

Article

Phytochemical Profile and Biological Activities of *Allium longistylum* Sprouts

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Abstract

Allium longistylum is a relatively understudied species whose phytochemical composition and biological activities remain largely unexplored. In this study, the first true leaf (FTL) and the second true leaf (STL) of *A. longistylum* were compared with respect to phenolic composition, antioxidant capacity, antimicrobial activity, and quorum-sensing (QS) inhibition. Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically, while antioxidant activity was evaluated using ABTS and DPPH radical scavenging assays. Antimicrobial and anti-QS activities were assessed against *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Chromobacterium violaceum*. STL exhibited significantly higher TPC and TFC than FTL, consistent with its stronger radical scavenging activity. Both extracts showed moderate antimicrobial activity and reduced violacein production in *C. violaceum*, indicating interference with QS. UPLC-Q-Orbitrap-ESI-MS/MS profiling tentatively identified several phenolic acids and flavonoid derivatives. HPLC analysis confirmed the presence of selected phenolic compounds, although several prominent peaks in the chromatograms remained unidentified. Many of the compounds detected by UPLC-Q-Orbitrap-ESI-MS/MS and HPLC have previously been reported to exhibit antioxidant, antimicrobial, and anti-QS activities; their presence may therefore contribute to the bioactivities observed in both extracts. However, their contribution to the observed effects remains speculative and requires further validation through targeted isolation and bioactivity testing. The results suggest that *A. longistylum* is a promising source of phenolic compounds with antioxidant and antimicrobial properties.

Keywords: phenolics; UPLC-Q Orbitrap-ESI-MS/MS; flavonoids; phytochemical profiling



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1. Introduction

The genus *Allium* represents one of the most diverse and economically significant groups within the family Amaryllidaceae, encompassing more than 900 species distributed across temperate regions of the Northern Hemisphere [1]. Several members of this genus, including *A. sativum* and *A. cepa*, are widely consumed as vegetables and used in traditional medicine [2]. *Allium* species are well known for their diverse phytochemical composition,

particularly organosulfur compounds, phenolic acids, and flavonoids, which contribute to their wide range of biological activities [3]. Therefore, increasing attention has been directed toward lesser-known *Allium* species as potential sources of bioactive compounds.

Phenolic acids and flavonoids are key plant secondary metabolites involved in defense against oxidative stress and are major contributors to the antioxidant capacity of plant extracts [4]. Reactive oxygen species, generated during metabolism and further elevated under active photosynthesis, require efficient antioxidant systems in plant tissues [5]. Radical-scavenging assays are widely used to evaluate antioxidant potential and are strongly correlated with phenolic content. The accumulation of these metabolites is often developmentally regulated. The first true leaf represents an initial photosynthetically active stage, while the second true leaf reflects a more developed and metabolically active tissue [6]. The progression from the first to the second true leaf is associated with metabolic reprogramming, including activation of the phenylpropanoid pathway responsible for phenolic and flavonoid biosynthesis [7]. Consequently, phytochemical composition and antioxidant capacity may vary across developmental stages, although such comparisons remain limited for many underexplored *Allium* species.

A. longistylum, commonly known as riverside chive, is a relatively understudied species that has been primarily documented in floristic and taxonomic surveys [8,9]. Despite its classification within a genus rich in bioactive phytochemicals, information regarding its chemical composition and biological properties remains limited. In particular, the phenolic and flavonoid constituents of this species, developmental stage-dependent variation in metabolite accumulation, and their associated biological activities have not been systematically investigated.

In this study, the phytochemical composition and biological activities of the first true leaf (FTL) and the second true leaf (STL) of *A. longistylum* were examined. Total phenolic content (TPC) and total flavonoid content (TFC) were compared between the two tissues, and antioxidant potential was evaluated using ABTS and DPPH radical scavenging assays. Antimicrobial activity was assessed against representative Gram-positive and Gram-negative bacteria using disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays, while quorum-sensing (QS) inhibitory activity was also investigated. In addition, metabolite profiles were characterized and quantified using ultra-performance liquid chromatography–quadrupole orbitrap electrospray ionization tandem mass spectrometry (UPLC-Q-Orbitrap-ESI-MS/MS) and high-performance liquid chromatography (HPLC). To the best of our knowledge, this study provides the first comprehensive evaluation of the phytochemical composition and multifunctional bioactivity of *A. longistylum*, with particular emphasis on developmental stage-dependent differences.

2. Materials and Methods

2.1. Plant Materials and Cultivation Conditions

A. longistylum Baker was collected and authenticated by C.-H. Choi at the Gyeonggi-do Forestry Environment Research Center, Osan, Republic of Korea. Voucher specimens (GFERC 202504) were deposited at the same institution. Seeds of *A. longistylum* were collected in mid-October 2024 from the Yeosu experimental field of the Gyeonggi-do Forestry Environment Research Center and sown in early April 2025 at the Osan experimental field. The germination rate was approximately 75%. Following germination, the plants were grown for approximately 20 days to allow the development of true leaves. FTL and STL were then harvested and used for subsequent experiments (Figure 1).

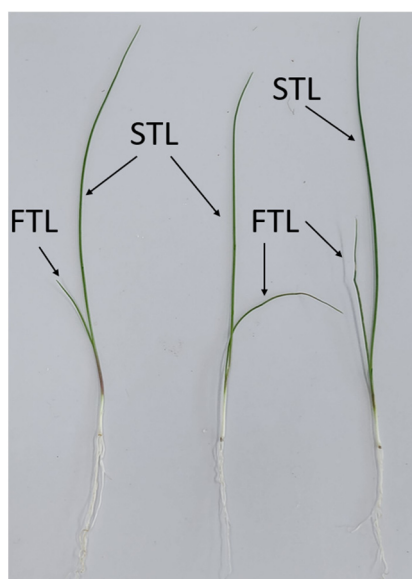


Figure 1. Morphological appearance of *A. longistylum* sprouts showing FTL and STL.

2.2. Instruments and Reagents

All solvents used for chromatographic analyses were of HPLC grade and obtained from commercial suppliers. Methanol (MeOH) and water were purchased from Honeywell Burdick & Jackson (Charlotte, NC, USA). Phosphoric acid and formic acid were obtained from Thermo Fisher Scientific (Waltham, MA, USA), while acetonitrile (ACN) was supplied by Scharlau (Sentmenat, Barcelona, Spain). Ethanol (95%, EtOH), used as the extraction solvent, was obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Gyeonggi, Republic of Korea). Resazurin was acquired from Sigma-Aldrich (St. Louis, MO, USA). Standards used for quantitative analysis included caffeic acid (1), kaempferol 3,4'-di-O-glucoside (2), *p*-coumaric acid (3), ferulic acid (4), hirsutrin (5), and astragalin (6), which were provided by the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 24 February 2026), Anseong, Republic of Korea (Figure 2).

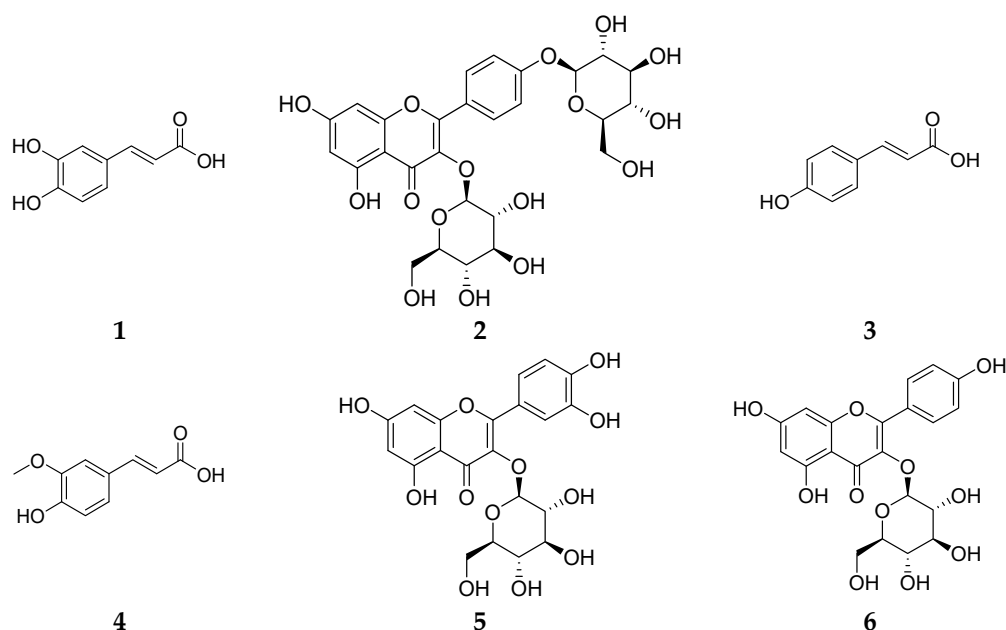


Figure 2. Chemical structures of caffeic acid (1), kaempferol 3,4'-di-O-glucoside (2), *p*-coumaric acid (3), ferulic acid (4), hirsutrin (5), and astragalin (6).

2.3. Crude Extraction

Metabolites from *A. longistylum* tissues were extracted using a reflux method. Dried powdered FTL and STL (1 g each) were transferred into extraction flasks containing 300 mL of absolute ethanol. The mixtures were heated under reflux at 80 °C for 3 h, and this extraction step was repeated three times to maximize compound recovery. After completion of the extraction cycles, insoluble plant residues were removed by filtration. The combined filtrates were then concentrated under reduced pressure using a rotary evaporator to remove the solvent, yielding crude extracts for subsequent analyses.

2.4. TPC Assay

The TPC of FTL and STL extracts was determined using a previously reported method with minor modifications [10]. Briefly, 60 µL of each extract solution was added to the wells of a 96-well microplate, followed by 40 µL of Folin–Ciocalteu reagent (Sigma-Aldrich). The mixture was gently mixed to ensure uniform reaction. Subsequently, 100 µL of 7.5% (*w/v*) sodium carbonate (Sigma-Aldrich) solution was added to initiate color development. The plate was incubated in the dark at room temperature for 30 min to allow complete formation of the blue-colored complex produced by the reduction in the Folin–Ciocalteu reagent by phenolic compounds. After incubation, absorbance was measured at 760 nm using a microplate reader (Epoch; BioTek, Winooski, VT, USA). TPC was quantified using a tannic acid calibration curve prepared at different concentrations, and the results were expressed as milligrams of tannic acid equivalents per gram of extract (mg TAE/g extract).

2.5. TFC Assay

The TFC of FTL and STL extracts was determined using an aluminum chloride colorimetric method with minor modifications [10]. In brief, 100 µL of each extract solution was mixed with 100 µL of 2% aluminum chloride hexahydrate (Sigma-Aldrich) solution in a 96-well microplate. The mixture was gently agitated to ensure uniform mixing and then incubated at room temperature for 10 min to allow the reaction to proceed. During this incubation period, flavonoids in the extracts formed stable complexes with aluminum ions, resulting in the development of a yellow-colored chromophore. Absorbance was measured at 430 nm using the same microplate reader. Quantification was performed using a calibration curve constructed from quercetin standard solutions at various concentrations. The total flavonoid content was calculated based on this curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

2.6. DPPH Radical Scavenging Assay

The antioxidant capacity of FTL and STL extracts was evaluated using the DPPH radical scavenging assay with minor modifications of a previously described protocol [11]. A working DPPH (Waltham, MA, USA) solution (0.2 mM) was prepared by diluting a stock solution with 95% ethanol. For the assay, 10 µL aliquots of each extract solution were dispensed into the wells of a 96-well microplate in triplicate. The reaction was initiated by adding 200 µL of the DPPH working solution to each well. After mixing, the plate was incubated in the dark at room temperature for 30 min to allow the reaction to proceed while minimizing light-induced degradation of the DPPH radical. Absorbance was then measured at 514 nm using a microplate reader, with decreases in absorbance values reflecting the radical scavenging activity of the extracts. Scavenging activity was calculated relative to a control and used to construct concentration–response curves. Ascorbic acid was used as a reference antioxidant standard.

2.7. ABTS Radical Scavenging Assay

Radical scavenging activity against the ABTS⁺ cation was determined using the ABTS decolorization assay with slight modifications to a previously reported method [11]. The ABTS radical cation was generated by preparing a stock solution from the ABTS powder (Thermo Fisher Scientific) and allowing the reaction to proceed under appropriate conditions to form the ABTS⁺ species. Prior to analysis, the stock solution was diluted with distilled water to obtain a working solution with a suitable absorbance. For measurement, 10 µL of each extract was transferred into the wells of a 96-well microplate in triplicate, followed by the addition of 200 µL of the ABTS⁺ working solution. The contents were gently mixed to ensure uniform distribution of reagents, and the plate was incubated in the dark at room temperature for 30 min. During this period, antioxidant compounds in the extracts reduced the ABTS⁺ radical cation, resulting in a decrease in absorbance. After incubation, absorbance was measured at 734 nm using a microplate reader. The percentage of radical scavenging activity was calculated relative to a control lacking the extract. Ascorbic acid was used as a reference antioxidant standard to construct calibration curves and enable comparison of antioxidant capacity.

2.8. Preparation of Microbial Inoculum

Staphylococcus aureus (ATCC BAA-1708) and *Acinetobacter baumannii* (ATCC BAA-1605) were subcultured on Mueller–Hinton agar (MHA) (Sigma-Aldrich), while *Chromobacterium violaceum* (ATCC 12472) was subcultured on Luria–Bertani (LB) agar (Sigma-Aldrich). All cultures were incubated at 37 °C for 18–24 h. Bacterial inocula were prepared according to antimicrobial susceptibility testing procedures recommended by the Clinical and Laboratory Standards Institute (CLSI). Briefly, a loopful of freshly grown culture was transferred into sterile 0.85% normal saline solution. The bacterial suspensions were adjusted to match a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.

2.9. Antimicrobial Activity by Disc Diffusion Assay

Antimicrobial activity was evaluated using the agar well diffusion method, following CLSI recommendations for diffusion-based susceptibility testing with slight modifications. Standardized bacterial inocula were evenly swabbed onto the surface of MHA plates. Wells (6 mm in diameter) were aseptically punched into the agar using a sterile cork borer. Each well was loaded with 30 µL of extract solution (100 mg/mL). Streptomycin was used as the positive control, while 10% dimethyl sulfoxide served as the negative control. The plates were incubated at 37 °C for 18–24 h. After incubation, the diameters of the inhibition zones were measured in millimeters, including the diameter of the well. All assays were performed in triplicate, and results were expressed as mean inhibition zone diameters.

2.10. Anti-QS Activity Assay

Anti-QS activity was evaluated using *C. violaceum* as a biosensor strain, following the agar well diffusion method described by Padayao et al. (2023) with slight modifications [12]. A standardized 0.5 McFarland suspension of *C. violaceum* was uniformly swabbed onto LB agar plates. Each well was loaded with 30 µL of extract (100 mg/mL), and the plates were incubated at 30 °C for 24 h. Anti-QS activity was indicated by the formation of a turbid but colorless halo surrounding the well, signifying inhibition of violacein production without affecting bacterial growth. In contrast, a clear zone around the well was interpreted as antibacterial activity. The diameter of the violacein inhibition zone was measured in millimeters. All assays were performed in triplicate, and results were expressed as mean inhibition zone diameters. The anti-QS zone diameter was calculated by subtracting the

diameter of the antibacterial (clear) zone from the total diameter of the violacein inhibition zone, as shown in the following equation:

$$\text{Anti-QS zone (mm)} = D_{\text{total}} - D_{\text{clear}}$$

2.11. MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were evaluated using a resazurin-based (Sigma-Aldrich, St. Louis, MO, USA) broth microdilution method in accordance with CLSI guidelines and the procedure described by Padayao et al. (2025), with slight modifications [13]. Briefly, two-fold serial dilutions of the extracts (1.56–100 mg/mL) were prepared in 96-well microplates. Bacterial suspensions were standardized spectrophotometrically to 10^6 CFU/mL (OD_{600}) prior to inoculation. Following incubation at 37 °C for 18–24 h, the MIC was defined as the lowest extract concentration that showed no visible bacterial growth. To determine the MBC, aliquots from wells without visible growth were subjected to a confirmatory spot assay by plating onto MHA and incubating for an additional 24 h. The MBC was defined as the lowest concentration at which no colony formation was observed. Streptomycin (10 µg/mL) served as the positive control.

2.12. Phytochemical Profiling by UPLC-Q-Orbitrap-ESI-MS/MS

Chromatographic separation was performed using a Waters Cortecs C18 column (2.1×150 mm, 1.6 µm), which was maintained at 45 °C and operated at a flow rate of 0.3 mL/min. The elution system consisted of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B), delivered under a programmed gradient starting at high aqueous conditions (95% A), gradually decreasing to 5% A between 50 and 55 min, followed by re-equilibration to the initial composition until 60 min. Detection was carried out using a mass spectrometer equipped with a heated electrospray ionization (H-ESI) source functioning in both positive and negative ionization modes, applying spray voltages of 3.5 kV and 3.0 kV, respectively. The ion source parameters included sheath, auxiliary, and sweep gas settings of 50, 10, and 1 arbitrary units, while the ion transfer tube temperature was set at 320 °C. Data acquisition covered an m/z range of 100–1500, with MS^1 and MS^2 scans collected at resolutions of 70,000 and 17,500, respectively. Fragmentation data were obtained using a data-dependent TopN ($n = 10$) method with stepped normalized collision energies of 10, 30, and 50.

2.13. Phytochemical Quantification by HPLC

The extracts and standards were dissolved in HPLC-grade MeOH to prepare stock solutions at the desired concentrations. The solutions were sonicated and filtered through a 0.45 µm PVDF membrane. Quantitative analysis of selected phenolic compounds was performed using a Waters Alliance e2695 HPLC system equipped with a 2489 UV/Visible detector. Separation was achieved on a YMC-Pack Pro C18 column (4.6×250 mm, 5 µm) maintained at 30 °C. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B), delivered at a flow rate of 1.0 mL/min. The gradient program was as follows: 95% A (0–10 min), 67% A (20 min), 0% A (40–45 min), and 5% B (50–65 min). The injection volume was 10 µL, and detection was performed at 330 nm.

2.14. Determination of Linearity

Calibration curves for quantitative analysis were constructed using the reference standards caffeic acid (1), kaempferol 3,4'-di-O-glucoside (2), *p*-coumaric acid (3), ferulic acid (4), hirsutrin (5), and astragalin (6). Each standard compound was serially diluted to generate at least five concentration levels, which were used to construct calibration

curves for the respective analytes. Method validation included determination of the limits of detection (LOD) and limits of quantification (LOQ), which were calculated based on the standard deviation (SD) of the response (σ) and the slope of the calibration curve (S), using the equations $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$. Linearity was evaluated based on the coefficient of determination (R^2), and the concentration of each target compound was calculated using the corresponding calibration equation. In these models, the x -axis represents concentration ($\mu\text{g/mL}$), and the y -axis corresponds to peak area. Values were expressed as mean $\pm$ SD ($n = 3$).

2.15. Statistical Analysis

GraphPad Prism 10.2.3 (GraphPad Software, Boston, MA, USA) was used for statistical analysis and data visualization. Differences were considered statistically significant at $p < 0.05$. Prior to analysis, the data were assessed for normality and log-normal distribution. Comparisons between the two independent groups (FTL and STL) were conducted using an unpaired Student's t -test. Homogeneity of variances was assessed using an F -test, and equal variance was assumed for the analysis. All results are presented as mean $\pm$ SD.

3. Results and Discussion

3.1. TPC and TFC

Significant differences were observed between FTL and STL in terms of phenolic and flavonoid content. The TPC of STL (65.11 mg tannic acid equivalents (TAE)/g extract) was markedly higher than that of FTL (38.77 mg TAE/g extract) (Figure 3a). Similarly, the TFC was greater in STL (0.97 mg quercetin equivalents (QE)/g extract) than in FTL (0.83 mg QE/g extract) (Figure 3b). These findings indicate enhanced accumulation of phenolic and flavonoid compounds in true leaves compared with cotyledons.

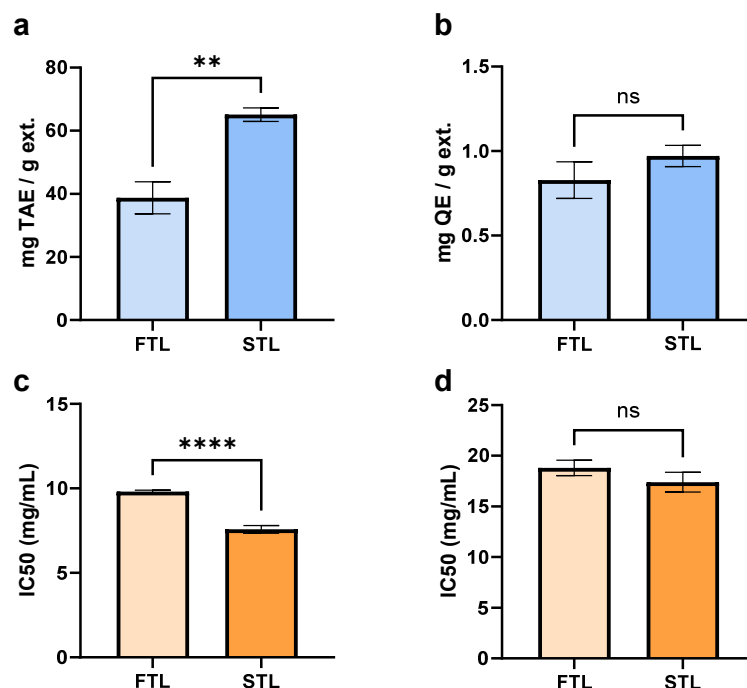


Figure 3. Comparison of TPC (a), TFC (b), ABTS (c), and DPPH (d) radical scavenging activities between FTL and STL extracts. Statistical significance between groups was determined using Student's t -test (** $p < 0.01$; **** $p < 0.001$; ns, not significant).

The present study demonstrates clear differences between FTL and STL in terms of phenolic composition and associated biological activities. These results suggest that the

developmental stage may influence the accumulation and functional roles of secondary metabolites in this species [14]. Similar stage-dependent variations in phenolic accumulation have been reported in other plant species, where metabolite production increases as tissues become fully photosynthetically active [15]. The higher TPC and TFC observed in STL indicate that phenolic biosynthesis increases as the leaf progresses from an earlier to a more developed true leaf stage [16]. Increases in phenolic content between successive leaf developmental stages have also been documented in other medicinal plants, supporting the association between phenolic biosynthesis, tissue maturation, and environmental exposure [17]. This pattern may reflect the ecological role of phenolic metabolites in protecting exposed leaf tissues from oxidative stress, ultraviolet radiation, and microbial attack. FTL represents an earlier developmental stage with comparatively lower metabolic activity, whereas STLs are metabolically active organs that must respond to environmental stressors, which may explain the enhanced production of phenolic compounds in STL [18].

3.2. ABTS and DPPH

Consistent with the higher phenolic and flavonoid levels, STL exhibited stronger radical scavenging activity. In the ABTS assay, STL showed a lower IC_{50} value (7.58 mg/mL) than FTL (9.82 mg/mL) (Figure 3c). Similarly, the DPPH assay indicated superior activity in STL, with a lower IC_{50} value (17.39 mg/mL) compared with FTL (18.80 mg/mL) (Figure 3d). Although STL demonstrated relatively better performance than FTL, it is important to note that the IC_{50} values for both extracts are relatively high, indicating weak antioxidant activity compared to standard antioxidants such as ascorbic acid.

The observed trend therefore reflects a relative difference between the two plant tissues rather than strong intrinsic antioxidant potency. Because lower IC_{50} values indicate higher antioxidant activity, the results of both ABTS and DPPH assays demonstrate that STL has a greater radical scavenging capacity than FTL. Phenolic compounds act as electron or hydrogen donors that stabilize reactive oxygen species; therefore, their higher concentration in STL is likely to contribute to the enhanced activity [19]. However, the relatively low potency observed suggests that either the concentration of active compounds is limited or that highly active constituents are present at low abundance in the crude extracts. Such relatively high IC_{50} values are not uncommon in crude plant extracts, where the presence of inactive or interfering compounds may dilute the overall activity compared to purified standards. This positive relationship between phenolic content and antioxidant capacity has been widely reported in studies of plant extracts rich in phenolics [20]. Interestingly, although the difference in TFC between the two extracts was relatively small, the antioxidant capacity showed a clearer improvement in STL. This further suggests that qualitative differences in phenolic composition, rather than total quantity alone, may play a more critical role in determining antioxidant efficiency. Previous studies have shown that variations in the composition of flavonoids and phenolic acids can significantly affect antioxidant activity, even when total flavonoid levels are similar [21]. The presence of specific phenolic acids and flavonoid glycosides with strong redox properties may further enhance antioxidant effects, even when overall flavonoid concentrations differ only slightly [22].

3.3. Microbial Inhibitory Activity

The disc diffusion method was used to evaluate the antimicrobial activity of FTL and STL against *S. aureus* and *A. baumannii*. Against *S. aureus*, FTL exhibited a mean zone of inhibition (ZOI) of 11.66 ± 0.41 mm, which was slightly larger than that of STL (10.62 ± 0.82 mm) (Figure 4a). In contrast, both extracts showed identical inhibition zones (6.00 ± 0.02 mm) against *A. baumannii* (Figure 4b), indicating relatively weak antibacterial

activity against the Gram-negative strain. This reduced susceptibility may be attributed to the unique cell structure of Gram-negative bacteria, particularly the presence of an outer membrane that limits the penetration of antimicrobial agents [23,24]. As a result, bioactive metabolites that are effective against Gram-positive bacteria often exhibit reduced activity against Gram-negative species. A previous study evaluated the inhibitory effects of *A. sativum* ethanolic extracts against *S. aureus* [25]. Although no inhibition zones were observed for *S. aureus* in the disc diffusion assay, the same study reported measurable MIC and MBC values against this strain, indicating that the extract possesses antibacterial activity under broth conditions. This discrepancy highlights the limitations of agar diffusion assays, which depend on compound diffusibility, and suggests that certain bioactive constituents may be present but exhibit limited diffusion in solid media.

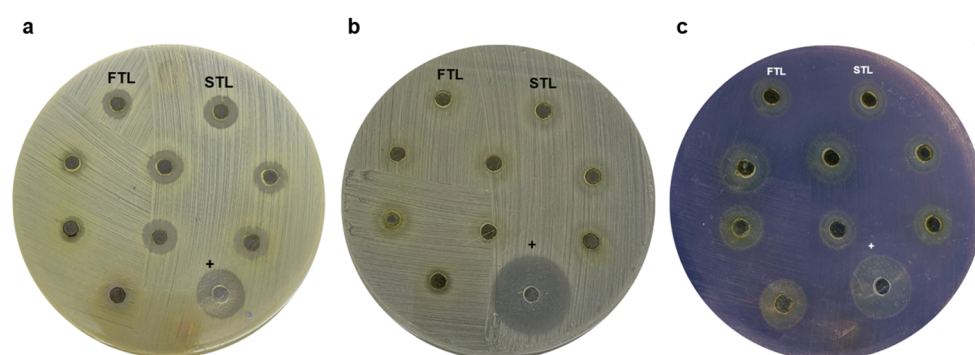


Figure 4. Disc diffusion assay showing the antibacterial activity of FTL and STL against *S. aureus* (a) and *A. baumannii* (b), and anti-QS activity against *C. violaceum* (c). Agar plates inoculated with the test microorganisms were treated with sterile discs containing FTL or STL, and the resulting inhibition zones were recorded after incubation. The (+) symbol indicates the positive control.

A contrasting trend was observed in the antimicrobial disc diffusion assay, where FTL produced a slightly larger inhibition zone against *S. aureus* than the STL extract. This observation suggests that certain antimicrobial metabolites may accumulate preferentially in earlier stages of seedling development [26]. Such tissue-specific distribution of defensive metabolites has been reported in various plant species, where early developmental organs accumulate protective compounds to safeguard seedlings during vulnerable stages of growth [27]. From an ecological perspective, this distribution may be advantageous, as young seedlings are particularly susceptible to soil-borne pathogens immediately after germination. The accumulation of antimicrobial compounds in early-stage true leaves may therefore serve as an early defensive barrier to protect developing tissues. Furthermore, the first true leaf functions as an emerging photosynthetic organ that may also contribute to early-stage defense prior to the full maturation of subsequent leaves [28]. Consequently, the presence of antimicrobial metabolites in these tissues may represent an adaptive strategy to reduce microbial infection during early plant growth.

3.4. QS Inhibition Assay

Anti-QS activity was assessed using *C. violaceum* (Figure 4c). Both extracts exhibited measurable inhibition of violacein production. The antimicrobial inhibition zones were comparable between FTL (13.71 mm) and STL (13.77 mm). For QS inhibition specifically, STL showed slightly higher activity (2.28 ± 0.26 mm) than FTL (2.11 ± 0.29 mm), suggesting marginally greater anti-virulence potential in the true leaves. Although the difference between the two extracts was not statistically significant ($p > 0.05$), this observation indicates that metabolites present in the true leaves may more effectively interfere with the QS regulatory system responsible for violacein production.

The ability of the extracts to inhibit QS in *C. violaceum* indicates potential anti-virulence activity. QS regulates the expression of virulence factors and biofilm formation in many pathogenic bacteria, and disruption of this communication pathway has been proposed as an alternative strategy for managing bacterial infections while minimizing the selective pressure that drives antimicrobial resistance [29,30]. Although the inhibition zones associated with QS suppression were relatively modest, the presence of measurable activity suggests that *A. longistylum* contains compounds capable of modulating bacterial communication pathways. Phenolic acids and flavonoids identified in the metabolite profiling analysis have been reported to interfere with QS signaling molecules, such as acyl-homoserine lactones, providing a possible mechanistic explanation for the observed effects [31].

3.5. MIC and MBC Assay

The antimicrobial activity of FTL and STL against *S. aureus*, *A. baumannii*, and *C. violaceum* was further evaluated by determining the MIC and MBC (Figure 5a). The FTL extract showed MIC values of 25 mg/mL against both *S. aureus* and *A. baumannii*, whereas a lower concentration of 12.5 mg/mL was sufficient to inhibit *C. violaceum*. The corresponding MBC values were 50 mg/mL for *S. aureus* and *A. baumannii*, and 25 mg/mL for *C. violaceum*. In contrast, STL exhibited lower MIC values across all tested microorganisms. The MIC for *S. aureus*, *A. baumannii*, and *C. violaceum* was 12.5 mg/mL, while the MBC was 25 mg/mL for all three strains. Visual confirmation of antimicrobial activity was obtained in the microdilution assay using resazurin as a viability indicator (Figure 5b). Wells containing effective inhibitory concentrations remained blue, indicating suppressed bacterial metabolic activity, whereas wells showing a pink color indicated bacterial growth [32]. The observed color changes were consistent with the MIC values determined for each extract. Despite the observed differences between FTL and STL, the MIC values obtained in this study (12.5–25 mg/mL) are relatively high, indicating weak antimicrobial activity in absolute terms when compared to commonly reported thresholds for plant extracts. In general, MIC values below 8 mg/mL are considered indicative of strong antimicrobial potential [33], suggesting that the extracts evaluated here exhibit only limited potency. However, such relatively high MIC values are not unexpected for crude plant extracts, which consist of complex mixtures of bioactive and non-active constituents [34]. The presence of inactive compounds may dilute the overall antimicrobial effect, while active constituents may be present at low concentrations, thereby reducing apparent potency.

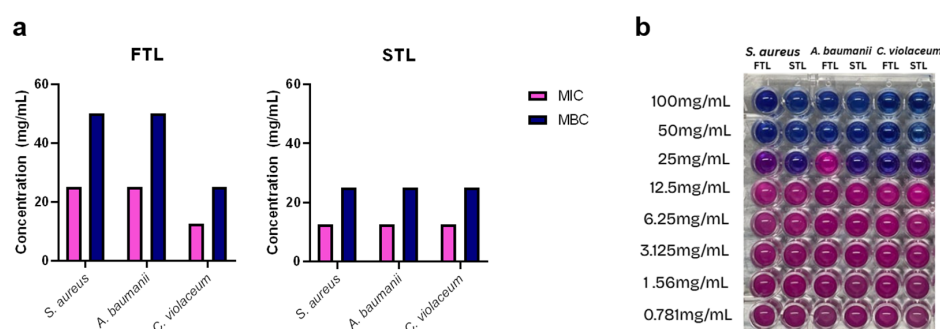


Figure 5. MIC and MBC of the extracts against *S. aureus*, *A. baumannii*, and *C. violaceum*, determined using the broth microdilution method (a). Representative microdilution assay plate using resazurin as a viability indicator (b), where blue wells indicate inhibition of bacterial metabolic activity and pink wells indicate bacterial growth at extract concentrations ranging from 100 to 0.781 mg/mL.

The MIC and MBC results indicate that STL exhibited lower inhibitory concentrations against all tested microorganisms. This suggests a relatively higher abundance or effective-

ness of antimicrobial constituents in STL compared to FTL, although the overall activity remains weak. The relatively high MIC values may also be partially influenced by the intrinsic resistance of the tested strains, particularly *A. baumannii*, a member of the ESKAPE group of multidrug-resistant pathogens [35]. In addition, discrepancies between the disc diffusion and broth microdilution results may be attributed to differences in compound diffusibility, polarity, and solubility in agar-based systems, which can limit the apparent activity of certain phytochemicals in solid media. Such differences have been widely reported in studies of plant-derived antimicrobials [36]. Taken together, these findings suggest that while *A. longistylum* exhibits measurable antimicrobial activity; its potency in crude extract form is limited. Further fractionation and isolation of active constituents may enhance the observed bioactivity and provide a clearer understanding of their antimicrobial potential.

3.6. UPLC-Q-Orbitrap-ESI-MS/MS Profiling

The phytochemical profiles of FTL and STL were investigated using UPLC-Q-Orbitrap-ESI-MS/MS. Base peak chromatograms recorded in both positive and negative ionization modes revealed that the two extracts contained a diverse and complex array of metabolites (Figure 6).

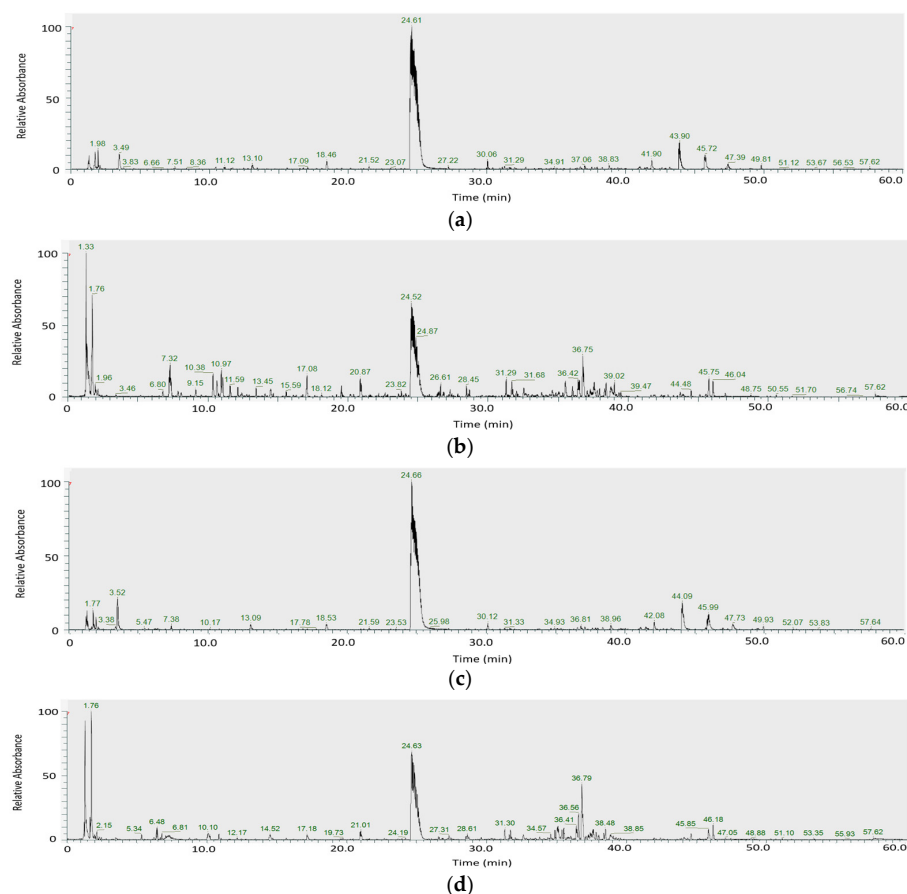


Figure 6. Base peak chromatograms of FTL (a,b) and STL (c,d) in positive and negative ionization modes.

Multiple peaks with varying intensities were observed throughout the chromatographic runs, indicating the presence of numerous secondary metabolites. Putative identification of these compounds was achieved by comparing accurate mass data along with their MS/MS fragmentation patterns [37]. This analysis led to the detection of several phytochemicals, primarily belonging to phenolic acids, flavonoids, flavonoid glycosides, and related aromatic compounds (Tables 1 and 2).

Table 1. Proposed structures of phytochemicals identified in FTL by UPLC-Q-Orbitrap-ESI-MS/MS in negative and positive ionization modes.

t_R (min) ^a	[M-H] ^b	Molecular Weight	Tentative Identity	Ionization Mode
10.22	C ₁₅ H ₁₈ O ₈	326.1	Coumaric acid O-glucoside	negative
10.35	C ₉ H ₆ O ₄	178.0	Esculetin	negative
10.41	C ₁₅ H ₁₈ O ₈	326.1	Coumaroyl hexoside	negative
14.48	C ₉ H ₈ O ₃	164.0	<i>p</i> -Coumaric acid	negative
16.78	C ₂₇ H ₃₀ O ₁₇	626.1	Herbacetin 3,8-di-O-glucoside	positive
17.08	C ₂₇ H ₃₀ O ₁₆	610.2	Kaempferol 3,4'-di-O-glucoside	negative
18.47	C ₁₁ H ₁₆ O ₃	196.1	loliolide	positive
20.92	C ₂₁ H ₂₀ O ₁₁	448.1	Astragalin	negative
21.07	C ₁₅ H ₁₂ O ₆	288.1	Aromadendrin	negative
24.12	C ₁₁ H ₁₂ O ₄	208.1	Ethyl <i>trans</i> -caffeate	negative
25.96	C ₁₅ H ₁₀ O ₆	286.0	Kaempferol	positive

^a Retention time in minutes; ^b molecular formula.**Table 2.** Proposed structures of phytochemicals identified in STL by UPLC-Q-Orbitrap-ESI-MS/MS in negative and positive ionization modes.

t_R (min) ^a	[M-H] ^b	Molecular Weight	Tentative Identity	Ionization Mode
10.10	C ₉ H ₈ O ₄	180.0	Caffeic acid	negative
10.87	C ₂₂ H ₃₀ O ₁₄	518.2	Arillatose B	negative
12.17	C ₂₂ H ₃₀ O ₁₄	518.2	Sibiricose A5	negative
12.49	C ₁₈ H ₂₆ O ₁₀	402.2	Kelampayoside A	negative
14.51	C ₉ H ₈ O ₃	164.0	<i>o</i> -Coumaric acid	negative
14.72	C ₂₇ H ₃₀ O ₁₆	610.2	Kaempferol 3,7-di-O-glucoside	negative
17.17	C ₂₇ H ₃₀ O ₁₆	610.2	Kaempferol 3,4'-di-O-glucoside	negative
17.21	C ₁₀ H ₁₀ O ₄	194.1	Ferulic acid	negative
18.52	C ₁₁ H ₁₄ O ₂	178.1	Hydrocinnamic acid ethyl ester	positive
19.73	C ₂₁ H ₂₀ O ₁₂	464.1	Hirsutrin	negative
21.00	C ₂₁ H ₂₀ O ₁₁	448.1	Astragalin	negative

^a Retention time in minutes; ^b molecular formula.

The chromatographic profiles further indicated that most detected compounds were more readily observed under negative ionization mode, consistent with the ionization behavior of phenolic compounds containing acidic functional groups [38]. Nevertheless, several compounds were also detected in positive ionization mode, demonstrating the presence of structurally diverse metabolites within the extracts. The use of EtOH as the extraction solvent may have influenced the observed phytochemical profile, as its intermediate polarity favors the extraction of phenolic compounds and flavonoids [39], while potentially limiting the recovery of highly non-polar or highly polar constituents. This selective extraction could partially account for the dominance of certain metabolites and the presence of unresolved peaks in the chromatographic profiles.

Untargeted metabolite profiling using UPLC-Q-Orbitrap-ESI-MS/MS provided further insight into the chemical basis underlying the observed biological activities. The identified compounds primarily belong to classes widely recognized for their antioxidant and antimicrobial properties. Phenolic acids are known to disrupt microbial membranes and interfere with enzymatic systems [40], whereas flavonoids can bind to cellular macromolecules such as proteins and nucleic acids, thereby suppressing microbial proliferation [41]. The predominance of these metabolites in the extracts therefore provides a strong chemical rationale for the biological activities observed in this study.

The biological effects observed may be partially attributed to specific compounds identified during metabolite profiling. Phenolic acids such as caffeic acid, *p*-coumaric acid,

and ferulic acid are well-established antioxidants due to their ability to neutralize reactive oxygen species through hydrogen atom or electron donation [42–45]. Similarly, flavonoids, including kaempferol and its glycosides, contain multiple hydroxyl groups capable of stabilizing free radicals, which likely contribute to the radical scavenging activity observed in the ABTS and DPPH assays [46]. These compounds have also been reported to exhibit antimicrobial activity through mechanisms such as disruption of microbial membranes, alteration of membrane permeability, and interference with essential enzymatic processes, which may explain the antibacterial effects observed against *S. aureus* and *A. baumannii* [47].

In addition to their antioxidant and antimicrobial properties, certain phenolic acids and flavonoids have been reported to interfere with bacterial QS systems [48]. These compounds may disrupt bacterial communication by inhibiting the synthesis of signaling molecules or by competitively interacting with QS receptors, thereby suppressing processes such as pigment production, virulence factor expression, and biofilm formation [49]. The presence of these metabolites in *A. longistylum* therefore provides a plausible explanation for the inhibition of violacein production observed in *C. violaceum*.

3.7. HPLC Quantification

The HPLC method achieved excellent separation of compounds 1–6 (Figure 7). All standard compounds exhibited strong linearity over the concentration range of 7.81–125 µg/mL, with high coefficients of determination, indicating robust linear relationships between peak area and concentration.

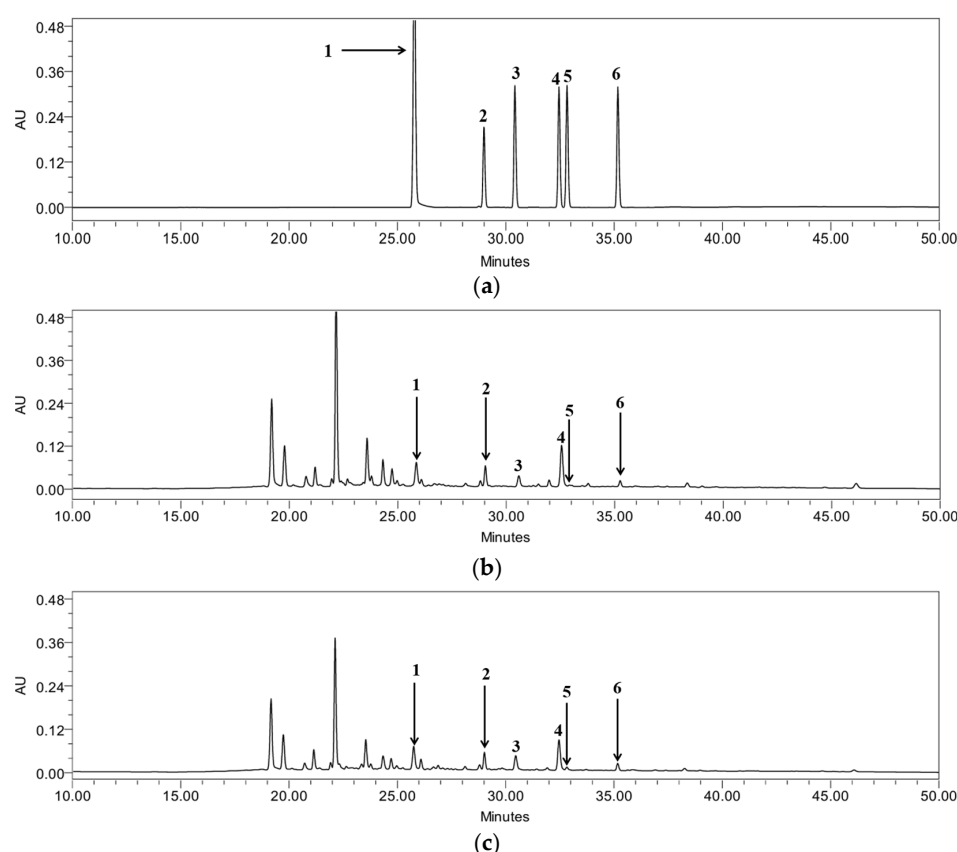


Figure 7. Representative HPLC chromatograms showing compounds 1–6 (a), FTL extract (b), and STL extract (c). The peaks correspond to caffeic acid (1), kaempferol 3,4'-di-O-glucoside (2), *p*-coumaric acid (3), ferulic acid (4), hirsutrin (5), and astragalin (6).

The LOD ranged from 0.86 to 1.73 µg/mL, while the LOQ ranged from 2.62 to 5.24 µg/mL, confirming the sensitivity and reliability of the analytical method (Table 3).

Among the quantified compounds, kaempferol 3,4'-di-O-glucoside (2) was the most abundant constituent in both samples, with a higher concentration observed in FTL than in STL (Table 4). Caffeic acid (1) and *p*-coumaric acid (3) were also detected at appreciable levels, again showing higher concentrations in FTL. In contrast, ferulic acid (4) exhibited substantially greater accumulation in STL compared to FTL. Similarly, hirsutrin (5) and astragalin (6) were slightly more abundant in STL.

Table 3. Linearity, LOD, and LOQ of compounds 1–6.

Compound	t _R ^a (min)	Range (µg/mL)	Calibration Equation	R ² ^b	LOD ^c (µg/mL)	LOQ ^d (µg/mL)
1	25.64	7.81–125	y = 43,454x + 55,815	0.9998	1.64	4.96
2	28.94	7.81–125	y = 10,642x + 18,632	0.9998	1.54	4.66
3	22.18	7.81–125	y = 28,610x + 34,824	0.9999	0.90	2.76
4	22.81	7.81–125	y = 45,068x + 57,472	0.9999	0.86	2.62
5	32.70	7.81–125	y = 32,193x + 48,254	1.0000	1.73	5.24
6	35.06	7.81–125	y = 16,221x + 35,852	0.9998	1.54	4.68

^a Retention time; ^b coefficient of determination; ^c limit of detection; ^d limit of quantification. Compounds: caffeic acid (1), kaempferol 3,4'-di-O-glucoside (2), *p*-coumaric acid (3), ferulic acid (4), hirsutrin (5), and astragalin (6).

Table 4. Quantitative content of compounds 1–6 in FTL and STL extracts.

Sample	Content (mg/g Extract)						Total
	1	2	3	4	5	6	
FTL	0.49 ± 0.01	1.26 ± 0.02	0.52 ± 0.03	0.31 ± 0.25	0.07 ± 0.01	0.20 ± 0.01	2.85
STL	0.46 ± 0.01	1.02 ± 0.02	0.44 ± 0.35	0.88 ± 0.05	0.11 ± 0.01	0.22 ± 0.01	3.13

Compounds: caffeic acid (1), kaempferol 3,4'-di-O-glucoside (2), *p*-coumaric acid (3), ferulic acid (4), hirsutrin (5), and astragalin (6).

Targeted HPLC quantification further highlighted distinct differences in the distribution of phenolic compounds between FTL and STL. While kaempferol 3,4'-di-O-glucoside (2) predominated in FTL, ferulic acid (4), hirsutrin (5), and astragalin (6) were relatively enriched in STL. Notably, the chromatographic profiles also revealed several prominent unidentified peaks, suggesting the presence of additional metabolites that were not characterized in the present study. These peaks were generally more abundant in FTL, as reflected by their higher peak intensities compared to STL, suggesting a greater accumulation of these unidentified constituents in FTL. Although UPLC-Q-Orbitrap-ESI-MS/MS analysis was performed, these major peaks could not be confidently annotated based on the available spectral data and databases, indicating that they may represent less commonly reported or structurally distinct compounds. Based on their retention behavior and UV absorption characteristics, they are tentatively presumed to be structurally related flavonoid glycosides or phenolic derivatives commonly found in *Allium* species. These unidentified constituents may contribute to, and potentially underlie, the slightly stronger antibacterial activity of FTL against *S. aureus* observed in the disc diffusion assay [50]. Thus, the comparatively higher bioactivity of FTL may be attributed, at least in part, to the presence and greater abundance of these unresolved compounds.

Meanwhile, the higher levels of ferulic acid (4) and flavonoid glycosides such as astragalin (6) in STL may contribute to its superior radical scavenging capacity, as these compounds are widely recognized for their strong antioxidant properties.

Taken together, the results suggest that FTL and STL employ distinct chemical strategies for defense. FTL appears to accumulate phenolic compounds that provide immediate antimicrobial protection during early plant development, whereas STL contains higher levels of antioxidants and specific flavonoid derivatives that help mitigate oxidative stress

and environmental exposure. This functional differentiation highlights the importance of the plant developmental stage in determining phytochemical composition and biological activity [51].

Overall, these findings expand the current understanding of the chemical diversity and biological properties of *A. longistylum*. The identification of phenolic acids and flavonoid glycosides associated with antioxidant, antimicrobial, and QS inhibitory activities indicates that this species may serve as a valuable source of naturally occurring bioactive compounds. Furthermore, the observed differences between first and second true leaves emphasize the importance of considering the developmental stage when evaluating the pharmacological and functional potential of plant-derived extracts.

4. Conclusions

This study compared the phytochemical composition and biological activities of FTL and STL extracts. STL contained higher levels of total phenolics and flavonoids than FTL, which corresponded with stronger antioxidant activity in the ABTS and DPPH assays, highlighting the important role of phenolic compounds in protecting photosynthetically active tissues from oxidative stress. Both extracts exhibited moderate antibacterial activity against *S. aureus* and *A. baumannii* and demonstrated QS inhibitory effects in *C. violaceum*, suggesting potential anti-virulence properties. Metabolite profiling using UPLC-Q-Orbitrap-ESI-MS/MS revealed a diverse array of phenolic acids and flavonoid derivatives. Differences in the accumulation of these metabolites between FTL and STL indicate that the plant developmental stage strongly influences secondary metabolite composition and associated bioactivities. Overall, *A. longistylum* contains phenolic compounds associated with antioxidant, antimicrobial, and anti-QS activities, highlighting its potential as a natural source of bioactive constituents for food, pharmaceutical, and functional applications. Future studies should focus on isolating unidentified metabolites and elucidating their mechanisms of action.

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