

Comparison of growth, phytochemical content and antioxidant activities of the halophyte, *Limonium tetragonum* under saline and non-saline conditions

Min Gyu Park

Gyeongsang National University

Yun Na Kim

Gyeongsang National University

Jae Sun Lee

Gyeongsang National University

Won Jin Jung

Gyeongsang National University

Yu Jung Kim

Gyeongsang National University

Seon Min Lee

Korea Institute of Toxicology

Min Hye Yang

Pusan National University

Bong-Oh Kwon

Kunsan National University

Jung-Rae Rho

Kunsan National University

Eun Ju Jeong

`jeong.ej@gnu.ac.kr`

Gyeongsang National University

Article

Keywords: *Limonium tetragonum*, Halophyte, Salinity, Soil, LC-MS, Metabolites, Antioxidant activity

Posted Date: April 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9339182/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on July 2nd, 2026. See the published version at <https://doi.org/10.1038/s41598-026-58129-2>.

1 Comparison of growth, phytochemical content and antioxidant
2 activities of the halophyte, *Limonium tetragonum* under saline and
3 non-saline conditions

4
5 Min Gyu Park^{a,†}, Yun Na Kim^{b,†}, Jae Sun Lee^b, Won Jin Jung^a, Yu Jung Kim^b, Seon Min Lee^{a,c},
6 Min Hye Yang^d, Bong-Oh Kwon^e, Jung-Rae Rho^{e,*}, and Eun Ju Jeong^{a,*}

7
8 ^a*Department of Green Bio Science (BK 21), Gyeongsang National University, Jinju 52828,*
9 *Korea*

10 ^b*Agri-Food Bio Convergence Institute, Gyeongsang National University, Jinju 52725, Korea*

11 ^c*Center for BioHealth Research Center, Gyeongnam Bio-Environment Research Head Quarter,*
12 *Korea Institute of Toxicology (KIT), Jinju 52834, Korea*

13 ^d*College of Pharmacy, Pusan National University, Busan 46241, Republic of Korea*

14 ^e*Department of Oceanography, Kunsan National University, Gunsan 54150, Republic of Korea*

15
16 ^{*}*Corresponding authors: jrrho@kunsan.ac.kr; Tel.: +82-63-469-4606 (J.-R.R),*
17 *jeong.ej@gnu.ac.kr; Tel.: +82-55-772-3224(E.J.J)*

18 [†]*Equal contribution: These authors contributed equally to this work.*

Abstract

Soil salinization threatens global agriculture, increasing interest in salt-tolerant halophytes like *Limonium tetragonum* for their remarkable bioactivities. However, cultivation strategies enabling their industrial utilization remain underdeveloped. This study assessed the cultivation potential of *L. tetragonum* under conventional agricultural conditions by comparing plants grown in saline soil (SS; high EC, Na⁺, Mg²⁺) and non-saline soil (NSS; higher organic matter, available P, Ca²⁺). Growth parameters (leaf number, leaf length, biomass) were measured, five major secondary metabolites (catechin and flavonoids) were quantified via LC-MS/MS, and antioxidant activities (DPPH, ABTS, ROS assays) were evaluated. Principal component analysis determined correlations among soil traits, plant growth, and metabolite profiles. Plants grown in NSS exhibited significantly superior growth, higher accumulation of major bioactive compounds (including total anthocyanins and carotenoids), and stronger antioxidant capacities compared to those in SS. Correlation analysis revealed that elevated organic matter and available P in NSS likely enhanced metabolite biosynthesis and physiological activity. These results demonstrate that *L. tetragonum* is readily cultivable under non-saline conditions, challenging the paradigm that salinity maximizes secondary metabolite production, and providing a scientific basis for the sustainable utilization of bioactive halophytes.

Keywords:

Limonium tetragonum, Halophyte, Salinity, Soil, LC-MS, Metabolites, Antioxidant activity

1. Introduction

Soil salinization refers to the accumulation of water-soluble salts in the soil, a process that can significantly impact agricultural productivity and ecosystem health¹. According to existing publications, soil salinization is a growing global issue that now affects more than 1×10^8 hectares across over 100 countries worldwide, with approximately 6% of agricultural land impacted by salt stress, making salinity a significant environmental factor limiting plant growth^{2,3,4}. Recent FAO assessments confirm this escalation, with salt-affected soils covering 1,381 million ha (10.7% of global land) and impacting 10% of irrigated and rainfed croplands⁵. Halophytes are generally characterized as plant species capable of completing their entire life cycle in soils containing a minimum of 200 mM NaCl. It is estimated that halophytes comprise no more than 2% of all terrestrial plant species⁶. Halophytes are indigenous to saline soils characterized by high concentrations of Na⁺ and Cl⁻. These plant species possess inherent mechanisms that enable them to tolerate multiple abiotic stresses commonly encountered in their natural environments, including salt excretion via specialized salt glands and salt bladders, osmotic adjustment through compatible solutes like proline, and enhanced antioxidant systems^{7,8}. Salinity stress substantially alters secondary metabolite profiles in halophytes growing in salt affected soils. Recent systematic reviews highlight halophytes' potential for cultivation and ecological restoration in saline regions⁹, yet practical applications remain limited by insufficient data on species like *Limonium tetragonum*.

L. tetragonum (Thunb.) Bullock is a biennial herbaceous halophyte within the Plumbaginaceae family, and it predominantly inhabits saline coastal ecosystems such as salt marshes and muddy seashores¹⁰. *L. tetragonum* exhibits ecological adaptability to high-salinity environments through specialized salt-secreting structures (e.g., salt bladders) and osmotic regulation via proline accumulation⁸. These traits support its cultivation potential in saline environments. In addition to its ecological properties, a variety of biological activities of *L. tetragonum* have been reported including hepatoprotective¹¹, osteogenic & anti-osteoporotic^{12,13}, anti-obesity¹², and endurance-enhancing activity¹⁴. Phytochemical studies have identified that *L. tetragonum* contains flavonoids (myricetin and quercetin glycosides) and catechins (epigallocatechin gallate) as major constituents^{15,16}.

The industrial exploitation of halophytic plants rich in bioactive compounds necessitates the development of cultivation strategies that enable large-scale production. Soils that halophytes typically inhabit are characterized by high salinity and low Organic Matter (OM) content

relative to conventional agricultural soils. To date, research on halophytic plants has focused on salt-adaptive mechanisms under high-salinity conditions, such as ion homeostasis, osmolyte synthesis, and salt secretion etc. However, studies aimed at elucidating the correlations among plant growth characteristics, secondary metabolite production, and associated changes in biological activities in response to soil environmental conditions remain limited. In the present study, plant growth characteristics, the production of secondary metabolites, and antioxidant activities were compared under different soil environments (saline soil from native habitat and conventional agricultural soil) using the bioactive halophyte, *L. tetragonum*. The contents of total carotenoid and anthocyanin of plants were determined using spectrophotometric colorimetric assays. The contents of five major constituents of *L. tetragonum* including (–)-epigallocatechin-3-gallate, myricetin-3-*O*- β -D-galactopyranoside, myricetin-3-*O*- α -L-rhamnopyranoside, 5,7,2',5'-tetrahydroxy flavanone-3-*O*- β -D-galactoside and myricetin-3-*O*-(2''-O-galloyl)- α -L-rhamnoside were determined using ESI-LC-MS. In addition, principal component analysis (PCA) was employed to evaluate the correlations among the parameters.

2. Materials and methods

2.1. Plant material and Soil collection

Seeds of *L. tetragonum* were collected in 2022 from Gohyeon-myeon, Namhae-gun, Gyeongsangnam-do, Republic of Korea. Plant identity was authenticated by Prof. Min Hye Yang at Pusan National University, Republic of Korea. A voucher specimen (voucher number GNT-68) was deposited at the Laboratory of Pharmacognosy, Gyeongsang National University, Republic of Korea. In 2023, the seeds were germinated under natural outdoor conditions at the Naedong Campus of Gyeongsang National University (GNU) and randomly divided into two groups. The first group (saline soil group, SS) was transplanted to a coastal site in Gohyeon-myeon, Namhae-gun, the native habitat of *L. tetragonum* (Saline Soil, 34.8746073°N, 127.8977219°E). The second group (non-saline soil group, NSS) was transplanted to an experimental farm at the Naedong Campus of GNU (Non-Saline Soil, 35.1573381°N, 128.0820927°E). Both groups were grown until August, prior to the flowering stage, and then harvested for subsequent analyses.

2.2. Collection of Soils

Soil samples were collected from the respective cultivation sites. In each plot, soil samples were obtained using a soil auger. Each plot was divided into three sections (A, B, and C), and five soil cores were collected at regular intervals within each section. Soil samples from each section were thoroughly homogenized to produce composite samples, and at least three composite samples were obtained per treatment.

2.3. Determination of Soil Physicochemical Properties

Soil texture (particle size distribution) was determined by the pipet method in accordance with the USDA Soil Survey Laboratory Methods Manual and relevant literature¹⁷. Air-dried soil samples (10 g) were pretreated with 30% H₂O₂ to remove OM, dispersed in 5% sodium hexametaphosphate solution, and equilibrated. After thorough mixing and sedimentation, aliquots were extracted at specified depths and times with a pipet, dried, and weighed to quantify the percentages of sand, silt, and clay fractions. The results were expressed as percentages by weight. pH and EC were determined in the laboratory using air-dried soil samples (5 g) mixed with 25 mL of distilled water (1:5 w/v ratio) in 50 mL tubes. The suspensions were agitated for 1 hour and filtered through filter paper. pH was measured using a calibrated pH meter (HM-30R, DKK-TOA, Japan), and EC was measured with a conductivity EC meter (CM-25R, DKK-TOA, Japan). OM content was determined by quantifying total organic carbon (TOC) using an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, USA) via combustion at 1,000°C. OM content was calculated by multiplying the TOC value by the van Bemmelen factor (1.724). Exchangeable cations (K⁺, Ca²⁺, Mg²⁺, Na⁺) were extracted from 2.5 g of air-dried soil with 30 mL of 1 M ammonium acetate (NH₄OAc, pH 7.0) in 50 mL tubes. The suspensions were shaken for 8 hours, filtered, and the concentrations of K⁺, Ca²⁺, Mg²⁺, and Na⁺ were determined by inductively coupled plasma optical emission spectroscopy (ICP-730-ES, VARIAN, USA). Available phosphorus (P) was measured using the Bray No. 1 method¹⁸. Soil samples (2.85 g) were extracted with 20 mL of Bray-1 solution (0.03 M NH₄F + 0.025 M HCl) in 50 mL centrifuge tubes, shaken for 1 minute, and immediately filtered. A 5 mL aliquot of the filtrate was mixed with 5 mL of ascorbic acid reagent in a 50 mL volumetric flask, and absorbance was measured at 720 nm after 10 minutes using a UV-Vis spectrophotometer (Cary 100, Agilent, USA).

2.4. Instrumentation and Reagents

LC-ESI-MS/MS analysis was performed using an Agilent HPLC 1100 series (Santa Clara, CA, USA) coupled to an AB Sciex API 3200 mass spectrometer (Applied Biosystems, MDS Sciex, Concord, Canada) equipped with an ESI source, and data were processed using Analyst® software (version 1.6.3). The analytical column was a YMC-Triart C₁₈ (50 × 2.1 mm i.d., 3 µm, 12 nm, YMC Co., Ltd., Japan). MTT reagent, penicillin/streptomycin, DMSO, H₂O₂, L-ascorbic acid, and sodium acetate buffer (pH 4.5) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, acetone, methanol, and water (HPLC grade) were obtained from Honeywell Burdick & Jackson (Muskegon, MI, USA), and formic acid (HPLC grade) from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Immortalized human HaCaT keratinocytes were kindly provided by KB Cosmetics (Jinju, South Korea). DMEM (CM002-050) was from GenDEPOT (Katy, TX, USA), fetal bovine serum (FBS) from Gibco (Grand Island, NY, USA), and the Muse® MitoPotential Kit from EMD Millipore (Burlington, MA, USA). Cell viability was measured with a microplate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland), and mitochondrial membrane potential with a Guava® Muse Cell Analyzer (Luminex Corporation, Seattle, WA, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), and Trolox were obtained from Thermo Fisher Scientific. Potassium persulfate and butylated hydroxytoluene (BHT) were purchased from Daejung Chemicals & Metals (Siheung, South Korea). Commercial buffer solution at pH 1.0 was obtained from Taelim Chemical (Korea).

2.5. Investigation of growth characteristics of *L. tetragonum*

To investigate the growth characteristics of *L. tetragonum*, leaf length, leaf width, root length, and number of leaves were measured prior to the flowering stage. For each individual, the length (mm) of the longest leaf and the width (mm) of the widest leaf among all leaves were recorded. The root length (cm) was measured after harvesting by carefully washing the roots to remove soil, and then measuring the length of the primary root using a ruler. The number of leaves was investigated by the number of leaves per individual. *L. tetragonum* leaves used for yield determination were obtained from freeze-dried leabes.

2.6. Preparation of *L. tetragonum* extract

For the extraction, 100 mg of dried *L. tetragonum* was extracted with 10 mL of 50% (v/v) or 100% ethanol at 70°C for 4 h using a water bath extraction. The extract was filtered through a 0.45 μ m PVDF filter and the solvent was removed *in vacuo*.

2.7. DPPH radical scavenging assay

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity was measured with modifications to the method described by Amalia *et al.*, 2020. A 1 M DPPH stock solution was diluted with methanol to prepare a 0.05 mM working solution. For the assay, 20 μ L of sample extract was mixed with 230 μ L of the DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. Radical scavenging activity (%) was calculated by comparing the absorbance reduction between the sample-treated group and the negative control (without sample). The IC₅₀ value, representing the sample concentration required to scavenge 50% of DPPH radicals, was determined from the dose-response curve of concentration versus scavenging activity.

2.8. ABTS radical scavenging assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) radical scavenging activity was analyzed following Zhang *et al.*, 2020 with modifications. An ABTS stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate in a 1:1 ratio and allowing the reaction to proceed in the dark at 4°C for at least 12 hours. The stock solution was diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 50 μ L of sample extract was reacted with 200 μ L of the diluted ABTS solution in the dark at room temperature for 30 minutes. Absorbance was measured at 734 nm. Scavenging activity (%) was calculated relative to the negative control (without sample). The IC₅₀ value, indicating the sample concentration needed to scavenge 50% of ABTS radicals, was derived from the concentration-scavenging activity curve.

2.9. Measurement of Total Anthocyanin Content

The content of total anthocyanin was measured following the method described by Lee *et al.*, 2005, with some modifications. First, 0.1 g of the sample was mixed with 5 mL of methanol containing 0.1% formic acid and sonicated for 20 minutes. The supernatant was collected, and

the extraction was repeated three times. 100 µL of the extract was mixed separately with 2 mL of buffer solutions at pH 1.0 and pH 4.5. The absorbance of each mixture was recorded at 520 nm and 700 nm using a spectrophotometer (Ultraspec 2100pro, Amersham Co.). The difference in absorbance between the two pH conditions was used to calculate the total anthocyanin content, expressed as cyanidin-3-O-glucoside equivalents, based on the following formula.

$$\text{Total anthocyanin content (mg/100 g)} = \frac{A \times Mw \times D F \times V \times F}{\epsilon \times l}$$

$A = [(OD_{520nm} - OD_{700nm}) \text{ at pH 1.0}] - [(OD_{520nm} - OD_{700nm}) \text{ at pH 4.5}]$, Mw = molecular weight of cyanidin-3-glucoside (449.2), DF = dilution factor, V = total volume of extract (mL), F = conversion factor for sample weight, ϵ = molar absorptivity of cyanidin-3-glucoside ($26,900 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$), l = path length

2.10. Measurement of Total Carotenoid Content

Total carotenoid content was determined using a modified version of the method described by Al-Farsi *et al.*, 2005. Briefly, 1 g of sample was extracted with acetone:ethanol (1:1, v/v) under dark conditions at 4°C for 24 hours in an incubator. The extracts were centrifuged at 12,000 rpm for 10 minutes at 4°C, and the supernatant was filtered using Whatman No.42 filter paper. The absorbance of the filtrate was measured at 470 nm using a spectrophotometer (U-2900, HITACHI Ltd., Japan). The total carotenoid content was calculated according to the following equation.

$$\text{Total carotenoid content (mg/g)} = \frac{A_{470} \times V \times 10^6}{A^{1\%} \times 100G}$$

A is the absorbance at 470 nm, V is the total volume of extract, $A^{1\%}$ is the extinction coefficient for a 1% mixture of carotenoids at 2,500, and G denotes sample weight (g).

2.11. Cell culture and Viability determination

Immortalized human HaCaT keratinocytes were maintained in DMEM containing 10% (v/v) fetal bovine serum and 1% (v/v) antibiotic (100 U mL^{-1} penicillin and $100 \mu\text{g mL}^{-1}$ streptomycin) mixture at 37°C in a 5% CO₂-humidified incubator. Cell viability was determined

using the colorimetric MTT assay, which is based on the reduction of MTT to formazan by cellular dehydrogenase. HaCaT cells were seeded at densities of 1×10^4 cells/well in 96-well plates and incubated for 24 h. Then, the extracts of *L. tetragonum* were treated at concentrations of 0, 5, 10, 20 and 40 $\mu\text{g/mL}$ and cultivated for 24 h. MTT (2 mg/mL) in distilled water was added to each well for 3 h at 37°C. Subsequently, the insoluble formazan precipitate was dissolved in DMSO, and the absorbance was measured at 540 nm using a microplate reader.

2.12. Measurement of intracellular ROS production in HaCaT cells

HaCaT keratinocytes were seeded at a density of 2×10^5 cells/mL and then treated with 20 $\mu\text{g/mL}$ of *L. tetragonum* extracts for 20 h prior to stimulation with 2,000 μM H_2O_2 for 4 h. The cells were washed with ice-cold PBS and incubated with Muse® MitoPotential Kit for 30 min. Mitochondrial membrane depolarization was measured by Guava® Muse Cell Analyzer.

2.13. LC-ESI-MS/MS analysis of *L. tetragonum* Extract

LC-ESI-MS/MS analyses were performed on an Agilent HPLC 1100 series (Santa Clara, CA, USA). A YMC-Triart C₁₈ (50 $\times$ 2.1 mm I.D. S-3 μm , 12 nm, Japan) was used and maintained at room temperature. The mobile phase consisted of DW (0.1% formic acid) (A) and MeOH (0.1% formic acid) (B) at a flow rate of 0.5 mL/min, with a gradient elution as follows: 0-18 min, 95-5% A; 18-20 min, 5%. The injection volume was 1.0 μL . MS/MS analysis was performed using MRM (Multiple Reaction Monitoring) mode after optimization to derive the optimal analysis conditions for compound **1-5**. Ionization was performed using ESI (electrospray ionization) using Z-spray, argon gas was used as collision gas, and nitrogen gas was used as nebulizer gas. Ion source temperature was set at 500°C, nebulizer gas flow at 50 L/min, curtain gas flow at 20 L/min, auxiliary gas flow at 55 L/min, and Ion spray voltage at 4.5 kV. ESI was operated in the positive ion mode. Full-scan spectra were acquired over the range of m/z 100-1000. *L. tetragonum* extracts were diluted to 50 $\mu\text{g/mL}$ with 10 % acetonitrile in 0.1% formic acid solvent and then injected into LC-ESI-MS/MS. (–)-epigallocatechin-3-gallate (**1**), myricetin-3-O- β -D-galactopyranoside (**2**), myricetin-3-O- α -L-rhamnopyranoside (**3**), 5,7,2',5'-tetrahydroxy flavanone-3-O- β -D-galactoside (**4**) and myricetin-3-O-(2''O-galloyl)- α -L-rhamnoside (**5**) were set as major compounds, and the experiment was conducted by diluting in the range of 62.5-10,000 ng/mL using 10% acetonitrile containing 0.1% formic acid.

2.14. Statistical analysis

Growth characteristics, dry yield, and quantitative data were analyzed using GraphPad Prism 10 (GraphPad Software, Inc., USA). Significant differences ($p < 0.05$) between groups were assessed using Duncan's multiple range test (for multiple comparisons), one-way ANOVA, and Student's t-test (for pairwise comparisons).

Principal component analysis (PCA) was performed in R (version 4.5.0; R Core Team, Vienna, Austria) using the `prcomp` function on standardized data to explore relationships among variables and visualize the distribution of samples in reduced dimensions. For antioxidant indices (ROS⁺, DPPH, ABTS), lower values indicate higher activity; therefore, these variables were sign reversed prior to PCA so that higher scores represented greater antioxidant capacity.

3. Results

3.1. Soil physicochemical characteristics of saline and non-saline soils used for *L. tetragonum* cultivation

Soil texture analysis revealed clear differences between SS and NSS. SS were dominated by sand, with sand content ranging from 89.32% to 90.28%, silt content from 1.40% to 2.40%, and clay content from 7.76% to 9.16%. According to the USDA soil classification, these soils were categorized as sand or loamy sand. In contrast, NSS exhibited a lower sand fraction (60.16%-64.08%), higher silt (22.32%-25.76%), and higher clay content (13.64%-14.24%), and were classified as sandy loam (Fig. 1). The physicochemical characteristics of SS and NSS are summarized in Table 1. Significant differences were observed between the two groups for all parameters measured, except for pH and K⁺ content. SS had EC and Na⁺ concentrations that were significantly 32 and 70 times higher, respectively, than those in NSS. In contrast, the contents of OM, Ca²⁺, and available P in NSS were 11, 7, and 4 times higher, respectively, than those in SS, and these differences were statistically significant. Both SS and NSS showed pH values between 6.6 and 6.8, confirming that both soils were slightly acidic.

3.2. Growth characteristics of *L. tetragonum* cultivated under saline and non-saline soils

The growth performance of *L. tetragonum* was compared between plants cultivated in SS and NSS (Fig. 2; Table 2). Plants grown in NSS had a significantly greater leaf width ($p < 0.001$), which was 1.1 times higher than that observed in SS. Similarly, the number of leaves in NSS was 1.2 times higher than in SS, also showing a significant difference ($p < 0.001$). No

significant differences were observed in leaf length and root length between the two soil conditions. Biomass production also differed markedly between habitats (Table 2). The fresh weight of plants cultivated in NSS was 1.7 times higher than that in SS, while the dry weight was 1.8 times higher. Both differences were statistically significant ($p < 0.001$). However, dry yield did not differ significantly between the two groups. These results indicate that non-saline soil conditions promote vegetative growth and biomass accumulation in *L. tetragonum*, primarily through increases in leaf width, leaf number, and overall plant weight, whereas the proportion of dry matter relative to fresh weight remained unchanged.

3.3. Antioxidant potentials of *L. tetragonum* extract cultivated under saline and non-saline soils

To select an extraction solvent that enhances the recovery of antioxidant compounds from *L. tetragonum*, plant materials were extracted using 50% or 100% ethanol. The extraction yields were higher for 100% ethanol extracts (SS: $11.98 \pm 1.37\%$; NSS: $11.95 \pm 1.38\%$) compared to 50% ethanol extracts (SS: $9.03 \pm 1.65\%$; NSS: $8.07 \pm 1.41\%$), regardless of soil type. Despite lower yields, the 50% ethanol extracts exhibited higher antioxidant activities than the 100% ethanol extracts, as reflected by consistently lower IC_{50} values in both DPPH and ABTS assays (Fig. 3). In DPPH assay, the IC_{50} value of the 50% ethanol extract from plants cultivated in NSS ($138.40 \pm 19.36 \mu\text{g/mL}$) was approximately 1.3-fold lower than that of the corresponding extract from SS ($174.30 \pm 27.36 \mu\text{g/mL}$), indicating a significantly greater free radical scavenging activity in the NSS group ($p < 0.001$). Similarly, in ABTS assay, the IC_{50} value of the 50% ethanol extract from the NSS group ($59.15 \pm 18.07 \mu\text{g/mL}$) was approximately 1.5-fold lower than that of the SS group ($87.16 \pm 10.60 \mu\text{g/mL}$) ($p < 0.001$). Furthermore, the 50% ethanol extracts from both NSS and SS exhibited lower IC_{50} values than the positive control, Trolox ($124.50 \pm 3.21 \mu\text{g/mL}$), indicating strong radical scavenging activity of *L. tetragonum* extracts.

3.4. Effects of *L. tetragonum* extracts on HaCaT cell viability and ROS production

The antioxidant potential of *L. tetragonum* cultivated in SS and NSS was further compared in vitro. The effects of *L. tetragonum* extracts on the attenuation of ROS production were evaluated in H_2O_2 -induced HaCaT cells using flow cytometry. Prior to the activity assays, the cytotoxicity of *L. tetragonum* extracts was assessed using an MTT assay (Fig. 4). Treatment

with each extract showed no detectable cytotoxicity in HaCaT cells at concentrations ranging from 0 to 40 $\mu\text{g/mL}$. In flow cytometric analysis, the proportion of ROS-positive cells (M2 population) in untreated control cells (NC) was 3.6%, which increased to 45.7% following H_2O_2 exposure. Treatment with NAC, used as a positive control, significantly reduced the proportion of ROS-positive cells to 7.3% ($p < 0.001$ vs. H_2O_2 -treated group). Treatment with *L. tetragonum* extracts also resulted in a significant reduction in ROS-positive cell populations, to 29.7% and 30.8% for the SS and NSS extracts, respectively ($p < 0.001$ vs. H_2O_2 -treated group). The decrease in ROS-positive cells was accompanied by a corresponding increase in ROS-negative cells following treatment with *L. tetragonum* extracts. No significant difference was observed between SS and NSS extracts with respect to their effects on ROS attenuation in vitro (Fig. 5).

3.5. Accumulation of Carotenoid and Anthocyanin, Pigment Type Secondary Metabolites, in *L. tetragonum* cultivated in SS and NSS

The total anthocyanin and carotenoid contents accumulated in *L. tetragonum* were compared using colorimetric assays. As shown in Fig. 6, *L. tetragonum* cultivated in NSS (350.30 ± 87.20 mg/100 g) exhibited significantly higher total anthocyanin contents than those grown in SS (245.21 ± 33.14 mg/100 g), with mean values approximately 1.4-fold greater ($p < 0.01$). Total carotenoid contents were also higher in NSS (299.88 ± 16.17 mg/g), with average levels approximately 2.6-fold greater than those observed in SS (114.16 ± 20.90 mg/g) ($p < 0.001$).

3.6. Optimization of LC-ESI-MS/MS for analysis of target compounds in *L. tetragonum*

In a previous study by Lee *et al.*, 2017, a total of 13 compounds, including flavonoids, flavonoid glycosides, and catechins, were isolated from the aerial parts of *L. tetragonum*, and their structures were elucidated. Among these compounds, (–)-epigallocatechin-3-gallate (**1**), myricetin-3-O- β -D-galactopyranoside (**2**), myricetin-3-O- α -L-rhamnopyranoside (**3**), 5,7,2',5'-tetrahydroxy flavanone-3-O- β -D-galactoside (**4**) and myricetin-3-O-(2''O-galloyl)- α -L-rhamnoside (**5**), which were identified as the major constituents of *L. tetragonum*, were quantitatively analyzed in the present study using LC-ESI-MS/MS. Prior to analyzing the extracts using MRM mode to improve detection sensitivity, the detection conditions for five compounds were optimized, and the corresponding parameters were selected for the analysis. The precursor and product ions for compound **1** were set as m/z 457.1 $[\text{M}-\text{H}]^-$ and m/z 169.1 $[\text{M}-\text{C}_7\text{H}_5\text{O}_5-\text{H}]^-$, respectively; for compound **2**, m/z 479.2 $[\text{M}-\text{H}]^-$ and m/z 316.8 $[\text{M}-$

C₆H₁₀O₅–H][–]; for compound **3**, m/z 463.1 [M–H][–] and m/z 317.2 [M–C₆H₁₀O₄–H][–]; for compound **4**, m/z 462.9 [M–H][–] and m/z 300.3 [M–C₆H₁₀O₅–H][–]; and for compound **5**, m/z 615.3 [M–H][–] and m/z 317 [M–C₁₃H₁₄O₈–H][–] (Fig. 7; Fig. 8). The parameter values of declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell entrance potential (CEP), and cell exit potential (CXP) optimized for compound **1–5** were shown in Table 3. For quantitation of compound **1–5** in the extract of *L. tetragonum* calibration curves were obtained with different concentration ranges. Calibration data indicated the linearity ($R^2 > 0.9994$) of the detector response for all standard compounds. The limits of detection (LOD) were determined using the formula $3.3 \sigma / S$, where σ represents the standard deviation (SD) of the response, and S is the slope of the calibration curve. The limits of quantification (LOQ) were calculated based on the expression $10 \sigma / S$. LOD and LOQ for compound **1–5** were found to be in the range from 0.08 to 0.26 $\mu\text{g/mL}$ and 0.25 to 0.78 $\mu\text{g/mL}$, respectively (Table 4).

3.7. Quantitative analysis of compounds 1-5 in *L. tetragonum* cultivated in SS and NSS using ESI-LC-MS/MS

Quantitative analysis of five major secondary metabolites in *L. tetragonum* extracts was performed using LC–ESI–MS/MS. The quantitative results for compounds **1–5** in *L. tetragonum* extracts are summarized in Fig. 9. Among the five secondary metabolites analyzed, compounds **2** and **3** were the most abundant in both SS and NSS extracts. The content of compound **2** in NSS was approximately 1.3-fold higher than that in SS with no statistical significance. In contrast, compound **3** was significantly more abundant in NSS, exhibiting an approximately 1.7-fold increase compared with SS ($p < 0.05$). Among all analyzed compounds, compound **1** showed the largest difference between the two groups, with its content in NSS being approximately 3-fold higher than that in SS ($p < 0.001$). Overall, the concentrations of all five compounds were generally higher in NSS than in SS. The significantly higher levels of compounds **1**, **3**, **4**, and **5** observed in the NSS group suggest a possible association between NSS conditions and increased accumulation of these secondary metabolites.

3.8. Principal component analysis of soil properties, secondary metabolites and antioxidant activities in *L. tetragonum* cultivated in SS and NSS

Principal component analysis (PCA) was performed to examine multivariate relationships

among soil physicochemical properties (EC, pH, OM, Ca^{2+} , Na^+ , Mg^{2+} , K^+ , and P), pigment-type secondary metabolites (total anthocyanin and total carotenoid contents), major secondary metabolites (compounds 1–5), and antioxidant activities (ROS, DPPH, and ABTS) in *L. tetragonum* cultivated in SS and NSS. Samples clearly clustered into two distinct groups according to soil type, with the NSS group (blue) distributed on the positive side of PC1 and the SS group (red) on the negative side, indicating discernible differences in their overall phytochemical, antioxidant-related, and soil physicochemical properties. The first two principal components (PC1 and PC2) explained 68.5% and 10.3% of the total variance, respectively. Among the measured variables, the five major compounds, total anthocyanin, total carotenoid, and antioxidant activity indices (DPPH and ABTS) showed positive loadings on PC1, suggesting that these parameters were associated with the variance contributing to the separation between NSS and SS. In contrast, ROS levels exhibited a negative loading on PC1, indicating an inverse relationship with secondary metabolite contents and antioxidant activities across the groups. The orientation of the loading vectors in the biplot further indicates that higher levels of secondary metabolites and antioxidant activities were more closely associated with the NSS group, whereas increased ROS levels were more strongly associated with the SS group. In addition, soil EC, Na^+ , Mg^{2+} , and K^+ were oriented toward the SS side of PC1, whereas pH, OM, Ca^{2+} , and P were more closely aligned with the NSS side, implying that distinct soil chemical environments in SS and NSS were closely associated with the observed differences in secondary metabolites and antioxidant activities.

4. Discussion

Soil salinization refers to the accumulation of soluble salts, primarily ions such as sodium, calcium, magnesium, and chloride, accumulate within the soil profile. This process progressively alters soil physical and chemical properties, impairs fertility, and limits plant access to water and essential nutrients^{23,24}.

Halophytes are defined as plant species capable of completing their life cycle under salinity conditions that are typically lethal to most glycophytes, often tolerating salt concentrations equivalent to seawater or higher⁶. Despite the extensive global distribution of saline habitats, halophytes represent only a minor component of the world's vascular flora, comprising approximately 1–2% of known species²⁵. *L. tetragonum* is a biennial halophytic species in the family Plumbaginaceae that typically occurs in saline coastal environments such as tidal marshes and mudflats¹⁰. Within these habitats, *L. tetragonum* mitigates ionic and osmotic stress through leaf-based salt-secreting glands and the intracellular accumulation of compatible solutes, proline⁸. Salt tolerance in halophytes has been frequently associated with elevated biosynthesis of secondary metabolites and antioxidant compounds, which play key roles in osmoprotection and mitigation of oxidative stress¹⁵. Building on this concept, the present study cultivated *L. tetragonum* under SS, representing its natural coastal environment, and NSS, representing more conventional agricultural conditions. This experimental design enabled assessment of the species' cultivation potential beyond its native range and provided comparative insights into the influence of soil salinity on plant growth, secondary metabolism, and antioxidant capacity.

Analysis of soil physicochemical properties revealed clear contrasts between SS and NSS soils. Exchangeable cations, particularly Na^+ , were substantially higher in SS (2031.75 ± 196.72 mg/kg) than in NSS (29.21 ± 3.49 mg/kg), consistent with the characteristic ionic enrichment of salt-affected soils. EC in SS reflected this accumulation of soluble salts, mainly Na^+ and Mg^{2+} , while NSS contained greater levels of organic matter ($1.47 \pm 0.28\%$) and available phosphorus (153.26 ± 10.19 mg/kg), indicating relatively higher fertility and nutrient exchange potential. Soil salinity is often accompanied by concurrent increases in Na^+ and Mg^{2+} , and higher combined concentrations of these cations have been shown to elevate EC and correlate strongly with salinity indices²⁶. Consistent with this pattern, Wanga *et al.*, 2025 reported significant positive correlations between EC and both Na^+ and Mg^{2+} in saline soils, which agrees with the elevated values observed in SS in the present study. High salinity is

known to suppress OM accumulation and phosphorus availability, largely through reduced microbial activity and altered phosphorus precipitation equilibria^{28,29}. Excess Na⁺ may further exacerbate soil degradation by displacing exchangeable cations due to its large hydration radius, promoting clay dispersion and swelling, and thereby reinforcing a positive feedback loop of salt accumulation³⁰. In contrast, organic amendments such as compost or animal manure have been shown to enhance microbial biomass carbon and stimulate the release of organic acids and humic substances, collectively improving P availability within the soil-plant system^{31,32}. Wang *et al.*, 2021 additionally identified Ca²⁺ as a cation positively associated with OM content, suggesting its role in OM accumulation and stabilization. The concurrently higher levels of OM, Ca²⁺, and available P in NSS reflect these ecological and biochemical mechanisms, whereas their depletion in SS indicates reduced nutrient bioavailability and diminished soil fertility under saline conditions. Collectively, these physicochemical patterns demonstrate that salinity-driven enrichment of Na⁺ and Mg²⁺, combined with the depletion of Ca²⁺, OM, and P, establishes a soil environment less favorable for plant growth and metabolic functioning.

In accordance with these soil characteristics, the assessment of *L. tetragonum* growth response highlighted the biological consequences of salinity. Plants cultivated in NSS exhibited significantly greater leaf width and higher leaf numbers than those grown in SS, indicating that reduced salinity promotes more vigorous vegetative development^{34,35}. Fresh and dry biomass production was also substantially higher under non-saline conditions, although the dry yield percentage remained unaffected by soil salinity. By contrast, leaf and root lengths showed no significant variation, suggesting that salinity primarily influences leaf initiation and overall biomass rather than organ elongation. These findings indicate that *L. tetragonum* achieves optimal growth and productivity under lower salinity, underscoring its potential for agronomic cultivation in non-saline or moderately saline environments.

Primary metabolites are organic compounds involved in vital physiological processes within organisms. They are universally present across plant species and play key roles in growth, development, and reproduction³⁶. In contrast, secondary metabolites including flavonoid glycosides like myricetin and quercetin derivatives are not strictly required for basic life processes but are synthesized in response to diverse environmental factors such as salinity, drought, light, and pathogen attack. These compounds play crucial roles in plant adaptation, environmental stress tolerance, and ecological interactions^{37,38,39,40}.

Catechins and flavonoid glycosides have been previously identified as major secondary metabolites in *L. tetragonum* through both phytochemical characterization and biological studies^{8,16}. In the present study, quantitative analysis revealed that all five major compounds comprising myricetin glycosides and tetrahydroxy flavanone derivatives were present at significantly higher levels in plants grown in NSS than in SS. Among these, compounds **2** and **3** represented the most abundant constituents in both soil types, yet their accumulation was markedly greater under non-saline conditions. Consistent patterns were observed for compounds **1**, **4**, and **5**, collectively indicating that reduced salinity promotes secondary metabolite biosynthesis and accumulation. Comparable responses have been observed in other plant species. Yang *et al.*, 2022 demonstrated that *Tetrastigma hemsleyanum* initially increased flavonoid accumulation under short-term high-salinity exposure, but after 12 days, the highest concentrations occurred under low-salinity treatment. Similarly, Taffouo *et al.*, 2017 observed that flavonoid content in *Capsicum annuum* L. reduced with increasing NaCl concentrations and peaked in the absence of salt. Together, these results reinforce the emerging understanding that moderate or non-saline environments are more conducive to sustained flavonoid and related secondary metabolite production than high-salinity conditions.

Building on the patterns observed for flavonoid metabolites, the influence of soil salinity on pigment-type secondary metabolites was further examined by quantifying total anthocyanin and carotenoid contents (Fig. 4). Anthocyanins and carotenoids are well-known antioxidant pigments that play pivotal roles in photoprotection and oxidative stress mitigation by scavenging reactive oxygen species and modulating stress signaling pathways^{42,43,44}. Both pigments accumulated at significantly higher levels in *L. tetragonum* grown in NSS compared with SS ($p < 0.01$ for anthocyanins, $p < 0.001$ for carotenoids), paralleling the trends observed for flavonoid glycosides. These results are consistent with previous reports showing that pigment accumulation in many plant species declines under salt stress, underscoring the inhibitory effect of high salinity on pigment metabolism^{42,45}.

The elevated pigment levels under non-saline conditions corresponded with enhanced antioxidant activities measured by biochemical and cellular assays. In DPPH and ABTS assays, *L. tetragonum* extracts from NSS exhibited markedly higher radical scavenging capacities than those from SS. In HaCaT cells, ROS production induced H₂O₂ was attenuated by treatments with *L. tetragonum* from both SS and NSS. Although the cell-based ROS assay did not

distinguish a pronounced superiority of NSS over SS, the consistently stronger performance of NSS extracts in DPPH and ABTS assays highlights their greater intrinsic chemical antioxidant capacity. Previous studies have highlighted that discrepancies between in vitro chemical assays and cellular ROS readouts often arise from differences in assay principles and biological complexity^{46,47,48}. While DPPH and ABTS assays quantify direct free radical scavenging in a controlled chemical system, cellular assays reflect additional physiological factors such as membrane permeability, cellular uptake, metabolism, and interactions with intracellular targets⁴⁹.

The increased accumulation of secondary metabolites observed in *L. tetragonum* grown in NSS can be mechanistically explained by previous studies linking soil physicochemical properties with secondary metabolism in plants. NSS generally exhibits higher OM content, which functions as a reservoir and source of key macronutrients, particularly N and P^{50,51,52}. OM–Ca²⁺–P interactions enhance P retention and bioavailability, thereby promoting nutrient cycling and microbial activity that, in turn, support both primary and secondary metabolism and lead to increased accumulation of flavonoids, phenolics, and pigment compounds^{53,54}. P metabolism is especially critical, as ATP derived from P-containing compounds provides the energy required for the enzymatic reactions involved in phenolic biosynthesis⁵⁵. In accordance with this hypothesis, P deficiency has been shown to suppress the expression of flavonoid pathway genes⁵⁶, whereas P fertilization significantly enhances the contents of phenolics, flavonoids, carotenoids, and tannins in sorghum grain⁵⁷. Furthermore, recent work has reported that higher levels of OM in soil are associated with increased quercetin accumulation⁵⁸, which is in close agreement with the elevated levels of flavonoid glycosides, anthocyanins, and carotenoids observed in NSS-grown *L. tetragonum* in the present study. Taken together, these observations indicate that enhanced OM status and P availability under non-saline conditions are likely to contribute to the superior secondary metabolite profiles of *L. tetragonum*, and point to an important role for soil–plant nutrient interactions in modulating halophyte secondary metabolism and antioxidant capacity in conventional agriculture.

PCA was employed to elucidate multivariate relationships among soil physicochemical properties, major secondary metabolite contents, pigment-type metabolites and antioxidant activities in *L. tetragonum*. The biplot (Fig. 10) revealed distinct clustering of samples by soil type, with NSS-grown plants (blue circles) separated from SS-grown plants (red circles), reflecting contrasting physiological and biochemical profiles. PC1, explaining 68.5% of the

total variance, was primarily associated with high levels of secondary metabolites (compounds **1–5**), pigment metabolites (anthocyanins and carotenoids), and antioxidant capacities (DPPH and ABTS), all of which showed strong positive loadings aligned with the NSS group. Conversely, soil salinity indicators (EC, Na⁺, Mg²⁺, K⁺) exhibited positive loadings toward the SS group, while pH, OM, Ca²⁺, and available P were aligned with NSS, underscoring how edaphic factors drive metabolic differentiation. Anthocyanins and carotenoids co-varied with DPPH and ABTS activities, reinforcing their coordinated accumulation and contribution to antioxidant potential under non-saline conditions. PC1 also showed a strong negative loading for ROS, which clustered with SS samples, indicating heightened oxidative stress in saline environments. These patterns demonstrate that NSS promote biosynthetic pathways for antioxidant compounds and radical scavenging, whereas saline conditions elevate ROS signaling and suppress metabolite accumulation. Overall, the PCA results validated the differences between the NSS and SS groups, and identified soil salinity as a key environmental determinant of halophyte physiology and biochemistry.

5. Conclusions

This study provides evidence that soil physicochemical properties, particularly salinity, regulate plant growth, secondary metabolism, and antioxidant responses in the halophyte *L. tetragonum*. Under NSS conditions, plants displayed greater vegetative biomass, higher accumulation of major flavonoid glycosides, and anthocyanins and carotenoids, and enhanced antioxidant capacities as assessed by DPPH and ABTS assays, compared to plants grown in SS. Multivariate PCA revealed a clear separation between SS and NSS samples, with NSS associated with elevated secondary metabolite levels and antioxidant activities, whereas SS was associated with indicators of enhanced oxidative stress. Importantly, higher levels of soil OM, Ca^{2+} , and available P in NSS were closely associated with metabolic patterns, suggesting that the soil fertility parameters and related edaphic processes play a significant role in modulating the phytochemical profile of *L. tetragonum*. Taken together, these findings indicate that non-saline cultivation conditions can support both the agronomic performance and bioactive metabolite production of this halophyte, and they identify soil salinity as an important environmental factor governing halophyte physiology and metabolism in managed systems.

Funding

This research was funded by a grant from “Development of living shoreline technology based on blue carbon science toward climate change adaption” of the Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Ocean and Fisheries (KIMST-20220526) and by National Research Foundation of Korea (NRF) grants funded by the Ministry of Science, ICT and Future Planning (Grant Number NRF- 2023R1A2C1007448).

Competing interests

None declared.

Author contributions

Min Gyu Park: Writing - original draft, Formal analysis, investigation. **Yun Na Kim:** Investigation. Formal analysis. **Jae sun Lee:** Formal analysis. **Won Jin Jung:** Formal analysis. **Yu Jung Kim:** Formal analysis. **Seon Min Lee:** Formal analysis. **Min Hye Yang:** Investigation. Methodology. **Bong-Oh Kwon:** Funding acquisition, Project administration. **Jung-Rae Rho:** Methodology, Data curation, Supervision. **Eun Ju Jeong:** Conceptualization, Writing – original draft, Writing – review & editing, Data curation, Project administration, Supervision.

Data availability

All data generated or analysed during this study are included in this published article.

References

1. Li, J., Pu, L., Han, M., Zhu, M., Zhang, R. & Xiang, Y. Soil salinization research in China: Advances and prospects. *J. Geogr. Sci.* **24**, 943-960 (2014); <https://doi.org/10.1007/s11442-014-1130-2>.
2. Munns R. Genes and salt tolerance: bringing them together. *New Phytol.* **167**(3), 645-663 (2005); <https://doi.org/10.1111/j.1469-8137.2005.01487.x>.
3. Hassani, A., Azapagic, A. & Shokri, N. Predicting long-term dynamics of soil salinity and sodicity on a global scale. *PNAS.* **117**(52), 33017-33027 (2020); <https://doi.org/10.1073/pnas.2013771117>.
4. Ivushkin, K., Bartholomeus, H., Bregt, A.K., Pulatov, A., Kempen, B. & De Sousa, L. Global mapping of soil salinity change. *RSE.* **231**, 111260 (2019); <https://doi.org/10.1016/j.rse.2019.111260>.
5. FAO. FAO launches first major global assessment of salt-affected soils in 50 years. Food and Agriculture Organization of the United Nations <https://www.fao.org/newsroom/detail/fao-launches-first-major-global-assessment-of-salt-affected-soils-in-50-years/en> (2024).
6. Flowers, T.J. & Colmer, T.D. Salinity tolerance in halophytes. *New Phytol.* **179**, 945-963 (2008); <https://www.jstor.org/stable/25150520>.
7. Lutts, S. et al. Heavy metal accumulation by the halophyte species Mediterranean saltbush. *J. Environ. Qual.* **33**(4), 1271-1279 (2004); <https://doi.org/10.2134/jeq2004.1271>.
8. Jang, S. et al. Physiological and biochemical responses of *Limonium tetragonum* to NaCl concentrations in hydroponic solution. *Front. Plant Sci.* **14**, 1159625 (2023); <https://doi.org/10.3389/fpls.2023.1159625>.
9. Abdellaoui, R., Elkelish, A., El-Keblawy, A., Mighri, H., Boughalleb, F. & Bakhshandeh, E. Halophytes: salt stress tolerance mechanisms and potential use. *Front. Plant Sci.* **14**, 1218184 (2023); <https://doi.org/10.3389/fpls.2023.1218184>.
10. Ihm, B., Lee, J. & Kim, J. Coastal vegetation on the western, southern, and eastern coasts of south Korea. *J. Plant Biol.* **44**, 163-167 (2001); <https://doi.org/10.1007/BF03030234>.
11. Kim, N., Sung, S., Heo, J. & Jeong, E. The extract of *Limonium tetragonum* protected liver against acute alcohol toxicity by enhancing ethanol metabolism and antioxidant

- enzyme activities. *NPS*. **21(1)**, 54-58 (2015).
12. Kim, J. et al. *Limonium Tetragonum* Enhances Osteoblastogenesis while Suppressing the Adipocyte Differentiation. *OPR*. **44(1)**, 29-38 (2022); <https://doi.org/10.4217/OPR.2022002>.
13. Shrestha, S., Cho, H., Kim, S., Jin, J. & Soh, Y. *Limonium tetragonum* Reduces Osteoclast Formation and Resorption through Mitogen-activated Protein Kinase-c-Fos-NFATc1 Signaling Pathways. *NPS*. **29(4)**, 323-329 (2023); <https://doi.org/10.20307/nps.2023.29.4.323>.
14. Lee, Y., Song, M., Cho, H., Jin, J., Park, B. & Bae, E. *Limonium tetragonum* promotes running endurance in mice through mitochondrial biogenesis and oxidative fiber formation. *Nutrients*. **14(19)**, 3904 (2022); <https://doi.org/10.3390/nu14193904>.
15. Lee, J., Kong, C., Jung, M., Hong, J., Lim, S. & Seo, Y. Antioxidant activity of the halophyte *Limonium tetragonum* and its major active components. *BBE*. **16**, 992-999 (2011). <https://doi.org/10.1007/s12257-011-0213-5>.
16. Lee, J. et al. Identification of hepatoprotective constituents in *Limonium tetragonum* and development of simultaneous analysis method using high-performance liquid chromatography. *Pharmacogn. Mag.* **13(52)**, 535 (2017); https://doi.org/10.4103/pm.pm_477_16.
17. Gee, G. & Or, D. 2.4 Particle-size analysis in Methods of soil analysis: Part 4 physical methods (eds Dane, J. H. & Topp, G. C.) 255-293 (Soil Science Society of America, 2002).
18. Bray, R. & Kurtz, L. Determination of total, organic, and available forms of phosphorus in soils. *Soil science*. **59.1**, 39-46 (1945).
19. Amalia, E., Diantini, A. & Subarnas, A. Water-soluble propolis and bee pollen of *Trigona* spp. from South Sulawesi Indonesia induce apoptosis in the human breast cancer MCF-7 cell line. *Oncol. Lett.* **20(5)**, 274 (2020); <https://doi.org/10.3892/ol.2020.12137>.
20. Zhang, X. et al. Three-dimensional printed cell culture model based on spherical colloidal lignin particles and cellulose nanofibril-alginate hydrogel. *Biomacromolecules*. **21(5)**, 1875-1885 (2020); <https://doi.org/10.1021/acs.biomac.9b01745>.
21. Lee, J., Durst, R. & Wrolstad, R. AOAC official method 2005.02: total monomeric

- anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method. *AOAC OMA*. **2** (2025).
22. Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M. & Shahidi, F. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *J. Agric. Food Chem.* **53(19)**, 7592-7599 (2005); <https://doi.org/10.1021/jf050579q>.
23. Shrivastava, P. & Kumar, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* **22(2)**, 123-131 (2015); <https://doi.org/10.1016/j.sjbs.2014.12.001>.
24. Zhao, C., Geng, R., Chi, T., Khiewngamdee, C. & Liu, J. Agricultural technology innovation and food security in china: An empirical study on coupling coordination and its influencing factors. *Agronomy*. **14(1)**, 123 (2024); <https://doi.org/10.3390/agronomy14010123>.
25. Flowers, T., Galal, H. & Bromham, L. Evolution of halophytes: multiple origins of salt tolerance in land plants. *Funct. Plant Biol.* **37(7)**, 604-612 (2010); <https://doi.org/10.1071/FP09269>.
26. Aadiwal, V., Meher, K., Kalidhas, A. & Mishra, A. Spatial Modeling of Soil Salinity and Its Impact on Nutrient Availability and Agricultural Productivity. *NESciences*. **10(1)**, 312-324 (2025); <https://doi.org/10.28978/nesciences.1648720>.
27. Wanga, M. et al. Impact of Paddy Fields on Soil Salinity and Sodicty at Kalimbeza Rice Project, Namibia. *OJSS*. **15(7)**, 477-495 (2025); <https://doi.org/10.4236/ojss.2025.157020>.
28. Su, R. et al. Interactive effects of phosphorus fertilization and salinity on plant growth, phosphorus and sodium status, and tartrate exudation by roots of two alfalfa cultivars. *Ann. Bot.* **129(1)**, 53-64 (2022); <https://doi.org/10.1093/aob/mcab124>.
29. Hassani, A., Smith, P. & Shokri, N. Negative correlation between soil salinity and soil organic carbon variability. *PNAS*. **121(18)**, e2317332121 (2024); <https://doi.org/10.1073/pnas.2317332121>.
30. Yan, S. et al. The higher relative concentration of K⁺ to Na⁺ in saline water improves soil hydraulic conductivity, salt-leaching efficiency and structural stability. *Soil*. **9(1)**, 339-349 (2023); <https://doi.org/10.5194/soil-9-339-2023>.

31. Muhammad, S., Müller, T. & Joergensen, R. Compost and P amendments for stimulating microorganisms and maize growth in a saline soil from Pakistan in comparison with a nonsaline soil from Germany. *J. Plant Nutr. Soil Sci.* **170(6)**, 745-752 (2007); <https://doi.org/10.1002/jpln.200625122>.
32. Marschner, P. The role of rhizosphere microorganisms in relation to P uptake by plants in The ecophysiology of plant-phosphorus interactions (eds White, P. J. & Hammond, J. P.) 165-176 (Springer, 2008).
33. Wang, S., Jia, Y., Liu, T., Wang, Y., Liu, Z. & Feng, X. Delineating the role of calcium in the large-scale distribution of metal-bound organic carbon in soils. *Geophys. Res. Lett.* **48(10)**, e2021GL092391 (2021); <https://doi.org/10.1029/2021GL092391>.
34. Naidoo, G., Somaru, R. & Achar, P. Morphological and physiological responses of the halophyte, *Odysea paucinervis* (Staph)(Poaceae), to salinity. *Flora.* **203(5)**, 437-447 (2008); <https://doi.org/10.1016/j.flora.2007.08.003>.
35. Alhaddad, F., Abu-Dieyeh, M., ElAzazi, E. & Ahmed, T. Salt tolerance of selected halophytes at the two initial growth stages for future management options. *Sci. Rep.* **11(1)**, 10194 (2021); <https://doi.org/10.1038/s41598-021-89462-3>.
36. Qaderi, M., Martel, A. & Strugnell, C. Environmental factors regulate plant secondary metabolites. *Plants.* **12(3)**, 447 (2023); <https://doi.org/10.3390/plants12030447>.
37. Wink, M. Introduction: biochemistry, physiology and ecological functions of secondary metabolites in Annual plant reviews volume 40: Biochemistry of plant secondary metabolism (ed. Wink, M.) 1-19 (Wiley-Blackwell, 2010).
38. Ashraf, M. et al. Environmental stress and secondary metabolites in plants: an overview in Plant metabolites and regulation under environmental stress (eds Ahmad, P. et al.) 153-167 (Academic Press, 2018).
39. Shomali, A. et al. Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. *Plants.* **11(22)**, 3158 (2022); <https://doi.org/10.3390/plants11223158>.
40. Yang, L. et al. Salt interferences to metabolite accumulation, flavonoid biosynthesis and photosynthetic activity in *Tetrastigma hemsleyanum*. *Environ. Exp. Bot.* **194**, 104765 (2022); <https://doi.org/10.1016/j.envexpbot.2021.104765>.
41. TAFFOUO, V., NOUCK, A., NYEMENE, K., TONFACK, B., MEGUEKAM, T. & YOUNBI, E., 2017. Effects of salt stress on plant growth, nutrient partitioning,

- chlorophyll content, leaf relative water content, accumulation of osmolytes and antioxidant compounds in pepper (*Capsicum annuum* L.) cultivars. *Not Bot Horti Agrobo.* **45**(2), 481-490. <https://doi.org/10.15835/nbha45210928>.
42. Jeon, D., Lee, S., Choi, S., Seo, S. & Kim, C. Effect of salt stress on the anthocyanin content and associated genes in *Sorghum bicolor* L. *KJOAS.* **47**(1), 105-117 (2020); <https://doi.org/10.7744/kjoas.20200003>.
43. Cerqueira, J. et al. Anthocyanins and reactive oxygen species: a team of rivals regulating plant development?. *Plant Mol. Biol.* **112**(4), 213-223 (2023); <https://doi.org/10.1007/s11103-023-01362-4>.
44. Shanaida, M. et al. Carotenoids for Antiaging: Nutraceutical, Pharmaceutical, and Cosmeceutical Applications. *Pharmaceuticals.* **18**(3), 403 (2025); <https://doi.org/10.3390/ph18030403>.
45. Batra, N., Kumari, N. & Sharma, V. Salt stress in plants and amelioration strategies: alleviation of agriculture and livelihood risks after the Covid-19 pandemic. *Vegetos.* **36**(1), 268-274 (2023); <https://doi.org/10.1007/s42535-022-00534-9>.
46. Honzel, D., Carter, S., Redman, K., Schauss, A., Endres, J. & Jensen, G. Comparison of chemical and cell-based antioxidant methods for evaluation of foods and natural products: generating multifaceted data by parallel testing using erythrocytes and polymorphonuclear cells. *J. Agric. Food Chem.* **56**(18), 8319-8325 (2008); <https://doi.org/10.1021/jf800401d>.
47. Liemburg-Apers, D., Willems, P., Koopman, W. & Grefte, S. Interactions between mitochondrial reactive oxygen species and cellular glucose metabolism. *Arch. Toxicol.* **89**(8), 1209-1226 (2015); <https://doi.org/10.1007/s00204-015-1520-y>.
48. Apak, R., Ozyurek, M., Guclu, K. & Capanoglu, E. Antioxidant activity/capacity measurement. 3. Reactive oxygen and nitrogen species (ROS/RNS) scavenging assays, oxidative stress biomarkers, and chromatographic/chemometric assays. *J. Agric. Food Chem.* **64**(5), 1046-1070 (2016); <https://doi.org/10.1021/acs.jafc.5b04744>.
49. Nwachukwu, I., Sarteshnizi, R., Udenigwe, C. & Aluko, R. A concise review of current in vitro chemical and cell-based antioxidant assay methods. *Molecules.* **26**(16), 4865 (2021); <https://doi.org/10.3390/molecules26164865>.
50. Marschner, H. Marschner's mineral nutrition of higher plants. Academic press (2011).

51. Aoyama, M. Functional roles of soil organic matter. *Humic Subs Res.* **12**, 21-28 (2015).
52. Audette, Y., Smith, D., Parsons, C., Chen, W., Rezanezhad, F. & Van Cappellen, P. Phosphorus binding to soil organic matter via ternary complexes with calcium. *Chemosphere.* **260**, 127624 (2020); <https://doi.org/10.1016/j.chemosphere.2020.127624>.
53. Wink, M. Plant secondary metabolism: diversity, function and its evolution. *Nat. Prod. Commun.* **3**(8), 1934578X0800300801 (2008); <https://doi.org/10.1177/1934578X0800300801>.
54. Chaouqi, S. et al. Effect of soil composition on secondary metabolites of moroccan saffron (*Crocus sativus* L.). *Plants.* **12**(4), 711 (2023); <https://doi.org/10.3390/plants12040711>.
55. Zulfiqar, S., Gu, R., Liu, Y. & Zhang, Y. From genes to traits: maximizing phosphorus utilization efficiency in crop plants. *Front. Plant Sci.* **16**, 1527547 (2025); <https://doi.org/10.3389/fpls.2025.1527547>.
56. Nezamivand-Chegini, M. et al. Nitrogen and phosphorus deficiencies alter primary and secondary metabolites of soybean roots. *BioRxiv.* 2022-03 (2022). <https://doi.org/10.1101/2022.03.14.484309>
57. Elsafy, M. et al. Unveiling the influences of P fertilization on bioactive compounds and antioxidant activity in grains of four sorghum cultivars. *Plos one.* **19**(10), e0311756 (2024); <https://doi.org/10.1371/journal.pone.0311756>.
58. Fonseca, S., Pereira, A. & Lopes, N. Secondary Metabolite Plasticity in *Eclipta prostrata* (L.) L.(Asteraceae) under Environmental and Biological Stressors. *ACS Omega.* **10**, 62916-62926 (2025); <https://doi.org/10.1021/acsomega.5c08697>.

Figure legends

Fig. 1. Classifications of soils in sampling sites. Soil samples from each group were separated by granulometric analysis, and the percentage of sand (0.063-2.0mm), silt (0.004-0.063mm), and clay (<0.004mm) was calculated. Data are presented as weight percent (%).

Fig. 2. Leaf length (A), Leaf width (B), Number of leaves (C) and Root length (D) of *L. tetragonum* grown in saline soils (SS, n=12) and non-saline soils (NSS, n=13). Growth characteristics were investigated prior to flowering. Asterisks indicate statistically significant differences between groups as determined by Student's t-test ($p < 0.001$).

Fig. 3. Antioxidant activities (IC_{50} values) of *L. tetragonum* extracts from plants grown in saline soil (SS) and non-saline soil (NSS) conditions, measured by DPPH (A) and ABTS (B) radical scavenging assays. Results were presented as mean $\pm$ SD (SS, n=11; NSS, n=14). L-ascorbic acid and Trolox were used as standard antioxidants. 50%E SS: 50% EtOH extract from saline soil, 50%E NSS: 50% EtOH extract from non-saline soil, 100%E SS: 100% EtOH extract from saline soil, 100%E NSS: 100% EtOH extract from non-saline soil. *** $p < 0.001$ vs standard.

Fig. 4. The cytotoxicities of *L. tetragonum* extracts from plants grown under saline soil (SS, n=11) (A) and non-saline soil (NSS, n=14) (B) against HaCaT cells. Cells were treated with each extract (0, 5, 10, 20, and 40 ug/ml) for 24 h, and cell viability was determined by MTT assay. Each data represents mean $\pm$ SD of three replicates per sample.

Fig. 5. Effects of *L. tetragonum* extracts from plants grown under saline soil (SS, n=11) and non-saline soil (NSS, n=14) conditions on H_2O_2 -induced oxidative stress in HaCaT cells. Intracellular ROS was measured by flow cytometry using the Muse® Oxidative Stress Kit. Shown are percentages of ROS^- (M1, blue) and ROS^+ (M2, red) cells (A). Quantification of ROS^- and ROS^+ cells in each group. Each data represents mean $\pm$ SD of three replicates per sample (B). NAC (5 mM) was used as a positive control. *** $p < 0.001$ vs H_2O_2 .

Fig. 6. Total anthocyanin (A) and total carotenoid (B) contents in *L. tetragonum* extracts from plants grown in saline (SS, n=11) and non-saline (NSS, n=14) soil conditions. Each data represents mean $\pm$ SD of three replicates per sample. Asterisks indicate statistically significance differences between groups as determined by Student's t-test (** $p < 0.01$, *** $p < 0.001$)

Fig. 7. Product ion mass spectra of compound 1 (A), compound 2 (B), compound 3 (C) compound 4 (D) and compound 5 (E) isolated from *L. tetragonum*

Fig. 8. Extracted ion chromatograms of analytes quantified compound 1 (A), compound 2 (B), compound 3 (C) compound 4 (D) and compound 5 (E) isolated from *L. tetragonum* in MRM mode of LC-ESI-MS/MS

Fig. 9. Quantitative analysis of (–)-epigallocatechin-3-gallate (A), myricetin-3-O- β -D-galactopyranoside (B), myricetin-3-O- α -L-rhamnopyranoside (C), 5,7,2',5'-tetrahydroxy flavanone-3-O- β -D-galactoside (D) and myricetin-3-O-(2''O-galloyl)- α -L-rhamoside (E) *L. tetragonum* extracts from plants grown under saline soil (SS) and non-saline soil (NSS) conditions, as determined by LC-ESI-MS/MS. Results are presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 10. PCA biplot of soil physicochemical properties (EC, pH, OM, Na⁺, Mg²⁺, K⁺, Ca²⁺, and P), pigment type secondary metabolites (total anthocyanin and total carotenoid contents), major secondary metabolite contents (compounds 1–5) and antioxidant activities (ROS, DPPH, and ABTS) in *L. tetragonum* extracts cultivated in saline soil (SS, red circles) and non-saline soil (NSS, blue circles).

Table 1. Comparison of physicochemical properties between saline and non-saline soils.

Habitats	pH	EC (ds/m)	Organic Matter (%)	Exchangeable (mg/kg)				Available Phosphorus (mg/kg)
				Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	
Saline soil	6.6 ± 0.17e	11.35 ± 1.42e	0.13 ± 0.07f	256.25 ± 58.91b	269.28 ± 35.29b	135.95 ± 21.78c	2031.75 ± 196.72a	43.36 ± 7.52d
Non- saline soil	6.8 ± 0.08e	0.35 ± 0.04f	1.47 ± 0.28f	1729.12 ± 107.96a	63.69 ± 3.40c	132.21 ± 14.24b	29.21 ± 3.49d	153.26 ± 10.19b

Data represent the mean values ± SD. Different letters indicate significant differences according the Tukey's Multiple Range test ($p < 0.05$).

839 **Table 2.** Dry yield of leaves according to the growth stages of *L. tetragonum*.

Habitats	Fresh weight (g)	Dry weight (g)	Dry yield (%)
Saline soil	4.95 ± 0.85b ^a	1.02 ± 0.22c	20.44 ± 1.44a
Non-saline soil	8.66 ± 1.72b	1.80 ± 0.42c	20.77 ± 2.21a
Significant ^b	***	***	NS

840 ^a Mean separation within column's by Tukey's multiple range test.

841 ^b Significant: NS = nonsignificant, $p < 0.001$ (***).

842

Table 3. Optimized parameters of compound **1-5** for Multiple reaction monitoring (MRM)-MS analysis.

Compounds	precursor ion (m/z)	product ion (m/z)	Optimized Parameters				
			DP	EP	CE	CEP	CXP
(-)-epigallocatechin-3-gallate (1)	457.1	169.1	-30	-9.5	-29.1	-30	-5
myricetin-3-O- β -D-galactopyranoside (2)	479.2	316.8	-85	-6.5	-20	-22	-6
myricetin-3-O- α -L-rhamnopyranoside (3)	463.1	317.2	-70	-5	-16	-22	-6
5,7,2',5'-tetrahydroxy flavanone-3-O- β -D-galactoside (4)	462.9	300.3	-60	-4	-18	-24	-6
myricetin-3-O-(2''O-galloyl)- α -L-rhamnoside (5)	615.3	317	-85	-7.5	-26	-30	-18

DP-declustering potential; EP-entrance potential; CE-collision energy; CEP-cell entrance potential;

CXP-cell exit potential

Table 4. Parameters of the developed method for quantitation of compound **1-5** in LC-ESI MS/MS analysis.

Compounds	Regression equation	R ²	Linearity range (ng/mL)	LOD (ug/mL)	LOQ (ug/mL)
(-)-epigallocatechin-3-gallate (1)	$y = 33.3x - 1.77 \times 10^3$	0.9999	62.5-10000	0.09	0.28
myricetin-3-O- β -D-galactopyranoside (2)	$y = 1.75x - 249$	0.9994	125-10000	0.26	0.78
myricetin-3-O- α -L-rhamnopyranoside (3)	$y = 2.71x - 266$	0.9999	62.5-10000	0.08	0.25
5,7,2',5'-tetrahydroxy flavanone-3-O- β -D-galactoside (4)	$y = 21.7x - 1.88 \times 10^3$	0.9997	62.5-10000	0.14	0.43
myricetin-3-O-(2''O-galloyl)- α -L-rhamnoside (5)	$y = 16x - 1.99 \times 10^3$	0.9994	62.5-10000	0.2	0.61

Fig. 1.

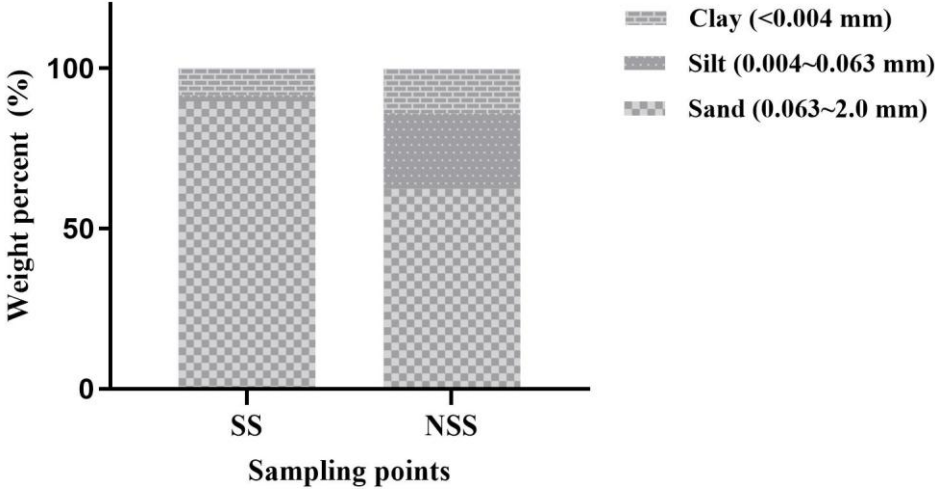


Fig. 2.

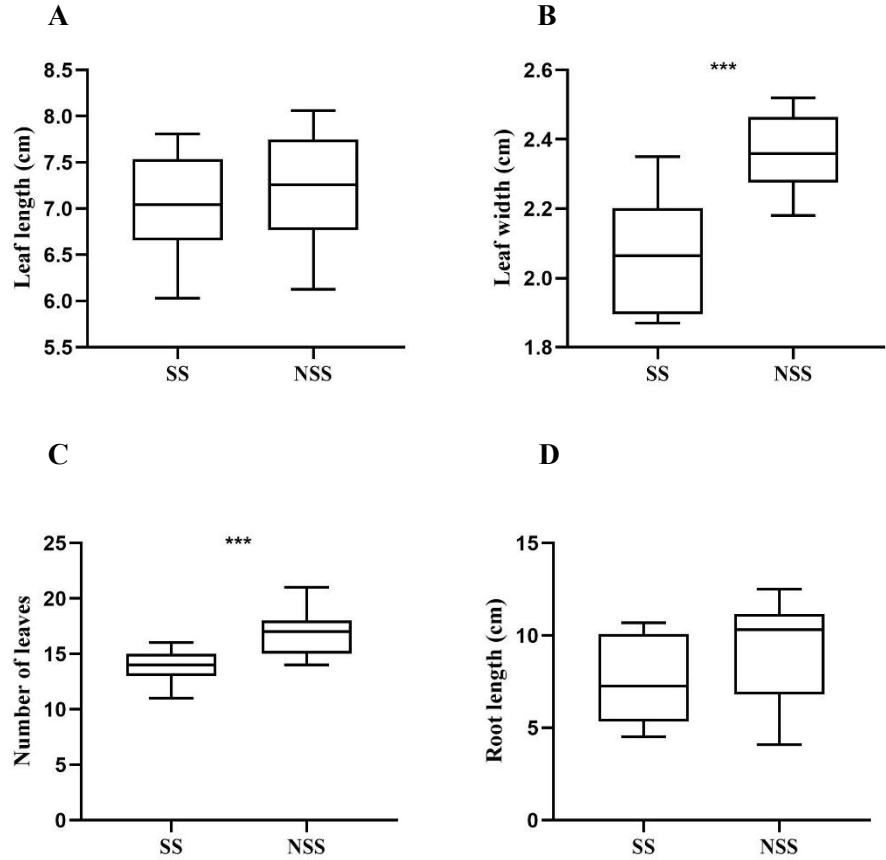
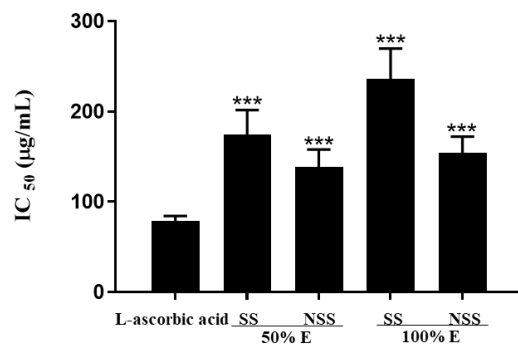


Fig. 3.

A



B

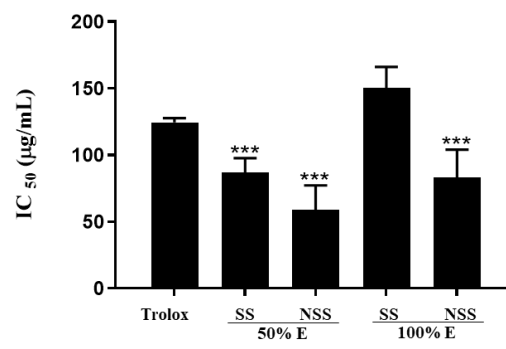
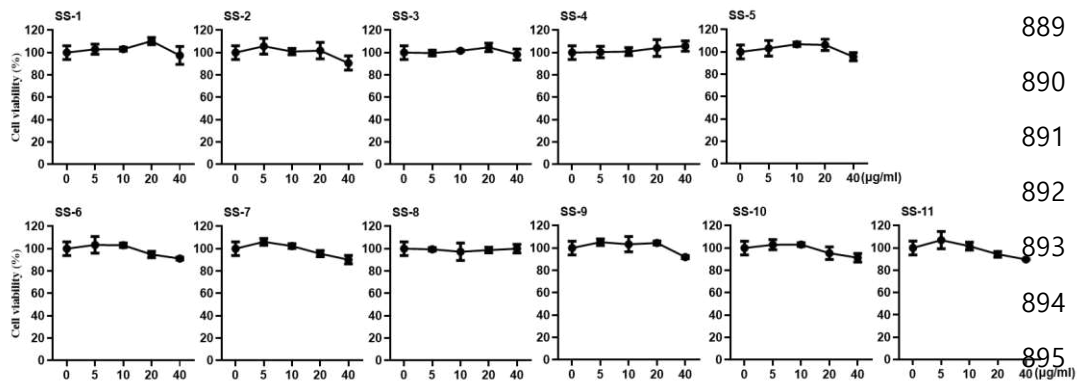


Fig. 4.

A



B

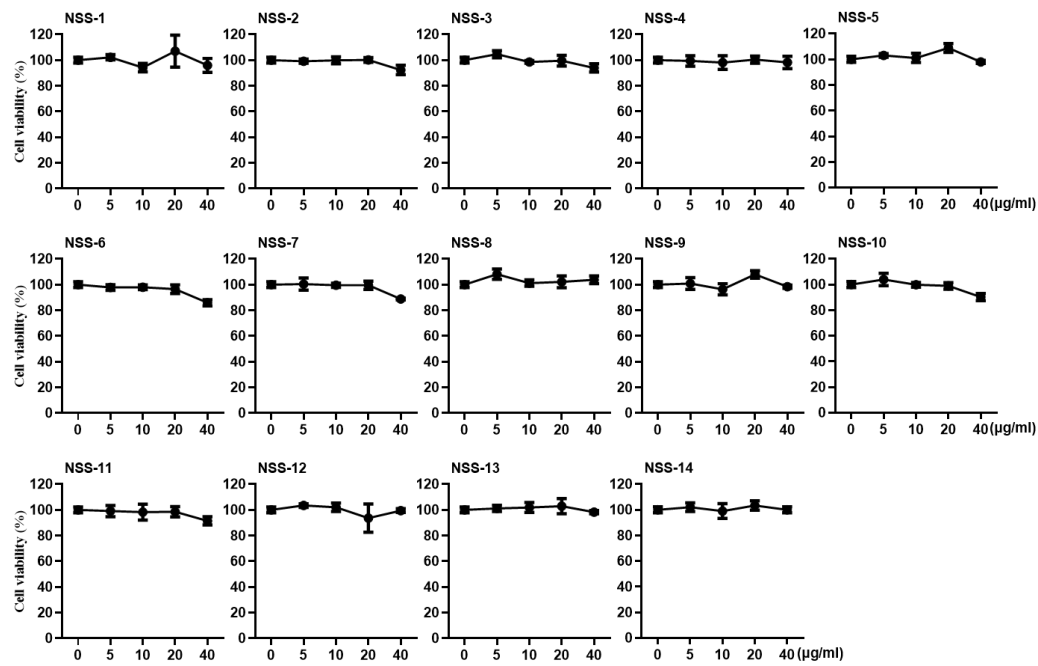
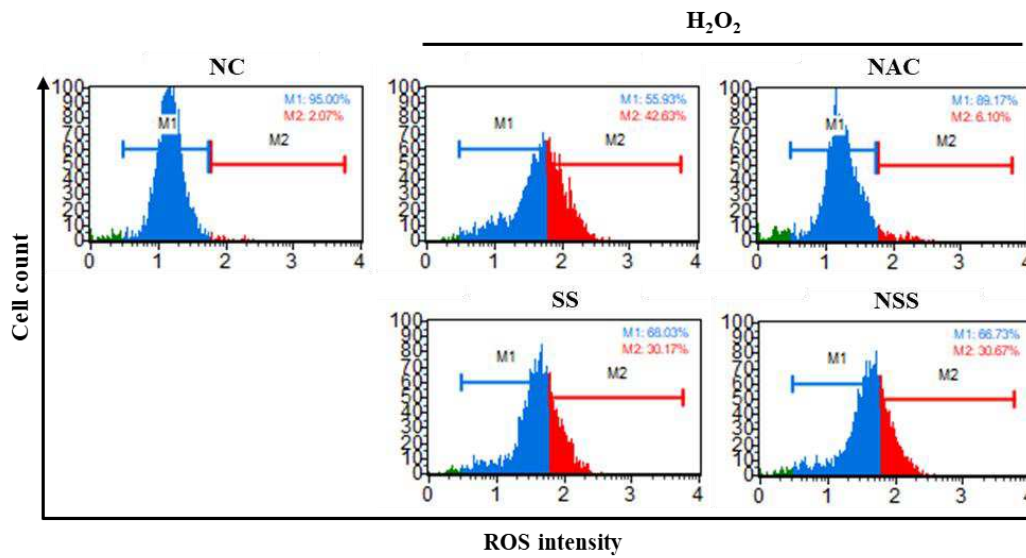
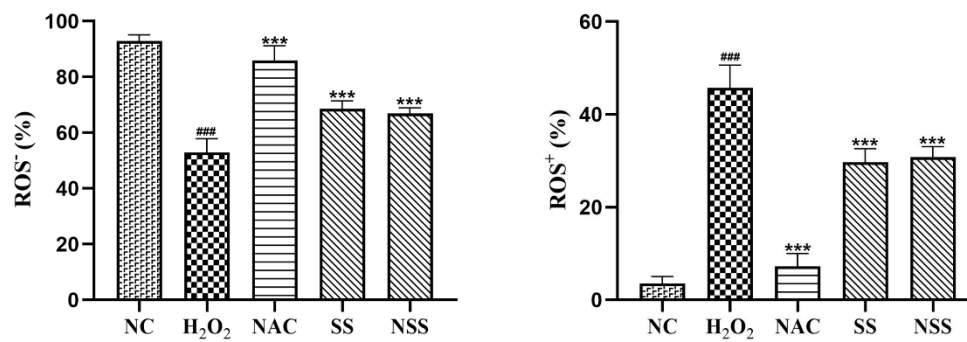


Fig. 5.

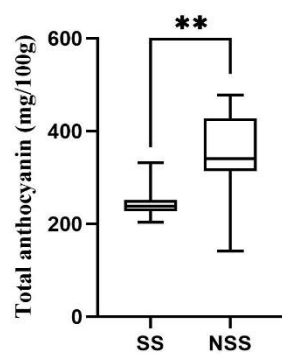
A



B



907 **Fig. 6.**
908 **A**



B

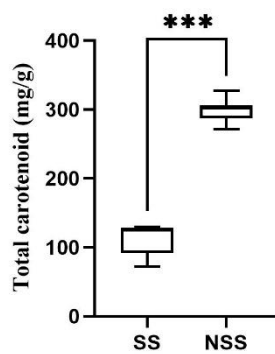
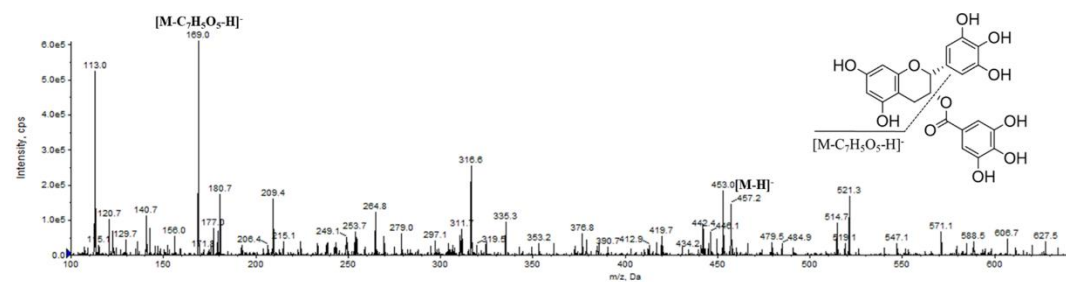
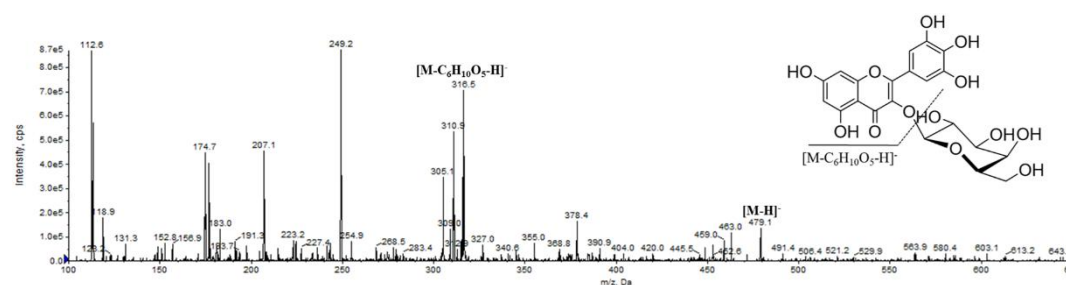


Fig. 7.

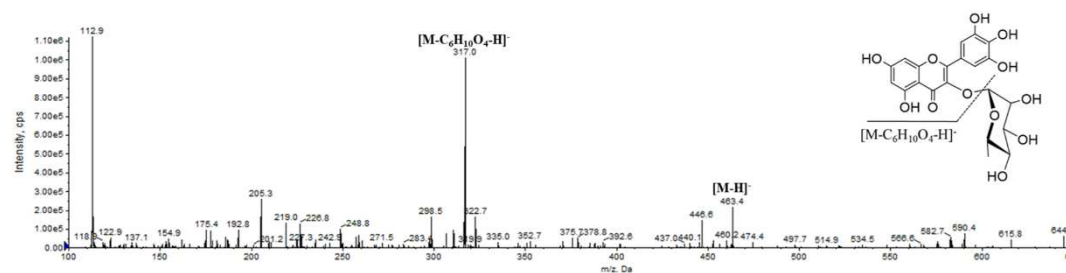
A



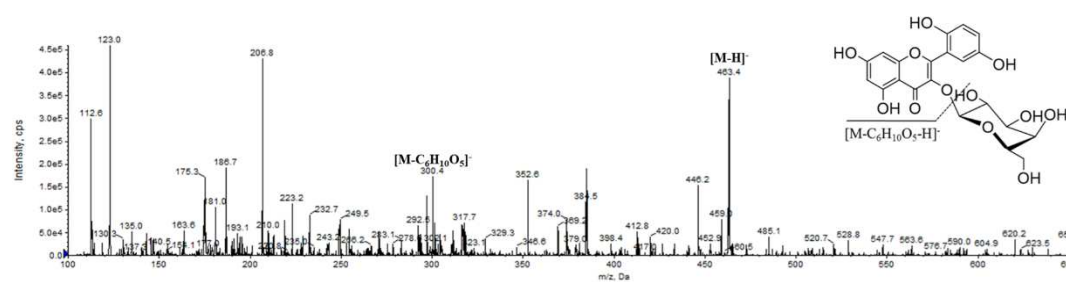
B



C



D



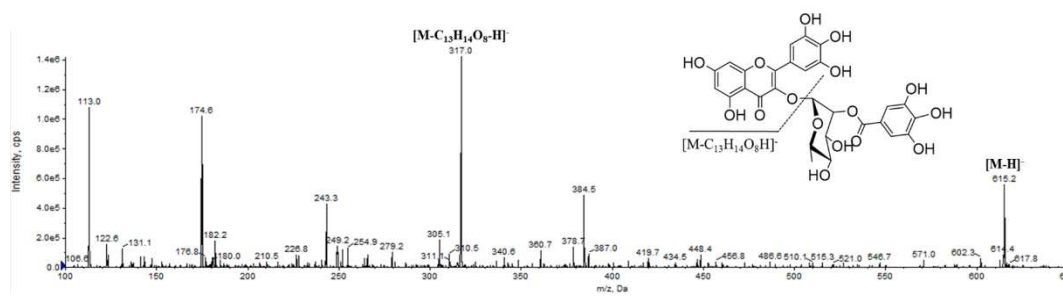
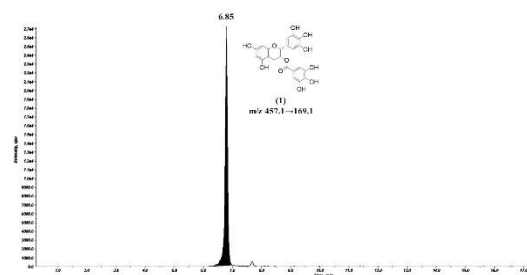
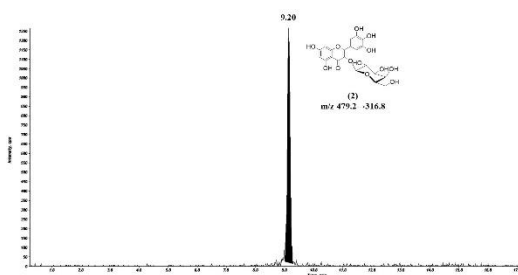


Fig. 8.

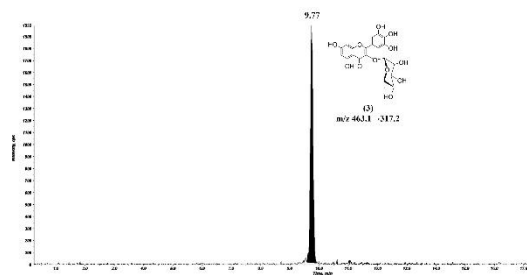
A



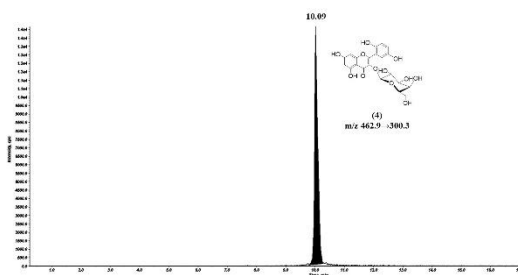
B



C



D



E

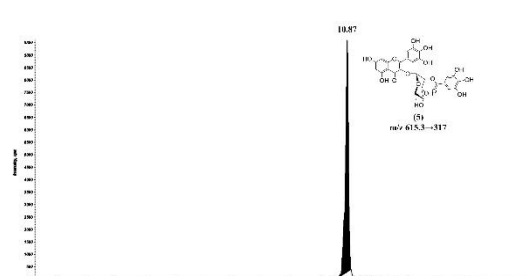
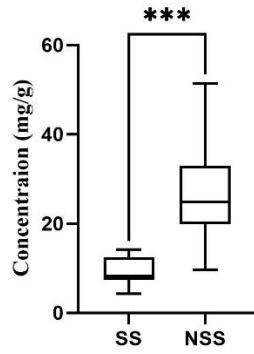
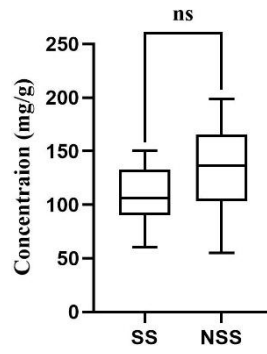


Fig. 9.

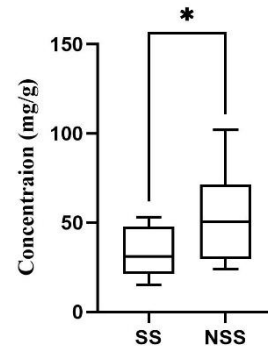
A



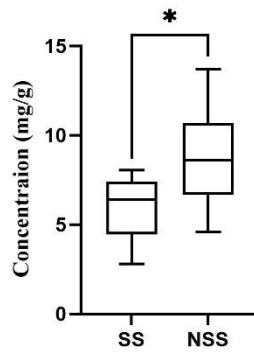
B



C



D



E

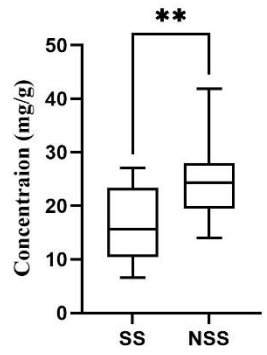


Fig. 10.

PCA - Biplot

