

Research Article

Effect of salinity stress on phytochemical characteristics of *Centella asiatica*

Sonal Gupta

Department of Biotechnology, AKS University, Satna (Madhya Pradesh), India

Ashwini A Wao* 

Department of Biotechnology, AKS University, Satna (Madhya Pradesh), India

*Corresponding author. Email: ashwiniwao@gmail.com

Article Info

<https://doi.org/10.31018/jans.v14i2.3387>

Received: March 12, 2022

Revised: June 9, 2022

Accepted: June 15, 2022

How to Cite

Gupta, S. and Wao, A. A. (2022). Effect of salinity stress on phytochemical characteristics of *Centella asiatica*. *Journal of Applied and Natural Science*, 14(2), 684 - 691. <https://doi.org/10.31018/jans.v14i2.3387>

Abstract

Salinity is one of the predominant abiotic stresses which affects plant growth by inducing excessive production of reactive oxygen species (ROS) that leads to oxidative damage of plant cells. Plants alleviate salinity stress by regulating intracellular concentrations of various phytochemicals like phenol, tannin, antioxidants, etc. The present work aimed to study the impact of salt stress on the production of various phytochemicals like phenol, tannin, flavonoids, antioxidants, total protein content, etc. The Salt stress response of the test plant *Centella asiatica* was studied by irrigating variant concentrations (50mM, 100mM, 150mM, 200mM, 250mM) of salt (NaCl). The phytochemical activity of the plants grown under salinity stress was estimated by using an appropriate biochemical assay. Comparative analysis of the photochemical activity of the test plants in comparison with the control revealed that various phytochemicals were increased in response to salt stress. Salt stress increased the levels of antioxidants from 10.79 to 14.31 µg/ml, phenol from 30.8 to 43.3 in µg/ml, flavonoids (from 490 to 683.33 in µg/ml), tannin from 55.5 to 64.5 in µg/ml, and proteins from 5720 to 6080 in µg/ml in the *C. asiatica* plants. To sum up, salt stress elicited phytochemical accumulation in the *C. asiatica* plant, thereby improving the plant's growth by enhancing its resistance to salt stress. This finding may play an important role in the sustainable cultivation of commercially important crops like *C. asiatica*.

Keywords: Biochemical analysis, *Centella asiatica*, Phytochemicals, Salinity stress, Stress tolerance

INTRODUCTION

Centella Asiatica is commonly known as Gotu kola, or Asiatic pennywort. It is a perennial herb belonging to the family *Apiaceae*. It is the native crop of wetlands in Asia. It is broadly used as a culinary vegetable and medicinal herb. It is a small trailing herb and the only species of *Centella* cultivated in India. The stem of the plant is glabrous, striated, and rooting at nodes (Shukurova *et al.*, 2021). The plant leaves are fleshy, dentate, and orbicular to reniform in shape. The petiole is long, smooth on the upper surface, and hairy below. Flowers are pink and white in fascicled umbels. The fruits are oblong, dull brown, laterally compressed, pericarp hard, thickened, and woody white (Singh *et al.*, 2010). *Centella* plant consists of pentacyclic triterpenoids (Hashim *et al.*, 2011), including asiaticoside (Wijeweera *et al.*, 2006), brahmoside, Asiatic acid, Brahmic acid, etc (James and Dubery 2009). Due to its phytochemical and medicinal properties (Brinkhaus *et al.*, 2000, Kun-

jumon *et al.*, 2022), the plant has wide economical and industrial importance and is used to treat various disorders and minor wounds, to encourage lactation, headache, nausea, and drowsiness, etc. (Kashmira *et al.*, 2010). Studies suggest that plant extract has significant importance in wound healing (Ruszymah *et al.*, 2012). It has the potential to monitor various heavy metal pollution levels in the soil. It also monitors and controls various species of weeds.

Like other biological forms, plant health is also influenced by its surrounding. Plant surroundings include many biotic and abiotic factors that affect plant growth and health. In all these factors, soil salinity or salinization is abiotic stress which highly affects plant growth. Soil salinization is a process in which water-soluble salts accumulate in the soil to extents that negatively affect various plant growth and productivity parameters. Nowadays, the concentration of salts in soil is continuously increasing by various human activities, which makes agricultural land saline and less productive for the crop (Hassan *et al.*, 2016). The high salt concentra-

tion is harmful to plants because it increases the osmotic potential gradient, further decreasing the plant's uptake of water and nutrient. Low water and nutrient availability affect various biological processes in the plant. Salt stress induces excessive production of oxidants like reactive oxygen species (ROS) that leads to damage of vital molecules (like protein, nucleic acids, etc) of plant cells. High salt concentration can also be responsible for an ionic imbalance in plant cells, resulting in cellular homeostasis disturbance and further cell death (Thaker *et al.*, 2021).

Plants can respond according to their surroundings. Plant remodels various metabolic pathways by switching gene expression levels accordingly, which leads to accumulation of various phytochemicals like phenol, tannin, carotenoids, flavonoids, antioxidants, etc. (Kumar *et al.*, 2017). The excessive concentration of such important phytochemicals helps plants to alleviate the negative impacts of abiotic stresses like soil salinity (Hirayama and Shinozaki 2010).

The primary aim of this study is to analyze the impact of salt stress on the concentration of various plant metabolites like antioxidants, phenols, flavonoids, tannins, and protein content in *Centella asiatica*.

MATERIALS AND METHODS

The study involved the preparation of the sample plant, which was further subjected to salinity stress. Afterwards, the phytochemical analysis was performed for leaf extracts of control and salt-treated plants. The methodology included the following steps

Collection and preparation of test plant

The plant sample, *C. asiatica*, was collected from the Botanical Garden of Indian Republic (BGIR), Noida (UP). After collecting the sample, it was authenticated from BGIR, Noida. Seeds of the test plant were purchased from a commercial supplier "MG Naturals" who is a certified manufacturer, retailer, and wholesaler of plant seeds. The seeds were kept at room temperature until used for further studies.

Treatment with salt stress

For this study, seeds were immersed in 5% sodium hypochlorite for 5 minutes for sterilization. Afterwards, the seeds were washed with distilled water. The sterilized seeds were sown in plastic pots of standard and uniform size, which were filled with a uniform mixture of soil, peat, and vermicompost (Devkota and Jha 2010). These pots were irrigated with water and kept under controlled conditions until germination. The salt stress treatment started three weeks after germination. These pots were divided into six sets, each containing three pots. One set was kept in control and irrigated with distilled water only. The remaining sets of pots were wa-

tered with variant concentrations of freshly prepared aqueous NaCl solutions (50 mM, 100mM, 150mM, 200mM, and 250mM) at two days intervals. All the pots were continuously treated with the salt solution for three weeks. Phytochemical analysis of the test plants in the reflex to salt stress was done with leaf materials collected from the salt-treated plants (for all the above-provided concentrations of NaCl) after one week, two weeks, and three weeks of salt treatment.

Phytochemical analysis

After salt treatment, various biochemical assays were performed to estimate the phytochemical compositions of leaf extracts. These quantitative assays were as follows

Preparation of aqueous plant extract

Dried finely crushed plant leaves were taken in a flask containing distilled water. The solution was boiled using a hot plate for 20 minutes with continuous stirring. After this, the solution was filtered with filter paper. The resultant filtrate was stored in a refrigerator and used for phytochemical analysis as per requirement (Sasidharan *et al.*, 2011).

Quantitative analysis of the aqueous plant extract

Quantitative analysis of various phytochemicals (like antioxidants, phenol, flavonoids, tannin, and total protein content) was performed on the aqueous extracts of *Centella asiatica* leaves by using standard protocols of spectroscopic methods of biochemical assays. Quantitative analysis of various phytochemicals was as follows-

Test for total antioxidant activity:

The stock solution was prepared by dissolving 24mg DPPH in 100ml methanol. The working solution was prepared by dilution of the DPPH stock solution with methanol to obtain an absorbance of about 1.011 at 517nm. In a test tube, 2.9 ml DPPH working solution was mixed with 100µl plant extract. The control was prepared with 3ml DPPH solution with methanol. The tubes were then placed in a dark place for 30 minutes. Then the absorbance was measured at 517nm. The results were interpolated (in µg/ml) using a standard curve (Katalinic *et al.*, 2006, Nurul *et al.*, 2008).

Test for total phenol content:

Total phenol content in the plant extract was estimated by the Folin-Ciocalteu method (Yadav and Agrawala, 2011). In this method, 2.5 ml of Folin-Ciocalteu reagent, 2ml of sodium carbonate were added to 500 microliters of the plant extract. The mixture was kept for 15 min at room temperature for incubation. The absorbance of the mixture was recorded at 765 nm. The amount of total phenol content (in µg/ml) was estimated

by using a standard curve which was prepared by using known concentrations of Gallic acid.

Test for total flavonoids

The aluminum chloride colorimetric method was used to estimate the total flavonoid content in the plant extract (Anna da Silva *et al.*, 2016). For this, 100 microliters of the plant, the extract was added to 4.9 ml of distilled water, 0.3 ml of 10% aluminum chloride, 0.3 ml of sodium nitrate, and 2ml of NaOH. The mixture was kept at room temperature for incubation then the absorbance was recorded at 510nm. The results were measured (in $\mu\text{g/ml}$) by using a standard curve prepared with standard concentrations of quercetin.

Test for tannin

The concentration of tannins was estimated by using ferric chloride (Ezeonu and Ejikeme 2016). 0.5 ml of the plant extract was added with 4.5 ml of distilled water and 2.0 ml of ferric chloride reagent. After 10 min of incubation, absorbance was measured at 605nm. The concentrations of tannin content ($\mu\text{g/ml}$) in given plant extracts were interpolated by using a standard curve prepared by known concentrations of tannin.

Test for total protein content

This study was performed by Lowry's method (Rangarajan and Sathiyavani 2014). For this 1.0 ml of the sample or plant extract was mixed with 4.5 ml of reagent 1 and 0.5 ml of reagent 2. The mixture was kept for 30 min at room temperature. The absorbance was measured at 660nm. The concentrations of total protein contents were calculated (in $\mu\text{g/ml}$) by using a standard curve which was plotted using known standard concentrations of reference protein; BSA (Bovine serum albumin).

After estimation of various phytochemicals quantitatively, the effects of salt stress on the molecular synthesis of these phytochemicals were studied. The study significantly revealed the role of these phytochemicals in the stress alleviation mechanism for salinity in the plant.

Statistical analysis

Analysis of variance (ANOVA) was conducted for this study which was performed in three replicates across the plant after three weeks of salt treatments (0 mM-250 mM). The ANOVA was performed to study the impact of salinity stress on the phytochemical activities of the test plant and the data was considered to be significant at $p < 0.05$. Multiple mean comparisons were performed by Post-hoc test (Tukey's HSD test).

RESULTS AND DISCUSSION

The combined data for quantitative estimations of various phytochemicals (antioxidants, phenols, flavonoids,

tannin, and total protein contents) in the *C. asiatica* plant leaf extracts are presented in Table 1. Data are expressed as the mean \pm SD. As shown in Table 1, total antioxidant activity in the leaf extract of *C. asiatica* was estimated as 10.79 $\mu\text{g/ml}$ in control at the first time of sampling (7th day). Under salt stress, the antioxidant activity was increased to 11.50 $\mu\text{g/ml}$, 12.07 $\mu\text{g/ml}$, 12.85 $\mu\text{g/ml}$, 13.53 $\mu\text{g/ml}$, 14.31 $\mu\text{g/ml}$ at 50mM, 100mM, 150mM, 200mM, 250mM respectively. The antioxidant activity was increased by 33% at the maximum salt concentration (250mM) as compared to the control. A similar pattern was shown on the second (14th day) and third time of sampling (21st day). The level of antioxidant activity was increased by 28% (from 11.39 to 14.54 $\mu\text{g/ml}$), and 30% (from 11.39 to 14.8 $\mu\text{g/ml}$), respectively, as compared to control. The increase was significant at $p < 0.05$ for all three times of sampling (7th, 14th, and 21st day).

The data concentrations of other phytochemicals like phenol, flavonoids, tannins, and total protein content were affected under salinity stress (0 to 250 mM) conditions. As is evident from Table 1, the total phenol content increased on all three times of sampling (7th, 14th, and 21st day) due to salt treatments. On the 7th day of sampling, the concentration of phenol was 30.8 $\mu\text{g/ml}$ in control, which was increased to 35.25 $\mu\text{g/ml}$ and 43.3 $\mu\text{g/ml}$ at 50mM and 250mM salt. Afterward, the concentration of phenol was decreased to 42.55 $\mu\text{g/ml}$, 29.33 $\mu\text{g/ml}$, and 20 $\mu\text{g/ml}$ in response to higher salt treatments of 150mM, 200mM, 250mM, respectively. The highest phenol concentration was found in the plants treated with 100mM salt. The level of total phenol was increased by 41% (at 100mM) as compared to control. Similar patterns of change in total phenol content were seen on the 14th and 21st days of sampling and the concentrations of total phenol were increased to 45% (from 31.0 to 45 $\mu\text{g/ml}$), 47% (from 31.5 to 46.25 $\mu\text{g/ml}$) respectively (at 100mM salt) as compared to control. However, the increase was not found to be significant at $p < 0.05$ for all three sampling times.

Flavonoids were increased in the extract of *C. asiatica* leaves in a dose-dependent manner with salt treatments (0 to 250mM), reaching the highest level at 100mM for all three times of sampling (7th, 14th, and 21st days). On the 7th day, the concentration of flavonoids was 490 $\mu\text{g/ml}$ in control. The concentration of flavonoids was increased to 585 $\mu\text{g/ml}$, and 683.33 $\mu\text{g/ml}$ at 50mM and 100mM salt concentrations, respectively. For higher salt concentrations, 150mM, 200mM, and 250 mM, the level of flavonoids was decreased to 662 $\mu\text{g/ml}$, 457 $\mu\text{g/ml}$, and 336 $\mu\text{g/ml}$, respectively. The flavonoid level was increased by 40% (at 100mM) as compared to the control. The same patterns of flavonoid increase were followed by the test plants on the 14th and 21st day of sampling and reached the highest concentration (705 $\mu\text{g/ml}$, 721.5 $\mu\text{g/ml}$, respectively) at

100mm salt. The increase in flavonoids was 36% and 31% on the 14th and 21st day of sampling, respectively, as compared to the control. The increase was found to be significant at $p < 0.05$ for all three times of sampling.

The next metabolite studied in the plants was tannin. The data shown in Table 1 revealed that the tannin concentration was increased up to 58.25 $\mu\text{g/ml}$ and 64.5 $\mu\text{g/ml}$ at 50mm and 100mm salt concentrations compared to control (55.5 $\mu\text{g/ml}$) for the 7th day of sampling. For the higher doses of salt treatments (150mM, 200mM, 250mM), the tannin level was lowered to 60, 52 $\mu\text{g/ml}$, and 33.45 $\mu\text{g/ml}$. The highest level of tannin was estimated at 100 mM as a 16% increase as compared to the control. For the 14th and 21st day of sampling, the highest tannin values 65.6 $\mu\text{g/ml}$ and 67 $\mu\text{g/ml}$, were found at 100mM salt, respectively, in special reference to the control 55 $\mu\text{g/ml}$ (on the 14th day of sampling), 55.3 $\mu\text{g/ml}$ (on the 21st day of sampling). The values showed a 19%, and 21% increase in tannin levels at 100mM as contrasted with control for the 14th and 21st day of sampling, respectively. ANOVA analysis showed that the increase in tannin levels under given salinity treatments was significant at $p < 0.05$.

The total protein content of the salt-treated plants is shown in Table 1. The concentration of total protein in the test plant first increased with the response of initial doses of salt treatments (50mM and 100mm) and then decreased with the higher doses of the salt treatments (150mM, 200mM, and 250mM) as compared to the control for all the three times of sampling (7th, 14th and 21st day). On the 7th day of sampling, the level of total protein found in control was 5720 $\mu\text{g/ml}$, which further increased to 5800 $\mu\text{g/ml}$, 6080 $\mu\text{g/ml}$ at 50mm and 100mM salt, respectively, then the concentration of total proteins was decreased to 5890 $\mu\text{g/ml}$, 5022 $\mu\text{g/ml}$, 4233 $\mu\text{g/ml}$ at 150mM, 200mm and 250mm salt respectively. On the 14th sampling day, the total protein concentration increased with the initial doses of salt (50mm and 100mm) and reached the highest concentration of 6250 $\mu\text{g/ml}$ at 100mM salt in contrast to the control (5795 $\mu\text{g/ml}$). The total protein concentration was decreased for higher doses of the salt treatments. This pattern was repeated on the 21st day of sampling, the total protein concentrations increased with initial doses (50mM and 100mM) of salt, and the highest concentration of the total protein as 6545 $\mu\text{g/ml}$ was found at 100mM salt as compared to the control (5790 $\mu\text{g/ml}$). For the higher ranges of salt concentrations (150mM, 200mM, and 250mM), the test plants' total protein concentration was decreased. The total protein concentration increased by 6.3%, 8%, and 13% at 100mM salt for the 7th, 14th, and 21st day of sampling. The increase in the total protein concentration was found significant at $p < 0.05$ for the 7th and 14th day of sampling, although the increase was not found significant at $p < 0.05$ for the 21st day of sampling.

The current study revealed that the plant tolerates salinity stress to a certain extent (50mM, 100mM). In the *C. asiatica* plant, some groups of phytochemicals are contributed to the plant's health under salinity stress: these are antioxidants, phenols, flavonoids, tannin, and proteins. Antioxidants are an important group of plant metabolites that protect plants against salinity stress. Therefore, it is not surprising that the salt treatments increased antioxidant levels in the present study. The antioxidant level was increased with salt treatments and the most effective salt concentration is the highest dose (250mM). This trend of increase in antioxidants level is also reported in the earlier study by Annamalai *et al.* (2016), who found a similar pattern of increase in certain phytochemicals, including antioxidants, in some green algae like *Chlorella vulgaris* and *Chlamydomonas reinhardtii*. Other authors also reported that the overall antioxidant properties of *Exiguobacterium oxidotolerans* help in ion leaching or scavenging action (Bharti *et al.*, 2013). The elevated concentrations of such phytochemicals as an antioxidant, also destroy ROS (reactive oxygen species), which is formed during salt stress. By destroying ROS, these phytochemicals defend the plant from oxidative damage (Hayat *et al.*, 2012, Khan *et al.*, 2014). Khan *et al.* (2014) explained the antioxidant and ion scavenging properties and the role of various sulfur-containing metabolites like methionine, cysteine, etc., in salinity stress tolerance by plants. Previous research also suggests that plants manipulate their physiological activities under salinity stress conditions, which leads to accumulations of certain stress-alleviating biomolecules and metabolites (Gill and Tuteja, 2010, Negrao *et al.*, 2017). Gill and Tuteja (2010) studied and concluded the role of various polyamines as stress messengers. These polyamines help to neutralize the effect of various abiotic stressors like drought, salinity, etc., through their antioxidant and anti-acid properties. Negrao *et al.* (2017) also showed that plants increase intracellular concentrations of various organic solutes like glycine and proline under salt stress conditions.

Table 1 shows that the molecular synthesis of antioxidants was significantly (at $p < 0.05$) changed under salt stress. The antioxidant activity was increased from 11.39 $\mu\text{g/ml}$ to 14.80 $\mu\text{g/ml}$ under 0mM to 250mM NaCl concentration on the 21st day of sampling. Similarly, Sharma and Ramawat (2013) reported a salt-induced increase in antioxidant activity in the callus of *Salvadora persica* (Sharma and Ramawat 2013). The present study also found that the plant has an inbuilt molecular mechanism for salinity tolerance by increasing antioxidant levels in response to higher salt concentrations. Table 1 explains that total phenol concentrations were increased with salinity stress from 39.50 $\mu\text{g/ml}$ to 46.25 $\mu\text{g/ml}$ under moderate salinity stress conditions (50mM to 100mM) and lowered down with higher ranges of salt

Table 1. Showing concentrations in groups of phytochemicals in *C. asiatica* under different salt treatments (Mean values± SD)

S. No	Name of test	Salt Conc. Day of analysis	Salt Concentration (NaCl)					
			Control (0 mM)	50mM	100mM	150mM	200mM	250mM
1.	Antioxidants (in µg/ml)	7 th day	10.79 ^f ±0.46	11.50±0.55	12.07±0.80	12.85±1.23	13.53±1.24	14.31 ^a ±1.30
		14 th day	11.39 ^f ±0.03	11.73±0.41	12.52±0.74	13.0±0.75	13.9±1.36	14.54 ^a ±1.09
		21 st day	11.39±0.71	11.84±0.79	12.52±1.07	13.15±0.92	14.05±1.41	14.8±1.26
2.	Phenol (in µg/ml)	7 th Day	30.8 ^{cd} ±0.88	35.25 ^{cd} ±1.14	43.3 ^{ab} ±1.27	42.55 ^{ab} ±1.70	29.33 ^{acdf} ±1.95	20.0 ^{abcde} ±1.39
		14 th Day	31.0 ^{bcd} ±0.84	37.55 ^{acef} ±1.37	45.0 ^{ab} ±1.59	42.5 ^{ae} ±2.13	27.55 ^{bcd} ±1.32	18.25 ^{abcde} ±1.68
		21 st Day	31.50 ^{bcd} ±2.75	39.5 ^{acef} ±1.61	46.25 ^{ab} ±1.56	44.25 ^{ae} ±1.40	24.50 ^{abcd} ±2.46	17.50 ^{abcde} ±0.99
3	Flavonoids (in µg/ml)	7 th Day	490±86.26	585 ^f ±80.51	683.33 ^f ±86.60	662 ^f ±58.76	457 ^f ±86.24	336 ^{bcd} ±22.84
		14 th Day	520 ^f ±50.65	610 ^{ef} ±57.0	705 ^{ef} ±79.81	582.4 ^f ±81.63	411.2 ^{bc} ±49.0	254.3 ^{abcd} ±26.35
		21 st Day	550 ^f ±83.74	654 ^{ef} ±46.72	721.5 ^{ef} ±41.21	516 ^f ±85.96	382 ^{bc} ±77.94	205.3 ^{abcd} ±51.29
4	Tannin (in µg/ml)	7 th Day	55.5±17.2	58.25±11.38	64.5 ^f ±6.06	60.0±6.80	52.0±6.53	33.45 ^c ±6.35
		14 th Day	55.0±11.60	60.5±8.15	65.6 ^f ±13.64	63.25±6.31	52.25±10.88	30.0 ^c ±8.36
		21 st Day	55.3 ^f ±12.42	62.25 ^f ±10.30	67.0 ^f ±7.53	65.0 ^f ±6.84	50.5±8.93	24.5 ^{abcd} ±6.53
5	Total Protein (in µg/ml)	7 th Day	5720±549.57	5800±843.76	6080 ^f ±558.17	5890±720.64	5022±249.26	4233 ^c ±332.30
		14 th Day	5795±929.37	6010 ^f ±262.80	6250±217.83	6075 ^f ±466.58	4890±220.72	4180 ^{abcd} ±211.29
		21 st Day	5790 ^{ef} ±656.28	6125 ^{ef} ±409.66	6545 ^{ef} ±236.22	6055 ^{ef} ±302.43	4520 ^f ±212.45	3685 ^{abcde} ±146.50

Mean values labeled with alphabet superscript (a-f) differed significantly at p<0.05 for each group of phytochemicals among all three times of sampling (7th, 14th, and 21st day); Statistical analysis was done for each row by multiple mean comparisons performed by Post-hoc test (Tukey's test)

concentrations. Valifard *et al.* (2014) also studied the effects of salt stress on antioxidants and the total phenolic content of *Salvia mirzayanii*, which also showed that these phytochemicals increased with certain extents of salt stress in the plant. The study agrees with the observations made by Razieh *et al.*, who found a significant increase in various biochemicals, including total phenols, in the leaves of certain genotypes of *Aegilop cylindrica* and wheat species (Razieh *et al.*, 2021). They also revealed that the phenols are the most important plant secondary metabolites, which act as osmoprotectants and help alleviate. The present study also reveals that flavonoid concentrations increased with salinity stress up to 100mM and gradually decreased with more increments in salt concentrations, Table 1. These results are in parallel with Zhou *et al.* (2018), who performed salt stress studies on *Schizonepeta tenuifolia* and revealed that the total flavonoid content increased with mild levels of salinity stress. Rezazadeh, *et al.* (2013) studied that flavonoid content influenced the leaves of *Cynara scolymus L* grown under many ranges of salt stress. Rajendra *et al.* (2019) also reported similar impacts of salt stress on flavonoid productivity in *Vigna unguiculata* (cowpea sprout). In the current study, the effect of salt stress on total tannin production by the plant is shown in Table 1, which describes that the tannin concentrations in the extracts gradually increased for 50mM and 100mM, then decreased for more concentrations of salt like 150mM, 200mM, and 250mM. The current results for tannin are supported by El-Lamey (2012), who also found similar impacts of salt stress on the total tannin content in *Leucaena leucocephala* and *Prosopis chilensis*. Falcinelli *et al* (2017) found the same observations on the total tannin content in early and late sprouts of *Brassica napus var oleifera Del* (rapeseeds) which were germinated under salinity stress conditions ranging from 50mM to 200mM. In the current study, data found that the total protein concentrations in the plant extracts increased with salt stress up to 100mM and then gradually decreased for higher concentrations (150mM, 200mM, 250mM) of salt, as shown in Table 1. Goudarzi and Pakniyat (2009) also found that salinity increases total protein content in wheat cultivars. Abdul Qados (2011) also reported that the total protein content increased under salinity stress in *Vicia faba* (L) or bean plants and revealed that there is a significant and positive relationship between the protein content of bean plants and salt stress ranging from 60mm to 240mM. The present study reported that the intracellular concentration of plant metabolites like antioxidants, phenol, flavonoids, tannin, and protein content was influenced by salt stress in *C. asiatica*. This increased level of these phytochemicals contributed to the plant's salinity stress tolerance mechanism and helped maintain the

plant's health and growth under salinity stress. The current finding provided a basis for the development of advanced control methods that can enhance the plant's inbuilt salt tolerance mechanism by increasing the levels of these phytochemicals. Such control methods can play a significant role in the sustainable productivity of various commercially valuable crops like *C. asiatica* even under salinity stress.

Conclusion

The present study showed that intracellular concentrations of various phytochemicals such as antioxidants, phenols, tannin, flavonoids, and total protein contents increase with the increment. The results were found significant at $p < 0.05$. The data revealed that under salt stress conditions, the *C. asiatica* plant regulates its metabolic pathways and increases the levels of the phytochemicals mentioned above. The increased concentrations of these phytochemicals further enhanced the tolerance mechanism against salt stress. The study confirmed that the above-mentioned plant metabolites or phytochemicals play an important role in the stress tolerance mechanisms and defend the plant against oxidative and osmotic stresses caused by salinity stress up to a certain range (50mm, 100mm). The current study offers an opportunity to increase the plant's inbuilt salt tolerance ability by increasing the concentrations of phytochemicals like phenols, flavonoids, tannin, and antioxidants and protect plant health from oxidative damage caused by salt-stressed conditions.

ACKNOWLEDGEMENTS

We are indebted to AKS University, the Department of biotechnology, Satna (M.P.), for providing all the assistance during the study. The authors appreciate Rapture Biotech lab, Noida (U.P.), for all the laboratory assistance.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abdul-Qados, A. M. S. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.) (2011). *Journal of Saudi Society and Agricultural Sciences*, 10 (1), 7-15 <https://doi.org/10.1016/j.jssas.2010.06.002>
2. Anna-da-Silva, A. P., Nascimento-da-Silva, L. C., Martins-da-Fonseca, C. S., De-Araujo, J. M., Dos-Santos-Correia, M. T., Da-Silva-Cavalcanti, M. & De-Menezes-Lima, V. L. (2016). Antimicrobial activity and phytochemical analysis of organic extracts from *Cleome spinosa* Jaqc. *Frontiers in Microbiology*, 7, Article 963. <https://doi.org/10.3389/>

- 2Ffmicb.2016.00963
3. Annamalai, J., Shanmugam, J. & Nallamuthu, T. (2016). Salt stress enhancing the production of phytochemicals in *Chlorella vulgaris* and *Chlamydomonas reinhardtii*. *Journal of Algal Biomass Utilization*, 7 (1), 7-44.
 4. Bharti, N., Yadav, D., Barnawal, D., Maji, D. & Kalra, A. (2013) *Exiguobacterium oxidotolerans*, a halotolerant plant growth-promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennell under primary and secondary salt stress. *World Journal of Microbiology and Biotechnology*, 29 (2), 379-387. <https://doi.org/10.1007/s11274-012-1192-1>
 5. Brinkhaus, B. M., Lindner, D., Schuppan, E. G. & Hahn. (2000). Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. *Phytomedicine*, 7, 427-448. [https://doi.org/10.1016/s0944-7113\(00\)80065-3](https://doi.org/10.1016/s0944-7113(00)80065-3)
 6. Devkota, A. & Jha, P. K. (2010). Seed germination responses of the medicinal herb *Centella asiatica*. *Brazilian Society of Plant Physiology*, 22 (2),143-150 <https://doi.org/10.1590/S1677-04202010000200008>
 7. El-Lamey, T. A. (2012). Effect of salinity on the tannin content of *Leucaena leucocephala* (Lam.) de wit. and *Prosopis chilensis* (Molina) Stuntz and techniques for their reduction. *Egypt Journal of Botany*, pp 51-63
 8. Ezeonu, C. S. & Ejikeme, C. M. (2016). Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Journal of Science*, <https://doi.org/10.1155/2016/5601327>
 9. Falcinelli, B., Sileoni, V., Marconi, O., Perretti, G., Quinet, M., Lutts, S. & Benincasa, P. (2017). Germination under moderate salinity increases phenolic content and antioxidant activity in rapeseed (*Brassica napus* var *oleifera* Del.) Sprouts. *Molecules*, 22 (8) <https://doi.org/10.3390/molecules22081377>
 10. Gill, S. & Tuteja, N. (2010). Polyamines and abiotic stress tolerance in plants. *Plant Signaling & Behavior*, 5 (1), 26-33. <https://doi.org/10.4161/psb.5.1.10291>
 11. Goudarzi, M. & Pakniyat, H. (2009). Salinity causes an increase in proline, protein contents, and peroxidase activity in wheat cultivars. *Journal of Applied Sciences*, 9 (2), 348-353 <https://dx.doi.org/10.3923/jas.2009.348.353>
 12. Hashim, P., Sidek, H., Helan, M., Sabery, A., Palanisamy, U. D. & MohdIlham. (2011). Triterpene composition and bioactivities of *Centella asiatica*. *Molecules*, 16 (2),1310-1322 <https://doi.org/10.3390%2Fmolecules16021310>
 13. Hassan, M. A., Pacurar, A., Lopez-Gresa, M. P., Donat-Torres, M. P., Llinares, J. V., Boscaiu, M. and Vicente, O. (2016). Effects of salt stress on three ecologically distinct *Plantago* Species. *PLoS ONE*, 11 (8). <https://doi.org/10.1371/journal.pone.0160236>
 14. Hayat, S., Hayat, Q., Alyemeni, M. N., Shafi Wani, A., Pichtel, J. & Ahmad, A. (2012). Role of proline under changing environments: A review. *Plant Signaling and Behaviour*, 7 (11), 1456-1466. <https://doi.org/10.4161/psb.21949>
 15. Hirayama, T. & Shinozaki, K. (2010). Research on plant abiotic stress responses in the post-genome era: past, present, and future. *The Plant Journal*, 61 (6), 1041–1052. <https://doi.org/10.1111/j.1365-313x.2010.04124.x>
 16. James, J. T. & Dubery, I. A. (2009). Pentacyclic Triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. *Molecules*, 14 (10), 3922-3941. <https://doi.org/10.3390/molecules14103922>
 17. Kashmira, J., Gohil, J., Patel, A. & Gajjar, A. K. (2010). Pharmacological review on *Centella asiatica*: A potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences*, 72 (5), 546-556. <https://doi.org/10.4103%2F0250-474X.78519>
 18. Katalinic, V., Milos, M., Kulisic, & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Elsevier*, 94 (4), 550-557. <https://doi.org/10.1016/j.foodchem.2004.12.004>
 19. Khan, N. A., Khan, M. I. R., Asgher, M., Fatima, M., Masood, A. & Saeed, S. (2014). Salinity tolerance in plants: Revisiting the role of sulfur metabolites. *Journal of Plant Biochemistry & Physiology*, 2 (1). <http://dx.doi.org/10.4172/2329-9029.1000120>
 20. Kumar, J., Singh, S., Singh, S., Srivastava, P. K., Mishra, R. K., Singh, V. P. & Prasad, S. M. (2017). Transcriptional regulation of salinity stress in plants: A short review. *Plant Gene*, 11 (part B),160-169. <http://dx.doi.org/10.1016%2Fj.plgene.2017.04.001>
 21. Kunjumon, R., Johnson, J. A. & Sabulal, B. (2022). *Centella asiatica*: Secondary metabolites, biological activities, and biomass sources. *Science Direct*, 2 (1) <https://doi.org/10.1016/j.phyplu.2021.100176>
 22. Negrao, S., Schomockel, S. M. & Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, 119 (1), 1-11 <https://doi.org/10.1093%2Faoab%2Ffmcw191>
 23. Nurul, M.H., Radzali, M., Johari, R., Syahida, A. and Maziah, M. (2008). Antioxidant Activities of Different Aerial Parts of Putat (*Barringtonia racemosa* L.). *Malaysian Journal of Biochemistry and Molecular Biology*, 16, 15-19
 24. Rajendra, F. M., Kristiani, L. S. & Ariviani, S. (2019). Elicitation under salinity stress increases flavonoid content and antioxidant activity in cowpea (*Vigna unguiculata*) sprouts. *Materials Science, and Engineering*, 633 (1). <http://dx.doi.org/10.1088/1757-899X/633/1/012034>
 25. Rangarajan, N. & Sathiyavani. (2014). Phytochemical screening and evaluation of protein content in the seed extracts of *Cucurbita maxima*. *International Journal of Pharmacy and Life Sciences*, 5 (7), 3637-3642
 26. Raziheh, K., Arzani, A. & Miramohammady-Maibody, S. A. M. (2021). Polyphenol, flavonoid, and antioxidant activity involved in salt tolerance in Wheat, *Aegilop cylindrica*, and their Amphidiploids. *Frontiers in Plant Science*. <https://doi.org/10.3389/fpls.2021.646221>
 27. Rezazadeh, A., Ghasemnezhad, A., Barani, M. & Telmardarrehai, T. (2013). Effect of salinity on phenolic composition and antioxidant activity in Artichoke (*Cynara scolymus* L.) leaves. *Research Journal of Medicinal Plants*, 6 (3), 245-252 <https://dx.doi.org/10.3923/rjmp.2012.245.252>
 28. Ruzzymah, B. H., Chowdhury, S. R., Manan, N. A., Fong, O. S., Adenan, M. I. & Saim, A. B. (2012). Aqueous extract of *Centella asiatica* promotes corneal epithelium wound healing in vitro. *Ethnopharmacol*, 140 (2), 333-338. <https://doi.org/10.1016/j.jep.2012.01.023>
 29. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. & Latha, L. Y. (2011). Extraction, isolation, and characterization of bioactive compounds from Plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8 (1), 1-10 <https://doi.org/10.4314/>

ajtcam.v8i1.60483

30. Sharma, V. & Ramawat, K. G. (2013) Salinity-induced modulation on growth and antioxidant activity in the callus cultures of miswak (*Salvadora persica*). *3 Biotech*, 3 (1) 11-17 <https://doi.org/10.1007/s13205-012-0064-6>
31. Shukurova, K. S., Myint, D., Yi, S. S., Saw, O. M. & Watanabe, K. N. (2021). Morphological description and ethnobotanical review of the orphan crop Myin-Hkwa (*Centella asiatica* L.) from Myanmar. *Frontiers in Sustainable Food System*. <https://doi.org/10.3389/fsufs.2021.680862>
32. Singh, S., Gautam, A., Sharma, A. & Batra, A. (2010). *Centella asiatica* (L.): A plant with immense medicinal potential but threatened. *International Journal of Pharmaceutical Sciences Review and Research*, 4 (2), 9-19.
33. Thaker, P. N., Brahmhatt, N. & Shah, K. (2021). A review: Impact of soil salinity on ecological, agricultural, and socio-economic concerns. *International Journal of Advanced Research*, 979-986. <http://dx.doi.org/10.21474/IJAR01/13200>
34. Valifard, M., Mohsenzahed, S., Kholadebarin, B. & Rowshan, B. (2014). Effects of salt stress on volatile compounds, total phenolic and antioxidant activities of *Salvia mirzayanii*. *South African Journal of Botany*, (93), 92-97. <https://doi.org/10.1016/J.SAJB.2014.04.002>
35. Wijeweera, P., Arnasona, J. T. & Koszycki Merali, Z. (2006). Evaluation of anxiolytic properties of Gotukola – (*Centella asiatica*) extracts and asiaticoside in rat behavioral models. *Phytomedicines*, 13 (9-10), 668-676. <https://doi.org/10.1016/j.phymed.2006.01.011>
36. Yadav, R. N. S. & Agrawala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3 (12), pp 10-14.
37. Zhou, Y., Tang, N., Huang, L., Zhao, Y., Tang, X. & Wang, K. (2018). Effects of salt stress on plant growth, antioxidant capacity, glandular trichome density, and volatile exudates of *Schizonepeta tenuifolia*. *International Journal of Molecular Sciences*, 19 (1), 252-266. <https://doi.org/10.3390%2Fijms19010252>