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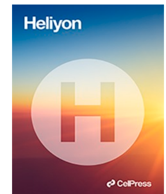

Research article

Determination of flavonoid content in *Grammatophyllum speciosum* and *in vitro* evaluation of their anti-skin cancer and antibacterial activities

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A R T I C L E I N F O

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A B S T R A C T

Grammatophyllum speciosum Blume, a plant of significant pharmacological and cultural importance in its native regions, has been the subject of traditional medicinal use. This study, however, delves deeper into the unique attributes of *G. speciosum* aerial part and root extracts, particularly their phytochemical content, antioxidant potential, antibacterial activity, and anticancer properties against human skin cancer cells. The results unveiled a promising aspect-higher flavonoid and phenolic compound levels in the aerial part compared to the root extracts. Both aerial part and root extracts demonstrated significant antioxidant activities, as evidenced by their ability to scavenge DPPH radicals and reduce ferric ions in the FRAP assay. Moreover, the ethanolic extract derived from *G. speciosum* aerial parts showed promising antibacterial activity against both gram-positive and gram-negative bacteria, hinting at its potential therapeutic efficacy. Notably, this extract also demonstrates a capacity to impede the viability of human skin cancer cells (A375). Collectively, these results demonstrated the potential applications of the *G. speciosum* aerial part extracts. Further investigation is imperative to elucidate the intricate molecular mechanisms underpinning these diverse effects, thereby contributing to a deeper understanding of the pharmacological potential of *G. speciosum* and its prospective applications in medicine and beyond.

1. Introduction

Grammatophyllum speciosum Blume (*G. speciosum*) is one of the largest orchid species, known for its impressive size and vibrant

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appearance. It belongs to the Orchidaceae family and is native to Southeast Asia, including Malaysia, Indonesia, Thailand, and the Philippines. Beyond its imposing stature and expansive rainforest habitat, *G. speciosum* is notable for the presence of compounds that have captured the interest of scientists [1,2]. These compounds harbor unique properties that potentially benefit human health, prompting researchers to investigate how these chemicals may enhance our well-being or combat specific illnesses. The substantial promise for our health and wellness offered by this exceptional orchid has been recognized [3]. Upon analyzing the *G. speciosum* extract, numerous compounds were discovered, such as glucosyloxybenzyl derivatives of (R)-2-benzyl-malic acid, (R)-eucomic acid, grammatophyllosides A-D, cronupapine, vandateroside II, gastrodin, vanilloloside, orcinol glucoside, and isovitexin, showcasing its complexity and diversity. This discovery propelled further studies on their potential applications, particularly in medicinal and pharmacological research [1,4].

Antioxidants are substances that can counteract the damaging effects of free radicals in cells. Free radicals are molecules that can cause cellular damage, leading to various health issues, including aging and chronic diseases [5]. Vitamins C and E, β-carotene, and other compounds found in fruits, vegetables, and certain beverages act as antioxidants [6]. Recent studies have reported antioxidant activity and α-glucosidase inhibitor activity in 6-year-old leaves, rhizomes, and root parts of *G. speciosum*, demonstrating particularly high DPPH free radical scavenging activity [7]. This orchid species has also demonstrated promising antiproliferative effects on breast cancer cells by impeding the AKT/β-catenin pathway [8].

In Asian region, *Grammatophyllum scriptum*, along with other orchids such as *Acriopsis javanica, Corymborchis longiflora, Epidendrum bifidum, Eria pannea, Nervilia arago-ana* and *Tropidia curculigoides*, have been traditionally used to treat various diseases, including postpartum recovery, malaria, scabies, skin diseases and intestinal tapeworms. Analysis of compounds and their biological activities reveals that polysaccharides, phenolics compounds (including flavonoids, stilbenes and bibenzyls), and alkaloids are the major bioactive compounds found in orchids. These bioactive compounds act as antimicrobial, anti-inflammatory, antidiabetic, anticancer, and antioxidant agents [9]. Among the studies on orchids, there are only a few reports on the bioactivity of the genus *Grammatophyllum*. The available reports also focused on leaves [10] and pseudobulb [8,11] but no other parts of the plants.

In this study, total flavonoid and phenolic contents were investigated in *G. speciosum* grown in Temporary Immersion Bioreactor (TIB) system. Furthermore, the biological activities including antioxidant, anti-bacterial, and anti-skin cancer, were evaluated.

2. Materials and methods

2.1. Chemicals, reagents, and cell lines

Gallic acid, quercetin, 2,2-Diphenyl-1-picrylhydrazyl, 2,3,5-Triphenyltetrazolium chloride were purchased from Sigma Aldrich, St. Louis, Missouri, USA. Folin-Ciocalteu reagent was obtained from Merck KGaA, Darmstadt, Germany. 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was purchased from AppliChem GmbH, Darmstadt, Germany. Bacteria, *Escherichia coli* (ATCC 25922), and methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC BAA-1690), as well as human tissue cell culture, A375 (ATCC® CRL-1619™) and Vero (ATCC ®, CCL-81) were purchased from the American Type Culture Collection (ATCC), Manassas, Virginia, USA. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Invitrogen™, California, USA. Fetal bovine serum (FBS) was purchased from Gibco, Thermo Fisher Scientific, Waltham, Massachusetts, USA. Other chemicals, reagents, and media were analytical and molecular biology grades.

2.2. Plant materials and growth conditions

The 1-year-old of *G. speciosum* were cultured in half-strength MS (½ Murashige and Skoog) media supplemented with 20 g/L sucrose and 1 g/L activated charcoal. The plantlets were maintained in TIB system (immersion 10 min/h) under white fluorescent for light/dark (16/8 h) photoperiod at 25 ± 1 °C and humidity of 51 ± 2 % for 2 weeks.

2.3. Phytochemical extraction

Approximately 300 mg of the lyophilized sample was homogenized with 30 mL of absolute ethanol (QRëC, Auckland, New Zealand) and sonicated in cool water using an ultrasonic bath for 30 min. After the sample was incubated at room temperature for 3 h, the sample was further centrifuged at 13,000 rpm at 4 °C for 20 min. The supernatant was concentrated using a vacuum centrifuge (Eppendorf, Germany) at 30 °C, subsequently dried using freeze dry. To analyze it further, the extracts were dissolved the ethyl alcohol to make a solution with a concentration of 1 mg/mL.

2.4. Determination of total flavonoid content

Total flavonoid measurement was performed by the aluminum chloride colorimetric assay with a slight modification [12]. Briefly, 50 μL of the extract was added into a 96-well microplate and combined with 10 μL of 10 % (v/v) aluminum chloride (KemAus Cherrybrook, NSW, Australia). Next, 150 μL of 60 % ethyl alcohol and 10 μL of 1 M potassium acetate (KemAus Cherrybrook, NSW, Australia) were added, respectively. The plate was left in the dark at room temperature for 40 min before measurement at 415 nm using a microplate reader (Tecan M200, Switzerland). The concentration of total flavonoid contents in the test sample was calculated as quercetin (Sigma-Aldrich, St. Louis, MO, USA) equivalent QE/mg of dry weight plant material.

2.5. Measurement of total phenolic content

The total phenolic content in aerial part and root extracts was determined using the Folin-Ciocalteau method, as previously reported [13]. Briefly, 20 μL of the extract solution was combined with 100 μL of 10 % (v/v) Folin-Ciocalteau reagent in a 96-well plate and incubated at room temperature for a few minutes. After that, 80 μL of 1 M sodium carbonate was added and further incubated for 20 min in the dark. The absorbance of the mixture was measured at 760 nm. The total phenolic contents in the extracts were calculated as gallic acid equivalent (GAE) from a calibration curve (0–200 μg/mL) and the results were expressed as μg of gallic acid equivalent per 1 g of dry weight plant tissue (μg GAE g^{-1} DW). All measurements were performed in triplicate.

2.6. Evaluation of antioxidant activities

2.6.1. Ferric reducing antioxidant power assay

This laboratory assay measures antioxidant capacity by reducing a colorless Fe^{3+} TPTZ (Sigma-Aldrich, St. Louis, MO, USA) complex to a blue-colored Fe^{2+} tripyridyl triazine complex under acidic conditions [14]. Briefly, 20 μL of the sample extract was placed in a 96-well plate. The FRAP working solution (280 μL) was added, mixed, and then covered. The mixture was incubated at 37 °C in darkness for 30 min. The absorbance at 593 nm was measured using a spectrophotometer and ascorbic acid (Fisher Scientific, Loughborough, UK) was served as a standard. The resulting FRAP value was reported as milligrams (mg) of ascorbic acid equivalent per gram of the sample. This value quantifies the antioxidant capacity of the sample.

2.6.2. DPPH radical scavenging capacity

For the DPPH radical scavenging assay, this assay is based on the reduction of the purple-colored DPPH radical to a yellow-colored compound in the presence of an antioxidant [15]. The samples extracts (25 μL) were mixed with 150 μM DPPH solution (Sigma-Aldrich, St. Louis, MO, USA) (200 μL) in a 96-well plate. Ascorbic acid was used as a standard to generate a calibration curve. The mixture reacted at room temperature in the dark for 30 min. Absorbance at 517 nm measured the reduction of DPPH. The ability to scavenge DPPH radicals was calculated using Herald et al.'s equation (2012) [12].

The percentage inhibition was calculated using the formula DPPH radical scavenging (%) = ((AB – AA)/AB) × 100, where AB was the absorbance of DPPH radical + ethanol and AA represented the absorbance of the DPPH radical when mixed with the plant extract and standard.

2.6.3. ABTS radical scavenging capacity

ABTS was modified from the previous report [16]. Briefly, ABTS·+ cation radicals (TCI, Tokyo, Japan) were generated by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate (AppliChem, Darmstadt, Garmany) (1:1) and stored the solution in the dark at room temperature for 12–16 h. The ABTS·+ solution was diluted with ethanol to achieve an absorbance of 1.1 ± 0.02 at 734 nm. To assess scavenging activity, 10 μL of plant extract was added to 190 μL of diluted ABTS·+ solution. Absorbance was measured for 30 min after mixing at 734 nm. A solvent blank was included for this assay and all measurements were performed at least three times and ascorbic acid was used as a standard to generate a calibration curve.

The percentage inhibition was calculated using this formula: ABTS·+ scavenging activity (%) = ((AB - AA)/AB) × 100, where AB represented the absorbance of the ABTS radical mixed with ethanol (Control) and AA represents the absorbance of the ABTS radical when mixed with the plant extract and standard.

2.7. Agar disc-diffusion method

The antibacterial activity of *G. speciosum* was evaluated by the agar disc-diffusion method [17]. Initially, filter papers (with a diameter of 0.5 cm) were soaked in various concentrations of *G. speciosum* ethanol extract. Bacterial cells were prepared at OD_{600} of approximately 0.6–0.7 Abs. Subsequently, 5 mL of bacterial cells were plated on LB agar media. The filter papers were then placed onto the agar. The LB agar plates were incubated for 16–18 h at 37 °C. Following incubation, an inhibition zone was observed. The inhibition zone was measured according to the Clinical & Laboratory Standards Institute.

2.8. Antibacterial assay

Bacteria, *E. coli* (ATCC 25922), and MRSA (ATCC BAA-1690) were cultured in LB broth. The assay was performed as described in Jindaruk et al., 2003 [18].

2.9. Cytotoxicity test using MTT assay

The cytotoxicity of *G. speciosum* extracts were investigated using human melanoma cell line (A375, ATCC® CRL-1619™), human keratinocyte cell line (HaCaT) including the normal cell line (Vero, CCL-81), through the MTT assay as described in Khamwut et al. 2023 [19].

2.10. Phytochemical analysis

LC-MS analysis was performed using a Thermo Q Exactive™ Plus mass spectrometer coupled to vanquish™ Horizon UHPLC. The samples were reconstituted in 100 % ethanol with 0.1 % formic acid prior to the separation of the chromatographic system. The analysts were separated using a Hypersil gold™Vanquish C18 column (2.1 mm × 100 mm, 1.9 μm) (Thermo Scientific, Lithuania). The mobile phases A and B were water with 0.1 % formic acid and acetonitrile with 0.1 % formic acid, respectively. Starting with 5 % B, the linear gradient was increased to 90 % B over 10 min. The column was flushed with 90 % B and reduced to 5 % B. The column was re-equilibrated before subsequent analysis. MS data was acquired under heated electrospray ionization (HESI). A positive and negative modes spray voltage was set as 3.5 kV and 2.5 kV. The rate of sheath gas, auxiliary gas, and sweep gas were 45, 10, and 2 arbitrary units, respectively. The capillary temperature was 320 °C. Full scan MS and data-dependent MS/MS were performed in the resolution of 120,000 and 30,000. The scan range was 100–1500 *m/z*. The raw data were acquired and processed with Xcalibur software (Thermo Scientific, CA, USA). The raw MS files were processed with Compound Discoverer v.3.3 software (ThermoFisher Scientific, Carlsbad, CA, USA). The peak area was then quantified. Metabolites were confirmed through online and In-house databases.

2.11. Statistical analysis

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

3. Results and discussion

3.1. Investigation of phytochemical content in G. speciosum

In this study, the Temporary Immersion Bioreactor (TIB) system was established for culturing *G. speciosum* (Fig. 1A). Plant growth was monitored over 3 weeks and the result showed an approximately 2-fold increase in biomass yield (Fig. 1B).

Plant tissue culture has revolutionized agriculture and biotechnology by enabling the propagation of plants under controlled conditions, free from environmental constraints. Among the various techniques employed, the TIB system stands out as a cutting-edge approach that offers unparalleled advantages in terms of efficiency, scalability, and resource utilization. Using this system, the explants are immersed directly into a liquid nutrient medium, facilitating optimal nutrient uptake and gas exchange, leading to healthier and more robust plant growth [20,21]. Additionally, the TIB system is particularly advantageous for difficult-to-culture plant species, allowing the conservation and propagation of endangered or economically valuable plant species that were previously challenging to culture successfully [22].

Ethanolic extracts of the aerial parts (AP) and roots (RP) were prepared and subsequently used in a colorimetric method for total flavonoid quantification by aluminium chloride (Fig. 2A). Higher flavonoid content was observed in the aerial parts (154.61 mg/g) than in the root (16.30 mg/g) (Fig. 2B). Flavonoids are secondary metabolites widely found in vegetables, fruits, stems, flowers, roots,

A

B

Plant growth (day)	0	3	7	21
Biomass (g)	1.29 ± 0.09	1.25 ± 0.01	1.52 ± 0.05	2.22 ± 0.10
Plant height (cm)	11.37 ± 0.42	11.70 ± 0.32	12.79 ± 0.10	13.51 ± 0.35
Leaf number	7.56 ± 0.16	7.89 ± 0.16	8.67 ± 0.27	9.44 ± 0.16
Root length (cm)	11.30 ± 0.40	11.50 ± 0.28	12.24 ± 0.28	13.31 ± 0.44

Fig. 1. *G. speciosum* grown in the Temporary Immersion Bioreactor (TIB) system (A) and plant growth measurement (B). The data was presented as mean ± standard error of mean from three independent experiments (n = 3).

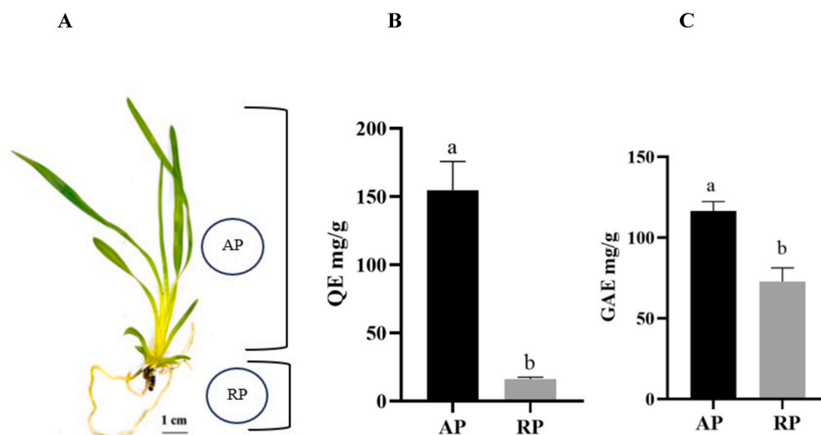

Fig. 2. The quantification of phytochemicals in distinct parts of *G. speciosum*, specifically the aerial part (AP) and root (RP). Plants grown in the Temporary Immersion Bioreactor (TIB) system were harvested and separated into aerial parts and roots (A). Total flavonoid (B) and total phenolic (C) contents in the aerial part and root extracts. The data was presented as mean ± standard error of mean from two independent experiments (n = 3).

and grains. These compounds have been regarded as an indispensable component in a variety of pharmaceutical, nutraceutical, and cosmetic applications due to their antioxidant, anti-inflammatory, and anti-carcinogenic properties [23]. Flavonoids are accumulated in different parts of the plant; however, in several plants, total flavonoid content was higher in leaves than in the roots [24,25].

Furthermore, total phenolic content (TPC) was assessed, revealing interesting insights into the comparative abundance of phenolic compounds between aerial part and root extracts. The result showed that aerial part extracts contained higher levels of total phenolics (116.53 mg/g) when compared to the root extracts (72.87 mg/g) (Fig. 2C). Phenolic compounds play a pivotal role in plant physiology, possessing redox properties responsible for antioxidant activity [26]. It is noteworthy that the extraction solvent, as well as the plant organ, significantly influenced the contents of phenolic compounds, highlighting the importance of extraction methods in preserving and quantifying these bioactive constituents [11,27,28].

3.2. Antioxidant activities of G. speciosum extract

The antioxidant activities of aerial part and root extracts were investigated, with ascorbic acid (Vit. C) serving as a positive control across all assays. Both aerial part and root extracts showed concentration-dependent increases in radical scavenging capacity. Specifically, at a concentration of 50 mg/mL, the aerial part extract exhibited superior inhibitory activity against DPPH in comparison to the root extract (Fig. 3A). This trend was similarly observed in the ABTS assays (Fig. 3B). Additionally, the reducing power of Fe^{2+} by aerial part and root extracts was assessed, revealing that the aerial part extract exhibited greater reducing antioxidant power than the root extract (Fig. 3C). Collectively, these findings suggest that the aerial part represents a promising natural source of antioxidants with notable activity. This conclusion is supported by the higher concentrations of total flavonoids and phenolics detected in the aerial part

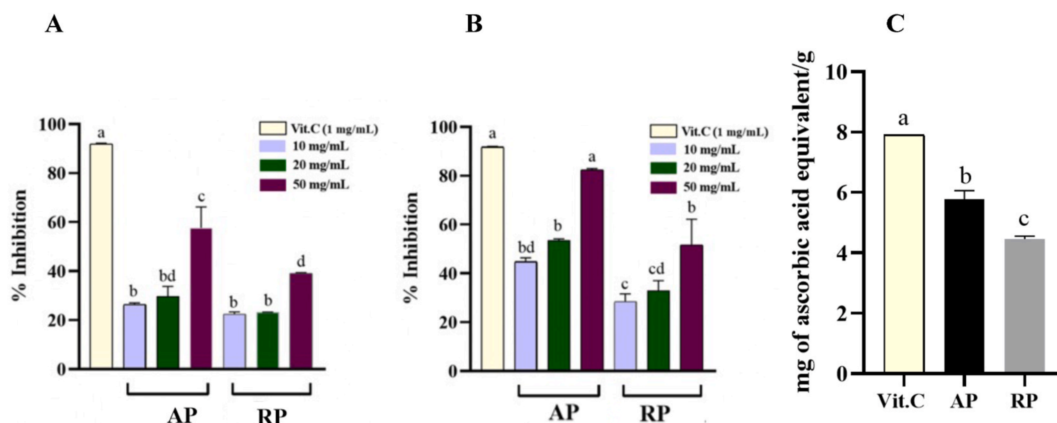

Fig. 3. Evaluation of antioxidant activities of *G. speciosum* extracts using DPPH (A), ABTS (B), and FRAP (C) assays. The experiments were performed in duplicates and measurements were applied in triplicate (n = 3). Tukey's multiple comparison test (One-way ANOVA) was used for testing the statistical difference among the samples ($p < 0.05$). AP: aerial part; RP: root part.

relative to the root.

Natural antioxidants from plants play a pivotal role in protecting against the action of free radicals. These include flavonoids whose chemical structure influences the antioxidant activity, depending on the number and position of free hydroxyl groups. The DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (ferric reducing antioxidant power) assays are commonly used methods to measure antioxidant activity in various substances, including food, beverages, and biological samples [16]. The assays provide quantitative measures of the antioxidant capacity of a sample by assessing its ability to neutralize or reduce different types of radicals. Combining these assays provides a comprehensive understanding of a sample's antioxidant potential.

3.3. Antibacterial activities of G. speciosum extract

Next, the antibacterial activity of *G. speciosum* aerial part ethanol extracts were evaluated using an agar disc diffusion assay. The results revealed that the extract, at a concentration of 1 mg/mL, could inhibit the growth of both gram-positive and *gram*-negative bacteria, with the inhibitory zone measuring lesser than 14 mm (Table 1).

Subsequently, different concentrations of *G. speciosum* aerial part ethanol extract, ranging from 0 to 3 mg/mL, were examined against *E.coli* and MRSA, representing gram negative and gram positive, respectively. The extract significantly suppressed the growth of both *E. coli* and MRSA with the IC_{50} values of 2.04 mg/mL and 1.64 mg/mL, respectively (Fig. 4).

Therefore, Fig. 4 evidently suggests potential antibacterial activity in the *G. speciosum* aerial part extract. This finding supports previous studies showing that orchids such as *Dendrobium crumenatum*, *Brasolia cattleya*, *Sobralia powellii* and *Elleanthus oliganthus* contain phenolic compounds and flavonoid glycosides with strong antibacterial activity against *S. aureus* bacteria [29,30]. From Table 2, flavone compounds found in the aerial part extract of *G. speciosum*, which have been reported for their antibacterial activity, include orientin [31] and vitexin [32] Additionally, another compound present in the extract known for its antibacterial activity is the alkaloid trigonelline [33].

3.4. Ethanol extracts from aerial part of G. speciosum possessed anti-cancer activities against human skin cancer cells (A375)

To investigate the anti-skin cancer activity of *G. speciosum* aerial part ethanol extract, A375 cells were treated with various concentrations of extract ranging from 0 to 375 μg/mL. As a result, the extract at 125 μg/mL significantly inhibited the viability of A375 cells, showing approximately 40 % inhibition. Conversely, the *G. speciosum* aerial part ethanol extract showed no cytotoxicity on keratinocytes (HaCaT) and normal epithelial cells (Vero) even at the higher concentration of 375 μg/mL (Fig. 5)

At concentrations lower than 100 μg/mL of *G. speciosum* aerial part ethanol extracts demonstrated no cytotoxicity on the keratinocyte cell line (HaCaT). Moreover, *G. speciosum* aerial part ethanol exhibited the ability to reduce reactive oxygen species (ROS), inflammatory responses and collagenase activities. Hence, it is suggested to have potential as promising cosmeceuticals agents [10]. According to Fig. 5, our results were consistent with the previous study, indicating that *G. speciosum* aerial part ethanol extract had no effect on keratinocyte cells. Additionally, our results demonstrated that the *G. speciosum* aerial part ethanol extract could significantly inhibit the cell viability of skin cancer cells from the concentration of 125–375 μg/mL. Accordingly, *G. speciosum* pseudobulb ethanol extract has been reported to suppress proliferation of human breast cancer cells (MCF-7) by reducing the levels of phosphorylated AKT and β-catenin [8]. Subsequently, further experiments are required to study the mode of action of the *G. speciosum* aerial part ethanol extract in skin cancer cells.

3.5. Qualitative phytochemical analysis

Phytochemical analysis by LC-MS/MS of aerial part and root extracts from *G. speciosum* revealed various compounds in both primary metabolites such as amino acids and secondary metabolites. However, this study focused on the secondary metabolites, so Table 2 shows secondary metabolite compounds identified by matching standard spectral libraries either positive ion or negative ion modes. The other metabolites annotated by literature review data. These compounds are found in both the arial part and root ethanol extracts of *G. speciosum* and are presented in order of retention time.

Table 1
Antibacterial effects of *G. speciosum* stem ethanol extract. "+" indicates a clear zone measuring lesser than 14 mm. The data represent the results of three independent experiments.

Bacteria (*gram*)	*Grammatophyllum* sp extract concentration (mg/mL)								
	0			0.1			1		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No.1	No. 2	No. 3
E. aerogenes (−)	–	–	–	–	–	–	+	+	+
E. coli (−)	–	–	–	–	–	–	+	+	+
B. subtilis (+)	–	–	–	–	–	–	+	+	+
S. epidermidis (+)	–	–	–	–	–	–	+	+	+
S. aureus (+)	–	–	–	–	–	–	+	+	+
MRSA (+)	–	–	–	–	–	–	+	+	+

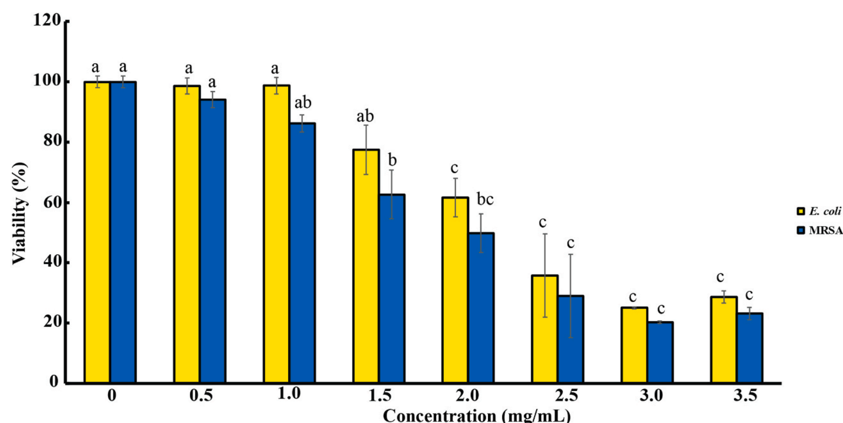

Fig. 4. Quantitative antibacterial activity of *G. speciosum* aerial part ethanol extract. The bar graphs represent mean ± standard error of mean from three independent experiments with duplicates (total n = 6).

Table 2
Liquid chromatography–mass spectrometry (LC–MS) identification of chemical constituents in *G. speciosum* extracts.

Compound	Retention time (min)	Chemical formula	Molecular weight	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	Biological activity	Reference
Orientin	7.81	$C_{21}H_{20}O_{11}$	449.1081	448.1008	447.0931	Antioxidant Anti-bacterial	An, 2012 [40] Lin, 2004 [31]
Tricin	8.92	$C_{17}H_{14}O_7$	331.0815	330.0742	329.0667	Anti-tumor Antioxidant	Lee, 1981 [34] Liu, 2018 [39]
Trigonelline	1.58	$C_7H_7NO_2$	138.0549	137.0476	–	Antioxidant Anti-viral Anti-bacterial Anti-cancer	Zhou, 2012 [33]
Isovitexin	8.15	$C_{21}H_{20}O_{10}$	433.1130	432.1057	–	Anti-cancer	Ninfali, 2007 [41]
Nootkatone	10.41	$C_{15}H_{22}O$	219.1744	218.1671	–	Anti-inflammatory Anti-cancer	Zhang, 2023
Oleamide	15.71	$C_{18}H_{35}NO$	282.2793	281.2720	–	Anti-cancer	Wisitpongpun, 2020 [46]
Nicotinamide	1.62	C6 H6 N2O	123.0554	122.0481	–	DNA repair Anti-cancer	Surjana, t2010 [47]
Vitexin	8.16	$C_{21}H_{20}\ O_{10}$	432.1052	–	431.0979	Antioxidant Anti-cancer Anti-microbial	Chen, 2016 Ninfali, 2007 [41] Das, 2016 [32]
Isorhamnetin	10.22	$C_{16}H_{12}O_7$	315.0508	–	316.0581	Antioxidant Anti-cancer	Pengfei, 2009 Gong, 2020
Piceatannol	8.47	$C_{14}H_{12}O_4$	244.0732	–	243.0659	Anti-cancer	Du, 2017 [38]

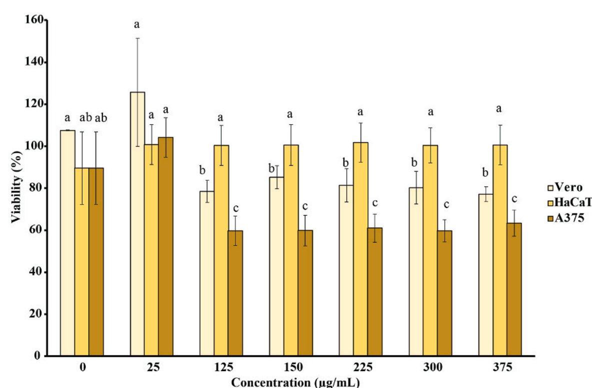

Fig. 5. Cytotoxicity effects of the ethanol extract from aerial part of *G. speciosum* on cells. The data was presented as mean ± standard error of mean from three independent experiments with quadruplicate.

The annotated compounds were classified into six main groups based on their chemical structures. The first group comprises flavones, including orientin, tricin [34], vitexin, isovitexin, and isorhamnetin [35,36] The second group consists of alkaloids, and the annotated compound is trigonelline. In the third group sesquiterpenoid are featured, with nootkatone. The fourth group represents fatty acids, with oleamide as the annotated compound. The fifth group comprises nicotinic acid, with niacinamide as the annotated compound. Lastly, the sixth group includes stilbenoid, with piceatannol [37,38] as the annotated compound (Table 2).

For the flavones, orientin, tricin, and vitexin have been reported for antioxidant activity [39,40], whereas tricin, vitexin, isovitexin, and isorhametin were previously reported for their anti-cancer activities. Isovitexin was demonstrated to inhibit the growth of a breast cancer cell line [41], while tricin inhibited the mortality of colon cancer cells and could suppress the growth and metastasis of lung cancer in a mouse model [42]. Isorhametin was reported to inhibit the viability of a wide range of cancer cell lines, including cervical, lung, colon, breast, pancreatic, nasopharyngeal, liver, and gastric cancer cells [43]. In addition, orientin and vitexin were reported to possess antimicrobial activity [31,32]. Notably, vitexin and orientin were previously identified in the water extract of *G. speciosum* fresh leaves and were reported to possess anti-inflammatory and anti-collagenase activities [10].

Trigonelline was also previously reported in water extract from fresh leaves of *G. speciosum* [10]. Our study also found trigonelline in the aerial part and root of *G. speciosum* ethanol extract. Trigonelline is a plant hormone that regulates plant cell cycle, plant survival, and oxidative stress. It is one of the well-known compounds found in coffee beans and fenugreek seeds [44]. Trigonelline has medical activity, including antioxidant activity, antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Bacillus subtilis*, *Candida albicans*, and *Candida parapsilosis*, and antiviral activity against herpes simplex virus (type 1). Moreover, trigonelline was reported to have anticarcinogenic activity in P-388 lymphocytic leukemia in mice [33].

Other compounds that were first reported in this study include nootkatone [45], oleamide [46], nicotinamide, and piceatannol. Nootkatone, a bicyclic conjugated sesquiterpene ketone, serves as a significant flavouring component in grapes. Renowned for its diverse beneficial properties, it exhibits antimicrobial, insecticidal, antioxidant, anti-inflammatory, anticancer, cardioprotective, neuroprotective, hepatoprotective, and nephroprotective effects [45] Nicotinamide, also referred to as niacinamide, is the amide form of nicotinic acid. Studies have shown its ability to inhibit the growth of transplanted murine breast adenocarcinoma in mice, along with its role in modulating DNA repair mechanisms and maintaining genomic stability [47]. Piceatannol, a stilbene compound and a hydroxyl derivative of resveratrol, has been documented to exhibit antiproliferative, antioxidant, anti-inflammatory effects, and possess chemopreventive properties [48]. It is noteworthy that discrepancies in the phytochemical profile compared to previous reports may be attributed to variances in growing conditions [49], especially considering that this study was the first to report the phytochemical profile of *G. speciosum* cultured *in vitro*.

4. Conclusion

This study provides compelling evidence of the diverse bioactive properties inherent in *G. speciosum* extracts. The distinct flavonoid profiles, antioxidant potential, antibacterial activity, and anticancer effects observed in the aerial part extract underscore its therapeutic potential across various applications. Further investigations are warranted to elucidate the complex molecular mechanisms underlying these effects and pave the way for developing novel therapeutic interventions. Additionally, the phytochemical content in the root of *G. speciosum* also suggests its potential utility as an alternative source of several important bioactive compounds. These findings collectively contribute to expanding our understanding of the pharmacological potential of *G. speciosum* and its prospects for future pharmaceutical and nutraceutical applications.

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Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Jiralapat Thamrongwatwongsa: Validation, Methodology, Investigation. **Jittraporn Chusrisom:** Methodology, Investigation. **Kittiphat Katemala:** Methodology, Investigation. **Siranat Tantasirin:** Methodology, Investigation. **Proudphat Jumnongjit:** Methodology, Investigation. **Pascha Nateerom:** Methodology, Investigation. **Weerasin Sonjaroon:** Resources, Methodology. **Pattama Tongkok:** Writing – review & editing, Resources, Project administration. **Panutchaya Pichaiyotinkul:** Investigation, Methodology. **Atchara Paemanee:** Investigation, Methodology, Writing – review & editing. **Nattanan Panjaworayan T-Thienprasert:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Wannarat Phonphoem:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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