



Tissue-specific accumulation of anti-inflammatory flavonoids in *Rhododendron micranthum* leaves

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ABSTRACT

Rhododendron micranthum is a plant of traditional medicinal significance, commonly used to treat various inflammation- and respiratory-related conditions. Despite its known therapeutic value, comprehensive phytochemical profiling of its different tissues remains limited. Therefore, this study aimed to analyze and compare the distribution of phytochemicals in the leaves and stems of *R. micranthum* and to validate their corresponding anti-inflammatory properties. The phytochemical distribution was analyzed using high-performance liquid chromatography and liquid chromatography–electrospray ionization mass spectrometry. The anti-inflammatory activity was assessed by measuring nitric oxide (NO) production in lipopolysaccharide-stimulated RAW 264.7 macrophage cells. The leaves were found to be significantly enriched in flavonoids, while the stems accumulated higher levels of triterpenoids and coumarins, and exhibited higher flavonoid content than stems. The anti-inflammatory potential of these compounds was validated in lipopolysaccharide-stimulated RAW 264.7 macrophages, where leaf-enriched compounds, particularly trifolin (3) and avicularin (4), significantly inhibited NO production. These findings highlight the metabolic specialization of *R. micranthum* leaves and suggest their greater potential as a source of bioactive compounds for nutraceutical and cosmetic application.

1. Introduction

The *Rhododendron* genus, part of the Ericaceae family, comprises approximately 1000 species distributed across Asia, Europe, and North America (Zhang et al., 2015). Many of these species have a long history of use in traditional medicine due to their diverse therapeutic properties, including anti-inflammatory, analgesic, antioxidant, and antimicrobial effects (Lee et al., 2025, 2024; Li et al., 2013; Popescu and Kopp, 2013). *Rhododendron micranthum*, an evergreen shrub within the Ericaceae family and *Rhododendron* genus, is primarily found in Korea, particularly in the Gyeongsang, Gangwon, and Chungcheong Provinces (Lee et al., 2025; Zhang et al., 2015). *R. mucronulatum* is another species widely distributed in Korea and is valued for both its ornamental and medicinal uses (Mok and Lee, 2013). Recent studies have identified significant bioactive compounds in these species, particularly flavonoids such as quercitrin and myricitrin, which exhibit strong antioxidant and aldose

reductase inhibitory activities. These findings suggest potential therapeutic applications, especially for managing diabetic complications (Park et al., 2022). Overall, the *Rhododendron* genus exhibits high genetic and phytochemical diversity, highlighting its value as a research resource in pharmacology, genetics, and horticulture.

One particular species, *R. micranthum*, commonly known as spike rosebay, is a semi-evergreen shrub native to northern and northeastern China, as well as parts of Korea (Lee et al., 2025; Zhang et al., 2015). In traditional Chinese medicine, its twigs and leaves have been used to treat conditions such as chronic bronchitis, menstrual pain, high blood pressure, and rheumatoid arthritis (Yang et al., 2022). Despite its widespread medicinal use, the plant's complete phytochemical profile remains largely unexplored. Previous research has identified several bioactive compounds in *R. micranthum*, primarily flavonoids (Liu et al., 2022; Zhang et al., 2013), along with diverse diterpenoids, including grayanane-, micranthane-, mollane-, and 1,5-seco-grayanane-type

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structures (Chang et al., 2011; Jin et al., 2021; Zhu et al., 2019). In particular, grayanane-type diterpenoids have been isolated and are known for their strong immunomodulatory and anti-inflammatory properties (Sun et al., 2019; Zhang et al., 2013). However, most studies have focused on specific chemical groups or individual bioactive molecules, leaving a gap in the scientific understanding of how these compounds are distributed across different parts of the plant. A more comprehensive analysis of the phytochemical variation within *R. micranthum* could offer deeper insights into its medicinal properties and support more effective applications in herbal medicine. Furthermore, while the genus *Rhododendron* is well-characterized phytochemically, the bioactivity of individual species, especially *R. micranthum*, is not yet fully understood (Liu et al., 2024). Notably, no studies to date have thoroughly investigated the anti-inflammatory potential of *R. micranthum*, particularly its ability to inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells, a key marker of anti-inflammatory efficacy. This study aims to fill this critical gap by analyzing and comparing the tissue-specific phytochemical profiles of *R. micranthum* and validating their anti-inflammatory activities. Such insights are essential to substantiate traditional uses, explore new therapeutic applications, and lay the groundwork for the development of *R. micranthum*-based nutraceuticals and pharmaceuticals.

Bioactive compounds were analyzed via liquid chromatography–electrospray ionization mass spectrometry (LC–ESI/MS) and high-performance liquid chromatography (HPLC), both of which are widely used techniques for identifying and quantifying phytochemicals. To interpret the resulting complex chemical data and uncover distinct tissue-specific signatures, this study applied a suite of multivariate statistical analyses, including metabolite set enrichment analysis (MSEA). Additionally, to correlate these phytochemical profiles with biological function and support the plant's ethnobotanical claims, we evaluated the anti-inflammatory potential of the extracts and purified compounds by measuring their inhibitory effects on NO LPS-stimulated RAW 264.7 macrophages. Through this integrated chemical and biological approach, we aim to establish a robust scientific foundation for the medicinal use of *R. micranthum*. Ultimately, the findings of this study will strengthen the scientific basis for its therapeutic applications and guide future research into its bioactive constituents.

2. Materials and methods

2.1. Plant materials

R. micranthum Turcz. (Ericaceae) samples were collected from the Korea National Arboretum (37.453°N, 127.102°E) in Pocheon, Korea, and from the Midongsan Arboretum (36.627°N, 126.665°E) in

Cheongju, Korea on September 10, 2024 (Figs. 1 and 2). All samples were authenticated by Dr. Kyung Choi, Forest Biodiversity Research Division, Korea National Arboretum, Pocheon, Korea. Leaf and stem samples from the Korea National Arboretum were designated as KAL and KAS, respectively, while those from the Midongsan Arboretum were labeled MAL and MAS, respectively.

2.2. Instruments and reagents

Samples were analyzed using an HPLC system consisting of an Agilent 1260 Infinity II Quaternary Pump, autosampler, and variable wavelength detector (Agilent, Santa Clara, CA), equipped with a YMC Pack-Pro C18 column (4.6 × 250 mm, 5 µm; YMC Co., Ltd., Kyoto, Japan). HPLC-grade solvents, including water, acetonitrile (ACN), and methanol (MeOH), were purchased from J. T. Baker (Phillipsburg, PA). Ethanol (EtOH) and trifluoroacetic acid (TFA) were obtained from Samcheon Chemical (Pyeongtaek, Korea). LPS and Griess reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5) were obtained from the Natural Product Institute of Science and Technology (Anseong, Korea) (Fig. 3).

2.3. LC–ESI/MS conditions

Separations were performed using an Ultimate 3000 UHPLC system equipped with a Waters Cortex T3 column (150 mm × 2.1 mm, 1.6 µm), maintained at 45 °C. The mobile phase consisted of water with 0.1 % formic acid (eluent A) and ACN with 0.1 % formic acid (eluent B). The gradient program began at 3 % B, increased to 15 % B over 15 min, then to 100 % B over the next 35 min, and was held at 100 % B for an additional 5 min, resulting in a total run time of 55 min. The column was then re-equilibrated at 3 % B for 5 min. The flow rate was set to 0.25 mL/min. Mass spectrometry and tandem mass spectrometry (MS and MS/MS) detection were carried out using a Triple TOF 5600⁺ system (AB SCIEX, Concord, Canada) in both positive and negative ESI modes. Full-scan and information-dependent acquisition (IDA) modes were used, covering a mass range of m/z 100–2000. Experimental parameters included ion spray voltages of 5.5 kV (positive mode) and 4.5 kV (negative mode), with gas-1 and gas-2 pressures both set at 50 psi. Curtain gas was maintained at 25 psi, collision gas at 20 psi, and the source temperature was set to 500 °C. Collision energies were set at 35 ± 15 eV for the positive mode and −35 ± 15 eV for the negative mode. Compound identification was achieved by comparing the acquired mass spectra to reference spectra from the NIST and MoNA databases, as well as an in-house spectral library.

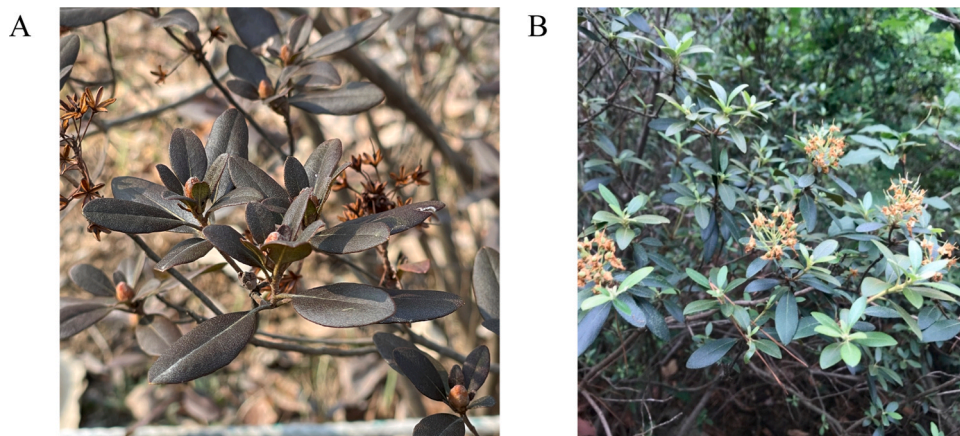


Fig. 1. Plant samples collected from the Korea National Arboretum (KAL/KAS) (A) and Midongsan Arboretum (MAL/MAS) (B).

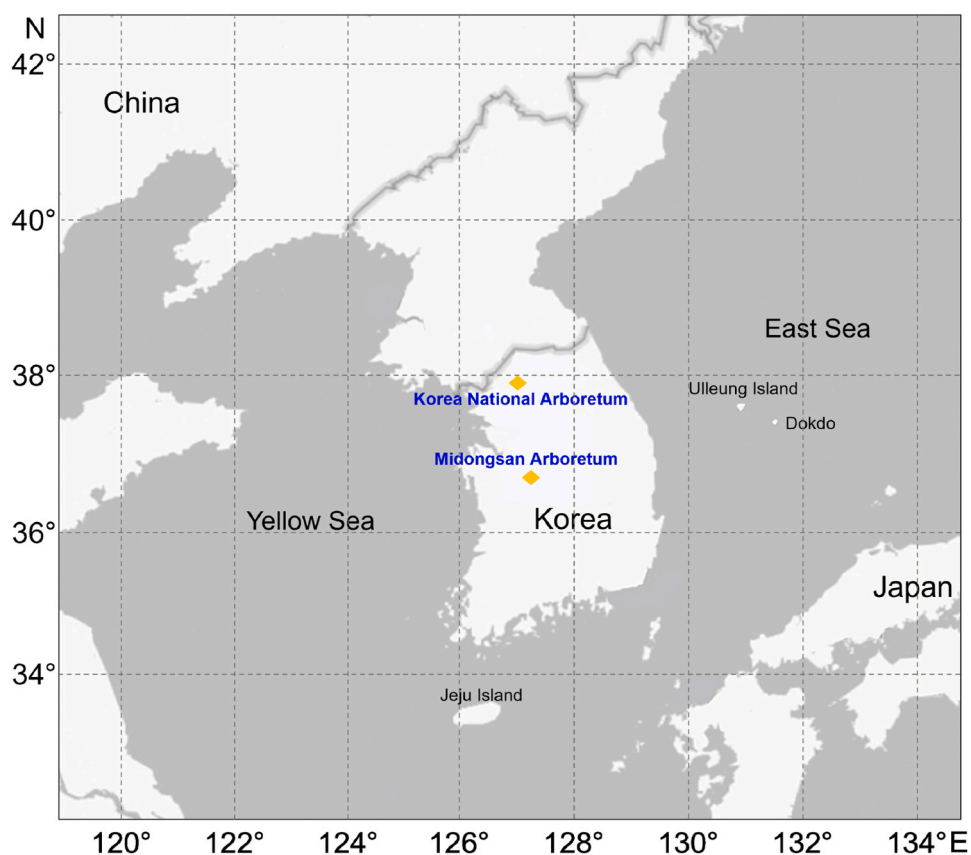


Fig. 2. Collection sites of *R. micranthum* samples from the Korea National Arboretum (KAL and KAS) and Midongsan Arboretum (MAL and MAS).

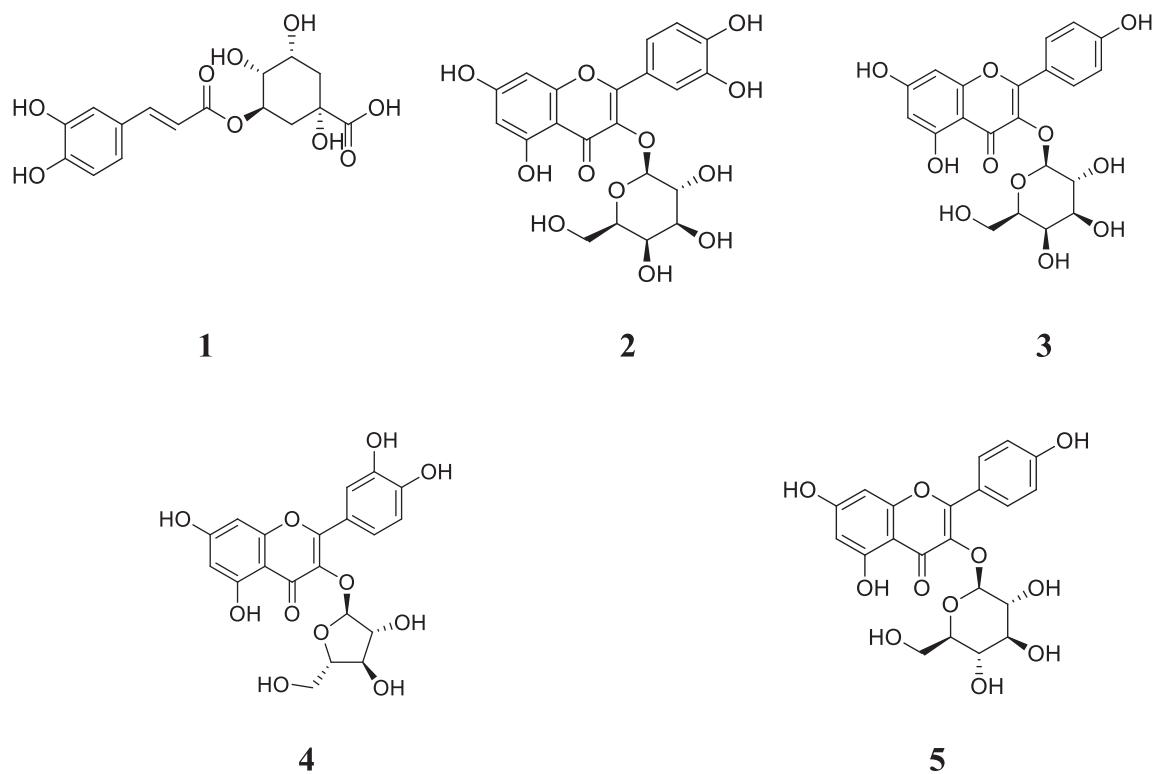


Fig. 3. Chemical structures of chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5).

2.4. Preparation of samples and standard solutions for HPLC

The KAL, KAS, MAL, and MAS samples were air-dried in the shade for two weeks prior to homogenization. Subsequently, ten grams of each sample were combined with 200 mL of 95 % EtOH and refluxed at 80 °C for three times. The resulting EtOH extracts were dissolved in MeOH at a concentration of 20 mg/mL and filtered through a syringe filter (PVDF, 0.20 µm). Stock solutions of the standard compounds were prepared by dissolving them in MeOH. Working solutions for standard calibration curves were prepared by diluting the stock solutions to concentrations ranging from 12.5 to 200 µg/mL.

2.5. HPLC conditions

HPLC analysis was performed on a reversed-phase system using a YMC Pack-Pro C18 column (4.6 × 250 mm, 5 µm), following conditions similar to previous studies (Choi et al., 2023; Hong et al., 2024; Lee et al., 2023). Detection was carried out at 254 nm, with an injection volume of 10 µL. The analysis was conducted at 30 °C with a flow rate of 0.8 mL/min using a gradient elution. The mobile phase consisted of 0.1 % TFA in water (A) and ACN (B). The gradient program was as follows: 13 % B from 0 to 10 min, 23 % B from 10 to 20 min, maintained at 23 % B from 20 to 25 min, 50 % B from 25 to 40 min, then increased to 100 % B from 41 to 45 min, and held at 100 % B until 60 min.

2.6. Calibration curves

Calibration curves were constructed by plotting the concentrations of the standard solutions against their corresponding peak areas. Linearity was assessed using the correlation coefficient (r^2), and standard concentrations in the samples were calculated from the calibration curves. Calibration equations were expressed based on peak area (Y) and concentration (X, mg/mL), with results reported as mean ± standard deviation ($n = 3$) (Table 1).

2.7. Cell culture

RAW 264.7 cells were purchased from the Korea Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 units/mL penicillin-streptomycin and 10 % fetal bovine serum. Cultures were maintained at 37 °C in a humidified incubator with 5 % CO₂.

2.8. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cells were seeded into 96-well plates at a density of 5×10^5 cells/mL and incubated overnight at 37 °C. The following day, cells were treated with various concentrations of the test samples and incubated for 24 h. After incubation, 5 mg/mL MTT solution was added to each well and allowed to react for 4 h. Formazan crystals formed in viable cells were solubilized using dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader (Thermo Fisher, Waltham, MA).

Table 1
Calibration curve equations for compounds 1–5.

Compound	t_R	Calibration equation ^a	Correlation factor, r^2
1	11.8	$Y = 2427.3X - 0.6$	0.9999
2	25.5	$Y = 4578.0X + 0.2$	0.9999
3	28.1	$Y = 4524.1X + 1.2$	0.9999
4	29.3	$Y = 4514.5X - 0.1$	1.0000
5	29.6	$Y = 4928.5X - 1.2$	0.9999

t_R , retention time;

^a Y = peak area, X = concentration of the standard (µg/mL); r^2 , correlation coefficient for five calibration data points; Compounds: chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5)

2.9. Measurement of NO production

NO production was assessed by measuring nitrite accumulation in the culture medium using the Griess reagent. Briefly, cells were seeded into 96-well plates at a density of 5×10^5 cells/mL and incubated overnight at 37 °C. Cells were pre-treated with the test samples for 2 h, followed by the addition of 1 µg/mL LPS. After 24 h, 100 µL of the cell culture supernatant were mixed with 100 µL of Griess reagent in a 96-well plate. Nitrite levels were quantified by measuring absorbance at 560 nm using a microplate reader.

2.10. Statistical analysis

All results are presented as mean ± standard error (SE, $n = 3$) from three independent experiments. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons, using GraphPad Prism (version 8.0.2; GraphPad Software, Boston, MA). A p -value of < 0.05 was considered statistically significant. MSEA was conducted using the enrichment analysis module in MetaboAnalyst 6.0 (Pang et al., 2024) [<https://www.metaboanalyst.ca/> (accessed on 22 July 2025)].

3. Results

3.1. LC-ESI/MS profiling and MSEA for chemical structure classification of *R. micranthum*

To characterize the chemical constituents of *R. micranthum*, LC-ESI/MS profiling was performed on leaf (Fig. 4) and stem (Fig. 5) extracts under both positive and negative ionization modes. A total of 23 compounds were tentatively identified from the leaf extract (Table 2) and 35 compounds from the stem extract (Table 3), based on their retention times, molecular weights, and elemental compositions. The identified compounds included a diverse array of flavonoids, phenolic acids, proanthocyanidins, and diterpenoids, underscoring the chemical complexity and pharmacological potential of this species. Notably, the leaf extract (Table 2) was rich in flavonol glycosides, such as chlorogenic acid, myricetin 3-galactoside, hyperin, avicularin, astragalin, quercetin, and kaempferol, all of which are widely known for their anti-inflammatory, anticancer, and skin-protective properties.

Metabolite profiles between the leaf and stem at the group level were compared via MSEA (Fig. 6). The analysis revealed that in the leaf, flavonoid-related subgroups, including flavonoid glycosides, flavones, and flavans, were the most significantly enriched sets. This finding is consistent with the identification of numerous flavonoid compounds listed in Table 2. In contrast, the stem exhibited a distinct chemical profile, where, in addition to flavonoid glycosides, significant enrichment was observed in biflavonoids and polyflavonoids, triterpenoids, and coumarin-related groups (including coumarin glycosides and hydroxycoumarins). This suggests that compounds uniquely detected in the stem extract, such as esculin, fraxin, ursolic acid, and betulin (Table 3), contributed to the enrichment of these metabolite classes. Collectively, the results from LC-ESI/MS profiling and MSEA demonstrate that the leaf and stem of *R. micranthum* accumulate distinct major classes of metabolites. Particularly, the leaf is characterized by a chemical composition dominated by diverse flavonoids, whereas the stem contains a distinct profile rich in triterpenoids and coumarins, alongside flavonoids.

3.2. HPLC analysis

Quantitative analysis of chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5) in the KAL, KAS, MAL, and MAS samples is presented in Table 4. The total concentration of the five compounds varied significantly across different plant parts (Fig. 7). The highest content was observed in KAL, with a total of 66.44 mg/g,

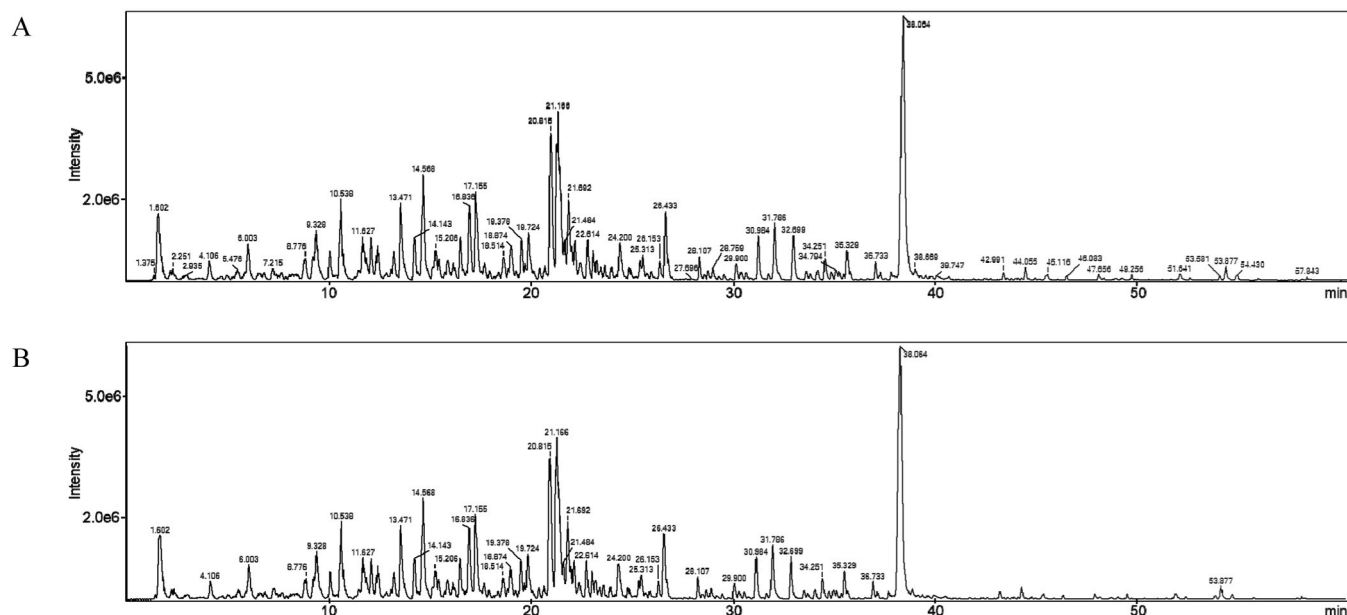


Fig. 4. Total ion chromatograms of *R. micranthum* leaf extract in positive (A) and negative (B) ionization modes.

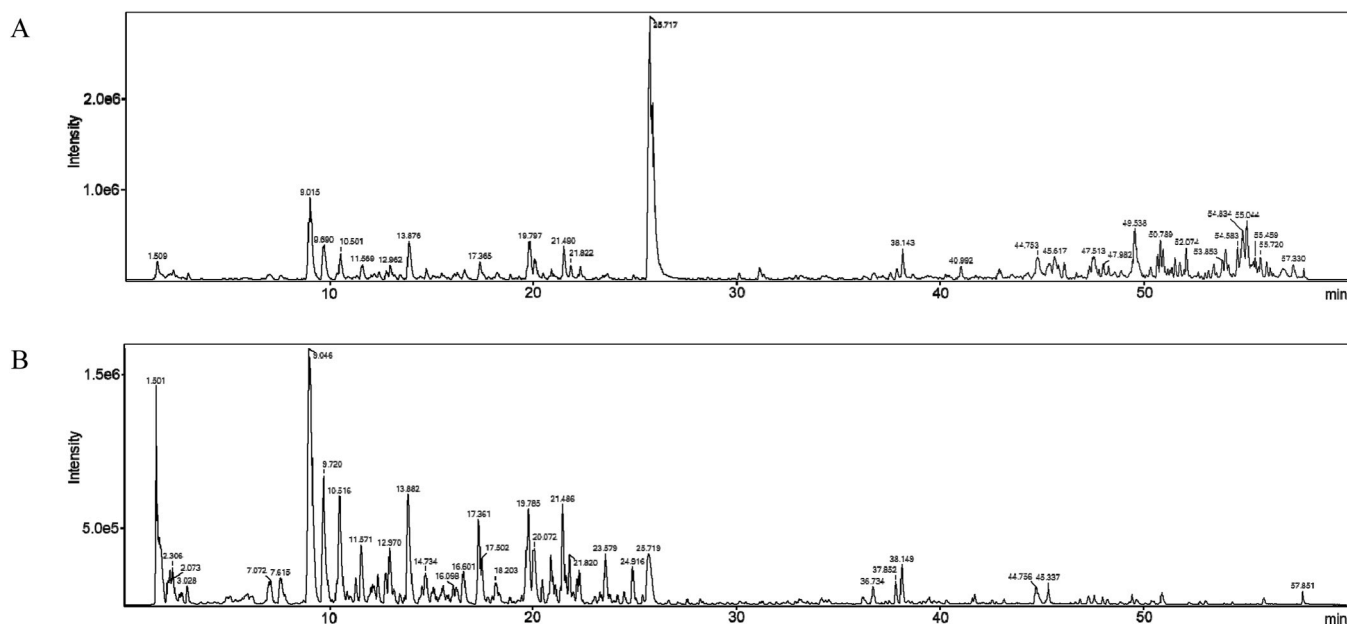


Fig. 5. Total ion chromatograms of *R. micranthum* stem extract in positive (A) and negative (B) ionization modes.

primarily consisting of hyperin (2, 25.24 mg/g) and chlorogenic acid (1, 16.54 mg/g). MAL also exhibited a high total content of 60.48 mg/g, with hyperin (2, 25.11 mg/g) and chlorogenic acid (1, 18.35 mg/g) as the dominant compounds. In contrast, KAS and MAS contained substantially lower total amounts at 3.68 mg/g and 2.82 mg/g, respectively. Both KAS and MAS samples contained very low levels of all five compounds, with trifolin (3) and astragalin (5) being the least abundant. Overall, leaf samples (KAL and MAL) exhibited significantly higher flavonoid content compared to stem samples (KAS and MAS), indicating that the leaves serve as the primary storage organs for these bioactive compounds in *R. micranthum*. These findings highlight that the concentrations of chlorogenic acid (1) and flavonoids vary among different plant parts, which may contribute to differences in their pharmacological and medicinal properties.

3.3. Anti-inflammatory activity of *R. micranthum* extract and identification of its active compounds

The results from MTT assay show that *R. micranthum* has no cytotoxicity to RAW 264.7 cells at concentrations lower than 100 µg/mL, thus non-toxic concentrations ranging from 12.5 to 50 µg/mL were selected for further investigation. Its active compounds did not have any toxic effect at concentration of 1 µg/mL (Fig. 8). To assess the anti-inflammatory potential of *R. micranthum* extract and its five compounds, NO levels were determined by Greiss assay in LPS-stimulated RAW 264.7 macrophages. As shown in Fig. 9, LPS treatment markedly increased NO production (318.22 %), compared to non-treatment (100 %), indicating that LPS induces inflammatory response. However, treatment with *R. micranthum* leaf extract at a concentration of 50 µg/mL significantly suppressed NO production to 287.75 %,

Table 2

LC-ESI/MS profiling of *R. micranthum* leaf extracts in positive and negative ionization modes.

t _R (min)	MW	Elemental composition	Proposed compound
5.63	306.1	C ₁₅ H ₁₄ O ₇	Epigallocatechin
7.23	354.1	C ₁₆ H ₁₈ O ₉	Neochlorogenic acid
9.33	306.1	C ₁₅ H ₁₄ O ₇	Gallocatechin
10.04	290.1	C ₁₅ H ₁₄ O ₆	Catechin
10.55	354.1	C ₁₆ H ₁₈ O ₉	Chlorogenic acid
12.34	578.1	C ₃₀ H ₂₆ O ₁₂	Procyanidin B2
13.51	338.1	C ₁₆ H ₁₈ O ₈	Coumaroylquinic acid
14.33	480.1	C ₂₁ H ₂₀ O ₁₃	Quercetagitrin
15.35	866.2	C ₄₅ H ₃₈ O ₁₈	Procyanidin C1
16.84	480.1	C ₂₁ H ₂₀ O ₁₃	Myricetin 3-galactoside
17.14	412.2	C ₂₂ H ₃₆ O ₇	Grayanotoxin I
17.19	480.1	C ₂₁ H ₂₀ O ₁₃	Gossypin
19.37	464.1	C ₂₁ H ₂₀ O ₁₂	Hirsutrin
19.73	464.1	C ₂₁ H ₂₀ O ₁₂	Hyperin
20.87	434.1	C ₂₀ H ₁₈ O ₁₁	Avicularin
21.07	448.1	C ₂₁ H ₂₀ O ₁₁	Astragalin
21.25	448.1	C ₂₁ H ₂₀ O ₁₁	Trifolin
21.70	318.0	C ₁₅ H ₁₀ O ₈	Myricetin
23.27	446.1	C ₂₂ H ₂₂ O ₁₀	Sissotrin
24.25	302.0	C ₁₅ H ₁₀ O ₇	Quercetin
26.44	286.0	C ₁₅ H ₁₀ O ₆	Kaempferol
29.91	300.1	C ₁₇ H ₁₆ O ₅	Farrerol
34.81	314.1	C ₁₈ H ₁₈ O ₅	4-Hydroxy-2',4',6'-trimethoxychalcone

t_R, retention time; MW, molecular weight.

Table 3

LC-ESI/MS profiling of *R. micranthum* stem extracts in positive and negative ionization modes.

t _R (min)	MW	Elemental composition	Proposed compound
9.70	340.1	C ₁₅ H ₁₆ O ₉	Esculin
10.37	306.1	C ₁₅ H ₁₄ O ₇	Epigallocatechin
10.89	290.1	C ₁₅ H ₁₄ O ₆	Catechin
11.03	354.1	C ₁₆ H ₁₈ O ₉	Chlorogenic acid
11.57	178.0	C ₉ H ₆ O ₄	Esculetin
12.06	354.1	C ₁₆ H ₁₈ O ₉	Cryptochlorogenic acid
12.77	578.1	C ₃₀ H ₂₆ O ₁₂	Procyanidin B4
12.96	578.1	C ₃₀ H ₂₆ O ₁₂	Procyanidin B2
13.06	370.1	C ₁₆ H ₁₈ O ₁₀	Fraxin
13.88	372.1	C ₁₇ H ₂₄ O ₉	Syringin
14.44	290.1	C ₁₅ H ₁₄ O ₆	Epicatechin
14.72	354.1	C ₁₆ H ₁₈ O ₉	1-Caffeoylquinic acid
15.46	480.1	C ₂₁ H ₂₀ O ₁₃	Quercetagetin-7-O-glucoside
17.29	866.2	C ₄₅ H ₃₈ O ₁₈	Procyanidin C1
17.80	480.1	C ₂₁ H ₂₀ O ₁₃	Myricetin-3-O-galactoside
18.19	576.1	C ₃₀ H ₂₄ O ₁₂	Procyanidin A1
19.71	576.1	C ₃₀ H ₂₄ O ₁₂	Procyanidin A2
20.00	464.1	C ₂₁ H ₂₀ O ₁₂	Hirsutrin
20.81	464.1	C ₂₁ H ₂₀ O ₁₂	Hyperin
21.11	434.1	C ₂₀ H ₁₈ O ₁₁	Guaiaverin
21.46	434.1	C ₂₀ H ₁₈ O ₁₁	Avicularin
21.79	448.1	C ₂₁ H ₂₀ O ₁₁	Astragalin
21.97	478.1	C ₂₂ H ₂₂ O ₁₂	Isorhamnetin 3-galactoside
22.32	318.0	C ₁₅ H ₁₀ O ₈	Myricetin
23.62	436.1	C ₂₁ H ₂₄ O ₁₀	Phlorizin
24.50	448.1	C ₂₂ H ₂₄ O ₁₀	Isosakuranin
34.21	302.0	C ₁₅ H ₁₀ O ₇	Quercetin
36.23	488.4	C ₃₀ H ₄₈ O ₅	Pygenic acid B
36.68	488.4	C ₃₀ H ₄₈ O ₅	Caulophyllogenin
37.85	472.4	C ₂₀ H ₂₈ O ₃	Echinocystic acid
45.04	488.4	C ₃₀ H ₄₈ O ₄	Pygenic acid B
45.52	456.4	C ₃₀ H ₄₈ O ₅	Ursolic acid
46.90	442.4	C ₃₀ H ₄₈ O ₃	Betulin
47.30	454.3	C ₃₀ H ₅₀ O ₂	Wilforlide A
55.20	472.4	C ₃₀ H ₄₆ O ₃	Corosolic acid

t_R, retention time; MW, molecular weight.

compared to cells treated with LPS alone. Among the five tested compounds, trifolin (3), avicularin (4), and astragalin (5) significantly reduced LPS-induced NO production. Notably, trifolin (3) and avicularin (4) inhibited NO production comparable to the positive control, dexamethasone. These findings suggest that *R. micranthum* extract may attenuate inflammatory responses, potentially mediated, at least partially, by its active constituents, trifolin (3) and avicularin (4).

4. Discussion

This study revealed clear tissue-specific metabolic profiles in *R. micranthum*, with a marked accumulation of flavonoids and phenolic acids in leaf tissues compared to the stem. Through an integrated approach combining LC-ESI/MS profiling, HPLC quantification, and multivariate statistical analysis, our findings demonstrated that the leaf of *R. micranthum* serves as the primary reservoir of key secondary metabolites with pharmacological relevance. These findings are consistent with the plant's ethnomedicinal use in treating inflammation and provide a scientific basis for tissue-specific therapeutic applications. The initial LC-ESI/MS profiling uncovered a rich and complex array of phytochemicals, highlighting the plant's metabolic diversity.

One particularly notable finding was the distinct metabolic profile of the stem, as revealed by the MSEA results (Fig. 6). The prominent accumulation of flavonoids in the leaf aligns with previous reports in various plant species, where these compounds are associated with protective roles against light-induced and environmental stress (Du et al., 2022; Hao et al., 2023; Lee et al., 2020; Zeng et al., 2025). In contrast, the enrichment of triterpenoids in the stem is consistent with studies showing the preferential accumulation of terpenoids in non-leaf organs, where they serve structural, storage, or defensive functions (Lee et al., 2020; Yu et al., 2024; Zeng et al., 2025). Although coumarin enrichment in stems is less widely reported, it has been observed in targeted analyses of other plant species, suggesting it may represent a species-specific metabolic adaptation (Kabbash et al., 2023; Lee et al., 2020). In this context, the distribution of terpenoids in *R. micranthum* stems parallels the diosgenin enrichment observed in *Tribulus terrestris* stems and fruits (Zahedi et al., 2023), further supporting the idea that terpenoid biosynthesis is often prioritized in supportive or reproductive organs. Conversely, the flavonoid-rich profile of *R. micranthum* leaves mirrors the accumulation of rutin and rosmarinic acid in *T. terrestris* leaves and flowers, as well as the high levels of gallic acid and rutin in *Quercus brantii* galls (Shakuri et al., 2025). The convergence of these results with findings in *Q. brantii* and *T. terrestris* suggests that, despite interspecific differences in compound identity, leaves across diverse taxa consistently emerge as hotspots of pharmacologically relevant phenolics and flavonoids. This cross-species trend reinforces the suitability of leaf-derived extracts for nutraceutical, cosmetic, and therapeutic applications. Collectively, these organ-specific patterns of metabolite accumulation support the well-established principle of differential chemical specialization among plant organs to fulfill distinct physiological roles (Hao et al., 2023; Lee et al., 2020; Yu et al., 2024; Zeng et al., 2025).

The results of our quantitative HPLC analysis further confirmed the presence of five major compounds—chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5)—across all tissue samples. Notably, leaf tissues (KAL: 66.44 mg/g; MAL: 60.48 mg/g) exhibited significantly higher total compound content than stem tissues, with chlorogenic acid (1) and hyperin (2) as the predominant constituents (Table 4). In contrast, the stem samples displayed much lower concentrations, reinforcing the notion of organ-specific metabolic capacity. These results are consistent with the known physiological functions of leaves, which serve as the primary sites of photosynthesis and are exposed to greater environmental stress, thus requiring higher levels of antioxidants and protective metabolites (Wang et al., 2023). This distribution likely reflects metabolic specialization in different organs (Lee et al., 2020), where the photosynthetically active leaves accumulate greater levels of secondary metabolites to counteract UV radiation and

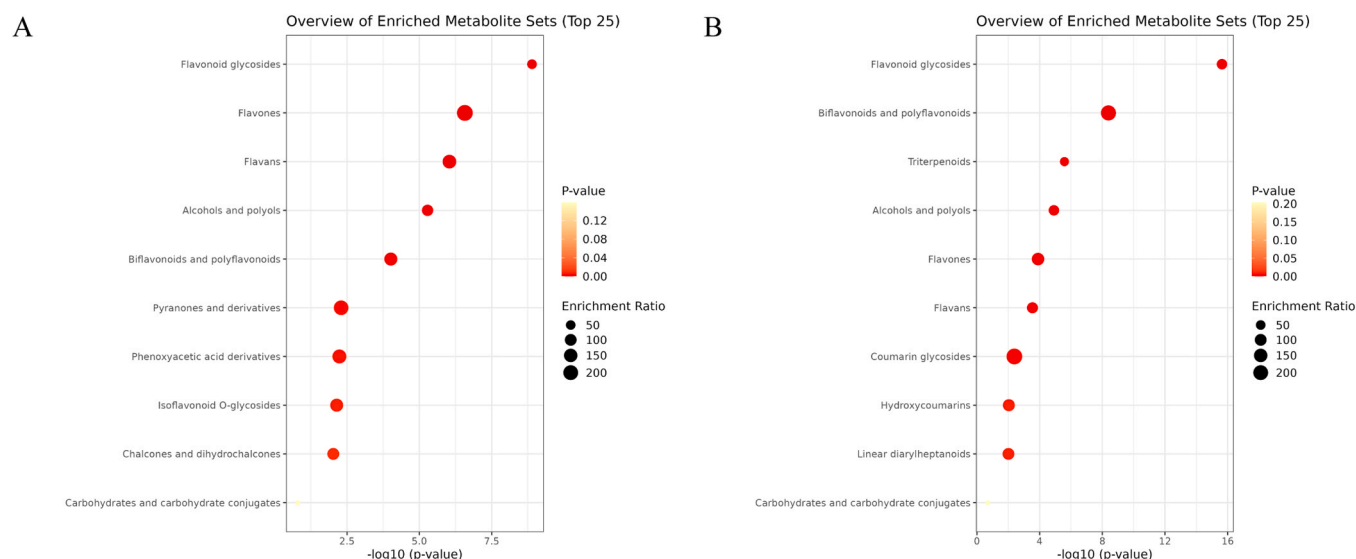


Fig. 6. MSEA-based chemical structure sub-class classification (1250 sub-chemical class metabolite or lipid sets) for *R. micranthum* leaf extract (A) and *R. micranthum* stem extract (B).

Table 4

Quantitative analysis of compounds 1–5 in different samples (KAL, KAS, MAL, and MAS) of *R. micranthum*.

Sample	Content (mg/g extract)****					Total
	1	2	3	4	5	
KAL	16.54 ± 0.01 ^b	25.24 ± 0.03 ^{NS}	4.45 ± 0.01 ^b	10.06 ± 0.01 ^a	10.15 ± 0.01 ^a	66.44
KAS	0.31 ± 0.01 ^{NS}	2.37 ± 0.06 ^a	0.40 ± 0.01 ^a	0.26 ± 0.02 ^b	0.34 ± 0.03 ^a	3.68
MAL	18.35 ± 0.35 ^a	25.11 ± 0.08 ^{NS}	4.54 ± 0.02 ^a	7.10 ± 0.01 ^b	5.38 ± 0.02 ^b	60.48
MAS	0.28 ± 0.01 ^{NS}	1.71 ± 0.01 ^b	0.24 ± 0.01 ^b	0.38 ± 0.03 ^a	0.21 ± 0.05 ^b	2.82

^{a,b} Different letters indicate significant differences; ^{NS} not significant; **** significant at 0.0001. Compounds: chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5).

insect predation (Anjali et al., 2023). Among the identified compounds, chlorogenic acid (1) and hyperin (2) were the most abundant in KAL and MAL samples, suggesting potentially greater antioxidant and anti-inflammatory activity in leaf-derived extracts. Trifolin (3), avicularin (4), and astragalin (5) followed the same accumulation trend, further supporting the superior functional profile of leaf extracts compared to those from the stem.

The high accumulation of chlorogenic acid (1) and hyperin (2) in leaf tissues is particularly noteworthy, given their broad pharmacological potential. This accumulation pattern suggests coordinated biosynthetic regulation tailored to the leaf's protective functions, likely in response to UV radiation, herbivory, and microbial attack (Panche et al., 2016). Chlorogenic acid (1) is well-documented for its neuroprotective, anti-diabetic, and hepatoprotective effects, primarily through modulation of metabolic and oxidative pathways (Guo et al., 2018; Xin et al., 2013). Similarly, hyperin (2) exhibits potent anticancer and anxiolytic activities, making its high concentration in leaf tissues especially significant (Patel et al., 2018).

These observations strongly suggest that the leaves of *R. micranthum* represent a superior source of bioactive compounds for nutraceutical or cosmetic applications compared to stem tissues. Moreover, this study experimentally validated the anti-inflammatory potential of *R. micranthum* leaf extracts. Beyond their higher phytochemical content, leaf extracts and major compounds demonstrated significant anti-inflammatory activity, as shown by the suppression of LPS-induced

NO production in RAW 264.7 macrophages (Fig. 9). Notably, trifolin (3), avicularin (4), and astragalin (5) significantly reduced NO production. Previous studies have reported that avicularin (4) and astragalin (5) mitigate inflammatory responses by inhibiting mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) signaling pathways in both LPS-stimulated RAW 264.7 macrophages and *in vivo* inflammation models (Lee et al., 2016; Soromou et al., 2012; Vo et al., 2012). Although the present study evaluated anti-inflammatory activity of *R. micranthum* and its active compounds only through NO inhibition in LPS-stimulated macrophages, further investigations on pro-inflammatory cytokine release and inflammation-related protein expression (e.g., NF-κB, MAPK, and JAK/STAT signaling pathways) should be examined. Nevertheless, these findings support the traditional pharmacological use of *R. micranthum* in treating inflammatory and respiratory conditions, highlighting the leaf's superior phytochemical richness and biological efficacy. These findings support the traditional pharmacological use of *R. micranthum* in treating inflammatory and respiratory conditions, highlighting the leaf's superior phytochemical richness and biological efficacy.

Practically, these results underscore the importance of selecting appropriate plant tissues for *R. micranthum* formulation development. While stem tissues may still provide structural or supporting phytochemicals not examined in this study, the leaf is clearly the more promising source for nutraceuticals, functional foods, and botanical therapeutics. This conclusion aligns with findings from other members of the Ericaceae family, where leaves generally accumulate the majority of pharmacologically active secondary metabolites. In *R. micranthum*, the leaf similarly emerges as the primary reservoir. Overall, the multivariate chemometric approach employed herein not only identified tissue-specific phytochemical markers but also demonstrated the value of integrative profiling to guide bioactivity-driven detection and product development. Future research should explore seasonal variation, bioavailability, and synergistic effects of these compounds to fully realize the therapeutic potential of this underutilized medicinal plant.

5. Conclusions

This study presents the first comparative phytochemical investigation of different tissue types of *R. micranthum* using HPLC and LC-ESI/MS techniques. The results reveal that leaves are the primary site for the accumulation of bioactive flavonoids and phenolic acids, whereas stems are enriched in triterpenoids and coumarins. Leaf tissues consistently

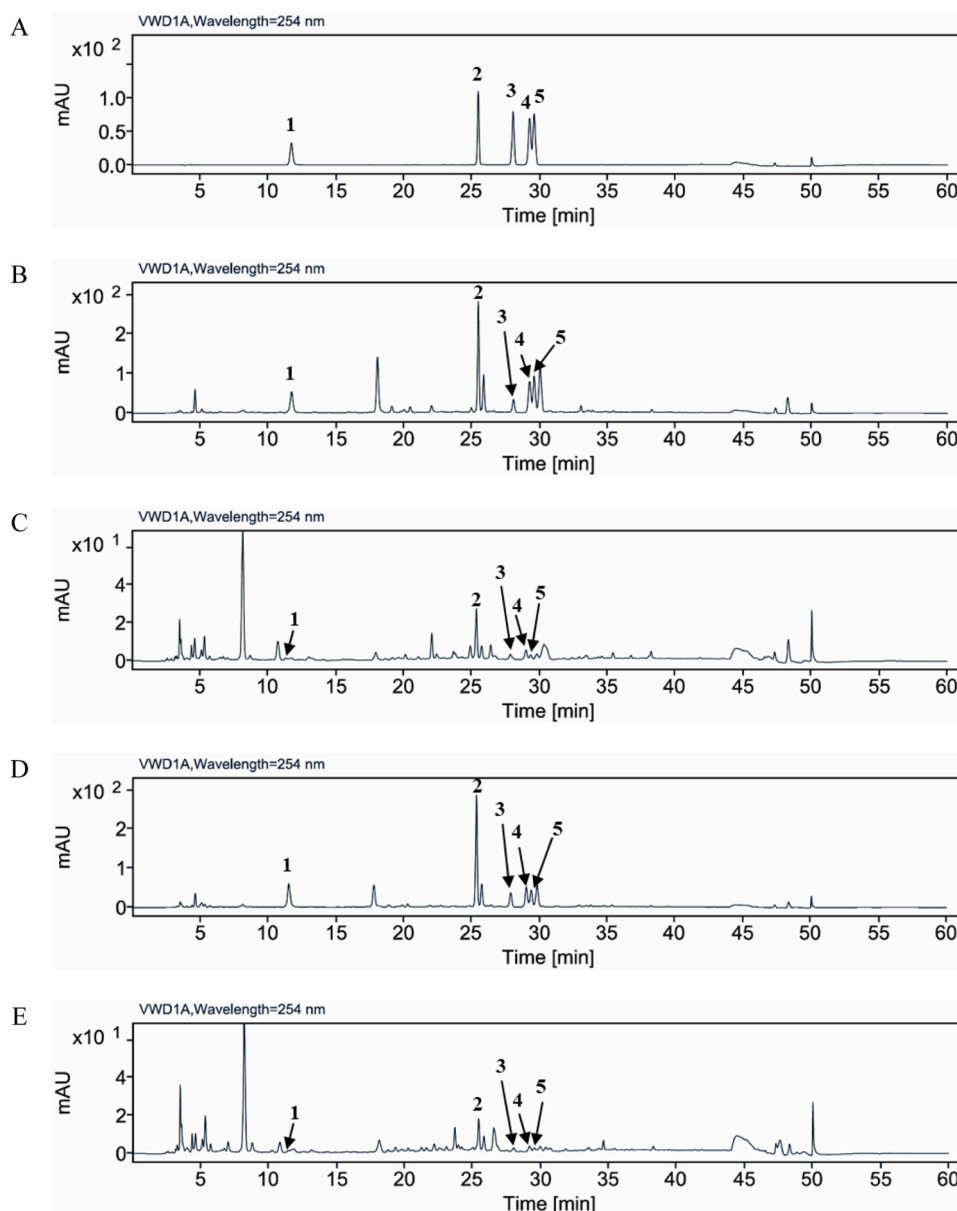


Fig. 7. HPLC chromatograms of different parts of *R. micranthum* showing compounds 1–5: standard mixture of compounds (A), KAL (B), KAS (C), MAL (D), and MAS (E). Identified compounds: chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5).

showed higher concentrations of the targeted compounds—chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5)—compared to stem tissues. The elevated levels of phytochemicals such as phenolics and flavonoids in leaf tissues support their functional significance and validate the plant's traditional use in medicine. This chemical superiority was further demonstrated through the leaf extract's greater ability to reduce NO production in LPS-stimulated macrophages. While this observation suggests anti-inflammatory potential, it should be noted that NO inhibition alone does not fully capture the complexity of inflammatory processes. Additional assays will be required to confirm broader pharmacological relevance. These findings highlight the metabolic specialization of *R. micranthum* leaves and provide supportive evidence for their ethnomedicinal application in traditional treatments of inflammatory and respiratory conditions. Furthermore, the predominance of trifolin (3), avicularin (4), and astragalin (5) supports their potential use as functional markers for quality control and therapeutic evaluation. Overall, this study lays the groundwork for future exploration of *R. micranthum* as a natural antioxidant source and offers

promising prospects for its development into botanically derived health-promoting products, nutraceuticals, and skincare products.

CRediT authorship contribution statement

Chang-Dae Lee: Writing – original draft, Investigation. **Ah Young Lee:** Investigation, Data curation. **Hak-Dong Lee:** Investigation, Data curation. **Sanghyun Lee:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Daeho Choi:** Resources, Data curation. **Kyung Choi:** Resources, Project administration, Data curation. **Mi-Jin Jeong:** Resources, Data curation. **Yong-Woo Park:** Resources, Data curation. **Jungmok Kang:** Resources, Data curation.

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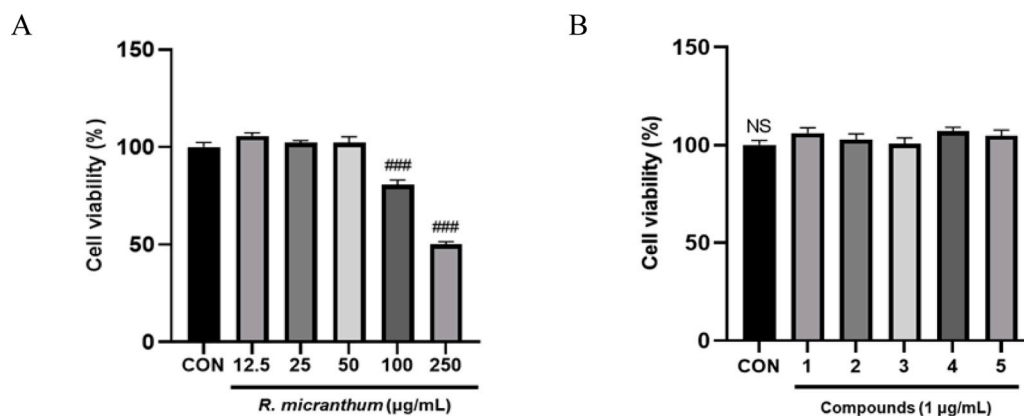


Fig. 8. Effects of different concentrations of *R. micranthum* extract (A) and compounds 1–5 (B) on RAW 264.7 cells. Cells were pre-treated with samples for 24 h. Cell viability was assessed using the MTT assay. Results are expressed as mean $\pm$ SE. The experiment was performed using six biological replicates for each sample. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. ### P < 0.001 vs. CON; NS, not significant. CON, control. Compounds: chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5).

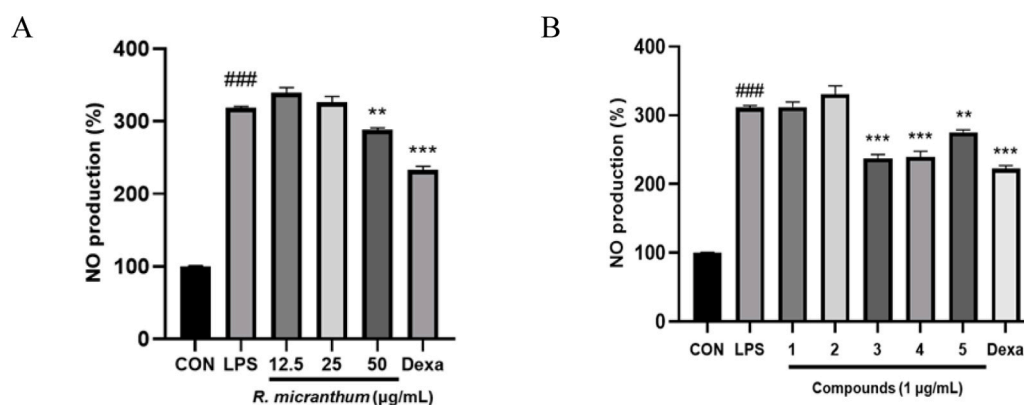


Fig. 9. Inhibitory effects of *R. micranthum* extract (A) and compounds 1–5 (B) on NO production in LPS-stimulated RAW 264.7 cells. Cells were pre-treated with samples for 2 h, followed by stimulation with LPS (1 μ g/mL) for 24 h. NO production was measured using the Griess assay. Results are presented as mean $\pm$ SE. The experiment was performed using six biological replicates for each sample. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. ### P < 0.001 vs. CON; ** P < 0.01, *** P < 0.001 vs. LPS-stimulated cells. CON, control; Dexa, dexamethasone (0.5 μ g/mL). Compounds: chlorogenic acid (1), hyperin (2), trifolin (3), avicularin (4), and astragalin (5).

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Data availability

Data will be made available on request.

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