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RESEARCH ARTICLE

Impact of salinity stress on phytochemical profiles in *Salvia officinalis*

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Abstract

This study examines how varying salinity levels (5, 25, 50, 100, 150 and 200 mM NaCl) affect the phytochemical composition of *Salvia officinalis*. Using LC-MS analysis, we observed significant alterations in secondary metabolite production under salt stress. Monoterpenes, such as thujone and borneol, increased notably at 100-200 mM NaCl, indicating an upregulation of terpenoid biosynthesis as a protective response. Sesquiterpenes like Ledene and Humulene rose under moderate salinity (50-150 mM NaCl) but declined at 200 mM, possibly due to disrupted biosynthetic pathways. Diterpenes showed enhanced yields under moderate to severe stress, suggesting a role in augmenting plant resilience. Phenolic compounds, known for antioxidant properties, peaked at 100 mM NaCl, while flavonoid content decreased under higher salinity, implying suppression of their biosynthesis. Unsaturated fatty acids, including oleic and arachidonic acids, increased under stress levels of 150-200 mM NaCl, aiding in membrane stability. Additionally, under salt stress (50-200 mM NaCl), elevated levels of β-carotene, beta-sitosterol and malic acid were observed, supporting their roles in osmotic adjustment and mitigating oxidative stress. These findings underscore *S. officinalis* adaptive mechanisms to salinity through the accumulation of protective metabolites and metabolic adjustments. Future research should delve into the molecular mechanisms driving these changes to develop salt-tolerant crops and optimize sage cultivation in saline environments.

Keywords: adaptive responses; metabolites; oxidative stress; salt stress; *Salvia officinalis*

Introduction

Salinity stress is a significant abiotic factor that adversely affects plant growth, development and metabolic processes, ultimately influencing the production of secondary metabolites. *Salvia officinalis* (common sage), a widely cultivated medicinal plant, is renowned for its rich phytochemical profile, including phenolic acids, flavonoids, terpenoids and essential oils, which contribute to its antioxidant, antimicrobial and anti-inflammatory properties (1, 2). However, under salinity stress, plants often undergo physiological and biochemical changes to adapt to adverse conditions, which can alter the synthesis and accumulation of these bioactive compounds (3).

Understanding the impact of salinity stress on the phytochemical composition of *S. officinalis* is crucial for optimizing its cultivation in saline-prone areas and ensuring the quality of its medicinal properties. Recent studies have shown that moderate salinity stress can enhance the production of certain secondary metabolites as a defense mechanism. In contrast, excessive salinity may lead to oxidative damage and reduced phytochemical content (4, 5).

Analysis of phytochemical changes in sage leaves under saline stress using LC-MS techniques reveals significant changing patterns in metabolites associated with salinity adaptive responses. These changes are aligned with previous studies on Salvia species and highlight the impact of environmental factors on secondary metabolites (6, 7). Metabolome profile-modulation under stress conditions is crucial to understanding plant resilience (8).

The LC-MS profile also supports the biochemical adaptations observed in other plants under similar stress (9, 10). In addition, phytochemical variations can correlate with enhanced antioxidant properties, influencing ecological interactions (6, 11, 12). As shown in hydroponic systems studies, controlled salinity stress affects bioactive metabolome and morphological characteristics (13, 14). Moreover, salinity significantly affects both the yield and composition of essential oil in sage. Moderate salinity levels, such as up to 75 mM NaCl, can enhance essential oil production, whereas higher concentrations, like 100 mM NaCl, result in a reduction. Additionally, salt stress modifies the composition of essential oil constituents. Under normal conditions, viridiflorol is the dominant component, but at 50 and 75 mM NaCl, 1,8-cineole becomes the primary constituent. At 100 mM NaCl, manool emerges as the most abundant compound (11). Therefore, integrating these ideas into the study of salvia species under saline stress is essential for future agricultural practices and conservation strategies.

This work aims to explore the effects of elevation of salinity stress and the duration of exposure on the phytochemical profiles of *S. officinalis*, highlighting the potential mechanisms underlying these changes and their implications for the plant's medicinal value.

Material and Methods

Plant growth condition

The *S. officinalis* seedlings undergo cultivation in controlled conditions, exposed to 14 hrs of approximately 80 μmolm^{2}s^{-1} light at 21 °C and 10 hrs of darkness at 20 °C, maintaining a relative humidity of 55-60 %. Subsequently, they were transplanted into a mixture of peat moss, perlite and vermiculite in a ratio of 2:1:1 (v/v/v). Following six weeks of growth, the plants were subjected to salinity treatments for varying durations.

Salinity stress treatment

To induce salinity stress, 6-week-old growing seedlings were used in salinity treatments as designed previously (15). 6-weeks-old plants irrigated with a 5 (Control samples irrigated with tap water), 25, 50, 100, 150, or 200 mM NaCl solution three times per week for up to 8 days. Interleaves samples were collected at 2, 4, 6and 8 days of treatment.

Phytochemical profiles analysis

To evaluate the phytochemical profiles, 50 mg of *S. officinalis* leaf tissue was extracted using a solution composed of 86 % methanol and 10 % acetonitrile. The sample was homogenized and vortexed for 3 min before being centrifuged at 4000 rpm for 2 min. The supernatant obtained was evaporated using nitrogen gas and the residue was reconstituted in 500 μL of the HPLC mobile phase. HPLC separation was performed using a gradient method with two solvents: Solvent A (90 % acetic acid -water) and Solvent B (10 % methanol). The gradient started with 100 % Solvent B for 20 min, followed by another 15 min of 100 % Solvent Band then re-equilibration. The analysis was conducted on an Agilent Zorbax Eclipse XDB-C18 column (4.6 x 125 mm, 5 μm particle size) maintained at 35 °C, with an injection volume of 10 μL. The eluent was analysed using a Shimadzu LC-MS 8030 system equipped with an electrospray ionization mass spectrometer (ESI-MS) operating in positive ion mode. The mass range scanned was 50 to 1200 *m/z*, with an ESI voltage of 125V and a SKIMMER setting of 65V. High-purity nitrogen (99.999 %) was used as the drying gas at a flow rate of 200 μL/min. The fragment nebulizer pressure was set at 45 psi and the capillary temperature was maintained at 350 °C. A 0.1 % formic acid solution was used as a blank (16).

Results

Phytochemical screening of *S. officinalis* leaves

In the methanolic extract of our samples, most of the *m/z* values ranged from 134 to 610. Terpenoid compounds, such as monoterpenes, sesquiterpenes and diterpenoids were the most prevalent and displayed higher intensity compared to other compounds. Phenolic compounds, flavonoids and fatty acids were also identified but were present in lower amounts (Table 1) and (Fig. 1).

Terpenoid compounds

Non-stressed sage plants showed a gradual decline in monoterpene yield over an extended growth period, with percentages of 30.5 %, 29 %, 26.7 % and 25.3 % after 2, 4, 6 and 8 days, respectively (Table 2). Under low salinity (25 mM NaCl), the monoterpene yield remained constant at 28 % across all exposure durations (Table 3). Moderate salt stress (50 mM NaCl) resulted in fluctuating yields of 23.4 %, 25 %, 31.1 % and 25.9 % after 2, 4, 6 and 8 days, respectively (Table 4). Higher moderate stress (100 mM NaCl) further altered yields to 31 %, 29.6 %, 34.1 % and 30.7 % over the same period (Table 5). Severe salt stress (150 mM NaCl) caused a reduction to 28.2 %, 28 %, 23.9 % and 29.3 % (Table 6), while extreme stress (200 mM NaCl) maintained a similar reduction

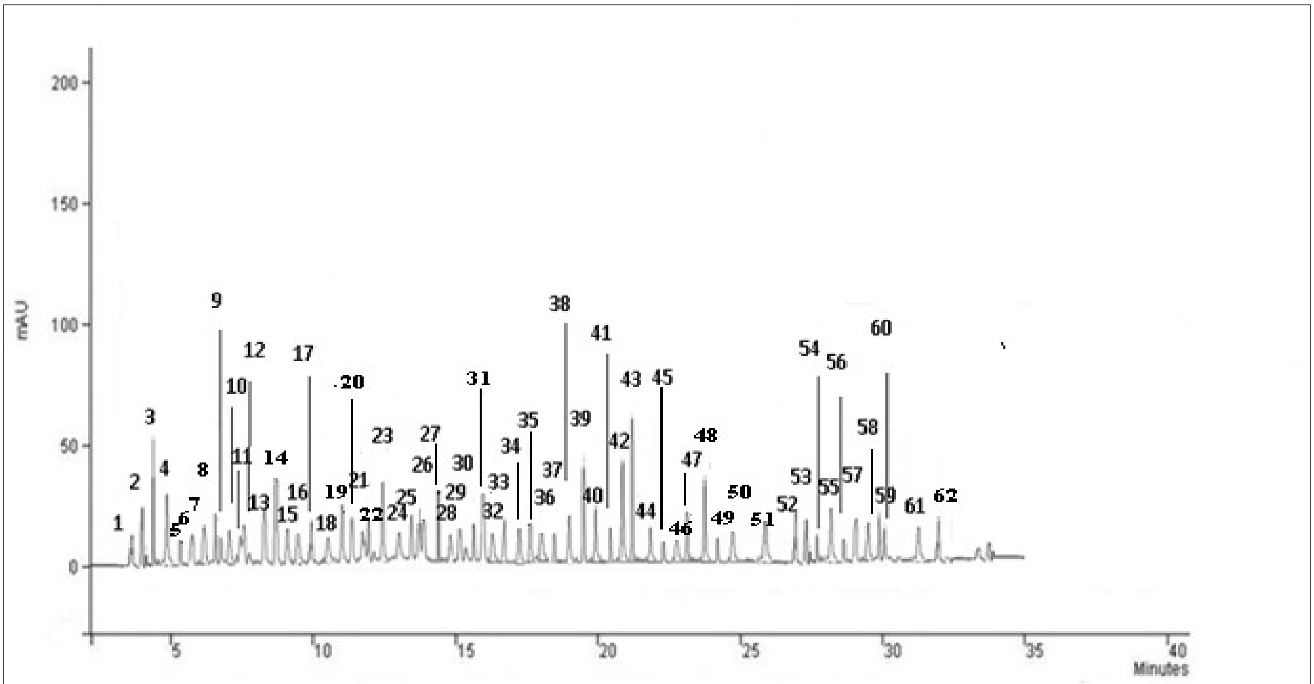

Fig. 1. Phytochemical chromatogram of *S. officinalis* leaves.

Table 1. Identification of compounds in *S. officinalis* leaves based on their LC-MS spectral characteristics in positive ion mode

Peak no.	Compounds	Molecular formula	Molecular weight	Retention time (min)
1	Malic acid	$C_4H_6O_5$	134.09g/mol	2.22
2	Cymene	$C_{10}H_{14}$	134.22g/mol	3.2
3	Pinene	$C_{10}H_{16}$	136.23g/mol	4.1
4	Camphene	$C_{10}H_{16}$	136.23g/mol	4.9
5	Sabinene	$C_{10}H_{16}$	136.23g/mol	5.2
6	Beta-myrcene	$C_{10}H_{16}$	136.23g/mol	5.6
7	Limonene	$C_{10}H_{16}$	136.23g/mol	6.2
8	Terpinolene	$C_{10}H_{16}$	136.23g/mol	6.9
9	Protocatechualdehyde	$C_7H_6O_3$	138.12g/mol	7.2
10	Coumarin	$C_9H_6O_2$	146.14g/mol	7.6
11	Carvacrol	$C_{10}H_{14}$	150.22g/mol	8.2
12	thymol	$C_{10}H_{14}$	150.22g/mol	8.6
13	Vanillin	$C_8H_8O_3$	152.15g/mol	8.9
14	Camphor	$C_{10}H_{16}O$	152.23g/mol	9.1
15	Thujone	$C_{10}H_{16}O$	152.23g/mol	9.6
16	Borneol	$C_{10}H_{18}O$	154.25g/mol	9.8
17	Geraniol	$C_{10}H_{18}O$	154.25g/mol	9.9
18	Terpinene	$C_{10}H_{18}O$	154.25g/mol	10.3
19	1,8-Cineole	$C_{10}H_{18}O$	154.25g/mol	11.1
20	Linalool	$C_{10}H_{18}O$	154.25g/mol	11.2
21	Terpineol	$C_{10}H_{18}O$	154.25g/mol	11.6
22	Vanillic p-hydroxy benzoic acid	$C_8H_8O_4$	168.15g/mol	12.2
23	Gallic acid	$C_7H_6O_5$	170.12g/mol	13.3
24	Caffeic acid	$C_9H_8O_4$	180.16g/mol	13.6
25	Quinic acid	$C_7H_{12}O_6$	192.17g/mol	14.2
26	Myristicin	$C_{11}H_{12}O_3$	192.21g/mol	14.6
27	Ferulic acid	$C_{10}H_{10}O_4$	194.18g/mol	14.8
28	Linalyl acetate	$C_{12}H_{20}O_2$	196.29g/mol	14.9
29	Geranyl acetate	$C_{12}H_{20}O_2$	196.29g/mol	15.1
30	Danshensu	$C_9H_{10}O_5$	198.17g/mol	15.3
31	Caryophyllene	$C_{15}H_{24}$	204.35g/mol	15.6
32	Ledene	$C_{15}H_{24}$	204.35g/mol	16.3
33	Humulene	$C_{15}H_{24}$	204.35g/mol	16.7
34	Elemene	$C_{15}H_{24}$	204.35g/mol	17.2
35	Cadinene	$C_{15}H_{26}$	206.37g/mol	17.7
36	Mirin	$C_{10}H_8N_2O_2S$	220.25g/mol	18.2
37	Spathulenol	$C_{15}H_{24}O$	220.35g/mol	18.5
38	Ledol	$C_{15}H_{26}O$	222.37g/mol	19.2
39	viridiflorol	$C_{15}H_{26}O$	222.37g/mol	19.6
40	farnesol	$C_{15}H_{26}O$	222.37g/mol	19.9
41	Cinametic acid	$C_{12}H_{14}O_5$	238.24g/mol	20.3
42	Apigenin	$C_{15}H_{10}O_5$	270.24g/mol	20.7
43	Naringenin	$C_{15}H_{12}O_5$	272.25g/mol	21.3
44	Oleic acid	$C_{18}H_{34}O_2$	282.5g/mol	21.8
45	Scutellarein	$C_{15}H_{10}O_6$	286.24g/mol	23.1
46	Fisetin	$C_{15}H_{10}O_6$	286.24g/mol	23.7
47	Ferruginol	$C_{20}H_{30}O$	286.5g/mol	24.3
48	catechin	$C_{15}H_{14}O_6$	290.27g/mol	24.5
49	Phytol	$C_{20}H_{40}O$	296.5g/mol	24.7
50	Hispidulin	$C_{16}H_{12}O_6$	300.26g/mol	24.9
51	Ellagic acid	$C_{14}H_6O_8$	302.19g/mol	25.9
52	Quercetin	$C_{15}H_{10}O_7$	302.23g/mol	27.2
53	Arachidonic acid	$C_{20}H_{32}O_2$	304.5g/mol	27.6
54	Cirsimaritin	$C_{17}H_{14}O_6$	314.29g/mol	27.9
55	Carnosol	$C_{20}H_{26}O_4$	330.4g/mol	28.5
56	Carnosic acid	$C_{20}H_{28}O_4$	332.4g/mol	29.1
57	Chlorogenic acid	$C_{16}H_{18}O_9$	354.31g/mol	29.6
58	Rosmarinic acid	$C_{18}H_{16}O_8$	360.3g/mol	29.9
59	Beta-sitosterol	$C_{29}H_{50}O$	414.7g/mol	30.2
60	Luteolin-7-glucoside	$C_{21}H_{20}O_{11}$	448.4g/mol	30.5
61	ß-carotene	$C_{40}H_{56}$	536.9g/mol	31.8
62	Rutin	$C_{27}H_{30}O_{16}$	610.5g/mol	32.2

Table 2. Phytochemical screening of *S. officinalis* leaves subjected to 5 mM NaCl salt stress and different exposure time points (2, 4, 6 and 8 days)

			Exposure time points (days)	2	4	6	8
Compounds	**Phytochemicals classification**	**Molecular formula**	**Molecular weight (g/mol)**	**%**	**%**	**%**	**%**
Cymene	Monoterpene	$C_{10}H_{14}$	134.22	2.02	2.39	1.07	1.33
pinene		$C_{10}H_{16}$	136.23	5.79	3.77	4.28	3.31
Camphene		$C_{10}H_{16}$	136.23	3.86	3.12	2.04	2.53
Sabinene		$C_{10}H_{16}$	136.23	1.84	1.09	1.13	0.6
Beta-myrcene		$C_{10}H_{16}$	136.23	1.01	0.74	0.49	1
Limonene		$C_{10}H_{16}$	136.23	2.11	0.38	0.1	0.12
Terpinolene		$C_{10}H_{16}$	136.23	1.38	4.78	4.97	3.82
camphor		$C_{10}H_{16}O$	152.23	2.48	2.68	3.39	4.1
Thujone		$C_{10}H_{16}O$	152.23	1.65	1.2	0.49	0.6
borneol		$C_{10}H_{18}O$	154.25	0.64	0.74	0.39	0.48
Geraniol		$C_{10}H_{18}O$	154.25	0.83	0.72	0.68	0.84
Terpinene		$C_{10}H_{18}O$	154.25	0.61	0.46	0.29	0.36
1,8-Cineole		$C_{10}H_{18}O$	154.25	1.65	4.2	4.86	5.03
Linalool		$C_{10}H_{18}O$	154.25	2.94	2.1	1.81	0.7
Terpineol		$C_{10}H_{18}O$	154.25	0.83	0.31	0.39	0.1
Linalyl acetate		$C_{12}H_{20}O_2$	196.29	0.55	0.24	0.14	0.17
Geranyl acetate		$C_{12}H_{20}O_2$	196.29	0.28	0.18	0.19	0.24
Total				30.47	29	26.71	25.33
Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.35	2.02	4.78	5.05	6.27
Ledene		$C_{15}H_{24}$	204.35	0.74	0.53	0.39	0.48
Humulene		$C_{15}H_{24}$	204.35	0.64	0.67	0.78	0.96
Elemene		$C_{15}H_{24}$	204.35	1.65	1.52	1.94	0.5
Cadinene		$C_{15}H_{26}$	206.37	1.29	4.2	4.24	1.4
Spathulenol		$C_{15}H_{24}O$	220.35	0.51	0.56	0.58	0.72
Ledol		$C_{15}H_{26}O$	222.37	2.67	2.1	2.23	2.77
Viridiflorol		$C_{15}H_{26}O$	222.37	3.31	2.61	2.91	3.62
Farnesol		$C_{15}H_{26}O$	222.37	3.03	2.39	1.94	2.41
Total				15.86	19.36	20.06	19.13
Ferruginol	Diterpenoid	$C_{20}H_{30}O$	286.5	1.01	1.67	1.86	2.5
Phytol		$C_{20}H_{40}O$	296.5	0.18	0.58	0.29	0.36
Carnosol		$C_{20}H_{26}O_4$	330.4	3.31	2.46	2.32	1.57
Total				4.5	4.71	4.47	4.43
Coumarin	Other polyphenols	$C_9H_6O_2$	146.14	0.37	0.23	0.4	0.84
carvacrol		$C_{10}H_{14}O$	150.22	0.3	0.2	0.1	0.12
thymol		$C_{10}H_{14}O$	150.22	0.26	0.12	0.1	0.12
vanillin		$C_8H_8O_3$	152.15	1.75	1.52	0.78	0.96
Danshensu		$C_9H_{10}O_5$	198.17	0.74	0.24	0.1	0.12
Carnosic acid		$C_{20}H_{28}O_4$	332.4	0.64	0.31	0.19	1.3
Total				4.06	2.62	1.67	3.46
Apigenin	Flavonoids	$C_{15}H_{10}O_5$	270.24	5.52	4.35	3.01	3.74
Naringenin		$C_{15}H_{12}O_5$	272.25	6.44	5.07	4.86	6.03
Scutellarein		$C_{15}H_{10}O_6$	286.24	0.68	0.54	4.96	4.15
Fisetin		$C_{15}H_{10}O_6$	286.24	0.81	0.24	3.89	2.8
Catechin		$C_{15}H_{14}O_6$	290.27	4.05	3.99	3.73	1.2
Hispidulin		$C_{16}H_{12}O_6$	300.26	0.28	0.8	0.78	0.96
Quercetin		$C_{15}H_{10}O_7$	302.23	1.01	1.23	0.97	1.21
Cirsimaritin		$C_{17}H_{14}O_6$	314.29	0.61	0.22	0.28	0.12
Luteolin-7-glucoside		$C_{21}H_{20}O_{11}$	448.4	0.74	0.62	0.49	0.6
Myristicin		$C_{11}H_{12}O_3$	192.21	0.55	1.96	1.94	2.41
Rutin		$C_{27}H_{30}O_{16}$	610.5	1.65	2.03	1.58	1.21
Total				22.34	21.05	26.49	24.43
Protocatechualdehyde	Phenolic acids	$C_7H_6O_3$	138.12	0.64	0.48	0.29	0.36
Vanillic p-hydroxy benzoic acid		$C_8H_8O_4$	168.15	0.97	1.38	1.41	2.77
Gallic acid		$C_7H_6O_5$	170.12	2.48	3.19	2.91	3.62
Caffeic acid		$C_9H_8O_4$	180.16	0.71	0.67	0.49	0.6
Quinic acid		$C_7H_{12}O_6$	192.17	1.56	1.59	1.81	1.21
Ferulic acid		$C_{10}H_{10}O_4$	194.18	1.29	2.39	3.11	1
Cinametic acid		$C_{12}H_{14}O_5$	238.24	2.57	2.03	1.07	2.2
Ellagic acid		$C_{14}H_6O_8$	302.19	1.65	2.1	2.04	2.53
Chlorogenic acid		$C_{16}H_{18}O_9$	354.31	2.3	1.3	0.97	1.21
Mirin		$C_{10}H_8N_2O_2S$	220.25	0.87	1.3	2.37	1.33
Rosmarinic acid		$C_{18}H_{16}O_8$	360.3	1.65	2.49	1.94	2.41
Total				15.82	17.62	16.04	17.91
Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	2.02	1.59	0.49	0.6
Arachidonic acid		$C_{20}H_{32}O_2$	304.5	0.97	0.87	0.87	1.09
Total				2.99	2.46	1.36	1.69
Beta-sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	0.46	0.25	0.19	0.24
Malic acid	Carboxylic acid	$C_4H_6O_5$	134.09	2.11	1.09	0.28	0.72
ß-carotene		$C_{40}H_{56}$	536.9	0.51	0.48	0.37	1.33

Table 3. Phytochemical screening of *S. officinalis* leaves subjected to 25 mM NaCl salt stress and different exposure time points (2, 4, 6 and 8 days)

			Exposure time points (days)	2	4	6	8
Compounds	Phytochemicals classification	Molecular formula	Molecular weight (g/mol)	%	%	%	%
Cymene	Monoterpene	$C_{10}H_{14}$	134.22	1.1	1.1	1.1	1.1
pinene		$C_{10}H_{16}$	136.23	4.41	4.41	4.4	4.44
Camphene		$C_{10}H_{16}$	136.23	2.11	2.11	2.1	2.12
Sabinene		$C_{10}H_{16}$	136.23	3.11	3.11	3.1	3.13
Beta-myrcene		$C_{10}H_{16}$	136.23	0.5	0.5	0.5	0.5
Limonene		$C_{10}H_{16}$	136.23	0.1	0.1	0.1	0.1
Terpinolene		$C_{10}H_{16}$	136.23	4.01	4.01	4	4.04
camphor		$C_{10}H_{16}O$	152.23	4.11	4.11	4.1	4.2
Thujone		$C_{10}H_{16}O$	152.23	0.5	0.5	0.5	0.5
borneol		$C_{10}H_{18}O$	154.25	0.4	0.4	0.4	0.4
Geraniol		$C_{10}H_{18}O$	154.25	0.7	0.7	0.7	0.7
Terpinene		$C_{10}H_{18}O$	154.25	0.3	0.3	0.3	0.3
1,8-Cineole		$C_{10}H_{18}O$	154.25	5.01	5.01	5	5.13
Linalool		$C_{10}H_{18}O$	154.25	1.1	1.1	1.1	1.14
Terpineol		$C_{10}H_{18}O$	154.25	0.4	0.4	0.4	0.4
Linalyl acetate		$C_{12}H_{20}O_2$	196.29	0.14	0.14	0.1	0.14
Geranyl acetate		$C_{12}H_{20}O_2$	196.29	0.2	0.2	0.2	0.2
Total				28.2	28.2	28.1	28.54
Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.35	5.21	5.21	5.2	5.4
Ledene		$C_{15}H_{24}$	204.35	0.4	0.4	0.4	0.41
Humulene		$C_{15}H_{24}$	204.35	0.8	0.8	0.8	0.83
Elemene		$C_{15}H_{24}$	204.35	2.01	2.01	2	2.1
Cadinene		$C_{15}H_{26}$	206.37	3.01	3.01	3	3.13
Spathulenol		$C_{15}H_{24}O$	220.35	0.6	0.6	0.6	0.57
Ledol		$C_{15}H_{26}O$	222.37	2.31	2.31	2.3	2.22
Viridiflorol		$C_{15}H_{26}O$	222.37	3.01	3.01	3	2.89
Farnesol		$C_{15}H_{26}O$	222.37	2.01	2.01	2	1.9
Total				19.36	19.36	19.3	19.45
Ferruginol	Diterpenoid	$C_{20}H_{30}O$	286.5	3.01	3.01	3	2.8
Phytol		$C_{20}H_{40}O$	296.5	0.3	0.3	0.3	0.28
Carnosol		$C_{20}H_{26}O_4$	330.4	1.3	1.3	1.3	1.27
Total				4.61	4.61	4.6	4.35
Coumarin	Other polyphenols	$C_9H_6O_2$	146.14	0.7	0.7	0.7	0.7
carvacrol		$C_{10}H_{14}O$	150.22	0.1	0.1	0.1	0.1
thymol		$C_{10}H_{14}O$	150.22	0.1	0.1	0.1	0.1
vanillin		$C_8H_8O_3$	152.15	0.8	0.8	0.8	0.82
Danshensu		$C_9H_{10}O_5$	198.17	0.1	0.1	0.1	0.1
Carnosic acid		$C_{20}H_{28}O_4$	332.4	0.2	0.2	0.2	0.19
Total				2	2	2	2.01
Apigenin	Flavonoids	$C_{15}H_{10}O_5$	270.24	3.11	3.11	3.1	2.99
Naringenin		$C_{15}H_{12}O_5$	272.25	5.01	5.01	5	4.8
Scutellarein		$C_{15}H_{10}O_6$	286.24	5.11	5.11	5.1	4.9
Fisetin		$C_{15}H_{10}O_6$	286.24	4.01	4.01	4	3.8
Catechin		$C_{15}H_{14}O_6$	290.27	2.21	2.21	2.2	2.12
Hispidulin		$C_{16}H_{12}O_6$	300.26	0.8	0.8	0.8	0.77
Quercetin		$C_{15}H_{10}O_7$	302.23	1	1	1	0.96
Cirsimaritin		$C_{17}H_{14}O_6$	314.29	0.1	0.1	0.1	0.09
Luteolin-7-glucoside		$C_{21}H_{20}O_{11}$	448.4	0.5	0.5	0.5	0.49
Myristicin		$C_{11}H_{12}O_3$	192.21	2.01	2.01	2	2.08
Rutin		$C_{27}H_{30}O_{16}$	610.5	1	1	1	0.98
Total				24.86	24.86	24.8	23.98
Protocatechualdehyde	Phenolic acids	$C_7H_6O_3$	138.12	0.3	0.3	0.3	0.3
Vanillic p-hydroxy ben-zoic acid		$C_8H_8O_4$	168.15	2.31	2.31	2.3	2.4
Gallic acid		$C_7H_6O_5$	170.12	3.01	3.01	3	3.1
Caffeic acid		$C_9H_8O_4$	180.16	0.5	0.5	0.5	0.5
Quinic acid		$C_7H_{12}O_6$	192.17	1	1	1	1.04
Ferulic acid		$C_{10}H_{10}O_4$	194.18	3.21	3.21	3	3.34
Cinametic acid		$C_{12}H_{14}O_5$	238.24	1.1	1.1	1.1	1.06
Ellagic acid		$C_{14}H_6O_8$	302.19	2.11	2.11	2.1	2.02
Chlorogenic acid		$C_{16}H_{18}O_9$	354.31	1	1	1	0.98
Mirin		$C_{10}H_8N_2O_2S$	220.25	1.1	1.1	1.1	1.06
Rosmarinic acid		$C_{18}H_{16}O_8$	360.3	2.01	2.01	2	1.96
Total				16.55	16.55	16.3	16.7
Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	0.5	0.5	0.5	0.48
Arachidonic acid		$C_{20}H_{32}O_2$	304.5	0.9	0.9	0.9	0.88
Total				1.4	1.4	1.4	1.36
Beta-sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	0.2	0.2	0.2	0.19
Malic acid	Carboxylic acid	$C_4H_6O_5$	134.09	0.6	0.6	0.69	0.6
ß-carotene		$C_{40}H_{56}$	536.9	1.1	1.1	1.1	1.08

Table 4. Phytochemical screening of *S. officinalis* leaves subjected to 50 mM NaCl salt stress and different exposure time points (2, 4, 6 and 8 days)

		Exposure time points (days)		2	4	6	8
Compounds	**Phytochemicals classification**	**Molecular formula**	**Molecular weight (g/mol)**	**%**	**%**	**%**	**%**
Cymene	Monoterpene	$C_{10}H_{14}$	134.22	0.61	2.61	3.11	2.62
pinene		$C_{10}H_{16}$	136.23	3.66	2.14	1.15	2.19
Camphene		$C_{10}H_{16}$	136.23	2.8	1.8	1.43	1.17
Sabinene		$C_{10}H_{16}$	136.23	0.51	1.12	1.68	1.5
Beta-myrcene		$C_{10}H_{16}$	136.23	1.58	0.75	1.96	1.07
Limonene		$C_{10}H_{16}$	136.23	2.14	2.14	0.92	1.43
Terpinolene		$C_{10}H_{16}$	136.23	0.97	1.19	2.24	1.07
camphor		$C_{10}H_{16}O$	152.23	1.63	1.43	2.24	1.83
Thujone		$C_{10}H_{16}O$	152.23	1.42	2.99	2.45	1.4
borneol		$C_{10}H_{18}O$	154.25	0.61	1.43	0.56	1.68
Geraniol		$C_{10}H_{18}O$	154.25	0.15	0.44	1.45	0.64
Terpinene		$C_{10}H_{18}O$	154.25	0.39	0.75	1.1	1.12
1,8-Cineole		$C_{10}H_{18}O$	154.25	1.12	1.46	2.6	2.24
Linalool		$C_{10}H_{18}O$	154.25	2.19	0.75	1.07	2.6
Terpineol		$C_{10}H_{18}O$	154.25	0.61	1.12	1.48	1.17
Linalyl acetate		$C_{12}H_{20}O_2$	196.29	2.7	2.31	2.83	1.5
Geranyl acetate		$C_{12}H_{20}O_2$	196.29	0.31	0.64	1.84	0.66
Total				23.4	25.07	30.11	25.89
Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.35	3.2	2.78	3.62	2.55
Ledene		$C_{15}H_{24}$	204.35	2.24	2.14	3.9	2.17
Humulene		$C_{15}H_{24}$	204.35	1.68	0.78	1.33	1.61
Elemene		$C_{15}H_{24}$	204.35	0.56	2.99	0.08	1.12
Cadinene		$C_{15}H_{26}$	206.37	1.83	2.34	0.38	2.01
Spathulenol		$C_{15}H_{24}O$	220.35	1.47	1.19	2.19	1.68
Ledol		$C_{15}H_{26}O$	222.37	2.34	2.92	1.86	2.52
Viridiflorol		$C_{15}H_{26}O$	222.37	1.68	1.29	1.35	1.15
Farnesol		$C_{15}H_{26}O$	222.37	2.8	2.55	1.61	2.24
Total				17.8	18.98	16.32	17.05
Ferruginol	Diterpenoid	$C_{20}H_{30}O$	286.5	2.8	1.87	1.35	1.02
Phytol		$C_{20}H_{40}O$	296.5	1.12	2.58	0.31	2.27
Carnosol		$C_{20}H_{26}O_4$	330.4	3	2.07	1.91	1.43
Total				6.92	6.52	3.57	4.72
Coumarin	Other polyphenols	$C_9H_6O_2$	146.14	0.51	0.85	1.12	1.4
carvacrol		$C_{10}H_{14}O$	150.22	0.25	1.87	2.27	1.68
thymol		$C_{10}H_{14}O$	150.22	0.28	2.24	2.6	2.24
vanillin		$C_8H_8O_3$	152.15	1.17	1.8	1.96	1.4
Danshensu		$C_9H_{10}O_5$	198.17	0.92	0.81	1.35	0.97
Carnosic acid		$C_{20}H_{28}O_4$	332.4	4.17	3.12	2.37	2.6
Total				7.3	10.69	11.67	10.29
Apigenin	Flavonoids	$C_{15}H_{10}O_5$	270.24	3.56	3.36	0.31	2.68
Naringenin		$C_{15}H_{12}O_5$	272.25	4.07	2.55	0.2	2.24
Scutellarein		$C_{15}H_{10}O_6$	286.24	0.45	0.37	1.84	1.66
Fisetin		$C_{15}H_{10}O_6$	286.24	0.56	1.49	2.37	1.99
Catechin		$C_{15}H_{14}O_6$	290.27	2.24	1.22	0.59	1.68
Hispidulin		$C_{16}H_{12}O_6$	300.26	1.27	2.14	1.4	2.52
Quercetin		$C_{15}H_{10}O_7$	302.23	1.58	1.63	1.35	1.15
Cirsimaritin		$C_{17}H_{14}O_6$	314.29	1.17	1.9	1.35	1.76
Luteolin-7-glucoside		$C_{21}H_{20}O_{11}$	448.4	1.17	1.78	0.59	1.68
Myristicin		$C_{11}H_{12}O_3$	192.21	2.24	1.22	1.12	1.43
Rutin		$C_{27}H_{30}O_{16}$	610.5	1.17	1.82	1.12	0.56
Total				19.48	19.48	12.24	19.35
Protocatechualdehyde	Phenolic acids	$C_7H_6O_3$	138.12	0.46	0.51	1.35	0.64
Vanillic p-hydroxy benzoic acid		$C_8H_8O_4$	168.15	1.04	1.12	1.61	1.4
Gallic acid		$C_7H_6O_5$	170.12	1.78	0.75	0.56	1.43
Caffeic acid		$C_9H_8O_4$	180.16	0.45	0.24	0.84	0.33
Quinic acid		$C_7H_{12}O_6$	192.17	1.12	1.49	2.24	1.99
Ferulic acid		$C_{10}H_{10}O_4$	194.18	1.83	1.8	0.84	1.96
Cinametic acid		$C_{12}H_{14}O_5$	238.24	3.36	1.56	0.57	1.68
Ellagic acid		$C_{14}H_6O_8$	302.19	2.64	0.78	2.24	0.84
Chlorogenic acid		$C_{16}H_{18}O_9$	354.31	1.78	2.65	3.11	3.19
Mirin		$C_{10}H_8N_2O_2S$	220.25	0.28	0.24	0.59	0.64
Rosmarinic acid		$C_{18}H_{16}O_8$	360.3	2.14	1.87	2.27	1.68
Total				16.6	12.77	15.63	15.14
Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	1.83	1.46	1.5	1.4
Arachidonic acid		$C_{20}H_{32}O_2$	304.5	3.08	2.82	2.63	1.96
Total				4.91	4.28	4.13	3.36
Beta-sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	0.61	0.78	2.63	1.83
Malic acid	Carboxylic acid	$C_4H_6O_5$	134.09	2.29	0.75	1.99	1.4
ß-carotene		$C_{40}H_{56}$	536.9	0.46	0.41	1.1	0.31

Table 5. Phytochemical screening of *S. officinalis* leaves subjected to 100 mM NaCl salt stress and different exposure time points (2, 4, 6 and 8 days).

		Exposure time points (days)		2	4	6	8
Compounds	**Phytochemicals classification**	**Molecular formula**	**Molecular weight (g/mol)**	**%**	**%**	**%**	**%**
Cymene		$C_{10}H_{14}$	134.22	2.41	1.09	2.91	1.22
pinene		$C_{10}H_{16}$	136.23	1.16	1.75	3.31	1.83
Camphene		$C_{10}H_{16}$	136.23	1.86	2.74	1.48	2.41
Sabinene		$C_{10}H_{16}$	136.23	2.67	1.34	1.13	0.67
Beta-myrcene		$C_{10}H_{16}$	136.23	2.93	1.75	0.97	1.83
Limonene		$C_{10}H_{16}$	136.23	1.42	1.07	2.66	2.09
Terpinolene		$C_{10}H_{16}$	136.23	1.86	2.4	2.1	1.25
camphor		$C_{10}H_{16}O$	152.23	0.57	1.34	1.99	0.35
Thujone	Monoterpene	$C_{10}H_{16}O$	152.23	0.93	1.12	1.13	2.46
borneol		$C_{10}H_{18}O$	154.25	1.35	0.56	1.51	2.12
Geraniol		$C_{10}H_{18}O$	154.25	1.86	2.96	2.99	3.57
Terpinene		$C_{10}H_{18}O$	154.25	0.85	2.4	2.23	2.49
1,8-Cineole		$C_{10}H_{18}O$	154.25	3.16	1.53	2.77	2.7
Linalool		$C_{10}H_{18}O$	154.25	2.56	2.06	1.94	0.96
Terpineol		$C_{10}H_{18}O$	154.25	1.71	1.87	2.21	1.51
Linalyl acetate		$C_{12}H_{20}O_2$	196.29	3.18	1.7	2.42	2.52
Geranyl acetate		$C_{12}H_{20}O_2$	196.29	0.57	1.94	0.35	0.75
Total				31.05	29.62	34.1	30.73
Caryophyllene		$C_{15}H_{24}$	204.35	0.31	0.56	2.23	2.09
Ledene		$C_{15}H_{24}$	204.35	3.18	3.23	3.04	2.99
Humulene		$C_{15}H_{24}$	204.35	2.64	2.33	2.37	3.54
Elemene		$C_{15}H_{24}$	204.35	0.13	0.15	0.05	0.17
Cadinene	Sesquiterpene	$C_{15}H_{26}$	206.37	0.18	0.24	0.16	0.2
Spathulenol		$C_{15}H_{24}O$	220.35	1.89	0.8	1.05	1.54
Ledol		$C_{15}H_{26}O$	222.37	1.71	0.07	2.21	1.83
Viridiflorol		$C_{15}H_{26}O$	222.37	0.57	0.8	0.46	0.67
Farnesol		$C_{15}H_{26}O$	222.37	0.08	2.31	2.29	2.78
Total				10.69	10.49	13.86	15.81
Ferruginol		$C_{20}H_{30}O$	286.5	2.49	1.89	2.4	0.64
Phytol	Diterpenoid	$C_{20}H_{40}O$	296.5	1.09	0.32	0.08	0.03
Carnosol		$C_{20}H_{26}O_4$	330.4	2.64	1.82	1.94	2.87
Total				6.22	4.03	4.42	3.54
Coumarin		$C_9H_6O_2$	146.14	2.28	2.99	1.7	2.96
carvacrol		$C_{10}H_{14}O$	150.22	1.63	2.5	1.05	3.57
thymol	Other polyphenols	$C_{10}H_{14}O$	150.22	2.02	2.14	0.67	0.96
vanillin		$C_8H_8O_3$	152.15	2.15	2.4	1.56	0.64
Danshensu		$C_9H_{10}O_5$	198.17	0.6	2.26	1.48	1.19
Carnosic acid		$C_{20}H_{28}O_4$	332.4	3.18	2.5	2.99	0.64
Total				11.86	14.79	9.45	9.96
Apigenin		$C_{15}H_{10}O_5$	270.24	0.05	0.53	0.89	1.33
Naringenin		$C_{15}H_{12}O_5$	272.25	0.23	0.8	0.62	0.64
Scutellarein		$C_{15}H_{10}O_6$	286.24	2.15	1.36	2.5	3.54
Fisetin		$C_{15}H_{10}O_6$	286.24	2.67	1.63	0.62	3.86
Catechin		$C_{15}H_{14}O_6$	290.27	2.15	2.21	0.62	0.96
Hispidulin	Flavonoids	$C_{16}H_{12}O_6$	300.26	2.23	0.68	0.24	0.09
Quercetin		$C_{15}H_{10}O_7$	302.23	1.89	1	0.99	1.22
Cirsimaritin		$C_{17}H_{14}O_6$	314.29	1.89	1.53	2.58	2.55
Luteolin-7-glucoside		$C_{21}H_{20}O_{11}$	448.4	2.28	2.5	0.04	0.67
Myristicin		$C_{11}H_{12}O_3$	192.21	1.63	1.07	0.48	2.81
Rutin		$C_{27}H_{30}O_{16}$	610.5	0.57	0.53	0.59	0.64
Total				17.74	13.84	10.17	18.31
Protocatechualdehyde		$C_7H_6O_3$	138.12	1.14	2.5	2.58	1.54
Vanillic p-hydroxy benzoic acid		$C_8H_8O_4$	168.15	1.42	0.56	2.58	0.67
Gallic acid		$C_7H_6O_5$	170.12	1.14	1.07	0.32	0.09
Caffeic acid		$C_9H_8O_4$	180.16	2.28	1.34	0.43	0.26
Quinic acid	Phenolic acids	$C_7H_{12}O_6$	192.17	1.42	2.02	3.98	2.96
Ferulic acid		$C_{10}H_{10}O_4$	194.18	1.14	1.53	3.26	0.96
Cinametic acid		$C_{12}H_{14}O_5$	238.24	0.23	0.29	0.62	0.96
Ellagic acid		$C_{14}H_6O_8$	302.19	1.09	0.87	0.62	1.91
Chlorogenic acid		$C_{16}H_{18}O_9$	354.31	3.68	2.99	2.77	1.04
Mirin		$C_{10}H_8N_2O_2S$	220.25	0.93	1.34	0.08	2.38
Rosmarinic acid		$C_{18}H_{16}O_8$	360.3	0.57	3.2	0.59	2.87
Total				14.11	16.37	17.75	13.26
Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	1.37	1.04	1.78	0.38
Arachidonic acid		$C_{20}H_{32}O_2$	304.5	0.57	1.26	2.23	0.75
Total				1.94	2.3	4.01	1.13
Beta-sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	1.71	3.45	0.08	0.46
Malic acid	Carboxylic acid	$C_4H_6O_5$	134.09	2.12	2.26	2.75	1.54
ß-carotene		$C_{40}H_{56}$	536.9	1.63	1.53	3.31	2.99

Table 6. Phytochemical screening of *S. officinalis* leaves subjected to 150 mM NaCl salt stress and different exposure time points (2, 4, 6 and 8 days).

			Exposure time points (days)	2	4	6	8
Compounds	**Phytochemicals classification**	**Molecular formula**	**Molecular weight (g/mol)**	**%**	**%**	**%**	**%**
Cymene	Monoterpene	$C_{10}H_{14}$	134.22	2.23	1.44	0.25	0.68
pinene		$C_{10}H_{16}$	136.23	2.02	1.87	0.41	1.37
Camphene		$C_{10}H_{16}$	136.23	1.75	2.46	0.8	0.68
Sabinene		$C_{10}H_{16}$	136.23	2.14	0.33	1.04	1.83
Beta-myrcene		$C_{10}H_{16}$	136.23	1.28	1.44	1.24	2.28
Limonene		$C_{10}H_{16}$	136.23	0.65	1.62	1.62	3.42
Terpinolene		$C_{10}H_{16}$	136.23	2.17	0.59	1.87	4.57
camphor		$C_{10}H_{16}O$	152.23	0.65	0.28	3.46	3.2
Thujone		$C_{10}H_{16}O$	152.23	1.37	1.83	3.74	0.53
borneol		$C_{10}H_{18}O$	154.25	1.25	2.87	1.15	0.25
Geraniol		$C_{10}H_{18}O$	154.25	1.22	2.13	0.03	0.66
Terpinene		$C_{10}H_{18}O$	154.25	1.28	2.36	0.09	1.99
1,8-Cineole		$C_{10}H_{18}O$	154.25	1.54	0.82	0.49	0.66
Linalool		$C_{10}H_{18}O$	154.25	1.75	3.9	2.25	1.8
Terpineol		$C_{10}H_{18}O$	154.25	1.87	1.31	2.47	1.83
Linalyl acetate		$C_{12}H_{20}O_2$	196.29	2.37	1.92	0.25	0.66
Geranyl acetate		$C_{12}H_{20}O_2$	196.29	2.67	2.85	2.78	2.88
Total				28.21	28.02	23.94	29.29
Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.35	0.59	1.62	0.69	0.43
Ledene		$C_{15}H_{24}$	204.35	0.89	2.54	1.81	0.59
Humulene		$C_{15}H_{24}$	204.35	2.14	2.1	1.18	2.74
Elemene		$C_{15}H_{24}$	204.35	3.03	0.1	0.6	0.96
Cadinene		$C_{15}H_{26}$	206.37	3.56	0.21	3.57	2.28
Spathulenol		$C_{15}H_{24}O$	220.35	2.37	2.56	2.42	0.25
Ledol		$C_{15}H_{26}O$	222.37	2.14	2.31	2.23	0.66
Viridiflorol		$C_{15}H_{26}O$	222.37	1.28	2.05	2.06	2.19
Farnesol		$C_{15}H_{26}O$	222.37	1.87	1.79	1.79	1.42
Total				17.87	15.28	16.35	11.52
Ferruginol	Diterpenoid	$C_{20}H_{30}O$	286.5	0.77	1.97	2.47	2.05
Phytol		$C_{20}H_{40}O$	296.5	1.66	1.72	0.55	0.46
Carnosol		$C_{20}H_{26}O_4$	330.4	1.19	0.67	1.81	1.44
Total				3.62	4.36	4.83	3.95
Coumarin	Other polyphenols	$C_9H_6O_2$	146.14	0.15	0.92	2.91	6.85
carvacrol		$C_{10}H_{14}O$	150.22	0.06	2.46	0.6	4
thymol		$C_{10}H_{14}O$	150.22	0.36	0.56	2.36	2.81
vanillin		$C_8H_8O_3$	152.15	1.87	2.1	3.05	1.28
Danshensu		$C_9H_{10}O_5$	198.17	0.3	2.13	0.3	0.07
Carnosic acid		$C_{20}H_{28}O_4$	332.4	1.57	2.13	1.98	1.19
Total				4.31	10.3	11.2	16.2
Apigenin	Flavonoids	$C_{15}H_{10}O_5$	270.24	1.25	1.28	0.88	2.28
Naringenin		$C_{15}H_{12}O_5$	272.25	0.98	1.03	2.72	4.34
Scutellarein		$C_{15}H_{10}O_6$	286.24	0.68	0.51	1.98	0.59
Fisetin		$C_{15}H_{10}O_6$	286.24	0.39	0.26	0.52	0.34
Catechin		$C_{15}H_{14}O_6$	290.27	1.28	2.23	1.18	1.14
Hispidulin		$C_{16}H_{12}O_6$	300.26	2.32	1.36	0.44	1.64
Quercetin		$C_{15}H_{10}O_7$	302.23	2.78	1.23	1.95	2.24
Cirsimaritin		$C_{17}H_{14}O_6$	314.29	1.48	1.85	1.54	1.62
Luteolin-7-glucoside		$C_{21}H_{20}O_{11}$	448.4	1.87	1.79	2.17	1.37
Myristicin		$C_{11}H_{12}O_3$	192.21	1.78	0.59	0.6	0.1
Rutin		$C_{27}H_{30}O_{16}$	610.5	1.25	1.97	0.63	0.91
Total				16.06	14.1	14.61	16.57
Protocatechualdehyde	Phenolic acids	$C_7H_6O_3$	138.12	0.24	1.62	2.69	5.71
Vanillic p-hydroxy benzoic acid		$C_8H_8O_4$	168.15	2.02	0.51	2.75	0.25
Gallic acid		$C_7H_6O_5$	170.12	2.17	0.85	3.3	0.07
Caffeic acid		$C_9H_8O_4$	180.16	1.19	7.44	2.47	0.21
Quinic acid		$C_7H_{12}O_6$	192.17	1.48	2.79	1.92	0.14
Ferulic acid		$C_{10}H_{10}O_4$	194.18	2.08	1.15	0.08	0.27
Cinametic acid		$C_{12}H_{14}O_5$	238.24	1.63	1.54	0.05	2.74
Ellagic acid		$C_{14}H_6O_8$	302.19	2.64	1.62	2.45	1.44
Chlorogenic acid		$C_{16}H_{18}O_9$	354.31	0.39	0.51	0.16	2.05
Mirin		$C_{10}H_8N_2O_2S$	220.25	3.27	1.08	2.14	0.53
Rosmarinic acid		$C_{18}H_{16}O_8$	360.3	1.63	0.77	0.36	1.83
Total				15.47	18.8	16.23	14.71
Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	1.63	0.77	2.36	0.53
Arachidonic acid		$C_{20}H_{32}O_2$	304.5	1.66	1.36	2.36	1.9
Total				3.29	2.13	4.72	2.43
Beta-sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	2.37	1.79	2.42	1.6
Malic acid	Carboxylic acid	$C_4H_6O_5$	134.09	3.21	2.13	0.19	2.05
ß-carotene		$C_{40}H_{56}$	536.9	2.35	2.03	3.3	1.14

Table 7. Phytochemical screening of *S. officinalis* leaves subjected to 200 mM NaCl salt stress and different exposure time points (2, 4, 6 and 8 days).

			Exposure time points (days)	**2**	**4**	**6**	**8**
Compounds	**Phytochemicals classification**	**Molecular formula**	**Molecular weight (g/mol)**	**%**	**%**	**%**	**%**
Cymene	Monoterpene	$C_{10}H_{14}$	134.22	0.08	2.63	2.07	1.1
pinene		$C_{10}H_{16}$	136.23	0.83	1.31	2.15	4.4
Camphene		$C_{10}H_{16}$	136.23	1.23	0.26	2.37	2.1
Sabinene		$C_{10}H_{16}$	136.23	2.01	5.26	3.23	3.1
Beta-myrcene		$C_{10}H_{16}$	136.23	0.28	2.68	0.47	0.5
Limonene		$C_{10}H_{16}$	136.23	2.51	0.32	0.71	0.1
Terpinolene		$C_{10}H_{16}$	136.23	2.33	2.31	1.49	4
camphor		$C_{10}H_{16}O$	152.23	0.3	0.16	0.47	4.1
Thujone		$C_{10}H_{16}O$	152.23	2.23	0.18	0.24	0.5
borneol		$C_{10}H_{18}O$	154.25	1.88	2.18	1.9	0.4
Geraniol		$C_{10}H_{18}O$	154.25	1.58	0.58	1.55	0.7
Terpinene		$C_{10}H_{18}O$	154.25	1.05	2.6	1.77	0.3
1,8-Cineole		$C_{10}H_{18}O$	154.25	0.83	1.87	0.28	5
Linalool		$C_{10}H_{18}O$	154.25	2.06	0.6	2.15	1.1
Terpineol		$C_{10}H_{18}O$	154.25	1.78	0.68	2.37	0.4
Linalyl acetate		$C_{12}H_{20}O_2$	196.29	1.66	2.31	1.08	0.14
Geranyl acetate		$C_{12}H_{20}O_2$	196.29	4.27	2.68	0.47	0.2
Total				26.91	28.61	24.77	28.14
Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.35	3.26	0.58	2.13	5.2
Ledene		$C_{15}H_{24}$	204.35	2.76	2.1	1.08	0.4
Humulene		$C_{15}H_{24}$	204.35	2.51	1.92	0.86	0.8
Elemene		$C_{15}H_{24}$	204.35	2.26	1.66	0.65	2
Cadinene		$C_{15}H_{26}$	206.37	1.51	1.45	0.24	3
Spathulenol		$C_{15}H_{24}O$	220.35	2.01	0.29	3.45	0.6
Ledol		$C_{15}H_{26}O$	222.37	1.51	0.08	3.88	2.3
Viridiflorol		$C_{15}H_{26}O$	222.37	1	0.21	4.31	3
Farnesol		$C_{15}H_{26}O$	222.37	0.5	0.18	0.04	2
Total				17.32	8.47	16.64	19.3
Ferruginol	Diterpenoid	$C_{20}H_{30}O$	286.5	2.11	4.47	1.42	3
Phytol		$C_{20}H_{40}O$	296.5	0.3	3.15	0.95	0.3
Carnosol		$C_{20}H_{26}O_4$	330.4	2.21	2.1	3.02	1.3
Total				4.62	9.72	5.39	4.6
Coumarin	Other polyphenols	$C_9H_6O_2$	146.14	3.76	1.45	1.51	0.7
carvacrol		$C_{10}H_{14}O$	150.22	3.01	1.16	1.29	0.1
thymol		$C_{10}H_{14}O$	150.22	0.9	2.08	1.77	0.1
vanillin		$C_8H_8O_3$	152.15	0.55	0.05	1.36	0.8
Danshensu		$C_9H_{10}O_5$	198.17	3.01	1.45	0.39	0.1
Carnosic acid		$C_{20}H_{28}O_4$	332.4	1.3	1.05	2.59	0.2
Total				12.53	7.24	8.91	2
Apigenin	Flavonoids	$C_{15}H_{10}O_5$	270.24	0.58	0.53	3.02	3.1
Naringenin		$C_{15}H_{12}O_5$	272.25	4.52	0.26	0.04	5
Scutellarein		$C_{15}H_{10}O_6$	286.24	3.51	0.79	1.9	5.1
Fisetin		$C_{15}H_{10}O_6$	286.24	0.55	1.05	1.66	4
Catechin		$C_{15}H_{14}O_6$	290.27	0.83	2.63	1.18	2.2
Hispidulin		$C_{16}H_{12}O_6$	300.26	1.37	2.1	0.71	0.8
Quercetin		$C_{15}H_{10}O_7$	302.23	1.81	2.23	0.24	1
Cirsimaritin		$C_{17}H_{14}O_6$	314.29	1.05	1.55	1.53	0.1
Luteolin-7-glucoside		$C_{21}H_{20}O_{11}$	448.4	0.28	0.21	1.51	0.5
Myristicin		$C_{11}H_{12}O_3$	192.21	0.05	3.94	3.02	2
Rutin		$C_{27}H_{30}O_{16}$	610.5	0.5	0.13	0.43	1
Total				15.05	15.42	15.24	24.8
Protocatechualdehyde	Phenolic acids	$C_7H_6O_3$	138.12	5.02	1.73	1.72	0.3
Vanillic p-hydroxy benzoic acid		$C_8H_8O_4$	168.15	0.05	1.84	1.29	2.3
Gallic acid		$C_7H_6O_5$	170.12	0.18	2.1	3.23	3
Caffeic acid		$C_9H_8O_4$	180.16	0.48	2.37	2.59	0.5
Quinic acid		$C_7H_{12}O_6$	192.17	2.51	2.89	2.15	1
Ferulic acid		$C_{10}H_{10}O_4$	194.18	0.73	3.15	1.29	3.2
Cinametic acid		$C_{12}H_{14}O_5$	238.24	2.56	2.94	3.92	1.1
Ellagic acid		$C_{14}H_6O_8$	302.19	0.63	1.84	0.47	2.1
Chlorogenic acid		$C_{16}H_{18}O_9$	354.31	0.28	0.79	2.26	1
Mirin		$C_{10}H_8N_2O_2S$	220.25	0.75	1.37	2.15	1.1
Rosmarinic acid		$C_{18}H_{16}O_8$	360.3	0.63	0.53	1.36	2
Total				13.07	20.18	20.28	16.5
Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	5.52	1.58	2.13	0.5
Arachidonic acid		$C_{20}H_{32}O_2$	304.5	1.33	1.5	0.22	0.9
Total				6.85	3.08	2.35	1.4
Beta-sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	0.55	0.05	2.09	0.2
Malic acid	Carboxylic acid	$C_4H_6O_5$	134.09	0.55	3.94	0.47	0.6
ß-carotene		$C_{40}H_{56}$	536.9	1.83	1.92	1.72	1.1

pattern at 26.9 %, 28.6 %, 24.8 % and 28.1 % (Table 7).

Compared to non-stressed plants, salt stress induced significant changes in monoterpene synthesis, particularly increasing thujone levels. At 100 mM NaCl, thujone levels rose 2.3- and 4.1-fold after 6 and 8 days, respectively. At 150 mM NaCl, the increase was 1.5- and 7.6-fold after 4 and 6 days, while at 200 mM NaCl, a 1.4-fold rise was observed after 2 days. Similarly, borneol levels increased significantly under salt stress, reaching 3.9- and 4.4-fold at 100 mM NaCl after 6 and 8 days and 2-, 3.9- and 2.9-fold at 150 mM NaCl after 2, 4 and 6 days. At 200 mM NaCl, borneol accumulation rose 2.9-, 2.9-and 4.9-fold over the same period. Linalyl acetate also showed substantial increases, particularly at 100 mM NaCl, where levels rose 6-, 7-, 17-and 15-fold after 2, 4, 6and 8 days, respectively. Geranyl acetate accumulation peaked at 150 mM NaCl, with 10-, 16-, 15-and 12-fold increases after 2, 4, 6 and 8 days.

Sesquiterpene yields in non-stressed plants increased over time, starting at 15.9 % and reaching 19.1 % after 8 days (Table 2). Low salinity (25 mM NaCl) maintained yields at ~19.4 % (Table 3), while moderate stress (50 mM NaCl) caused slight reductions (Table 4). Higher stress levels (100-200 mM NaCl) further decreased yields (Tables 5-7). Among sesquiterpenes, Ledene and Humulene levels increased under salt stress, with Ledene showing significant rises at 50-150 mM NaCl and Humulene peaking at 50-150 mM NaCl.

Diterpene yields in non-stressed plants remained stable at ~4.5 % (Table 2). Low salinity (25 mM NaCl) did not alter yields, but moderate stress (50-100 mM NaCl) induced increases, particularly at 50 mM NaCl (Table 4). Severe stress (200 mM NaCl) also raised yields (Table 7).

Phenolic compounds and flavonoids

Phenolic compounds, including phenolic acids and polyphenols, were sensitive to salinity stress. Non-stressed plants maintained yields of ~20-22.7 % (Table 2), while low salinity (25 mM NaCl) kept yields at ~19.7 % (Table 3). Moderate stress (50-100 mM NaCl) increased yields, with 100 mM NaCl showing the highest rise (Table 5). Severe stress (150-200 mM NaCl) further elevated yields (Tables 6-7). On the other hand, flavonoid content in non-stressed plants varied over time (Table 2), but salt stress reduced yields, particularly at higher concentrations (Tables 4-7).

Fatty acids and other phytochemicals

Fatty acid content, however, increased under high salt stress (150-200 mM NaCl), with unsaturated fatty acids like oleic and arachidonic acids being prominent (Tables 2-7). Other phytochemicals, such as beta-sitosterol and malic acid, also increased under salt stress, with significant rises at 50-200 mM NaCl (Tables 4-7). ß-carotene levels similarly increased under moderate to severe salt stress (Tables 5-7). Overall, salt stress significantly altered the phytochemical profile of sage plants, with varying effects depending on stress intensity.

Discussion

The results of this study highlight the significant impact of salt stress on the phytochemical composition of sage plants, particularly on terpenoid compounds, phenolic compounds, flavonoids and fatty acids. The findings align with previous studies demonstrating that environmental stressors, such as salinity, can induce substantial changes in secondary metabolite production in plants (5, 17).

Terpenoid compounds

Terpenoids, including monoterpenes, sesquiterpenes and diterpenes were the most abundant compounds in the methanolic extract of sage plants. Monoterpenes, such as thujone, borneol, linalyl acetate and geranyl acetate, showed significant increases under salt stress, particularly at higher NaCl concentrations (100-200 mM). This is consistent with studies indicating that terpenoid biosynthesis is often upregulated under stress conditions as a protective mechanism (18). The biosynthesis regulation may involve methylerythritol phosphate pathway that is responsible for biosynthesis of the precursors of isoprenoid compounds for their role in oxidative stress responses (19) and increased expression of *PtDXS* gene which encode PtDXS a key enzyme in terpenoid biosynthesis to enhance stress resistance (20). For example, the 2.3- to 7.6-fold increase in thujone levels under moderate to severe salt stress suggests its role in stress adaptation. This accumulation may by the regulation of stress-responsive genes like *TPS* genes for terpene synthases lead to increased production of compounds like thujone and linalyl acetate (21). Similarly, accumulating borneol and linalyl acetate under salt stress may contribute to membrane stabilization and antioxidant activity (21, 22). Moreover, the increased yield of new compounds from *S. officinalis* under high salt stress (150 and 200 mM NaCl) such as borneol which is the precursor of camphor (23) suggests a positive influence of borneol, in these tested concentrations, to mitigate the toxic effect of salt ions, that is one of the mechanisms responsible for the tolerance to salinity. In addition to borneol, the increased yield of linalyl acetate and geranyl acetate suggests that *S. officinalis* activates the metabolic pathways to produce monoterpenes under salinity as one of the salt tolerance strategies (2).

In this work, plants subjected to salt stress showed a reduction of sesquiterpenes (Tables 3-7). This dynamic was already supposed to be a defense mechanism of the plants against hostile environments (24). Furthermore, sesquiterpenes, such as Ledene and Humulene, also exhibited increased levels under moderate salt stress (50-150 mM NaCl), which may be attributed to their role in plant defense mechanisms (25). However, higher stress levels (200 mM NaCl) reduced sesquiterpene yields, possibly due to the disruption of biosynthetic pathways under extreme conditions (17). On the other hand, diterpenes showed increased yields under moderate to severe salt stress, suggesting their potential role in enhancing plant resilience (26).

Phenolic compounds and flavonoids

Phenolic compounds, including phenolic acids and polyphenols, were sensitive to salinity stress, with yields increasing under moderate to severe salt stress. This aligns with the well-documented role of phenolics in scavenging reactive oxygen species generated under stress conditions (27). The highest rise in phenolic yields at 100 mM NaCl suggests an optimal stress level for phenolic biosynthesis (28, 29). Salt stress induced the expression of PAL1 (phenylalanine ammonia lyase 1), a key gene in the phenylpropanoid pathway, leading to increased PAL activity and phenolic accumulation (29). In contrast, flavonoid content decreased under salt stress, particularly at higher NaCl concentrations, which may indicate the suppression of flavonoid biosynthetic pathways under extreme stress (22).

Fatty acids and other phytochemicals

Fatty acid content, particularly unsaturated fatty acids like oleic and arachidonic acids, increased under salt stress. This is consistent with studies showing that unsaturated fatty acids play a crucial role in maintaining membrane fluidity and integrity under stress conditions (30). Additionally, the increase in beta-sitosterol and malic acid levels under salt stress may contribute to osmotic adjustment and stress tolerance (31, 32). The rise in ß-carotene levels under moderate to severe salt stress further supports its role as an antioxidant protecting against oxidative damage (33).

Implications and future directions

The findings of this study demonstrate that salt stress alters the phytochemical profile of sage plants, with varying effects depending on stress intensity and duration. These changes likely reflect adaptive responses to enhance stress tolerance and survival. However, the reduction in certain compounds, such as flavonoids, under extreme stress conditions, warrants further investigation into the underlying mechanisms and potential strategies to mitigate these effects.

Conclusion

This research demonstrates that salt stress alters the phytochemical profile of sage plants, with the extent of change dependent on the severity and duration of the stress. While stress induces the accumulation of compounds like monoterpenes and phenolic acids, flavonoid production is reduced. These changes likely represent the plant's adaptive strategies to mitigate the effects of salinity through increased synthesis of protective metabolites and adjustments in metabolic pathways. Future studies should focus on elucidating the molecular mechanisms governing terpenoid and phenolic biosynthesis under salt stress and on identifying the specific roles of compounds like thujone and linalyl acetate in stress adaptation through functional analyses. Ultimately, these insights could be leveraged to develop salt-tolerant crop varieties and optimize sage cultivation in saline environments.

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Authors' contributions

MSM performed the research. KYA designed the project, supervised and discussed the results and wrote the article. SAO discussed the results and wrote the article. Percentage participation is equal for all co-authors. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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