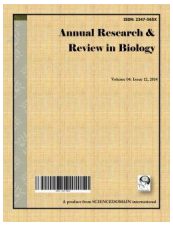

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Growth, Chlorophyll Fluorescence, Leaf Gas Exchange and Phytochemicals of *Centella asiatica* Exposed to Salinity Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MHI and NIS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NAMZ managed the analyses of the study. Author AAI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to investigate the effect of salinity by using a different concentration of sodium chloride (NaCl), on growth, chlorophyll fluorescence and secondary metabolites production of *Centella asiatica.*
Study Design: *Centella asiatica* plants were exposed to four different concentration of sodium chloride (0, 50, 100 and 150 mM). This research was conducted using a randomized complete block design 4 x 3 with three replications for each treatment and each treatment consists of 12 plants regarding four times harvesting.
Place and Duration of Study: Glasshouse of SLAM field, University Agriculture Park, Universiti

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Putra Malaysia from February to April 2015.
Methodology: Salinity stress was induced by irrigating the plants using four salinity levels (0, 50, 100 and 150 mM) of salt concentrations for 12 weeks. The leaves number were counted manually and the total plant biomass was taken by calculating the dry weight of root, stem, and leaf per seedling. The total chlorophyll content in the leaves was measured using a SPAD chlorophyll meter. Chlorophyll fluorescence was measured using Hansatech Pocket PEA, The leaf gas exchange were determined using a LI-6400XT portable photosynthesis system. Total phenolics and flavonoid was determined using Folin-Ciocalteu reagent. Phytochemical screening was conducted to determine the presence of tannin, terpenoids, phenolics, flavonoids, saponin, and alkaloids of plant samples under salinity stress.
Results: Increased in salinity levels from 0 > 150 mM, the number of leaves, total biomass and total chlorophyll content were gradually decreased. *Centella asiatica* exhibit a significant decrease in net photosynthesis (A), transpiration rate (E), maximum efficiency of photosystem II (f_v/f_m) and Performance index (PI) when the salinity level increased. However, it was noticed that salinity stress significantly enhanced the total phenolic and flavanoid content of *C. asiatica*. It was also observed, that under salinity there were more presence of phytochemicals (tannin, terpenoids, phenolics, flavonoids, saponin and alkaloids) compared to the control.
Conclusion: This study revealed that the increase in salinity level have greatly reduced the growth of *C. asiatica* but high salinity level also can enhance the production of secondary metabolites (total phenolic and flavonoid content) in *C. asiatica*.

Keywords: Salinity; Centella asiatica; secondary metabolites; sodium chloride.

1. INTRODUCTION

Nowadays, the exploitation of herbal plants has become extremely popular for cosmetics and health consumptions. This is due to the awareness of hazards and toxicity associated with the indiscriminate use of a chemical, synthetic drugs and antibiotics. Even today, it is estimated about 75% of the world's population use medicinal plants for traditional medicine [1]. According to Naik [2], the progress of micropropagation method for some of the medicinal plant species have been reported and needs to be considered in order to produce a large scale of plant products that can be used in pharmaceutical industry. The increase in interest of medicinal plants happened due to the content of biologically active compounds such as phenolic and flavonoids compound in plants that can give many beneficial effects to human health [3,4,5]. Other than that, the secondary metabolites of plants also can be used as agrochemicals, flavors, fragrances, colors, biopesticides and food additives [1]. Nowadays, many researchers and scientists have tried many ways to increase the accumulation of plant products such as by doing a cell culture or organ culture on the medicinal plants to ensure a continuous supply of high-quality medicinal herbs for pharmaceutical industry [1,6].

Secondary metabolite is natural product that usually have an ecological role in regulating the interactions between plants and their environments [7]. This phytochemicals are important for plant's survival and reproductive fitness as they can be a defensive substances such as phytoalexins and phytoanticipins, anti-feedants, attractants and pheromones [8,9]. Besides that, secondary metabolites is also an important source of active pharmaceutical [10]. From the previous studies, it is estimated that over 40% of medicines have derived from these active natural products [7]. The production of secondary metabolites has been achieved through the process of cultivation of medicinal plants due to the high demand for pharmaceutical, agriculture and industry [11,12].

Centella asiatica or locally known as pegaga in Malaysia is a perennial herb plant that inhabits a tropical and subtropical regions. *Centella asiatica* also known as Gotu Kola or Indian pennywort in Europe has been used as a medicinal plant since prehistoric times and has become an important commercial product [13,7]. *Centella asiatica* has been used to treat several disorders such as insanity, asthma, leprosy, ulcers, eczema and dermatological problems [4]. Other than that, it is also can help in improving cognition, relieving anxiety and as an anti-cancer agent [14,15]. According to World Health Organization (WHO), *C. asiatica* has been mentioned as one of the most crucial medicinal plant species that needs to be conserved and cultivated. *C. asiatica* contains large quantities of pentacyclic triterpenoid saponins, a secondary metabolite that has been widely used for various medicinal

and cosmetic purposes. During the last years, *C. asiatica* based pharmaceutical and cosmetic products have gained popularity worldwide [7,16,17]. Besides being used as a traditional medicine, it is also used as a nutraceutical vegetables and drinks in Malaysia, China and other parts of Asia [18,7]. In Malaysia, it is more popular known as a salad or an 'ulam' among Malay people rather than a medicinal plant.

As being told before, the demanding on the herbal plant has come to increase these couple of years due to its medicinal properties. *C. asiatica* is one of the examples of herbal plant that had caught a lot of attention among scientist and researcher for further research on the synthesis and accumulation of secondary metabolite on this plants. The cultivation technology has been used to increase the production of the desired compund in *C. asiatica* plants but unfortunately, the propagators are not able to produce a high quality of plantlets as a planting material for cultivation. According to James and Dubery [7], the triterpene component found in *C. asiatica* can be affected by location and various environmental conditions. Based on previous research, it shows that environmental stress can enhance the accumulation of several secondary metabolites in plants. The synthesis of secondary metabolites will occur when the plants try to overcome the stress conditions [1]. One of the examples of environmental stress is salinity stress. Heidari et al. [19] had been reported that abiotic environmental stress, especially salinity has the most effect on medicinal plants. Before this, a research has been conducted on the effect of fertilizer, light intensity and water stress but there are no fully report yet on the effect of salinity stress on this plants. Therefore, the study about this plant is conducted to investigate the effect of salinity by using a different concentrations of sodium chloride, on growth and secondary metabolites of *C. asiatica* and, to identify the best salinity environment for growth of *C. asiatica* and to understand the relationship between the growth and secondary metabolites of *C. asiatica* under salt stress.

2. 2. MATERIALS AND METHODS

2.1 Plant Material and Maintenance

The experiment was carried out in a glasshouse of SLAM field, Universiti Agriculture Park, Universiti Putra Malaysia at longitude 101º44'N and latitude 2º58' S, 68 m above sea level. The same or most identical in size *C. asiatica* was chosen to propagate in a tray that was loaded with a mixture of peat moss for about one week in the nursery. After that, the plants were transferred into a 16 cm x 30 cm size of polybag filled with a mixture of top soil and sand (ratio 3:1). The plants were left to grow in a glasshouse under a natural lighting for 12 weeks. Before the experiment initiated, preliminary experiment were conducted to determine the tolerance level of *C. asiatica* to salinity. The concentration of NaCl from 0–250 mM were used. Among the treatments, the application of NaCl below 200 mM have shown to significantly increased the plant dry weight and leaf number, however concentration more than 200 mM have shown to reduce the plant dry weight and leaf number. Hence the concentration < 200 mM were used in this study. The plants were watered manually with the different levels of salt treatment (0, 50, 100, 150 mM) using 100 ml pot when necessary. In the preliminary study, after four months of exposure to salinity stress most of the plant died so the current experiment was conducted for three months.The experiment was organized in a randomized complete block (RCBD) design with three replications and each treatment consists of 12 plants regarding to four times harvesting. So, the total of the plants were 96 plants for two set of experiments.

2.2 Leaf Numbers, Plant Biomass and Total Chlorophyll Content

The whole leaves of the plants were counted manually and total plant biomass was taken by calculating the dry weight of root, stem, and leaf per seedling. Plant parts were separated and placed in paper bags and oven dried at 80ºC until constant weight was reached before dry weights were recorded using the electronic weighing scale.SPAD-502 chlorophyll meter was used to measure the total chlorophyll content in the leaves. Three readings were taken at three spot on a leaf of each plant and the average readings were recorded. The leaf numbers, plant biomass and total chlorophyll content was measured every three weeks during the three months experimental duration.

2.3 Leaf gas Exchange Measurement

The leaf gas exchange was measured by using a close infra-red gas analyzer of LICOR 6400XT Portable Photosynthesis System. Before used, the instrument was warmed first for 30 minutes and calibrated with ZERO IRGA mode. Two steps are required in this calibration process

which is the initial zeroing process for the built-in flow meter and zeroing process for the infra-red gas analyzer. The measurement used optimal cuvette conditions for *C. asiatica* i.e. 400 µmol mol^{-1} CO_2, 30ºC temperature, 50-60% relative humidity with air flow rate set at 500 cm^3 min^{-1}, and light intensity of 800 µmol m^{-2} s^{-1} photosynthetically photon flux density (PPFD). The measurement of gas exchange was carried out between 9.00 to 11.00 am using fully expanded young leaves numbered three and four from plant apex to record net photosynthesis rate (A). The operation was automatic and the data were stored in the LI-6400 console and analyzed by the Photosyn Assistant software. The leaf surfaces were cleaned and dried using tissue paper before enclosed in the leaf cuvette Ibrahim et al. [20]. The leaf gas exchange measurement was taken at 12 weeks after treatments.

2.4 Chlorophyll Fluorescence Measurement

Measurements of chlorophyll fluorescence were taken from fully expanded leaf of the second leaves. Leaves were darkened for 15 min by attaching light-exclusion clips to the central region of the leaf surface. Chlorophyll fluorescence was measured using a portable chlorophyll fluorescence meter (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK). Measurements were recorded up for 5 S. The fluorescence responses were induced by emitting diodes. Measurement of f_O (initial fluorescence), f_M (maximum fluorescence), f_V (variable fluorescence) and PI (performance index) were obtained from this procedure. This data was measured during the last week of treatment.

2.5 Total Phenolics and Flavonoids Quantification

Total phenolics and flavonoids were measured at 12 weeks after treatment. For total phenolics content determination, it was determined using Folin-Ciocalteu reagent and use gallic acid as a standard. Initially, 0.1 g of ground tissue samples were extracted with 80% ethanol (10 ml) and were shaken in orbital shaker for 120 minutes at 50ºC. Then, the mixture was filtered with filter paper Whatman No. 1. The extract then was used for the quantification of total phenolics and flavonoids. After that, 200 µL of the sample extract were mixed with 1.5 µL Folin- Ciocalteu reagent and left for 5 minutes at 20ºC before adding 1.5ml $NaNO_3$ solution. After two hours, the absorbance of each reaction mixture was measured at 725 nm. Total phenolics was expressed as mg gallic acid equivalents (GAE). Then, for total flavonoids content determination, 1 mL of sample was mixed with 0.3 mL $NaNO_3$ in a test tube that covered with aluminium foil and left for 5 min. After that, 0.3 mL of 10% $AlCl_3$ was added and followed by 2 mL of 1 M NaOH. The absorbance of each reaction mixture was measured at 510 nm by using spectrophotometer with rutin as a standard. The results were expressed as mg rutin g^{-1} dry samples [21]. Fig. 1 shows the calibration curve for gallic acid and rutin.

2.6 Phytochemical Screening

For extraction five grams of dried powder was transferred to flask, then methanol (25 mL) was added to the mixture and shaken with a shaker at 100 rpm for 5 minutes. The mixture then filtered using Whatman filter no 1. The solvent kept

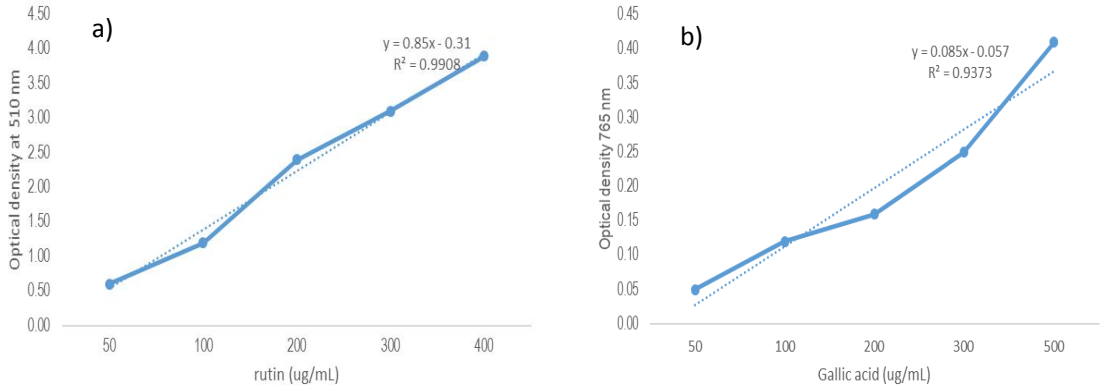

Fig. 1. Total phenolics (a) and total flavonoids (b) calibration curves in the experiment

at -20 for phytochemical analysis. Extract of *C. asiatica* was subjected to the different phytochemical test as described below. For tannins, the plant extract was treated with ferric chloride solution until blue colour appeared, that indicate the presence of tannin. For terpenoid screening, a mixture of chloroform and H_2SO_4 was added to 1 g of plant extract. The red color indicated the presence of terpenoids. For phenolics screening, 2 ml of plant extract were added to $FeCl_3$ in the water bath at the temperature of 40ºC. Green colour showed the presence of phenolics. For flavonoids determination, 4 ml of NaOH (10%) were added to the plant extract until yellow colour appear that indicate the presence of flavonoid. For saponin, 1 g of the dried plant samples was mixed with hot water (100ºC). The mixture then cooled to the room temperature and shaken vigorously, any froth appeared to indicate the presence of saponin. The alkaloid content was determined by using Hager's reagent. One gram of the plant samples was added to the reagent. Any precipitation in the test tubes indicates the presence of alkaloids [22,23]. Same with total phenolics and flavonoids this screening was conducted at 12 WAT.

2.7 Statistical Analysis

Statistical analysis was performed by using SPSS version 2.1. The mean separation test between treatments was compared using Duncan multiple range tests (DMRT) and standard error of differences between means was calculated with the assumption that data were normally distributed and equally replicated. A value of $p \leq 0.05$ was considered statistically significant [24].

3. RESULTS AND DISCUSSION

3.1 Leaf Number

In this study, the number of the leaf of *C. asiatica* was found to be influenced by the salinity level ($P \geq 0.05$; Fig. 2). A significant difference ($P \leq 0.05$) was observed in 3, 6, 9 and 12 weeks after treatments. In every three weeks of harvesting, the number of leaves was highest in control (0 mM) followed by 50 mM, 100 mM and lastly the lowest 150 mM. There was a decrease in the number of leaves at 50 mM, 100 mM and 150 mM concentration of salts after 9 weeks of giving treatments compared to control which always increase from the beginning. According to Volkmar et al. [25], salinity can enhance the senescence of older leaves. The decrease in the number of leaves at 50 mM, 100 mM and 150 mM of salt concentration might be due to the high concentration of sodium chloride that accumulates in the cell walls and cytoplasm of old leaves that have disrupt protein synthesis and interfere enzyme activity and leads to the leaf death [26,27]. These results agree with what Raul et al. [28] reported, that the leaves of tepary bean (*Phaseolus acutifolius* L.) have been reduced as the concentration of sodium chloride increase.

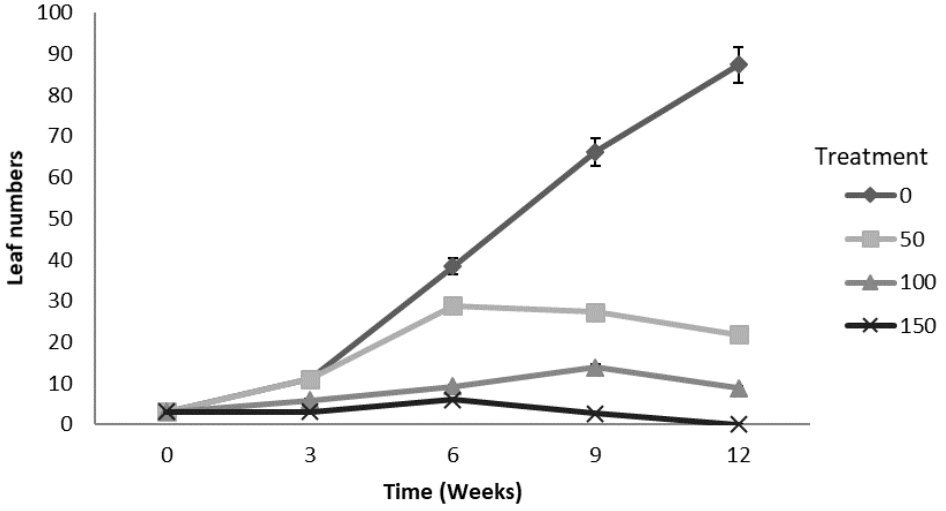

Fig. 2. The effect of salinity on leaf number of *C. asiatica* after 12 weeks of planting
Data are mean ± standard error of mean (SEM) N= 6

3.2 Total Plant Biomass

Based on Fig. 3, it shows that total biomass was dramatically influenced by salinity level ($P \leq 0.05$). There was a significant difference ($P \leq 0.05$) in week 6, 9 and 12 of harvesting while there was no significant difference ($P \geq 0.05$) in week 3 of harvesting. The general trend of this result was the total biomass has increased from week 0 until week 6 of and start falling off after week 6 of treatment. It has been reported that a stressful environment usually inhibits the biomass production of many plants [29]. According to Kirst [30] a declining in plant biomass might be due to a high accumulation of NaCl in chloroplast that reduces the photosynthesis process and affects the growth rate of *C. asiatica*.

3.3 Total Chlorophyll Content

The results showed that there was a clear effect of salinity on the chlorophyll content of *C. asiatica* (Fig. 4). A significant difference ($P \leq 0.05$) was observed in week 6 and 12 after giving treatment. In week 6, the highest chlorophyll content was found in 50 mM of salt concentration and the lowest was found in 150 mM of salt concentration. In week 12 of harvesting, the highest chlorophyll content was noticed in control plants (25.93) followed by 50 mM (18.76), 100 mM (9.84) and 150 mM (zero). For 150 mM, the

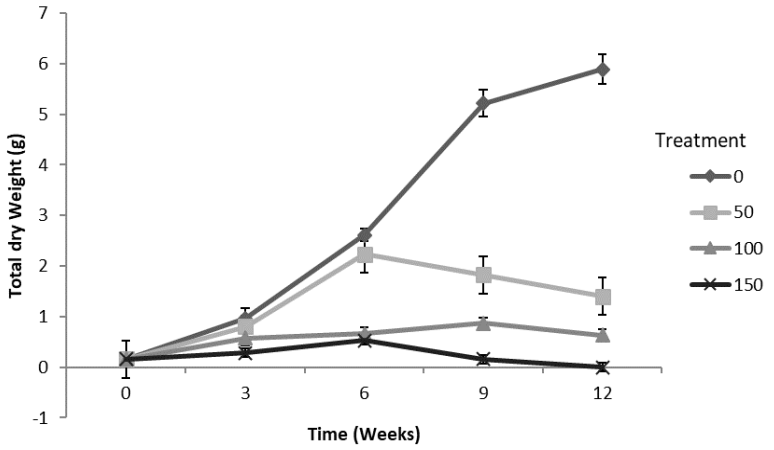

Fig. 3. The effect of salinity on total plant biomass of *C. asiatica* after 12 weeks of planting
Data are mean ± standard error of mean (SEM) N= 6

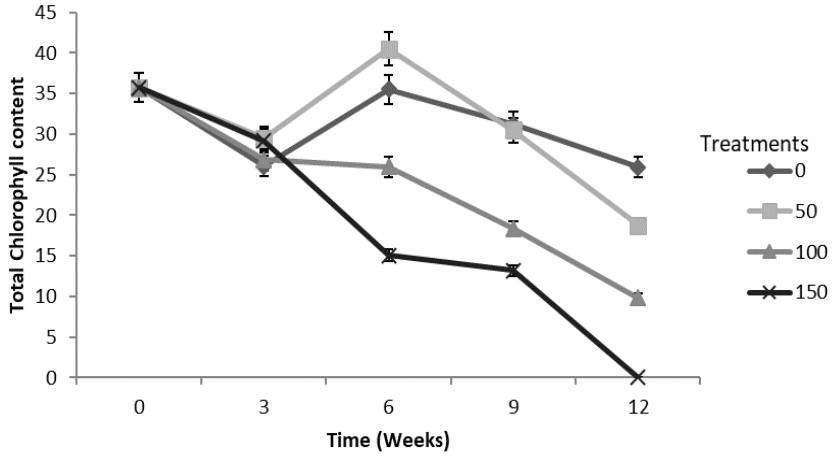

Fig. 4. The effect of salinity on chlorophyll content of *C. asiatica* after 12 weeks of planting
Data are mean ± standard error of mean (SEM) N= 6

chlorophyll content is zero because all plants have already died. According to Teulat et al. [31], salinity can reduce a synthesis of photosynthetic pigment in leaves. The reduction in leaf chlorophyll content occurred due to the destruction of chlorophyll pigments as the activity of chlorophyll-degrading enzymes chlorophyllase increased when plants under salinity stress [32,33]. The decrease in chlorophyll content also found in a medicinal plant, *Catharanthus roseus* (L.) when the plant is under salt-stressed [34].

3.4 Transpiration Rate (mmol/m^2/s)

Salinity level has shown to influence the transpiration rate of *C. asiatica* (Fig. 5). The significant difference ($P \leq 0.05$) was observed after 3 months of giving a treatment. In general, the results show that transpiration rate decreased as the salinity level intensifies. The maximum values of transpiration rate were noticed in untreated salt plants which is control plants (1.835 mmol m^{-2} s^{-1}) followed by 50 mM (1.490 mmol m^{-2} s^{-1}), 100 mM (1.2683 mmol m^{-2} s^{-1}) and 150 mM (0.9517 mmol m^{-2} s^{-1}) after 12 weeks of treatment. The decrease in transpiration rate might be due to the reduction in stomatal conductance as salt stress increases [35]. The reduction in stomatal conductance might be due to the chemical signal that comes from the low water content of the root and shoot [34]. According to O' Leary [37] and Prisco [38], it has been reported that high salinity would decrease the root hydraulic conductivity which can lead to a decrease in water flow from root to shoot, even in osmotically adjusted plants. This decrease in water flow might reduce the water content of the leaf so this is why the plants were tried to not transpire very much in order to maintain their low water content. The results regarding a decrease in transpiration rate, agree with what Sharma et al. [39] reported, that the high exposure of salt concentration to wheat (*Triticum aestivum* L.) has reduced the transpiration rate of this plants.

3.5 Photosynthesis Rate (µmol/m²/s)

Fig. 6 showed that the photosynthesis rate was dramatically influenced by the salt level. Generally, it was found that the photosynthesis rate decrease as the salt concentration increased from 0 mM to 150 mM. The lowest photosynthesis was 150 mM (1.8917 umol/m2/s) and the highest is control plant (5.8967 umol/m2/s). As mentioned before, ion toxicity has caused a reduction in the number of leaves, thus it has reduced the leaf area that was available for photosynthesis. Other than that, a decrease in photosynthesis rate might be due to the dehydration of cell membranes that reduce the permeability to CO_2 so the assimilation of CO_2 reduced [40]. In the mean time, salt stress also reduces the electron transport activities of photosynthesis and inhibit the activity of PS II [30,41]. Based on the previous research, salinity stress usually will lead to the continuous decline in photosynthesis rate, stomatal conductance, transpiration rate. These results agree with Huang et al. [40], where a decrease in photosynthesis rate, stomatal conductance and transpiration rate have been reported in ramie (*Boehmeria nivea* L.).

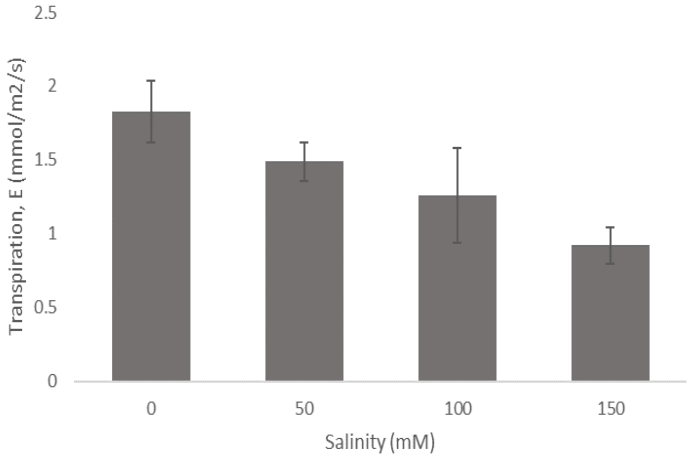

Fig. 5. The effect of salinity on transpiration rate of *C. asiatica* after 12 weeks of planting
N= 6. Bars represent standard error of differences between means (SEM)

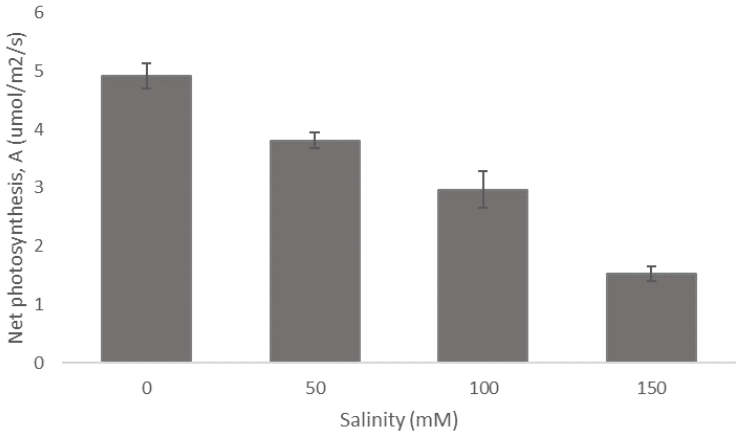

Fig. 6. The effect of salinity on photosynthesis rate of *C. asiatica* after 12 weeks of planting
N= 6. Bars represent standard error of differences between means (SEM)

3.6 Chlorophyll Fluorescence

The fv/fm represent the maximum quantum yield of photosystem II, which is correlated with the quantum yield of photosynthesis. The fv/fm values were influenced by salinity levels (P≤0.05: Table 1). As salinity levels increased from 0 to 150 mM the fv/fm reduced in C. asiatica. Fv/Fm is usually used as an indicator of the photoinhibitor or other injury caused to the photosystem II complex [42]. The values are 0.78–0.84 and almost constant for different plants measured under non-stressed conditions [43,44]. In the present study, the fv/fm values were less than 0.79 in 50, 100 and 150 mM. This suggests that the total amount of light energy transformed in the photosystem II reaction centres was decreased and that *C. asiatica* was stressed under these conditions. This showed that reduction in photochemical activity of PS II can contribute to the limitation in photosynthesis under water stress conditions [45]. The same tends followed also the PI of the plant. The performance index is a measure of plant vitality. It is a combined measurement of photosynthetic reaction centres (RC/ABS), the maximal energy flux which reaches the PSII centres and the electron transport at the onset of illumination [46]. The decreases in PI under salinity stress indicate that salinity stress decrease the numbers of reaction centres and capacity of electron carriers under JIP test that performed [47,48]. The reduction of fv/fm and PI was also observed by Arun et al. [46] on Syrian Barley exposed to abiotic stress. The current result suggests that salinity stress reduces the fv/fm and PI of *C. asiatica* under salinity stress.

3.7 Total Phenolics Content (mg GAE/g)

Fig. 7 showed the mean of absorbance values of the extract solutions reacted with Folin–Ciocalteu reagent compared with the gallic acid standard solutions. In this study, the production of total phenolics content was dramatically influenced by salinity levels ($P \leq 0.05$). A significant different was observed ($p \leq 0.05$) in total phenolic content of *C. asiatica* after 12 weeks of giving treatments. The application of 150 mM salt concentration has produced the highest production of total phenolic

Table 1. Impact of salinity stress on chlorophyll fluorescence parameters of *Centella asiatica*

Treatments salinity (mM)	fv/fm (maximum efficiency of photosystem II)	PI_{ABS} (Performance index)
0	0.85 ± 0.02^{a}	2.23 ± 0.12^{a}
50	0.76 ± 0.01^{b}	1.76 ± 0.10^{b}
100	0.72 ± 0.01^{b}	1.45 ± 0.02^{c}
150	0.67 ± 0.03^{c}	1.21 ± 0.01^{d}

Data are means ± standard error of means of nine replicates. Means not sharing a common single letter were significantly different at p ≤ 0.05

contents (1.8 mg GAE/g dry weight), followed by 100 mM (1.3583 mg GAE/g dry weight), 50 mM (1.0133 mg GAE/g dry weight) and lowest in 0 mM (0.6483 mg GAE/g dry weight). This also showed that total phenolics of control (0 mM) is 110% lower than the average total phenolics for the three salinity treatments. This result imply, that the production of gallic acid can be increased with the high concentration of salts compared to the low concentration of salts and control plants. Thus, it can be said that high concentration of salts has increased the medicinal plant properties. According to Mahajan and Tuteja [49] ionic and osmotic stress that created by salt stress in plants will lead to the accumulation or decline of secondary metabolites in plants. According to Bryant et al. [50] carbon will exchange to form a defensive secondary metabolite, when plants are under stressed. Additional carbon skeleton has provided for phenolic biosynthesis due to the new pattern of resource partitioning that resulted from a reduction in the growth of plants when the salt concentration increase [51]. Other results that support what has been shown here are those by Navarro et al. [52] where salinity induced significant increases in total phenolic content in pepper.

3.8 Total Flavonoids Content (mg rutin/g)

Fig. 8 showed the mean of total flavonoids of *C. asiatica* as influenced with salinity stress. A significant difference were observed in total flavonoid content of *C. asiatica* ($P \leq 0.05$). From the figure, it is clearly showed that the total flavonoid content in control plants of *C. asiatica* was relatively low compared to salt- treated plants. In 12 WAT, total flavonoids for the respective 50 mM, 100 mM and 150 mM was 60%, 138% and 200% higher than 0 mM (control). Total flavonoids content in the leaves of *C. asiatica* is increased with the increasing levels of salinity. Lin et al. [53] found that the increase in total flavonoids content in plants might be related to their protective role against oxidative stress due to high salt concentrations. One of the studies that support these results also is a study by Rajamane and Gaikwad [54]. They noticed that total flavonoid content in *Simarouba glauca* increase with the increasing concentration of salt treatments. Similarly, in Artichoke (*Cynara scolymus* L.), Rezazadeh et al. [55] reported that salt stress has induced significant increases in total flavonoid content of this plants also.

3.9 Phytochemical Screening

Table 2 shows phytochemical screening results for the impact of salinity on *C. asiatica*. It is apparent that salinity stress has influenced the phytochemical of *C. asiatica*. It is observed, that under control condition, only saponin was a presence. As salinity levels increased to 50 mM, tannin, terpenoids and phenolics were detected. As the level of salinity increased to 100 to 150 mM, all the five phytochemical was presence. This indicates that the presence of phytochemicals might be stimulated by the salinity stress imposed to *C. asiatica*. The production of the plant secondary metabolites may be the mechanism used by the plant to

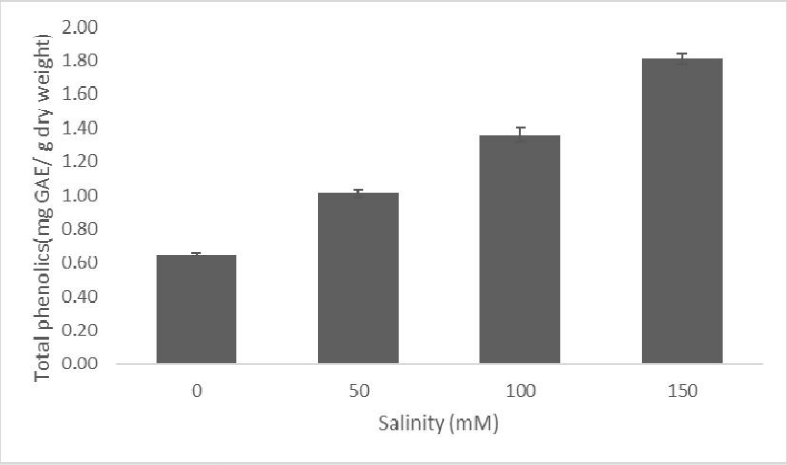

Fig. 7. The effect of salinity on total phenolics content of *C. asiatica* after 12 weeks of planting
N= 6Bars represent standard error of differences between means (SEM)

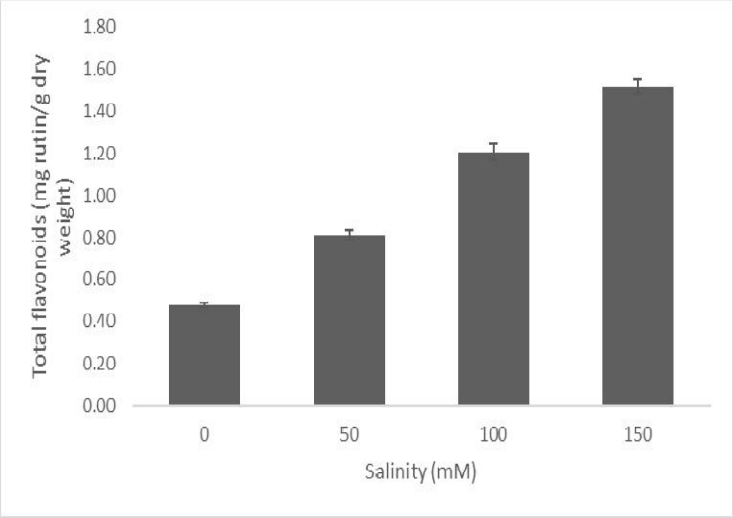

Fig. 8. The effect of salinity on total flavonoids content of *C. asiatica* after 12 weeks of planting
N= 6Bars represent standard error of differences between means (SEM). Means not sharing a common letter were significantly different at P ≤ 0.05 using Duncan Multiple Range Test

Table 2. Impact of salinity stress on phytochemical screening of *Centella asiatica*

Treatments salinity (mM)	Tannin	Terpenoids	Phenolics	Flavonoids	Saponins	Alkaloids
0	-	-	-	-	+	-
50	+	+	+	-	-	-
100	+	+	+	+	+	+
150	+	+	+	+	+	+

+ and – indicates presence and absence of phytochemicals

regulate osmotic balance and maintain membrane fluidity under salt stress condition [56]. The increase in phytochemical in a plant with salinity stress was also observed by [57] in Thyme where production of thymol and carvacrol increased when the plant exposed to 100 mM NaCl. Another study by Grimaldo et al. [58] showed that *Capsicum annum* exposed to saline condition (8 dS m^{-1}) produced higher levels of phenolics and capsaicinoids. The current result showed that imposition of salinity stress has the potential to increase the production of phytochemicals in *C. asiatica.*

4. CONCLUSION

In conclusion, the growth of *C. asiatica* has found to be influenced by salinity stress. It was noticed that salinity stress has caused a greater reduction in plant growth as all the growth variable was drastically decreased. The number of leaves, leaf area, total chlorophyll content, plants fresh weight and dry weight, plant total biomass were significantly reduced as the salt stress intensifies. Same results also found in leaf gas exchange characteristics, where the photosynthesis rate, transpiration rate, Fv/fm and PI index results in a minimum value as the salinity level increase. This reduction in the growth of *C. asiatica* may relate to the decrease in photosynthetic capacity when plants under salt stress. However, the production of total phenolic and flavanoid was found to be enhanced by salt stress. The result shows that the production of total phenolic and flavonoid content increases as salinity level increase to 150 mM. The maximum production of both secondary metabolites (total phenolic and flavonoid) was observed at 150 mM. It was also observed, that under salinity there were more presence of phytochemicals compared to the control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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