J Med Biosci Suppl 2025;7: S15 - S20 DOI: 10.58837/CHULA.JMBS.7.S1.3

Original article

Comparative analysis of root-derived ethanolic extracts from tissue-cultured vs. wild *Grammatophyllum speciosum*: antioxidant and antibacterial activities

Bhasyn Saraporn[1,#], Nathakorn Ekpanyaskul[1,#], Jittraporn Chusrisom[3], Proudphat Jumnongjit[2], Pascha Nateerom[2], Pattama Tongkok[4], Gadewara Matmarurat[3], Nawin Chanthra[1], Wannarat Phonphoem[2], Nattanan Panjaworayan T-Thienprasert[2,*]

[1]Department of Biochemistry and Genetic Engineering, Faculty of Medicine, Kasetsart University, Bangkok, Thailand
[2]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand
[3]Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand
[4]Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

#These authors are co-first authorship.

Abstract

Background: *Grammatophyllum speciosum*, the world's largest orchid, contains diverse phytochemicals with therapeutic potential. While antioxidant and antibacterial properties of aerial parts have been studied, comparative analysis of root extracts from wild versus tissue-cultured plants is lacking.

Objectives*:* This study aimed to compare antioxidant and antibacterial activities of ethanolic root extracts from wild and salt stress tissue-cultured *G. speciosum*, focusing on key phytochemicals.

Methods*:* Ethanol extraction (sonication, centrifugation, vacuum concentration) was performed on roots. High-performance liquid chromatography (HPLC) quantified catechin, coumaric acid, vitexin, and isovitexin. Antioxidant capacity was assessed via DPPH assay (vitamin C equivalents), and antibacterial activity was evaluated using agar disc-diffusion against plant and human pathogens.

Results*:* HPLC revealed distinct phytochemical profiles: wild roots contained catechin (absent in tissue-cultured roots), while tissue-cultured roots uniquely expressed isovitexin and vitexin. Antioxidant activity was significantly higher in tissue-cultured roots (4.5 ± 0.2 mg/g vitamin C equivalents) compared to wild roots (2.5 ± 0.3 mg/g). Tissue-cultured extracts inhibited all tested pathogens (inhibition zones: 8–14 mm at 1 mg/mL), including *Xanthomonas oryzae*, *Xanthomonas citri*, *Erwinia carotovora, Ralstonia solanacearum*, *Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli*, whereas wild root extracts showed no activity.

Conclusion: This study demonstrates that tissue-cultured roots of *G. speciosum* under salt stress yield extracts with superior antioxidant and broad-spectrum antibacterial properties, linked to specific phytochemicals. This supports tissue culture as a sustainable approach to enhance bioactive compounds in threatened orchids, potential cosmeceutical and agricultural applications.

Keywords*:* Antibacterial activity, antioxidant activity, *G. speciosum*, plant tissue culture,wild.

***Correspondence to: Nattanan Panjaworayan T-Thienprasert,** Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.

E-mail: fscinnp@ku.ac.th

Grammatophyllum speciosum Blume, commonly known as the "Tiger Orchid," is recognized as the largest orchid species in the world. Beyond its impressive size, its striking flowers—adorned with brown and yellow tiger-like stripes—have captivated botanists and orchid enthusiasts alike.[1] This species belongs to the family Orchidaceae and primarily grows as an epiphyte, occasionally as a lithophyte, in lowland forests near streams and rivers at elevations ranging from 100 to 550 meters. Native to Southeast Asia, *G. speciosum* is found in countries such as Malaysia, Indonesia, Thailand, and the Philippines.[2]

In recent years, *G. speciosum* has garnered increasing scientific interest due to its diverse phytochemical composition, which includes compounds such as isovitexin, vitexin, catechin, coumaric acid, grammatophyllosides, and glucosyloxybenzyl derivatives.[3, 4] These bioactive compounds have demonstrated promising therapeutic and clinical applications. For instance, extracts from *G. speciosum* pseudobulbs have shown significant anti-aging effects by improving skin viscoelasticity and reducing wrinkle volume in both *in vitro* and clinical studies.[5] Additionally, pseudobulb extracts have exhibited antiproliferative activity against human breast cancer cells (MCF-7).[5]

Further investigations into aerial and root extracts of *G. speciosum*, particularly those obtained from plants cultivated in Temporary Immersion Bioreactors (TIB), have revealed potent anti-skin cancer and antibacterial properties.[3] Building upon these findings, this study aimed to evaluate the antioxidant and antibacterial properties of ethanolic extracts derived from both wild *G. speciosum* roots and tissue culture roots. By comparing these extracts, the findings of this study will lead to a better understanding of their potential applications in medicine and biotechnology.

Materials and methods

Plant materials and growth conditions.

For wild plant material, *G. speciosum* roots were collected in mid-September 2024 from the Samut Sakhon Agricultural Occupation Promotion and Development Center (39 Moo 12, Tha Mai, Krathum Baen District, Samut Sakhon 74110, Thailand).

Phytochemical extraction

Approximately 300 mg of the ground sample was homogenized with 30 mL of absolute ethanol (QRëC, Auckland, New Zealand) and sonicated in cool water using an ultrasonic bath for 30 minutes. The sample was then incubated at room temperature for 3 hours, followed by centrifugation at 13,000 rpm at 4°C for 20 minutes. The supernatant was concentrated using a vacuum centrifuge (Eppendorf, Germany) at 30°C. For further analysis, the extracts were dissolved in 99% absolute ethanol at various dilutions to prepare different concentrations.

HPLC analysis

Dried and finely ground samples (0.1 g) were extracted with 2 mL of ethanol. The mixture was sonicated for 45 minutes using a sonicator. Centrifugation was performed at 12,000 rpm for 10 minutes to separate the supernatant from the pellet. The supernatant was filtered through a 0.45 μm syringe filter for High-performance liquid chromatography (HPLC) analysis. Catechin and coumaric acid were quantified by measuring absorbance at 270 nm, while vitexin and isovitexin were detected at 340 nm using an HPLC system (Nexera LC-40 series, Shimadzu, Japan). The stationary phase consisted of a C18 column (5 μm, 150 × 4.6 mm), with a mobile phase comprising 1% acetic acid in ultrapure water (solvent A) and acetonitrile (solvent B). The flow rate was set at 0.7 mL/min, starting with 10% solvent B and gradually increasing to 100%. A 10 μL injection volume was used for each sample. Statistical analysis was conducted using GraphPad Prism software.

DPPH radical scavenging capacity

The antioxidant capacity of the sample extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Sigma Aldrich, Missouri, USA). A 0.2 mM DPPH solution was prepared by dissolving 0.8 mg of DPPH crystals in 10 mL of 99% absolute ethanol. Sample extracts (1 mg/mL, 20 μL) were mixed with 100 μL of DPPH solution in a 96-well plate, with ethanol (EtOH) serving as the assay buffer to complete the remaining volume. Controls included Blank 1 (DPPH background), Blank 2 (ethanol background), and Sample background cut, with all mixtures prepared in quadruplicate. Ascorbic acid was used as a positive control to ensure proper experimental technique. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm. The percentage inhibition was calculated using the formula: DPPH radical scavenging (%)=[(Ac–As)/Ac)×100 where Ac represents the absorbance of Blank 1 minus Blank 2, and As represents the corrected absorbance of the sample calculated as follows:

As = ((Absorbance of sample–Sample background cut) – Blank 2)

Agar disc-diffusion method

The antibacterial activity of tested compounds was evaluated using the agar disc-diffusion method, following Clinical & Laboratory Standards Institute (CLSI) guidelines. Experiments were conducted in triplicate for each bacterial strain. Human pathogenic strains included *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 23235), and *Bacillus subtilis* (ATCC 23857), while plant pathogenic strains were isolated and characterized by the Plant Protection Research and Development Office, Bangkok, Thailand, which comprised *Xanthomonas axonopodis pv. citri, Xanthomonas oryzae*, *Ralstonia solanacearum*, and *Erwinia carotovora*. Bacterial suspensions were standardized to a turbidity of 0.4–0.6 OD at 600 nm. LB agar plates were inoculated with 300 µL of each suspension using a sterile spreader to ensure uniform distribution. Sterile filter paper discs (~6 mm diameter) were impregnated with ethanolic extracts of *G. speciosum* root (1 mg/mL). Discs were placed on inoculated plates, which were incubated at 37°C for 24 hours. Inhibition zones were measured post-incubation according to CLSI protocols.

Statistical analysis

All experiments were carried out with at least three biological replicates $P < 0.05$ was considered statistically significant. Tukey's multiple range test was employed to test the significant difference in each experimental group using GraphPad Prism software version 6.0 (GraphPad Prism software, San Diego, CA, USA).

Results

HPLC analysis

HPLC analysis was conducted using standards for vitexin, isovitexin, catechin, and coumaric acid (**Figure 1**) to identify major compounds in wild and tissue-cultured *G. speciosum* root extracts. The results revealed distinct compositional differences between the two plant sources. Wild-type root extracts contained detectable levels of catechin but showed no evidence of vitexin, isovitexin, or coumaric acid. Conversely, tissue-cultured root extracts exhibited detectable quantities of vitexin and isovitexin, while catechin and coumaric acid remained undetected (**Table 1**).

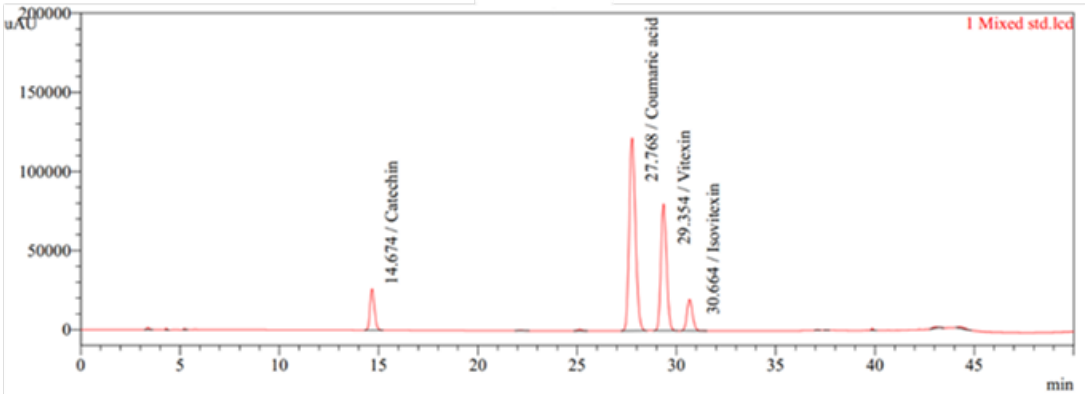

Figure 1. HPLC chromatogram of vitexin, isovitexin, catechin, and coumaric acid standards. Retention times and peak characteristics are labeled for reference.

Table 1. HPLC identification of chemical constituents in *G. speciosum* root extracts.

Compound	Retention time (min)	Molecular weight	Detection	
			Wild	Tissue culture
Catechin	14.674	290.270	+	-
Coumaric acid	27.768	164.047	-	-
Vitexin	29.354	432.105	-	+
Isovitexin	30.664	433.113	-	+

"+" denotes presence while "-" indicates absence

Antioxidant activities of G. speciosum root extracts

As shown in **Figure 2**, the antioxidant activity was significantly higher in tissue-cultured roots (4.5 ± 0.2 mg/g vitamin C equivalents) compared to wild roots (2.5 ± 0.3 mg/g).

Anti-bacterial activity of G. speciosum root extracts

Using the agar disc-diffusion assay, no inhibitory effects were observed for wild *G. speciosum* root extracts at 1 mg/mL against any tested bacteria. In contrast, tissue-cultured *G. speciosum* root extracts exhibited inhibition zones d"14 mm across all bacterial strains (**Table 2**).

Discussion

The comparative analysis of phytochemical profiles, antioxidant activity, and antibacterial properties of *G. speciosum* root extracts from wild and tissue-cultured plants highlights notable differences driven by cultivation methods and environmental conditions.

HPLC analysis revealed distinct phytochemical profiles between wild and tissue-cultured roots. Wild roots contained catechin, a flavonoid known for its antioxidant and antimicrobial properties, while tissue-cultured roots uniquely expressed vitexin and isovitexin, two C-glycosyl flavones with established antioxidant and anti-inflammatory activities.[3] The absence of catechin in tissue-cultured roots suggests that cultivation conditions, such as salt stress, may alter

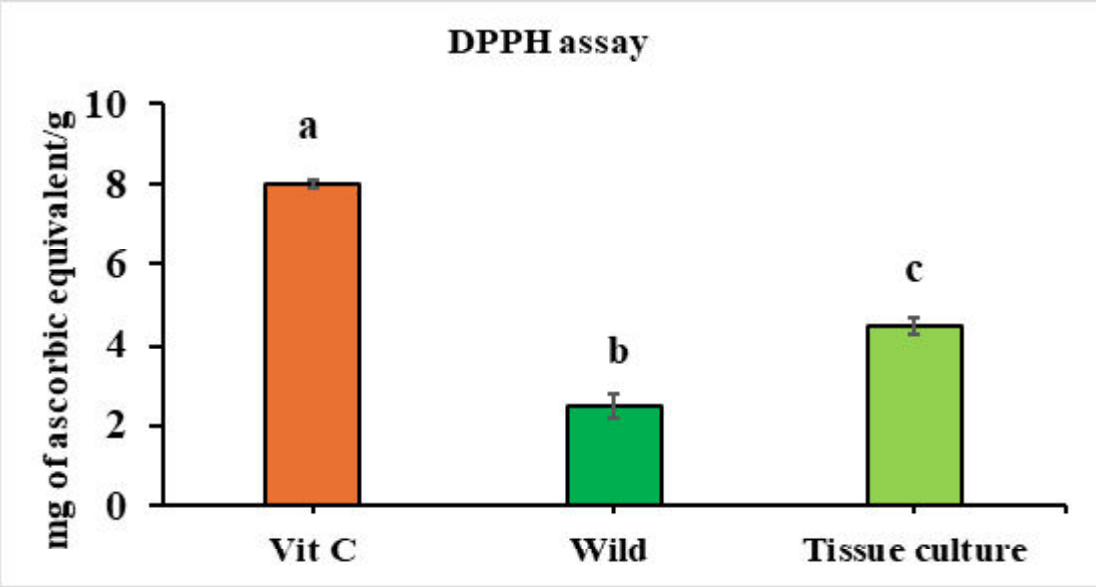

Figure 2. Evaluation of antioxidant activities of *G. speciosum root* extracts using DPPH assay. The experiments were performed in quadruplicate with two independent experiments. Tukey's multiple comparison test (One-way ANOVA) was used for testing the statistical difference among the samples ($P < 0.05$).

Table 2. Anti-bacterial activity of *G. speciosum* root extracts.

Root extract	Plant pathogens				Human pathogens		
(1 mg/mL)	*X. oryzae*	*X. citri*	*E. carotovera*	*R. solanosearum*	*E. coli*	*S. aureus*	*B. subtilis*
Wild	-	-	-	-	-	-	-
Tissue culture	+	+	+	+	+	+	+

"-" indicates absence of inhibition zones, on the other hand "+" indicates presence of inhibition zones of less than 14 mm.

secondary metabolite biosynthesis pathways. This aligns with prior studies showing environmental stressors can induce specific phytochemical production in orchids.[4]

The antioxidant capacity of tissue-cultured root extracts was significantly higher (4.5 ± 0.2 mg/g vitamin C equivalents) than wild root extracts (2.5 ± 0.3 mg/g). This enhanced activity correlates with the presence of vitexin and isovitexin in tissue-cultured roots, both of which exhibit strong radical-scavenging properties. [3, 4] In contrast, catechin, while effective as an antioxidant, may not contribute as substantially to the overall activity at the tested concentrations. These findings support the hypothesis that tissue culture under controlled stress conditions can optimize the production of bioactive compounds with superior antioxidant properties.[6]

Tissue-cultured root extracts demonstrated broad-spectrum antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli, X. oryzae, X. citri, E. carotovora*), with inhibition zones ranging from 8–14 mm at 1 mg/mL. In contrast, wild root extracts showed no inhibitory effects on any tested bacteria. The antibacterial efficacy of tissue-cultured extracts is likely driven by vitexin and isovitexin[3], which have been reported to disrupt bacterial membranes and inhibit key enzymatic pathways.[6] The absence of antibacterial activity in wild roots further underscores the role of specific phytochemicals in mediating these effects.

These results demonstrate that tissue culture is a viable method for enhancing the production of bioactive compounds in *G. speciosum*. By subjecting plants to controlled stress conditions, such as salinity, it is possible to induce the synthesis of desirable phytochemicals like vitexin and isovitexin while maintaining sustainable cultivation practices for this threatened orchid species.[3] The superior antioxidant and antibacterial properties of tissue-cultured root extracts also suggest potential applications in cosmeceutical formulations and agricultural biocontrol agents.

While the findings support the potential of tissue culture to enhance phytochemical production in *G. speciosum*, several limitations remain. Although HPLC identified key compounds, comprehensive metabolomic profiling (e.g., LC-MS/MS or NMR) would provide deeper insights into the full range of phytochemicals, including secondary metabolites and biosynthetic pathways influenced by cultivation conditions. Additionally, although tissue culture conditions were standardized, external factors affecting the wild samples (e.g., soil composition, microclimate) were not characterized, which may confound direct comparisons.

Conclusion

The study highlights the importance of cultivation methods in shaping phytochemical profiles and bioactivity in *G. speciosum*. Tissue culture not only enhances antioxidant and antibacterial activities but also provides a sustainable approach to preserving this valuable orchid species while maximizing its therapeutic potential. Further research should explore the molecular mechanisms underlying these effects and optimize cultivation protocols for targeted phytochemical production.

Acknowledgments

This research is supported by "Kasetsart University Research and Development Institute, KURDI, FF (KU) 32.66 and FF(KU) budget year 2569". This research was also funded by the Research Development Program for Medical Students, Faculty of Medicine, Kasetsart University.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data sharing statement

All data generated or analyzed during the present study are included in this published article. Further details are available for non-commercial purposes from the corresponding author upon reasonable request.

References

1. Schiff JL. Rare and exotic orchids: their nature and cultural significance. Cham: Springer; 2018.
2. Lemmens RHMJ, Bunyapraphatsara N. Plant resources of South-East Asia 12(3). medicinal and Poisonous plants 3. Leiden: Backhuys Publishers; 2003.
3. Thamrongwatwongsa J, Chusrisom J, Katemala K, Tantasirin S, Jumnongjit P, Nateerom P, et al. Determination of flavonoid content in *Grammatophyllum speciosum* and *in vitro* evaluation of their anti-skin cancer and antibacterial activities. Heliyon 2024;10:e33330.

4. Yingchutrakul Y, Sittisaree W, Mahatnirunkul T, Chomtong T, Tulyananda T, Krobthong S. Cosmeceutical potentials of Grammatophyllum speciosum extracts: anti-inflammations and anti-collagenase activities with phytochemical profile analysis using an untargeted metabolomics approach. Cosmetics 2021;8:116.
5. Chowjarean V, Phiboonchaiyanan PP, Harikarnpakdee S, Tengamnuay P. A natural skin anti-ageing serum containing pseudobulb ethanolic extract of *Grammatophyllum speciosum*: a randomized double-blind, placebo-controlled trial. Int J Cosmet Sci 2019;41:548–57.
6. Yingchutrakul Y, Krobthong S. Proteomics dataset for the analysis of the effects of *Grammatophyllum speciosum* extracts on RAW 264.7 cells. Data Brief 2023;47:108937.
7. Mu D, Xiang H, Dong H, Wang D, Wang T. Isovitexin, a potential candidate inhibitor of sortase a of *Staphylococcus aureus* USA300. J Microbiol Biotechnol 2018;28:1426-32.