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Original Article

Effects of abiotic factors on *Zingiber officinale* and *Glycyrrhiza glabra* to extract bioactive compounds under different time incubation and different salt concentrations

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Abstract

Zingiber officinale, commonly known as ginger, and Glycyrrhiza glabra, commonly known as licorice, are medicinal plants that are rich in bioactive compounds with various health benefits. This study aimed to investigate the effects of different growth durations and salt concentrations on the production of bioactive compounds by these plants. This experiment was conducted under natural conditions and the plants were subjected to salt stress at different stages of growth. This analysis focused on assessing the production of polysaccharides, flavonoids, ergosterol, adenine, adenosine, hypoxanthine, and guanosine by UV spectrophotometer and high-performance liquid chromatography (HPLC) in both plants. The results showed that Z. officinale exhibited the highest polysaccharide content at 20-d of growth with 3mM salt, whereas G. glabra showed slightly lower polysaccharide content. Similarly, Z. officinale had higher flavonoid content at 25-d of growth with 5 mM salt compared to G. glabra. Additionally, Z. officinale demonstrated higher concentrations of ergosterol, adenine, adenosine, hypoxanthine, and guanosine than G. glabra, particularly at 25-d of growth with 5 mM salt. This study provides valuable insights into the production of bioactive compounds in Z. officinale and G. glabra under different growth conditions, which can be beneficial for optimizing their cultivation and utilization in various applications including pharmaceuticals and functional foods.

Keywords: Abiotic factors, *Zingiber officinale*, *Glycyrrhiza glabra*, Bioactive compounds, Different incubation, Salt stress.

1. Introduction

Zingiber officinale, commonly known as ginger, comprises various components including 40–70% starch, 1.5–3% essential oil, 6–20% protein, 2–11% fixed oil, 9–12% water, and 8–10% ash, along with pungent principles and other saccharides [1]. Additionally, it is a rich source of antioxidants, antimicrobial polyphenols, and flavonoids [2]. Beyond its culinary uses, ginger root has demonstrated efficacy in reducing cholesterol, alleviating arthritis pain, addressing digestive issues, acting as an expectorant, and stimulating intestinal function [3]. Various diseases, including cardiovascular ailments, stroke, diabetes, common cold, rheumatism, asthma, catarrh, gingivitis, toothache, and constipation, have traditionally been treated with ginger in medicinal and therapeutic preparations because of its pharmacological effects, which include antiplatelet, immunomodulatory, anti-tumor, anti-apoptotic, anti-inflammatory, antiviral, antimicrobial, analgesic, antioxidant, and anti-hyperglycemic properties [4]. The main active constituents of ginger, namely gingerol, zingerone, paradol, and shogaol, contribute to its characteristic odor and flavor [5]. Gingerols exhibit potent antioxidant and anticancer properties, thereby offering potential benefits in disease prevention and treatment [6].

Glycyrrhiza glabra commonly known as licorice, belongs to the Leguminosae or Fabaceae families. It is valued as an ethnomedicinal sweetener and calming herb and is used in various applications. Licorice is increasingly being incorporated into numerous commercial products, including pharmaceuticals, foods, beverages, and cosmetics, serving as a flavoring agent [7]. Additionally, it has been recognized for its antioxidant and antimicrobial properties under the name mulethi [8]. Licorice contains two main classes of bioactive compounds: saponins and flavonoids, with glycyrrhizin and glabridin or glabrene as the major constituents. It also contains various nutrients such as proteins, amino acids, simple sugars, polysaccharides, carbohydrates, minerals (manganese and calcium), vitamins (E,

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B1, B2, B3, B5, and C), tannins, coumarins, phytosterols (stigmasterol and sitosterol), and glycosides [9]. Its roots have been reported to exhibit various beneficial properties, including antacid, anti-inflammatory, demulcent, diuretic, anti-ulcer, tonic, expectorant, sedative, and laxative effects [10]. Licorice derivatives can be used in food as encapsulated polyphenolic compounds, owing to their antioxidant and antimicrobial properties. However, it is important to note that the antioxidant activity of dried ginger may be affected by prolonged heating [11]. Therefore, freeze-drying the extracted active components of ginger and licorice is crucial for producing high-quality dried ginger and licorice products, despite higher initial equipment expenditures. This process can result in increased color, nutritional, and flavor stability, while maintaining the natural characteristics of the raw product [12] . This study aimed at the extraction of bioactive compounds from *Zingiber officinale* and *Glycyrrhiza glabra* which grow with different time incubation and different salt concentration.

2. Materials and methods

Zingiber officinale and *Glycyrrhiza glabra* were selected as experimental plants, and *Zingiber officinale* and *Glycyrrhiza glabra* were grown on a large scale in Khyber Pakhtunkhwa. The experiments were conducted under natural conditions at the Department of Botany, Bacha Khan University Charsadda, from 2022 to 2023, with an average day/night temperature of 37 ± 8°C and a photoperiod ranging from 10 to 13 hours. Seeds of two plants were obtained from the Surezai Research Station, Peshawar Khyber Pakhtunkhwa Pakistan, and were grown in 18 pots filled with soil and sand at a ratio of 3:1. *Zingiber officinale* (9 pots) and *Glycyrrhiza glabra* (9 pots) varieties were grown in triplicate. In contrast, salinity stress was applied at the vegetative stages and examined for 15-d (2mM salt), 20-d (3mM salt), 25-d (5 mM salt), 30-d (10mM salt), 35-d (20mM salt), 40-day (30mM salt), 45-d (40mM salt), 50-d (50mM salt), and 55-d (60mM salt). The objectives of the present study were to analyze different types of parameters under the consequence of benzoic acid foliar spraying under salinity stress.

2.2. Experimental species

Zingiber officinale and ***Glycyrrhiza glabra*** were selected for experimental evaluation due to their well-documented bioactive compounds and traditional medicinal uses, particularly their anti-inflammatory and analgesic properties.

2.3. Experimental chemicals

Glucose ($C_6H_{12}O_6$), Glycerin ($C_3H_8O_3$), Methanol (CH_4O or CH_3OH), Distilled water, Sodium Acetate trihydrate ($C_2H_3NaO_2$) (NaAc), acetic acid (CH_3COOH) (HAC), Aluminum Chloride Hexahydrate ($AlCl_3.6H_2O$), Sulfuric Scid (H_2SO_4), Anthrone ($C_{14}H_{10}O$), Potassium periodate (KIO_4), Hydrochloric acid (HCl), L-rhamnose ($C_6H_{12}O_5$), Nash solution (ammonium acetate [$NH_4CH_3CO_2$] plus Acetic Acid [CH_3COOH] plus Diacetone [$C_6H_{12}O_2$]), Potassium Hydroxide (KOH), sodium hydroxide (NaOH), Hydrogen chloride (HCl), and Dimethyl Sulfoxide ($(CH_3)_2$ (DMSO). Additionally, a buffer solution was prepared using Sodium Phosphate (Na_2HPO_4) and Sodium Phosphate (NaH_2PO_4)•H_2O), and Congo Red was used.

2.4. Instrumentation

Erlenmeyer flask, Different size of beakers, Test-tube, Pipette, BP 221 S Electronic Balance (Sartorius, Germany); 101 - 3 ES Electric Heating Blower Drying Oven (Beijing Yongguang Medical Instrument Co., Ltd.) company); ZWY-2102 Constant Temperature Culture Oscillator (Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd.); DFT-50 A universal high-speed pulverizer (Zhejiang Wenling Linda Machinery Co., Ltd.); simultaneous distillation extractor (Yunnan Ruisheng Technology Co., Ltd.) Ultraviolet (UV) radiation (UV–Vis Jasco V-730, Jasco, USA), HPLC™ Ultimate 3000, Shimadzu, Kyoto, Japan, Ultrasound machine.

2.5. Determination of the polysaccharides content

The total polysaccharide content was measured through anthrone sulfuric colorimetry [13]. Samples were placed in 0.300 g tube-shaped bottles, to which 5 mL of 80% methanol was added. Subsequently, the bottles were refluxed in an ultrasound machine for 45 min to facilitate the polysaccharide extraction. Following reflux, the samples were centrifuged for 5 min to separate the solid particles from the solution. A 1mL aliquot of the sample solution was then transferred to a test tube, and 1mL of water was added. A mixture comprising 6mL of 80% sulfuric acid and anthrone (1 g) was prepared. This mixture was added to a test tube containing the sample and water, and the resulting solution was allowed to stand for 20 min in a water bath maintained at 35°C. After incubation, the absorbance of the reaction mixture was measured at 625 nm using a spectrophotometer. The control solution was prepared by substituting the sample with distilled water in the same reaction mixture. A standard curve (y = 43.487 + 0.0909_005, R^2 = 0.9987) for polysaccharides was generated using a known polysaccharide standard, such as that from Sigma-Aldrich, USA. The polysaccharide content in the extract was determined based on the absorbance values obtained from the sample and standard curve.

2.6. Determination of the flavonoid content

The total flavonoid content of the extracts was assessed [14]. The samples were placed in tube-shaped bottles, each weighing 0.300 g. To each bottle, 5 mL of 70% methanol was added and the mixture was refluxed for 45 min using an ultrasound machine. Subsequently, the solution was centrifuged for 5 min to separate the sample solution, and 2 mL was transferred to a test tube. Then, 1 mL of buffer solution (prepared by mixing 100 mL distilled water, 2.72 g sodium acetate trihydrate, and 1.15 mL acetic acid to achieve a pH of 5.2) was added to each test tube, followed by 2 mL of chemical reagent (prepared by mixing 100 mL methanol with 1.34 g aluminum chloride hexahydrate) and shaking. Test tubes were allowed to stand for 10 min in a water bath at 40°C. The absorbance of the reaction mixture was then measured at 415 nm using a spectrophotometer (UV–Vis Jasco V-730, Jasco, USA), with distilled water used as the control. The flavonoid content of the extract was calculated using a standard curve (y = 43.487 + 0.0909, R^2 = 0.9987) generated from known concentrations of flavonoids (Sigma-Aldrich).

2.7. Quantification of the ergosterol content

The ergosterol content was determined using high-performance liquid chromatography (HPLC) [15]. Initially, a

standard curve was generated by precisely weighing 0.300 g of standard ergosterol and preparing a solution with a concentration of 0.04 mg/mL in pure methanol in a 50 mL volumetric flask. Subsequently, 1 mL of the sample solution was drawn with a syringe and manually filtered through a 0.45 µm micro-porous filter membrane into an HPLC sample vial. Different sample sizes were used for detection, according to the chromatographic conditions. The chromatographic parameters were as follows: Waters C_{18} column, pure methanol as the mobile phase, isocratic elution, column temperature of 30°C, detection wavelength set at 284 nm, flow rate of 1 mL/min, and total run time of 20 min. For the analysis of test samples, a liquid culture sample weighing 0.05 g was accurately weighed and placed in a 5 mL centrifuge tube. Next, 2 mL of pure methanol solution was added to the tube, followed by thorough mixing and oscillation. The samples were extracted for 3 h followed by ultrasonic extraction for 1 h. The resulting mixture was centrifuged at 4000 r/min for 5 min, and the supernatant was collected. Subsequently, 1 mL of the sample liquid was drawn with a syringe and filtered manually through a 0.45 µm micro-porous filter membrane into an HPLC sample vial. The samples were analyzed using the previously mentioned chromatographic conditions with a sample volume of 30 µL.

2.8. Measurement of the nucleosides and their analogs

Nucleosides and their analogs were quantified using high-performance liquid chromatography (HPLC), [16]. Standard curves were constructed for adenine, guanine, cytosine, thymine, adenosine, and guanosine. Each standard compound (10 mg) was dissolved in a 10 mL volumetric flask containing 20% methanol to achieve a fixed concentration of the standard solution. A 1 mL aliquot of the sample was then withdrawn using a syringe and manually filtered through a 0.45 µm micro-porous filter membrane into an HPLC sample vial. Subsequently, the sample was analyzed using an HPLC system (Ultimate 3000, Shimadzu, Kyoto, Japan) under the following conditions: Column: Waters Symmetry C_{18} column (4.6 mm × 250 mm, 5 µm); mobile phases: methanol (A) and water (B) gradient elution method: 0-10 min: 5-10% A, 10-15 min: 10-40% A, 15-25 min: 40-25% A, 25-27 min: 25-5% A, 27-30 min: 5% A Column temperature: 30 °C, detection wavelength: 260 nm, flow rate: 1 mL/min, total running time: 30 min. A standard regression curve was constructed by plotting the mass on the abscissa and the peak area on the ordinate. For the determination of test samples, a 0.05 g sample was accurately weighed and soaked in 2 mL of a 20% methanol solution for 1 h. The sample was subjected to ultrasonic extraction for 30 min, followed by centrifugation at 4000 rpm for 5 min to collect the supernatant. The extraction process was repeated and the solvent was added to achieve a constant volume of 5 mL with thorough mixing. A 1 mL sample solution was then withdrawn using a syringe and manually filtered through a 0.45 µm micro-porous filter membrane into an HPLC sample vial. The content of each component in the test sample solution was determined under the same chromatographic conditions with a sample volume of 40 µL.

2.9. Statistical analysis

In each of the experiments, a total of three distinct trials were conducted. The outcomes were reported as the mean ± standard error of the mean (SEM) derived from three distinct investigations (n = 3). Graph-Pad Prism 8 was utilized for creating bar graphs [17].

3. Result

3.2. Evaluation of various components production

The various components of *Zingiber officinale* and *Glycyrrhiza glabra*, such as polysaccharides, flavonoids, ergosterol, and nucleic acid derivatives, including adenine, adenosine, guanosine, and hypoxanthine, were examined over a period with salt of 15-d (2mM salt), 20-d (3mM salt), 25-d (5 mM salt), 30-d (10mM salt), 35-d (20mM salt), 40-day (30mM salt), 45-d (40mM salt), 50-d (50mM salt), and 55-d (60mM salt).

3.3. Determination of the polysaccharides content

The results in Figure 1 confirm that *Zingiber officinale* harvested at 20 days with 3mM salt exhibited the highest polysaccharide content at 17.22 mg/g, while *Glycyrrhiza glabra* showed slightly lower content (15.76 mg/g. Polysaccharide concentrations varied across different time points for both plants, with *Zingiber officinale* ranging from 15.55 mg/g to 14.09 mg/g and *Glycyrrhiza glabra* ranging from 15.96 mg/g to 12.80 mg/g over the 15 to 55-d period with 5-d intervals. The minimum polysaccharide content for both plants was observed at 50 and 55-d, indicating a decrease in polysaccharide concentration over time. These findings suggest that the response of polysaccharide content depends on the plant species, duration of growth, and salt concentration. Specifically, in *Zingiber officinale*, the polysaccharide content gradually decreased after the 20-d mark with 3 mM salt. Similarly, both plants exhibited the lowest polysaccharide content after 55-d of induction. Overall, the results indicate that *Zingiber officinale*, especially after 20 days with 3mM salt, tended to have higher polysaccharide content than *Glycyrrhiza glabra*.

3.4. Detection of the flavonoid content

The data presented in Figure 2 demonstrate that *Zingiber officinale* harvested at 25-d with 5 mM salt exhibited the highest flavonoid content at 6.96 mg/g, while *Glycyrrhiza glabra* showed a slightly lower content (4.04 mg/g. Flavonoid concentrations varied across different

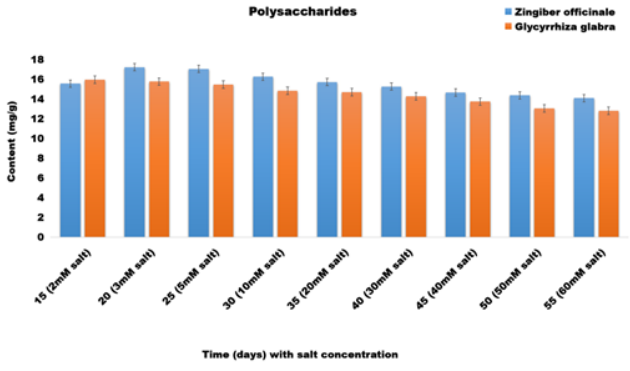

Fig. 1. For polysaccharide content, the X-axis shows incubation time with salt concentration and the Y-axis shows quantification of content (mg/g). Error bars indicate the mean ± standard error, as shown in the bar graph.

time points for both plants, with *Zingiber officinale* ranging from 5.24 mg/g to 2.93 mg/g and *Glycyrrhiza glabra* ranging from 3.55 mg/g to 2.36 mg/g over the 15 to 55-d period with 5-d intervals. The minimum flavonoid content for both plants was observed at 50 and 55-d, indicating a decrease in flavonoid concentration over time. These findings suggest that the response of flavonoid content depends on plant species, duration of growth, and salt concentration. Specifically, in *Zingiber officinale*, the flavonoid content gradually decreased after the 25-d mark with 5 mM salt. Similarly, both plants exhibited the lowest flavonoid content after 25-d of induction. Overall, the results indicate that *Zingiber officinale*, especially after 25-d with 5 mM salt, tended to have a higher flavonoid content than *Glycyrrhiza glabra*.

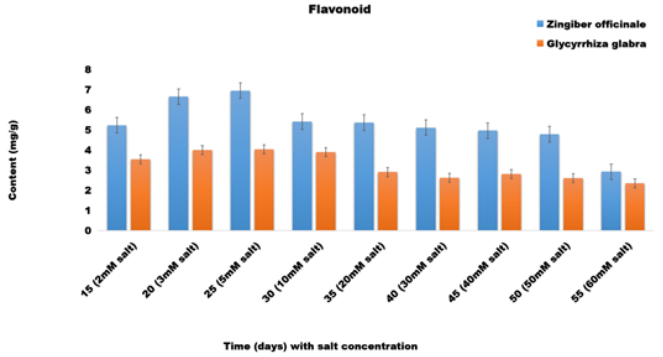

Fig. 2. For flavonoid content, the X-axis shows incubation time with salt concentration and the Y-axis shows quantification of content (mg/g). Error bars indicate the mean ± standard error, as shown in the bar graph.

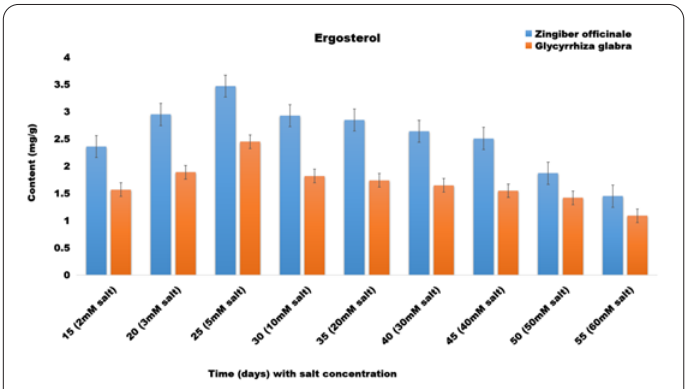

Fig. 3. For ergosterol content, the X-axis shows incubation time with salt concentration and the Y-axis shows quantification of content (mg/g). Error bars indicate the mean ± standard error, as shown in the bar graph.

3.5. Quantification of the ergosterol content

The results obtained from (Table 1) verified that the 25-d (5 mM salt) 2.263 mg/g *Zingiber officinale* and *Glycyrrhiza glabra* 0.9876 mg/g showed the maximum ergosterol content in both pants. The various concentrations of guanosine content in the *Zingiber officinale* part are 0.249, 1.840, 2.263, 1.889, 1.689, 1.379, 1.3724, 0.690, and 0.690 mg/g, and in the *Glycyrrhiza glabra* part, 0.7088, 0.7560, 0.9876, 0.9553, 0.8275, 0.7321, 0.6920, 0.6634, 0.5725 mg/g from 15 to 55-d with 5-d intervals. However, the minimum ergosterol content in both parts has been reported under 50-d and 55-d, which indicates that the concentration decreases with the passage of time. This implies that the response of ergosterol content pants and duration. Consequently, the ergosterol content of the *Zingiber officinale* indicates that after 25-d (5 mM salt), the ergosterol content decreased gradually. Similarly, the minimum ergosterol content in both body parts was reported after 25-d (5 mM salt) induction. The results clearly suggest that at 25-d (5 mM salt), the *Zingiber officinale* body parts had a higher ergosterol content than the *Glycyrrhiza glabra* body parts (Figure 3).

3.6. Adenine content

The data from Table 1 demonstrate that *Zingiber officinale* harvested at 25-d with 5 mM salt exhibited the highest adenine content at 3.4186 mg/g, while *Glycyrrhiza glabra* showed a slightly lower content of 2.4521 mg/g. Adenine concentrations varied across different time points for both plants, with *Zingiber officinale* ranging from 2.3622 mg/g to 0.9523 mg/g and *Glycyrrhiza glabra* ranging from 0.5725 mg/g to 1.0519 mg/g over the 15 to 55-d period at 5-d intervals. The minimum adenine content for both plants was observed at 50 and 55-d, indicating a decrease in adenine concentration over time, and salt concentration. These findings suggest that the response of adenine content depends on plant species, growth duration, and salt concentration. Specifically, in *Zingiber officinale*, adenine content gradually decreased after the 25-d mark with 5 mM salt. Similarly, both plants exhibited the lowest adenine content after 25-d of induction. Overall, the results indicate that *Zingiber officinale*, especially after 25-d with 5 mM salt, tended to have a higher adenine content than *Glycyrrhiza glabra*.

3.7. Adenosine content

The data presented in Table 1 confirm that *Zingiber officinale* harvested at 25-d with 5 mM salt exhibited the highest adenosine content at 3.4078 mg/g, while *Glycyrrhiza glabra* showed a slightly lower content of 2.4501 mg/g. Adenosine concentrations varied across different time points for both plants, with *Zingiber officinale* ranging from 2.3226 mg/g to 1.4655 mg/g and *Glycyrrhiza glabra* ranging from 0.6755 mg/g to 1.0529 mg/g over the 15-to 55-day period with 5-d intervals. The minimum adenosine content for both plants was observed at 50 and 55 days, indicating a decrease in adenosine and salt concentrations over time. These findings suggest that the response of adenosine content depends on the plant species, duration of growth, and salt concentration. Specifically, in *Zingiber officinale*, the adenosine content gradually decreased after the 25-d mark with 5 mM salt. Similarly, both plants exhibited the lowest adenosine content after 25-d of induction. Overall, the results indicate that *Zingiber officinale*, especially after 25-d with 5 mM salt, tended to have a higher adenosine content than *Glycyrrhiza glabra*.

3.8. Hypoxanthine content

The data presented in Table 1 confirm that *Zingiber officinale* harvested at 25 days with 5 mM salt exhibited the highest hypoxanthine content at 2.4627 mg/g, while *Glycyrrhiza glabra* showed a slightly lower content of 1.4547 mg/g. Hypoxanthine concentrations varied across different time points for both plants, with *Zingiber officinale* ranging from 1.3326 mg/g to 0.4555 mg/g and *Glycyrrhiza glabra* ranging from 0.5155 mg/g to 0.0649 mg/g over

Table 1. List of different nucleosides and their analogs and ergosterol in a *Zingiber officinale* and ***Glycyrrhiza glabra*** in mg/g.

		Zingiber officinale		
Time (days) with salt concentration	**Adenine (mg/g)**	**Adenosine (mg/g)**	**Hypoxanthine (mg/g)**	**Guanosine (mg/g)**
15 (2 mM salt)	2.3622	2.3226	1.3326	2.4556
20 (3mM salt)	2.9556	2.9565	1.9655	2.7875
25 (5 mM salt)	3.4186	3.4078	2.4627	3.4537
30 (10 mM salt)	2.0018	2.0330	1.2529	2.9523
35 (20 mM salt)	2.0013	2.0125	1.1965	2.8655
40 (30 mM salt)	1.1427	2.0074	1.0524	2.6628
45 (40 mM salt)	1.0013	1.9147	1.0001	2.5621
50 (50 mM salt)	1.0007	1.8767	0.8787	1.8657
55 (60 mM salt)	0.9523	1.4655	0.4555	1.4325
		Glycyrrhiza glabra		
Time (days) with salt concentration	**Adenine (mg/g)**	**Adenosine (mg/g)**	**Hypoxanthine (mg/g)**	**Guanosine (mg/g)**
15 (2mM salt)	0.5725	0.6755	0.5155	1.6023
20 (3mM salt)	1.0957	1.2387	0.9367	1.8967
25 (5 mM salt)	2.4521	2.4501	1.4547	2.4513
30 (10mM salt)	1.7862	1.8224	0.8274	1.8279
35 (20 mM salt)	1.7155	1.7465	0.7425	1.7423
40 (30 mM salt)	1.6230	1.6530	0.6523	1.6534
45 (40 mM salt)	1.5375	1.5865	0.5625	1.5875
50 (50 mM salt)	1.3982	1.4822	0.4322	1.4652
55 (60 mM salt)	1.0519	1.0529	0.0649	1.0789

the 15 to 55-d period with 5-d intervals. The minimum hypoxanthine content for both plants was observed at 50 and 55-d, indicating a decrease in hypoxanthine and salt concentrations over time. These findings suggest that the response of hypoxanthine content depends on both plant species and the duration of growth and salt concentration. Specifically, in *Zingiber officinale*, the hypoxanthine content gradually decreased after the 25-d mark with 5 mM salt. Similarly, both plants exhibited the lowest hypoxanthine content after 25-d of induction. Overall, the results indicate that *Zingiber officinale*, especially after 25-d with 5 mM salt, tends to have a higher hypoxanthine content than *Glycyrrhiza glabra*.

3.9. Guanosine content

The data provided in Table 1 confirm that *Zingiber officinale* harvested at 25-d with 5 mM salt exhibited the highest guanosine content at 3.4537 mg/g, while *Glycyrrhiza glabra* showed a slightly lower content of 2.4513 mg/g. Guanosine concentrations varied across different time points for both plants, with *Zingiber officinale* ranging from 2.4556 mg/g to 1.4325 mg/g and *Glycyrrhiza glabra* ranging from 1.6023 mg/g to 1.0789 mg/g over the 15–55-d period with 5-d intervals. The minimum guanosine content for both plants was observed at 50 and 55-d, indicating a decrease in guanosine concentration over time and salt concentration. These findings suggest that the response of guanosine content depends on both plant species and the duration of growth and salt concentration. Specifically, in *Zingiber officinale*, the guanosine content gradually decreased after the 25-d mark with 5 mM salt. Similarly, both plants exhibited the lowest guanosine content after 25-d of induction. Overall, the results indicate that *Zingiber officinale*, especially after 25-d with 5 mM salt, tends to have a higher guanosine content than *Glycyrrhiza glabra*.

4. Discussion

This study evaluated the production of various components of *Zingiber officinale* (ginger) and *Glycyrrhiza glabra* (licorice) under different salt concentrations and growth durations. The components examined included polysaccharides, flavonoids, ergosterol, and nucleic acid derivatives, such as adenine, adenosine, guanosine, and hypoxanthine. *Zingiber officinale* harvested at 20-d with 3mM salt exhibited the highest polysaccharide content, whereas *Glycyrrhiza glabr*a showed slightly lower polysaccharide content. Polysaccharide concentrations varied over time in both plants, with a gradual decrease observed after 20-d. *Zingiber officinale* (Ginger root) has long been used to alleviate and manage various common ailments, such as headaches, colds, nausea, and vomiting. Numerous bioactive compounds found in ginger, including phenolic and terpene compounds, contribute to its therapeutic properties. Among these, gingerols, shogaols, and paradols are the primary phenolic compounds responsible for the diverse biological activities of ginger [18]. Recent research has revealed the additional biological effects of ginger, including antioxidant [19], anti-inflammatory [20], antimicrobial [21], and anticancer properties [22].

Overall, *Zingiber officinale* tended to have a higher polysaccharide content than *Glycyrrhiza glabra*, especially after 20-d with 3mM salt. *Zingiber officinale* harvested at 25-d with 5 mM salt exhibited the highest flavonoid content, whereas *Glycyrrhiza glabra* showed a slightly lower flavonoid content. Flavonoid concentrations varied over time in both plants, with a gradual decrease observed after day 25-d mark. *Zingiber officinale* tended to have a higher flavonoid content than *Glycyrrhiza glabra*, especially after 25-d with 5 mM salt. Both *Zingiber offi-*

cinale and *Glycyrrhiza glabra* showed maximum ergosterol content at 25-d with 5 mM salt. The ergosterol content gradually decreased over time in both plants. Furthermore, emerging evidence suggests that ginger may play a role in the prevention and management of various diseases, including neurodegenerative diseases [23], cardiovascular diseases [24], obesity [25], diabetes mellitus [26], chemotherapy-induced nausea and vomiting [27], and respiratory disorders [28].

Zingiber officinale had a higher ergosterol content than *Glycyrrhiza glabra*, especially after 25-d with 5 mM salt. Nucleic Acid Derivatives (Adenine, Adenosine, Guanosine, Hypoxanthine) *Zingiber officinale* harvested at 25 days with 5 mM salt exhibited the highest content of adenine, adenosine, guanosine, and hypoxanthine. The concentrations of these derivatives varied over time, with a gradual decrease observed after day 25-d. The medicinal properties of *Glycyrrhiza glabra* roots have been recognized since ancient times, with populations in Rome, Greece, India, and China using them to treat respiratory ailments such as asthma and bronchitis [29, 30]. Pharmaceutical companies commonly incorporate *Glycyrrhiza glabra* into cough syrup preparations owing to its therapeutic effects [29].

Zingiber officinale tended to have a higher nucleic acid derivative content than *Glycyrrhiza glabra*, especially after 25-d with 5 mM salt. Furthermore, the sweet taste of the roots has garnered interest from various industries, including tobacco, confectionery, and flavoring, where it is utilized as a sweetener in products such as chewing gums, ice creams, and candies [29]. It is important to note that only certain species of *Glycyrrhiza glabra* possess a notably sweet taste [30]. This sweetness is attributed to the presence of glycyrrhizin, a triterpenoid saponin, and its primary phytochemical constituent, which typically constitutes 10–25% of the root extract [31]. Glycyrrhizin, present in the form of glycyrrhizic acid salt within the plant, serves as a quality marker in pharmacopeias of countries such as Japan and China [32].

In conclusion, the evaluation of the various compounds produced by *Zingiber officinale* and *Glycyrrhiza glabra* revealed interesting insights. The polysaccharide, flavonoid, ergosterol, adenine, adenosine, hypoxanthine, and guanosine contents were analyzed over a period ranging from 15 to 55-d with increasing salt concentrations. The polysaccharide content was highest in *Zingiber officinale* harvested at 20-d with 3mM salt, whereas *Glycyrrhiza glabra* showed slightly lower levels. Both plants exhibited a gradual decrease in polysaccharide content over time, with the lowest levels observed after 55-d. Similarly, *Zingiber officinale* harvested at 25-d with 5 mM salt exhibited the highest flavonoid content, with a gradual decrease observed over time. For ergosterol, adenine, adenosine, hypoxanthine, and guanosine, the highest concentrations were observed at 25-d with 5 mM salt in both plants, with levels decreasing as the duration of growth increased. *Zingiber officinale* tended to have higher contents of these components than *Glycyrrhiza glabra*, especially after 25-d with 5 mM salt. These findings suggest that the responses of these components to growth duration and salt concentration vary among plant species.

Author's contribution

All authors contributed to the study's conception and design. The paper was written by **Mustafa I. Almaghasla** and **Naima Kanwal, and the** grammar was checked by **Tahir Khan** and **Jameel M. Al-Khayri**. All authors read and approved the final manuscript.

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Availability of data and materials

All processed data used in this study can be obtained from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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