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CALENDULA OFFICINALIS MEDICINAL VALUE UNDER SALINE-STRESS AND SALICYLIC ACID TREATMENT

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Abstract

The present study investigated the effect of saline stress and salicylic acid (SA) treatment on growth, flowering, biochemical attributes, antioxidant properties, and antibacterial activity of *Calendula officinalis*. Plants were treated under two salinity levels (0 and 60 mM NaCl) and four SA concentrations (0, 0.01, 0.05, and 0.10 mM). Salinity caused a significant reduction in vegetative growth, flowering performance, carbohydrate accumulation, mineral content, and chlorophyll concentration. Salt-stressed plants showed reduced plant height, biomass production, flower yield, potassium, calcium, and total carbohydrate contents as compared to non-stressed plants. Conversely, salinity increased proline accumulation, total phenolic content, antioxidant activity, and antibacterial properties of leaf extracts. Foliar application of SA decreased the adverse effects of salinity and improved the plant performance under saline and non-saline conditions. Among the concentrations used, 0.05 mM SA produced the highest increases in growth, flower production, antioxidant activity, and biochemical constituents. SA application enhanced total phenolic content, DPPH scavenging activity, and the inhibition of β-carotene-linoleic acid and maintained higher chlorophyll and mineral contents under salinity. The strongest antibacterial activity of leaf extracts against the tested bacterial strains was observed under salinity and 0.05 mM SA treatments. Gram-positive bacteria were more sensitive to the extracts than Gram-negative bacteria. The results demonstrated that exogenous SA could alleviate the salt stress and improve the medicinal quality and biological activity of *C. officinalis* under saline conditions.

Key words: *Calendula officinalis;* Saline stress; Antioxidants; Salicylic acid, Antibacterial.

Introduction

The *Calendula officinalis* is one of the most important medicinal and ornamental plants of the Asteraceae family. It is widely grown around the world for its pharmacological relevance, gorgeous blossoms, and tolerance to varied environmental conditions. *C. officinalis* has long been utilized in traditional and modern medicine for its anti-inflammatory, antibacterial, antioxidant, antifungal, antiviral, wound-healing, and anticancer effects. The medicinal benefit of the species is due to several biologically active substances such as flavonoids, phenolic acids, carotenoids, triterpenoids, coumarins, quinones, and volatile oils present in extracts of flowers and leaves. These phytochemicals are mostly exploited in pharmaceutical, cosmetic, nutraceutical, and food industries. In recent years, the therapeutic efficacy of *C. officinalis* has been substantially increased due to the growing interest in natural antioxidants and plant-derived antibacterial agents.

Plant secondary metabolites, especially phenolics and flavonoids, have a strong ability to scavenge free radicals and play a role in the prevention of human oxidative stress-related disorders (Shahrajabian *et al*., 2023). Moreover, a recent study has reported the antibacterial activity of *C. officinalis* extracts against Gram-positive and Gram-negative pathogenic bacteria, indicating their potential as natural antimicrobial agents (Efstratiou *et al*., 2021). Environmental stress factors are very important in the growth, productivity, and phytochemical composition of medicinal plants. Salinity is one of the most damaging environmental abiotic stresses for the sustainability of agriculture worldwide. Soil salinization is progressively exacerbated by climate change, poor irrigation methods, over-fertilization, and low rainfall in arid and semi-arid regions (Hasanuzzaman *et al*., 2021).

The salt level in the root zone increases, which decreases the soil water potential and generates osmotic stress that inhibits water intake and plant growth. High levels of sodium (Na^+) and chloride (Cl^-) ions can also disrupt the balance of ions in cells, which may lead to a shortage of nutrients, cell membrane damage, metabolic failure, and oxidative stress. Salt stress negatively affects different physiological and biochemical activities such as photosynthesis, respiration, protein synthesis, the activity of enzymes, and the control of hormones. Salinity results in loss of chlorophyll, which affects light gathering and photosynthetic efficiency and hence diminishes biomass accumulation and productivity (Taibi *et al*., 2021). Salinity also influences the absorption and translocation of critical minerals such as potassium and calcium because of the rivalry of Na^+ ions with mineral uptake mechanisms in roots (Kaya *et al*., 2021). Potassium is a key player in osmotic regulation, enzyme activation, and stomatal function, whereas calcium has prominent functions in membrane stability and signalling pathways. Thus, the decrease of these nutrients is highly responsible for the growth suppression

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under saline circumstances. One of the principal effects of salinity stress is the overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. ROS buildup causes oxidative damage to lipids, proteins, nucleic acids, and cellular membranes (Hasanuzzaman *et al*., 2021). Plants have evolved sophisticated antioxidant defence systems, consisting of enzymatic and non-enzymatic components, to cope with oxidative stress. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) detoxify reactive oxygen species (ROS) and maintain cellular redox equilibrium (Verma *et al*., 2021). Moreover, plants collect suitable solutes such as proline, glycine betaine, and soluble sugars that help in osmotic adjustments and stabilization of cellular structures (Zulfiqar *et al*., 2020). The biosynthesis of secondary metabolites in medicinal plants is often altered in salinity conditions. Mild salinity can increase the production of phenolics, flavonoids, alkaloids, and terpenoids as adaptive defensive strategies (Farhangi-Abriz & Ghassemi-Golezani, 2021). These chemicals have antioxidant activity and help in stress tolerance via detoxification of ROS and preservation of membranes against oxidative damage. However, high salt stress produces metabolic disruption and cellular injury, which degrade plant yield and medicinal quality. Salicylic acid (SA) is a naturally occurring phenolic phytohormone involved in the regulation of plant development, photosynthesis, ion uptake, flowering, and responses to abiotic and biotic stresses. SA is a signaling molecule associated with the induction of defense pathways and stress adaptation processes. Exogenous application of SA has been regularly documented to enhance plant tolerance to salinity, drought, heat, cold, and heavy metal toxicity. SA-mediated stress tolerance is linked with improvement in membrane stability, photosynthetic efficiency, induction of antioxidant defense mechanisms and regulation of osmolyte accumulation. SA has been reported to promote salinity tolerance in plants by regulating antioxidant metabolism and maintaining ionic homeostasis. SA enhances potassium and calcium absorption while restricting the excessive buildup of sodium under saline circumstances. SA also induces the phenylpropanoid pathway, leading to the buildup of phenolic compounds and secondary metabolites.

Application of SA can boost antioxidant substances, which can improve medicinal quality and biological activities of plant extracts. Exogenous SA has been reported to enhance the growth, essential oil quality, antioxidant activity, and accumulation of bioactive chemicals under environmental stress conditions in medicinal and aromatic plants (Riaz *et al*., 2022). Salicylic acid (SA) treatment was also reported to improve chlorophyll stability and photosynthetic efficacy under salt, thereby decreasing stress-induced growth suppression (Nazar *et al*., 2011). Moreover, SA can affect the expression of genes in stress signaling pathways and antioxidant defense systems, which enhances plant adaptation to unfavorable environmental conditions (Zhou *et al*., 2022).

The influence of salinity and SA on plant stress physiology has been intensively researched; however, less information is available on their combined effect on growth, flowering, antioxidant metabolism, phenolic build up, and antibacterial activity of *C. officinalis*. These interactions are very important since the environmental conditions have a major influence on the quality of medicinal plants and phytochemicals. Therefore, the present study was done to explore the effects of salt stress and salicylic acid administration on vegetative growth, flowering behaviour, biochemical contents, antioxidant capabilities, and antibacterial activity of *C. officinalis*. Moreover, the study evaluated the possible use of SA in alleviating salt stress-induced oxidative stress and in enhancing the medicinal and pharmacological value of the species.

Materials and Methods

Plant material: Uniform juvenile specimens (about 7 cm in height) of *C. officinalis* L. were procured from commercial nurseries in Riyadh, Saudi Arabia, in 2024. The experiment was performed in a controlled greenhouse at King Saud University. Plants were relocated into 2.1 L containers filled with a blend of peat and perlite (3:1, w/w). After one week of growth establishment, the plants were supplied with a balanced fertilizer (Crystalon® 20:20:20 N:P:K) at a concentration of 2 g L^{-1}. Plants were acclimatized for three weeks under regulated climatic conditions (15.1°C–27.5°C, 58%–67% relative humidity, and around 1000 µmol m^{-2} s^{-1} PAR at noon), aligning with the best greenhouse culture standards for ornamental species. Irrigation (38–50 mL per plant) was administered every day throughout this time. Following acclimatization, the plants were exposed to two salinity levels (0 and 60 mM NaCl) for 75 days to replicate salt stress. SA solutions (degree of deacetylation > 95%; Sigma Aldrich, Germany) were administered to leaves at varying concentrations (0, 0.01, 0.05, and 0.10 mM) until runoff, one week before the imposition of stress, in accordance with previously established methods for biostimulant applications (Rojas-Pirela *et al*., 2024). The untreated plants served as controls. The plants were organized in a split-plot pattern using a randomized complete block design (RCBD). The primary plots were designated for irrigation treatments, whereas the subplot allocations pertained to SA concentrations. Each treatment was duplicated five times across three blocks.

Morphological and physiological measurements: Plants were collected after 75 days of treatment initiation. Growth characteristics, such as plant height and leaf count, were assessed. The leaf area was quantified by digital image analysis utilizing AutoCAD software. The total dry biomass was determined by oven-drying the plant samples at 30°C until a consistent weight was achieved. The duration to blooming was determined from the commencement of the experiment, while the flower count per plant was assessed at the conclusion of the trial. The bloom diameter, fresh flower weight, and total flower output per plant were assessed at the conclusion of the trial.

The total carbohydrate content in fresh leaves was measured according to Dubois *et al*., (1956). The mineral elements were quantified from leaf sap samples using inductively coupled plasma spectrophotometry (K^+ and Ca^{2+}). The proline content in leaves was quantified spectrophotometrically at 520 nm using the methodology of Bates *et al*., (1973). Leaf samples were air-dried, pulverized into a fine powder, and subjected to methanol (99%) extraction for 24 hours in darkness, adhering to the established protocol for phenolic compound extraction

(Elansary *et al*., 2020; Elansary *et al*., 2026; Al-Yafrsi *et al*., 2026). The antioxidant activity was assessed by the DPPH radical scavenging assay (Brand-Williams *et al*. 1995) and the β-carotene–linoleic acid bleaching technique (Elansary *et al*., 2020). The absorbance was measured at 517 and 470 nm. IC_{50} values were derived from the inhibition curves. Butylated hydroxytoluene (BHT) served as a positive control. The total phenolic content was quantified using the Folin–Ciocalteu technique and represented as gallic acid equivalents (mg GAE g-1 extract) (Singleton & Rossi, 1965). The chlorophyll content was quantified spectrophotometrically according to the methodology established by Moran & Porath (1980).

Antibacterial assays and medicinal properties: The antibacterial efficacy of methanolic leaf extracts against certain gram-positive and gram-negative bacteria, as well as fungal strains, was assessed. The microorganisms chosen for analysis included *Listeria monocytogenes* (clinical isolate), *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 6538), *Micrococcus flavus* (ATCC 10240), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 35210). The microdilution technique was used to assess antibacterial and antifungal effects (Elansary *et al*., 2018). The minimum inhibitory concentration (MIC) is the lowest concentration that prevents visible microbial growth, whereas the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) are the lowest concentrations that decrease the initial inoculum by 99.5%. Streptomycin and ampicillin served as positive controls for antibacterial tests, while fluconazole and ketoconazole were used as positive controls for antifungal assays; 5% dimethyl sulfoxide functioned as the negative control. All tests were performed in triplicate and repeated two times.

Statistical analysis

Data are presented as mean values ± standard deviation. A one-way analysis of variance (ANOVA) was used for statistical analysis, and the least significant difference (LSD) test at $p \leq 0.05$ was utilized to differentiate the means. We used SPSS (PASW Statistics, version 21) for the analyses.

Results

Vegetative growth parameters: Salinity significantly reduced vegetative growth parameters of *C. officinalis*, including plant height, number of branches, number of leaves, shoot dry weight, and root dry weight (Table 1). Plants without salicylic acid (SA) treatment and subjected to 60 mM NaCl showed the poorest growth performance. The plant height declined from 41.6 cm in the untreated control to 30.4 cm under salinity stress, a decrease of ~26.9%. The number of branches dropped from 8.1 to 5.6 branches plant^{-1} (30.9% reduction) while the number of leaves fell from 57.4 to 40.5 leaves plant^{-1} (29.4% reduction). Salinity also significantly lowered biomass accumulation. Shoot dry weight decreased in saline conditions from 18.3 to 11.0 g plant^{-1}, a decrease of 39.9%. Root dry weight decreased from 4.5 to 2.7 g plant$^{-1,}$ which was a 40.0% reduction of non-saline untreated plants. Vegetative growth was improved by the application of SA in saline and non-saline conditions. The enhancement was the highest at 0.05 mM SA of all the concentrations tested. The height of the plants under non-saline conditions increased from 41.6 cm in untreated plants to 50.1 cm after application of 0.05 mM SA, which was 20.4% increase. Under saline conditions, the shoot dry weight increased from 18.3 to 22.2 g plant^{-1} (21.3% increase), and the root dry weight increased by 22.2%. The 0.05 mM SA significantly ameliorated the inhibitory effects of salt on plant growth, with the plant height increasing from 30.4 to 39.8 cm (30.9% increase) and the shoot dry weight increasing from 11.0 to 17.2 g plant^{-1} (56.4% higher than that of untreated salt-stressed plants). Root dry weight also increased from 2.7 to 4.1 g plant^{-1} (51.9% increase).

Flowering characteristics: Salinity significantly affected flowering behavior and flower yield of *C. officinalis* (Table 2). Plants subjected to 60 mM NaCl without SA treatment required 70.2 days to flowering compared with 62.5 days in untreated control plants, indicating a delay of approximately 12.3%.

Salinity stress reduced the flower number considerably. The number of flowers plant^{-1} for untreated salt-stressed plants was 12.1 compared with 20.3 flowers plant^{-1} for the control treatment, a reduction of 40.4%. Flower diameter reduced from 6.6 to 5.0 cm (24.2% reduction) and fresh flower weight from 4.9 to 3.3 g flower^{-1} (32.7% reduction). Total flower yield was most reduced under salinity. Flower yield was reduced from 98.1 g plant^{-1} in control plants to 38.8 g plant^{-1} under salinity stress, a reduction of 60.4%.

Application of SA significantly improved flowering performance under both saline and non-saline conditions. The 0.05 mM SA treatment produced the highest flower yield and flower quality. Under non-saline conditions, flower yield increased from 98.1 to 149.4 g plant^{-1} (52.3% increase), while flower number increased from 20.3 to 25.7 flowers plant^{-1} (26.6% increase). Under salinity stress, 0.05 mM SA reduced flowering delay and improved flower production. Flower yield increased from 38.8 to 84.4 g plant^{-1}, representing a 117.5% increase compared with untreated salt-stressed plants. Flower number increased by 51.2%, while flower diameter increased from 5.0 to 6.3 cm (26.0% increase).

Biochemical composition: Salinity significantly reduced total carbohydrates, potassium, calcium, and chlorophyll contents in leaves of *C. officinalis* (Tables 3 and 4). Total carbohydrates decreased from 148.6 mg g^{-1} DW in control plants to 112.8 mg g^{-1} DW under salinity stress, corresponding to a 24.1% reduction.

Potassium concentration decreased from 3.21% to 2.14% under saline conditions, representing a reduction of 33.3%, while calcium concentration declined from 1.84% to 1.21% (34.2% reduction).

In contrast, proline accumulation increased markedly under salinity stress. Proline level increased from 4.8 µmol g^{-1} FW in control plants to 18.7 µmol g^{-1} FW in salt-stressed plants, and about 289.6% increase.

Application of SA has shown a significant improvement in biochemical characteristics under saline and non-saline conditions. The maximum carbohydrate and mineral accumulation was obtained with 0.05 mM SA. Total carbohydrate increased from 112.8 to 142.7 mg g^{-1} DW (26.5%) following treatment with 0.05 mM SA under salinity stress. Potassium increased from 2.14% to 2.98% (39.3%), while calcium also increased by 39.7%.

Table 1. Saline stress (0 and 60 mM NaCl) and SA effects on leaf number and area, dry weight, and plant height in *C. officinalis.* Values are expressed as means (± sd).

NaCl (mM)	SA (mM)	Plant height (cm)	Branches plant^{-1}	Leaves plant^{-1}	Shoot dry weight (g)	Root dry weight (g)
0	0.00	41.6 ± 2.1 c	8.1 ± 0.5 cd	57.4 ± 3.2 cd	18.3 ± 1.1 cd	4.5 ± 0.3 c
0	0.01	44.3 ± 2.0 b	8.9 ± 0.6 b	61.7 ± 3.5 b	19.7 ± 1.0 b	4.8 ± 0.2 b
0	0.05	50.1 ± 2.3 a	9.5 ± 0.4 a	67.2 ± 3.8 a	22.2 ± 1.2 a	5.5 ± 0.3 a
0	0.10	45.8 ± 2.4 b	9.0 ± 0.5 b	63.8 ± 3.1 b	20.5 ± 1.3 b	4.9 ± 0.4 b
60	0.00	30.4 ± 1.8 f	5.6 ± 0.4 f	40.5 ± 2.6 f	11.0 ± 0.8 f	2.7 ± 0.2 f
60	0.01	34.6 ± 2.0 e	6.3 ± 0.5 e	46.1 ± 2.8 e	13.4 ± 0.9 e	3.2 ± 0.2 e
60	0.05	39.8 ± 2.2 d	7.7 ± 0.5 d	54.7 ± 3.0 d	17.2 ± 1.0 d	4.1 ± 0.3 d
60	0.10	36.2 ± 2.1 de	7.0 ± 0.4 de	49.2 ± 2.9 de	15.0 ± 0.9 de	3.6 ± 0.2 de

*Means followed by different letters within columns are significantly different, based on the least significant difference test (p≤0.05)

Table 2. Saline stress (0 and 60 mM NaCl) and SA effects on days to flowering, number of flowers per plant, flower diameter, fresh flower weight, total flower yield per plant in *C. officinalis.* Values are expressed as means (± sd).

NaCl (mM)	SA (mM)	Days to flowering	Flowers plant^{-1}	Flower diameter (cm)	Fresh flower weight (g)	Total flower yield plant^{-1} (g)
0	0.00	62.5 ± 2.3 c	20.3 ± 1.1 c	6.6 ± 0.3 c	4.9 ± 0.3 cd	98.1 ± 4.5 d
0	0.01	60.8 ± 2.1 d	22.5 ± 1.2 b	7.0 ± 0.3 b	5.3 ± 0.3 b	114.3 ± 5.0 c
0	0.05	55.7 ± 1.9 f	25.7 ± 1.3 a	7.5 ± 0.4 a	5.9 ± 0.4 a	149.4 ± 6.1 a
0	0.10	59.4 ± 2.0 e	23.5 ± 1.1 b	7.1 ± 0.3 b	5.5 ± 0.3 b	127.3 ± 5.4 b
60	0.00	70.2 ± 2.5 a	12.1 ± 0.8 f	5.0 ± 0.2 f	3.3 ± 0.2 f	38.8 ± 3.0 f
60	0.01	67.4 ± 2.3 b	14.4 ± 0.9 e	5.3 ± 0.3 e	3.9 ± 0.2 e	55.0 ± 3.5 e
60	0.05	62.1 ± 2.1 c	18.3 ± 1.0 d	6.3 ± 0.3 d	4.7 ± 0.3 d	84.4 ± 4.2 d
60	0.10	64.7 ± 2.2 bc	16.0 ± 0.9 de	5.9 ± 0.3 de	4.2 ± 0.2 de	65.8 ± 3.8 de

*Means followed by different letters within columns are significantly different, based on the least significant difference test (p≤0.05)

Table 3. Saline stress (0 and 60 mM NaCl) and SA effects on total carbohydrate, K, Ca, and proline composition in the leaves *of C. officinalis leaves.* Values are means (± sd).

NaCl (mM)	SA (mM)	Total carbohydrates (mg g^{-1} DW)	K (%)	Ca (%)	Proline (µmol g^{-1} FW)
0	0.00	148.6 ± 6.4 cd	3.21 ± 0.12 bc	1.84 ± 0.08 bc	4.8 ± 0.3 g
0	0.01	156.9 ± 6.8 bc	3.38 ± 0.14 ab	1.92 ± 0.07 ab	5.3 ± 0.4 g
0	0.05	171.4 ± 7.2 a	3.61 ± 0.15 a	2.08 ± 0.09 a	6.4 ± 0.5 f
0	0.10	163.2 ± 6.9 ab	3.46 ± 0.13 ab	1.97 ± 0.08 ab	6.0 ± 0.4 f
60	0.00	112.8 ± 5.5 f	2.14 ± 0.10 f	1.21 ± 0.06 f	18.7 ± 1.1 a
60	0.01	124.5 ± 5.8 e	2.46 ± 0.11 e	1.38 ± 0.07 e	15.9 ± 0.9 b
60	0.05	142.7 ± 6.2 d	2.98 ± 0.12 cd	1.69 ± 0.08 cd	11.8 ± 0.7 d
60	0.10	135.1 ± 6.0 de	2.73 ± 0.11 d	1.54 ± 0.07 d	13.6 ± 0.8 c

*Means followed by different letters within columns are significantly different, based on the least significant difference test (p≤0.05)

Table 4. DPPH and β-Carotene-linoleic acid in leaf extracts, phenolic composition, and total chlorophyll of *C. officinalis.* Values are means of triplicate determinations ±sd.

NaCl (mM)	SA (mM)	DPPH scavenging activity (%)	β-carotene–linoleic acid inhibition (%)	Total phenolics (mg GAE g^{-1} DW)	Total chlorophyll (mg g^{-1} FW)
0	0.00	58.4 ± 2.3 e	54.6 ± 2.1 e	18.7 ± 0.9 e	2.14 ± 0.10 cd
0	0.01	63.2 ± 2.5 d	59.8 ± 2.3 d	21.3 ± 1.0 d	2.31 ± 0.11 bc
0	0.05	71.8 ± 2.8 a	68.7 ± 2.6 a	26.9 ± 1.2 a	2.68 ± 0.12 a
0	0.10	67.4 ± 2.6 b	64.2 ± 2.4 b	24.5 ± 1.1 b	2.49 ± 0.11 ab
60	0.00	61.5 ± 2.4 de	57.1 ± 2.2 de	23.8 ± 1.1 c	1.42 ± 0.08 f
60	0.01	66.1 ± 2.5 bc	62.4 ± 2.3 bc	26.1 ± 1.2 ab	1.71 ± 0.09 e
60	0.05	73.9 ± 2.9 a	70.8 ± 2.7 a	29.8 ± 1.3 a	2.18 ± 0.10 c
60	0.10	69.2 ± 2.7 ab	66.0 ± 2.5 ab	27.4 ± 1.2 a	1.94 ± 0.09 d

*Means followed by different letters within columns are significantly different based on the least significant difference test (p≤0.05)

Table 5. Effects of saline stress and salicylic acid on MIC of *C. officinalis* leaf extracts against tested bacteria (mg mL⁻¹).

Treatment	*Listeria monocytogenes*	*Bacillus cereus*	*Staphylococcus aureus*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Escherichia coli*
0 mM NaCl + 0 SA	2.40 ± 0.12	2.20 ± 0.11	2.60 ± 0.13	2.10 ± 0.10	3.80 ± 0.18	3.40 ± 0.17
0 mM NaCl + 0.01 mM SA	2.10 ± 0.10	1.95 ± 0.09	2.30 ± 0.11	1.90 ± 0.09	3.40 ± 0.16	3.10 ± 0.15
0 mM NaCl + 0.05 mM SA	1.45 ± 0.08	1.30 ± 0.07	1.60 ± 0.08	1.20 ± 0.06	2.60 ± 0.13	2.30 ± 0.11
0 mM NaCl + 0.10 mM SA	1.70 ± 0.09	1.55 ± 0.08	1.85 ± 0.09	1.45 ± 0.07	2.90 ± 0.14	2.60 ± 0.12
60 mM NaCl + 0 SA	1.85 ± 0.09	1.70 ± 0.08	2.00 ± 0.10	1.60 ± 0.08	3.10 ± 0.15	2.80 ± 0.13
60 mM NaCl + 0.01 mM SA	1.55 ± 0.08	1.40 ± 0.07	1.70 ± 0.08	1.30 ± 0.06	2.70 ± 0.13	2.40 ± 0.12
60 mM NaCl + 0.05 mM SA	0.90 ± 0.05	0.80 ± 0.04	1.00 ± 0.05	0.75 ± 0.04	1.90 ± 0.09	1.70 ± 0.08
60 mM NaCl + 0.10 mM SA	1.15 ± 0.06	1.00 ± 0.05	1.25 ± 0.06	0.95 ± 0.05	2.20 ± 0.10	1.95 ± 0.09
Streptomycin	0.12 ± 0.01	0.10 ± 0.01	0.15 ± 0.01	0.09 ± 0.01	0.25 ± 0.02	0.22 ± 0.02
Ampicillin	0.18 ± 0.01	0.16 ± 0.01	0.20 ± 0.02	0.14 ± 0.01	0.40 ± 0.03	0.35 ± 0.03

Table 6. Effects of saline stress and salicylic acid on MBC of *C. officinalis* leaf extracts against tested bacteria (mg mL⁻¹).

Treatment	*Listeria monocytogenes*	*Bacillus cereus*	*Staphylococcus aureus*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Escherichia coli*
0 mM NaCl + 0 SA	4.20 ± 0.20	4.00 ± 0.19	4.50 ± 0.21	3.80 ± 0.18	6.20 ± 0.28	5.80 ± 0.26
0 mM NaCl + 0.01 mM SA	3.80 ± 0.18	3.50 ± 0.16	4.00 ± 0.18	3.30 ± 0.15	5.70 ± 0.25	5.10 ± 0.23
0 mM NaCl + 0.05 mM SA	2.70 ± 0.13	2.40 ± 0.12	2.90 ± 0.14	2.20 ± 0.11	4.30 ± 0.20	3.90 ± 0.18
0 mM NaCl + 0.10 mM SA	3.00 ± 0.14	2.80 ± 0.13	3.30 ± 0.15	2.50 ± 0.12	4.80 ± 0.22	4.20 ± 0.19
60 mM NaCl + 0 SA	3.10 ± 0.15	2.90 ± 0.14	3.40 ± 0.16	2.70 ± 0.13	5.00 ± 0.23	4.50 ± 0.21
60 mM NaCl + 0.01 mM SA	2.60 ± 0.12	2.40 ± 0.11	2.90 ± 0.14	2.20 ± 0.10	4.40 ± 0.20	3.90 ± 0.18
60 mM NaCl + 0.05 mM SA	1.60 ± 0.08	1.40 ± 0.07	1.80 ± 0.09	1.20 ± 0.06	3.00 ± 0.14	2.60 ± 0.12
60 mM NaCl + 0.10 mM SA	2.00 ± 0.10	1.80 ± 0.09	2.10 ± 0.10	1.50 ± 0.07	3.40 ± 0.16	3.00 ± 0.14
Streptomycin	0.25 ± 0.02	0.22 ± 0.02	0.30 ± 0.02	0.18 ± 0.01	0.50 ± 0.03	0.45 ± 0.03
Ampicillin	0.35 ± 0.03	0.30 ± 0.02	0.40 ± 0.03	0.25 ± 0.02	0.70 ± 0.04	0.65 ± 0.04

SA treatment reduced the excessive proline accumulation under salinity. Proline decreased from 18.7 to 11.8 µmol g^{-1} FW following application of 0.05 mM SA, which represented a reduction of 36.9%.

Antioxidant activity and phenolic content: Salinity and SA treatments significantly affected antioxidant activity, phenolic accumulation, and chlorophyll content of *C. officinalis* (Table 4). Under salinity stress without SA treatment, total phenolic content increased from 18.7 to 23.8 mg GAE g^{-1} DW and represented 27.3% increase in comparison to untreated control plants.

DPPH scavenging activity increased from 58.4% in control plants to 61.5% under salinity stress, while β-carotene-linoleic acid inhibition increased from 54.6% to 57.1%. Application of SA significantly enhanced antioxidant activity and phenolic accumulation. The maximum antioxidant activity was recorded in plants treated with 60 mM NaCl and 0.05 mM SA. Under this treatment, DPPH scavenging activity reached 73.9%, representing 26.5% increase in comparison to untreated control plants. β-carotene-linoleic acid inhibition increased from 54.6% to 70.8% (29.7% increase), whereas total phenolics increased from 18.7 to 29.8 mg GAE g^{-1} DW, which is 59.4% increase.

Total chlorophyll content declined significantly under salinity stress. The chlorophyll concentration dropped from 2.14 to 1.42 mg g^{-1} FW under saline conditions, which is a decline of 33.6%.

Application of antibacterial activity: The leaf extracts of *C. officinalis* showed antibacterial activity against all tested strains of bacteria (Tables 5 and 6). The antimicrobial activity of the extracts was significantly enhanced by salinity and SA treatments. The extracts from plants treated with 60 mM NaCl and 0.05 mM SA showed the strongest antibacterial activity with the lowest MIC and MBC values among all the tested treatments. The MBC values were also significantly lower with salinity and SA treatment. For example, the MBC values against Staphylococcus aureus were reduced from 4.50 mg mL^{-1} in untreated control plants to 1.80 mg mL^{-1} under salinity and 0.05 mM SA treatment, a reduction of 60.0%.Similarly, MIC values against *Listeria monocytogenes* decreased from 2.40 to 0.90 mg mL^{-1} (62.5% reduction), while MIC values against *Micrococcus flavus* decreased from 2.10 to 0.75 mg mL^{-1} (64.3% reduction). The MIC values against Bacillus cereus were reduced from 2.20 mg mL -1 in untreated control plants to 0.80 mg mL -1 after salinity and 0.05 mM SA treatment, which is a decrease of 63.6%. The Gram-positive bacteria (*Bacillus cereus* and *Micrococcus flavus*) were more sensitive to the extracts than the Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). Salinity in combination with SA significantly enhanced the antibacterial potential of *C. officinalis* leaf extracts, but the streptomycin and ampicillin had lower MIC and MBC values than the plant extracts.

Discussion

The present study proved that salinity remarkably reduced vegetative growth, flowering performance, mineral uptake, and chlorophyll content of *C. officinalis*. Osmotic stress, ionic toxicity, nutrient imbalance, and oxidative damage are the main causes of the inhibitory effects of salinity, which together affect cellular

metabolism and plant development (Munns & Tester, 2008; Hasanuzzaman *et al*., 2021). The accumulation of high salt reduces the soil water potential, which leads to limitations in the water uptake and thus reduces cell expansion and division. Plant growth is usually inhibited by the salinity effects as found in previous studies (Taibi *et al*., 2021; Khalid *et al*., 2022).

Several morphological and physiological parameters had been affected negatively under saline stress, such as plant height, branch number and length, biomass accumulation, and photosynthetic operations (Gill & Tuteja, 2022). Salinity may disrupt the photosynthetic apparatus and degrade the chlorophyll composition, which ultimately reduces the efficiency of light harvesting, leading to lower carbohydrate assimilation and production (Gill & Tuteja, 2022). Sodium ions build up during saline conditions, leading to ionic toxicity that affects the overall enzyme activities within plant cells and influences the membrane stability, leading to lower physiological performance. Similar effects of salinity on growth and productivity were observed in several medicinal plants, including basil, peppermint, and marigold species (Farhangi-Abriz & Ghassemi-Golezani, 2021). Salinity also affected the flowering behavior and flower yield of C. officinalis. The delayed flowering as well as reduced flower production in stressed plants is commonly attributed to stress-induced metabolic disturbance resulting in disturbed assimilation, translocation, and hormonal imbalance (Riaz *et al*., 2022).

Flower initiation and development are highly sensitive to environmental stress, as reproductive processes require a high allocation of energy and nutrients. Thus, the lesser flower diameter and fresh flower weight under salinity might be associated with the reduced availability of photosynthate and reduced cell expansion. Salt stress has also been found to induce a reduction in reproductive performance in some ornamental crops (Kaya *et al*., 2021). Foliar application of salicylic acid effectively alleviated the adverse effects of salinity on vegetative growth and flowering performance. Among the applied concentrations, 0.05 mM SA was the most effective treatment in enhancing the plant growth and productivity. SA-treated plants showed improved nutrient absorption, hormonal regulation, and membrane stability (Khan *et al*., 2023). The SA application on different plants enhances stomatal function and performance and promotes carbon fixation and biomass building under abiotic stress (Nazir *et al*., 2022). Hayat *et al*. (2010) reviewed, and Nazar *et al*., (2011) reported that SA improved salt-stressed plants.

Salinity stress causes loss of potassium and calcium from plants. Na^+ builds up in excess and competes with the uptake of K^+ and Ca^{2+}, resulting in nutritional imbalance and membrane instability (Munns & Tester, 2008). Potassium has a role in enzyme activity, osmotic adjustment, and stomatal regulation, whereas Calcium acts in intracellular signal transduction and membrane integrity. Salinity reduces important nutrients and influences growth and metabolism. SA treatment enhanced potassium and calcium accumulation, suggesting its role in ionic homeostasis and membrane selectivity under stress conditions (Kaya *et al*., 2021). Saline stress leads to a decrease in carbohydrate accumulation because of a loss in photosynthetic activity and a rise in respiration. Carbohydrate synthesis declines, cutting energy for growth and flowering.

Similar decreases in carbohydrate content were recorded in medicinal plants subjected to NaCl stress (Parida & Das, 2005). However, the SA treatment enhanced carbohydrate accumulation, probably through the improvement of chlorophyll stability and photosynthetic efficiency. The significant enhancement in proline accumulation in salt-stressed plants confirms the activation of osmotic adjustment mechanisms. Proline is one of the major compatible solutes involved in osmoprotection, stabilization of proteins and membranes, maintenance of cellular redox balance, as well as scavenging of reactive oxygen species (Ahanger *et al*., 2020). The increased accumulation of proline under salinity stress is widely recognized as an adaptive response to osmotic stress; however, an excessive accumulation of proline may also be indicative of severe cellular injury and metabolic disturbance. The SA application reduced the excessive accumulation of proline, suggesting that the stress severity was mitigated through improved physiological regulation. Salinity stress causes excessive ROS generation, which is a major effect leading to oxidative stress. The increases found in the DPPH assay on saline-stressed plants are strongly associated with increases in damage to lipids and proteins by free radicals, which ultimately damage the photosynthetic apparatus (Hasanuzzaman *et al*., 2021). The increased antioxidant activities observed in this study may be a signal for activation of the plant defense systems under stress. Phenolic compounds are one of the most important non-enzymatic antioxidants responsible for ROS detoxification and membrane protection (Rouphael & Kyriacou, 2021). Salinity resulted in increment of total phenolic content, which may serve as a defense mechanism against oxidative stress. Phenylics metabolism and biosynthesis of secondary metabolites in several medicinal plants, such as *C. officinalis*, be stimulated by abiotic stress in general (Shahrajabian *et al*., 2023). The increases in phenol accumulation in leaves of medicinal plants subjected to salt stress have also been reported in thyme, basil, and rosemary (Khalid *et al*., 2022). Increased phenolic biosynthesis is associated not only with stress tolerance but also with improvement of medicinal quality and antioxidant properties. Application of SA further increased antioxidant activity and phenolic accumulation in *C. officinalis*. SA treatment in the current experiment increased the phenolic composition in *C. of*ficinalis plants; these results are strongly associated with a previous report that found that SA may enhance the activity of phenolic biosynthesis enzymes (Hayat *et al*., 2010). The increased DPPH scavenging activity and β-carotene-linoleic acid inhibition after SA treatment indicate the improved antioxidant capacity and ROS detoxification efficiency. A similar increase in antioxidant compounds was reported in several medicinal plants under abiotic stress conditions by treatment with salicylic acid (Farhangi-Abriz & Ghassemi-Golezani, 2021). The higher antibacterial activity of *C. officinalis* extracts under salinity and salicylic acid treatments could be due to the increased accumulation of phenolic compounds, flavonoids, and other secondary metabolites with antimicrobial activity. Phenolics exert antimicrobial activity by damaging microbial membranes, inhibiting enzyme activities, and interfering with nucleic

acid synthesis in pathogenic microorganisms (Efstratiou *et al*., 2021). The greater susceptibility of Gram-positive bacteria than Gram-negative bacteria in this study is probably due to the structural differences of the bacterial cell walls. Gram-negative bacteria have an outer membrane that is rich in lipopolysaccharides, which limits the penetration of antimicrobial compounds and thus increases the resistance (Burt, 2020).

The increase in antibacterial activity for the extracts of plants treated with salinity and SA suggests that moderate abiotic stress, along with appropriate hormonal treatment, may increase the pharmaceutical value of medicinal plants. Similar relationships between environmental stress, phenolic accumulation, and antimicrobial activity were reported by Elansary *et al*., (2020) and Shahrajabian *et al*., (2023). Thus, enhancement of medicinal properties under controlled stress conditions may represent an effective strategy for enhancing the quality of medicinal plant products.

In conclusion, the present results demonstrate negative effects of salinity on growth and productivity of *C. officinalis*, while exogenous salicylic acid was effective in alleviating stress damage and improving antioxidant metabolism, phenolic accumulation, and antibacterial activity. Application of 0.05 mM SA was more successful in enhancing plant performance and medicinal quality under saline conditions. These results highlight the potential use of SA as an effective strategy to enhance tolerance and pharmaceutical value of medicinal plants grown under saline conditions.

Conclusion

Salinity stress significantly impaired growth, flowering, mineral nutrition, and chlorophyll accumulation in *C. officinalis*, while simultaneously stimulating osmoprotective and antioxidant defense responses. Application of salicylic acid effectively alleviated the negative effects of salt stress by enhancing growth performance, nutrient balance, antioxidant enzyme activities, and accumulation of bioactive compounds. The 0.05 mM SA treatment was the most effective concentration, producing substantial improvements in vegetative growth, flower yield, phenolic content, antioxidant capacity, and antibacterial activity under saline conditions. Increased antimicrobial activity of leaf extracts under combined salinity and SA treatment was closely associated with enhanced phenolic accumulation and oxidative defense mechanisms. These findings indicate that exogenous SA can be used as an effective strategy to improve salinity tolerance and medicinal quality of *C. officinalis*, particularly under moderately saline environments.

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