al.*, 2025;

Kshirsagar *et al.*, 2025; Kumar *et al.*, 2025; Malwal *et al.*, 2025; Nayak *et al.*, 2025; Patra *et al.*, 2025; Sharma *et al.*, 2025; Yadav *et al.*, 2025).



Figure 2: Collected flowers of *D. aphyllum* for experimental work

Test for tannin: 1 ml of flower extract was taken in a test tube and added 3-5 drops of 10% lead acetate solution into it. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of the flowers extract was added to 1 ml of distilled water and shaken well. The formation of persistent froth was observed confirming the presence of saponin.

Test for flavonoids: About 1 ml of the flowers extract was taken. Added 2 ml of NaOH solution and 3-5 drops of dilute HCl to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless which confirmed the presence of flavonoids.

Test for terpenoids: To 1 ml of the flowers extract, 5-6 drops of chloroform were added. Then 4-6 drops of concentrated sulphuric acid were added into it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the flowers extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the flowers extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: To 1 ml of the flowers extract, added 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid into it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

Test for alkaloid: About 1 ml of the flowers extract was taken. Added 3-4 drops of Dragendroff's reagent to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of flowers extract was taken and added 3-4 drops of 2,4- Dinitrophenylhydrazine (DNPH) reagent. The formation of yellow crystal confirmed the presence of carbonyl compounds.

TLC (Thin layer chromatography) analysis: TLC plates were prepared on 6 cm glass slides using silica gel powder. Slides were washed with clinical laboratory

detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring, and finally was poured over the slides and slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50° C for 15-20 minutes before running the TLC. Methanol: chloroform (50%), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were marked, recorded the RF (Retention Factor) values, and visualized under UV chamber (Mishra and Bhatnagar, 2024).

RESULTS AND DISCUSSION

The phytochemical screening of *Dendrobium aphyllum* flowers revealed the presence of tannins, saponins, phenols, reducing sugars, and alkaloids in various extracts. Saponins and reducing sugars were detected in all extracts (n-hexane, ethanol, and aqueous), while tannins, phenols, and alkaloids were found in ethanol and aqueous extracts (Table 1; Figures 4-6). The TLC analysis of the hydroethanolic extract showed distinct Rf values for different mobile phases, indicating the separation of bioactive compounds. The Rf values ranged from 0.76 to 0.91, with multiple spots observed in some mobile phases, suggesting the presence of diverse phytochemicals in *D. aphyllum* flowers (Table 2).

Table 1: Qualitative phytochemical screening of *Dendrobium aphyllum* flowers using different extracts

Bioactive compounds	Extracts		
	N-Hexane	Ethanol	Aqueous
Tannin	Not detected	Detected	Detected
Saponin	Detected	Detected	Detected
Flavonoids	Not detected	Not detected	Not detected
Terpenoids	Not detected	Not detected	Not detected
Phenols	Not detected	Detected	Detected
Reducing sugar	Detected	Detected	Detected
Steroid	Not detected	Not detected	Not detected
Alkaloid	Not detected	Detected	Detected

Carbonyl compound	Not detected	Not detected	Not detected
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Table 2: TLC analysis of *D. aphyllum* flowers using different mobile phases

Mobile phases	Extracts	Rf value of spots	
		S ₁	S ₂
Chloroform: methanol (1:1)	Hydroethanolic	0.76	0.79
Ethyl acetate: chloroform (1:9)	Hydroethanolic	0.81	Nil
Ethyl acetate: water: acetic acid (8:1:1)	Hydroethanolic	0.87	0.91

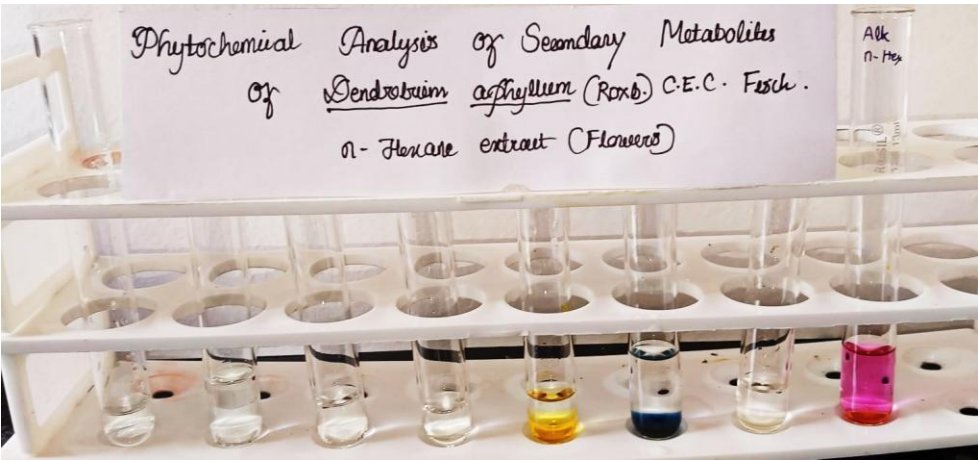


Figure 4: Detection of secondary metabolites of *D. aphyllum* flowers using n-hexane extract

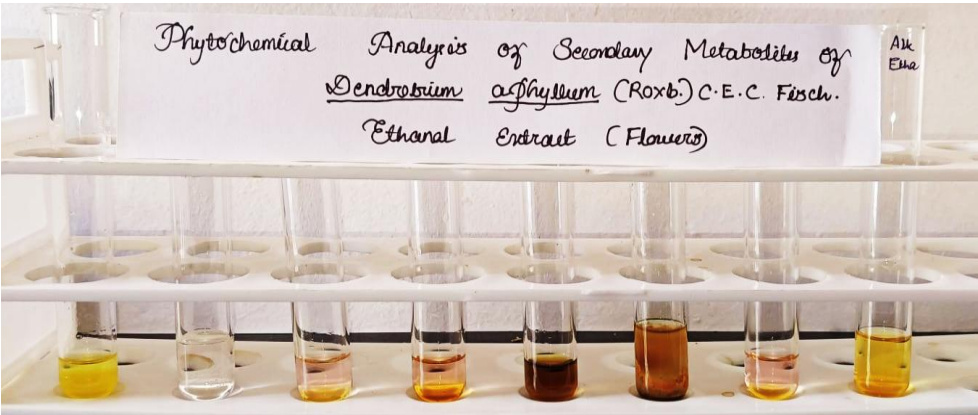


Figure 5: Detection of secondary metabolites of *D. aphyllum* flowers using ethanolic extract



Figure 6: Detection of secondary metabolites of *D. aphyllum* flowers using aqueous extract

CONCLUSION

The study provides valuable insights into the phytochemical composition of *Dendrobium aphyllum* flowers, highlighting the presence of bioactive compounds such as tannins, saponins, phenols, reducing sugars, and alkaloids. These findings suggest the potential medicinal properties of this orchid species, warranting further research to explore its therapeutic applications and pharmacological effects.

REFERENCES

- Ankari AB, Mahendru N, Paniyappanavara V, Jena N, Kumar S and Tripathi G. (2024). Evaluation of medicinal values of a fern species [*Thelypteris parasitica* (L.) Tardieu] of India. In: *Plants and Secondary Metabolites, Volume 2*. Ambika Prasad Research Foundation, India.
- Chakraborty A, Pal N, Thanvi HA, Dhal A, Debi C, Kumar S and Mishra S. (2022). Medicinally important common orchids of India. In: *Medico Biowealth of India, Volume 5*. Ambika prasad Research Foundation, India

- Darshani P, Sharma N, Kapil MJ, Uniyal R, Mahanta D, Jena N and Kumar S. (2025). Qualitative phytochemical analysis of fruits of *Shorea robusta* C.F. Gaertn. In: *Plants and Secondary Metabolites, Volume 4*. Ambika Prasad Research Foundation, India
- Das K, Jena N, Patra A, Kumar S and Rathore S. (2025). Phytochemical and cytotoxicity analysis of flowers of *Symphorema involucratum* (Lamiaceae). In: *Plants and Secondary Metabolites, Volume 4*. Ambika Prasad Research Foundation, India.
- Devi RS, Satapathy KB and Kumar S. (2023). Validation of tribal claims for formulation of future drugs through evaluation of ethnopharmacological values of *Ludwigia adscendens*. Medicinal Plants. 15(4): 691-697.
- Jayalakshmi K, Mudalkar S, Thakur K, Jena N and Kumar S. (2025). Qualitative analysis of bioactive compounds of bark of *Buchanania lanzan* Spreng.: a medicinal tree of India. In: *Plants and Secondary Metabolites, Volume 19*. Ambika Prasad Research Foundation, India.
- Jena N, Rout S, Devi RS and Kumar S. (2024). Phytochemical and cytotoxicity analysis of fruits of *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen: A minor fruit plant of Odisha, India. e-planet. 22(1): 62-70.
- Kshirsagar JJ, Malwal M, Nayak NR, Sharma BP, Jena N and Kumar S. (2025). Analysis of secondary metabolites of seeds of *Oroxylum indicum* (L.) Kurz (Bignoniaceae). In: *Plants and Secondary metabolites, Volume 4*. Ambika Prasad Research Foundation, India.
- Kumar A and Kumar S. (2020). Some medicinal orchid species of Jharkhand, India. Journal of Biodiversity and Conservation. 4(4): 398-404.
- Kumar G, Verma RK, Sable PD, Lal S, Jena N and Kumar S. (2025). Medicinal values and phytochemical screening of fruits of Indian franchincense (*Boswellia serrata*) Roxb. In: *Plants and Secondary Metabolites, Volume 4*. Ambika Prasad Research Foundation, India.
- Kumar S and Jena PK. (2014). Chromatographic, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. Plant Science Research. 36 (1&2): 83-90.
- Kumar S and Thorat SS. (2018). Addition of a host plant of an epiphytic orchid species (*Vanda testacea* (Lindl.) Rchb.f.) from Indo Burma Biodiversity Hotspot. Journal of Biodiversity and Conservation. 2(1): 108-110.

- Kumar S, Behera SP and Jena PK. (2013). Validation of tribal claims on *Dioscorea pentaphylla* through phytochemical screening and evaluation of antibacterial activity. Plant Science Research. 35: 55-61.
- Kumar S, Mishra AK, Singh RK, Kumar SN and Marndi S. (2022). Orchids of Bonai Forest Division. Bonai Forest Division, Odisha & Ambika Prasad Research Foundation, India.
- Kumar S, Mishra S and Mishra AK. (2021). Diversity of orchid species of Odisha state, India. With not on the medicinal and economic uses. Richardiana. 5: 1-26.
- Malwal M, Jena N, Gyani G, Lal S and Kumar S. (2025). Phytoconstituents of fruits of Marking nut tree (*Semecarpus anacardium* L.f.). In: *Plants and Secondary Metabolites, Volume 5*. Ambika Prasad Research Foundation, India.
- Mishra S and Bhatnagar S. (2024). Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. World Journal of Pharmaceutical Life Science. 10(2): 116-118.
- Nayak G, Roy BC, Jena N and Kumar S. (2025). Phytochemical screening of fruits and bark of *Cleistanthus collinus*: a wild biopesticide of India. In: *Medico-Biowealth of India, Volume 19*. Ambika Prasad Research Foundation, India.
- Patra A, Hossain E, Roy BC, Lal S, Jena N and Kumar S. (2025). Qualitative analysis of secondary metabolites in *Cordia myxa* L. fruits: a multipurpose tree species. In: *Plants and Secondary Metabolites, Volume 4*. Ambika Prasad research Foundation, India.
- Sharma BP, Patra A, Sahu BK, Jena N and Lal S. (2025). Phytochemical screening and TLC profiling of *Argemone mexicana* L. (Svarnakshiri) flowers. In: *Medico-Biowealth of India, Volume 19*. Ambika Prasad Research Foundation, India.
- Sharma BP, Saradar B, Choudhari SS, Sampatrao TS, Marndi S and Kumar S. (2024). Orchid Diversity in Mining Impacted Forest areas of Odisha: Documentation, Restoration and Conservation Aspect. Indian Forester. 150 (9): 876-881.
- Yadav A, Verma RK, Basole S, Tripathi G, Das K, Jena N and Kumar S. (2025). Phytochemical analysis of leaves, stem and root of Punarnava (*Boerhavia diffusa* L.): a medicinal herb of Nyctaginaceae. In: *Plants and Secondary Metabolites, Volume 5*. Ambika Prasad Research Foundation, India.