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RESEARCH ARTICLE
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Effect of Supplied Salt Concentrations in the Nutrient Solution during Hydroponic Production on Phytochemicals and Antioxidant Activity of Ice Plants (*Mesembryanthemum crystallinum* L.)

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ABSTRACT

The research aimed to evaluate the effects of different salt concentrations (0, 10, 30, 50, 70, and 100mM NaCl) applied during the cultivation process on the levels of phytochemical components (polyphenols, flavonoids, tannins, saponins, and alkaloids), color compounds (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), and antioxidant activity (DPPH and FRAP) in ice plants. The monitoring showed that ice plants grew well at salt concentrations between 30 and 100mM NaCl, without any visible negative effect. As salt concentration increased, the levels of bioactive compounds, color compounds, and antioxidant activity also increased. However, the appropriate salt concentration should be selected based on the intended use of the ice plant; For pharmaceutical or cosmetic applications, a concentration of 100mM NaCl is recommended, as it maximizes the accumulation of bioactive compounds, color compounds, and antioxidant capacity. Specifically, the ice plant contained polyphenols at 205.22mgGAE/100g FW, flavonoids at 32.02mgQE/100g FW, tannins at 62.74mgTAE/100g FW, saponins at 162.34mgSE/100g FW, and alkaloids at 234.02mgCE/100g FW. The total chlorophyll and carotenoids content were 11.74mg/g FW and 0.57mg/g FW. The antioxidant activities measured by DPPH and FRAP were 75.74% and 73.72mM FeSO4/100g FW, respectively. If the ice plant is intended for consumption as a food vegetable, a lower salt concentration of 30-70mM NaCl is recommended, as excessive salt accumulation in the plant could affect its taste and consumer health.

Keywords: Antioxidant activities, Bioactive compounds, Color compounds, *Mesembryanthemum crystallinum*

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INTRODUCTION

With the growing global population, the demand for food production will increase, adding more pressure on resources of water and land. Furthermore, climate change is causing higher soil salinity and drier conditions, which are among the most severe environmental challenges for plants, leading to substantial losses in plant yields worldwide. Consequently, understanding plant responses to salinity and drought is essential to identify the best conditions for plant production (Mndi et al., 2023; Zafar et al., 2024).

The ice plant (*Mesembryanthemum crystallinum* L.) is belonging to the group of halophytes (Loconsole et al., 2019). The plant can survive in various conditions and different climates, such as drought, salinity and prolonged cold (Bohnert and Cushman, 2000; Tembo-Phiri et al., 2019). The appropriate temperature for growth is from 12

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to 30°C and in favorable conditions, plants photosynthesize in the C_3 pathway, but when affected by stress such as poor nutrition, drought, and salinity, plants will strongly take the CAM photosynthetic pathway (Tembo-Phiri et al., 2019). Its medicinal benefits are noted in Tunisian traditional medicine, people used leaf extracts to cure diseases of eye, mouth and throat (Saad and Said, 2011). Additionally, the fresh leaf extract helps decrease illness about lung, urinary tract, respiratory and water retention. The leaves are rich in minerals, organic acids, vitamins and many bioactive compounds (alkaloid, polyphenols, carotenoids, flavonoids, saponins, tannins), and especially the active ingredient D-pinitol has the same function as insulin to help support and stabilize blood sugar (Essa & Elsebaie, 2018). Thus, this plant is regarded as a highly functional food (Mndi et al., 2023). The ice plant is used for the production of pharmaceuticals because of its rare nutrients and bioactive compounds, especially high level of antioxidant activity (Rodríguez-Hernández and Garmendia, 2022).

The ice plant is a salt-loving plant that can withstand salinity concentrations up to 500mM (Madhavi et al., 2022). However, plants will switch from the C3 to CAM photosynthetic pathway and induce premature flowering when exposed to high salt concentrations, and this condition caused reduces in plant productivity. In addition, high salt concentrations are associated with salt accumulation in leaf stems, which may be a concern for human health (Paz et al., 2023). According to Xia and Mattson (2022), ice plants grow optimally in saline conditions at concentrations from 50mM to 100mM. Therefore, in this experiment, the salt concentration was set at a maximum of 100mM.

The objective of the study was to determine the effects of NaCl concentration added in nutrient solution on the content of biological compounds, pigments and antioxidant capacity of semi-hydroponically cultivated ice plant in An Giang, Vietnam. Also, the process is suggested for growing ice plants as food vegetables.

MATERIALS & METHODS

Experimental Design

Ice plants are grown in a net house at An Giang University, the net house is closed/covered to prevent insects, the roof of the net house has a rain cover, the roof height is 4.5 m, the area is 200 m^2. The implementation period starts from December 2022 to March 2023. A completely randomized block design was applied with one factor; NaCl salt concentration with six levels of 0, 10, 30, 50, 70 and 100mM, with 3 repetitions. Each treatment corresponds to a 5 m^2 growing shelf for one replicate.

Prepare Seedlings

Ice plant seeds are sown on special coconut fiber substrate, 3-5 seeds are sown in each bulb, size of nursery bulb is 3 x 3 x 4cm, under appropriate temperature conditions (25-30˚C). The seven days after the seeds germinate, prune away small, weak plants and leave strong plants (1 plant/pot). Then moved the plants to the net house in the cool afternoon to gradually adapt to the environmental conditions. Provide nutrients when plants are 7 days old, once a week. The remaining days keep the plant moist, humidity is about 60-70%. The 28-day-old seedlings were transferred to shelves and planted in a semi-hydroponic style, the substrate used was smoked rice husks. Planting density is 40x20cm, 1 tree/hole (12.5 trees/m^2), planted in crocodile style.

Experimental Care

Nutrition is provided to plants in liquid form, mixed according to the formula of Van der Lugt et al. (2020) for leafy vegetables in a 50 L tank and watered by a circulatory system. An automatic drip irrigation equipment was used. Nutrition is supplemented with fresh mix, once a week. Salt was added to hydroponic nutrients at six levels of 0, 10, 30, 50, 70 and 100mM, respectively for each treatment. The added salt was in the form of NaCl (Co.op Select brand). Plants are watered at 7:00 am and 3:00 pm for 15min each time per day. Watered nutrients are recovered according to the royal circulation system. pH was maintained between 6.5-7.0 and EC=1.2. After 75 days of planting, harvest, analyze and evaluate research criteria (Fig. 2).

Preparation of Sample and Chemical

Ice plants were collected from the experimental practice area in An Giang University (Fig. 1) (latitude: 10°37'16.77'' N, longtitude: 105°43'20.98'' E), with growth time of 75 days after cultivation and taken to the laboratory ready for analysis (Fig. 2). The samples were prepared according to different methods to determine bioactive compounds, color substance and antioxidant activities.

Chemical used including Folin-ciocalteu, Folin-denis, Quercetin, Gallic, Tannic, Colchicine, Saponin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), Acetone, $FeSO_4$, $FeCl_3$, $AlCl_3$, Na_2CO_3, KCl, CH_3COONa, $HClO_4$, HCl, H_2SO_4, etc. were purchased from brands of Sigma® (Sigma Aldrich Co.) and Merck®.

Analytical Methods

Sample Preparation

Fresh whole plant was grounded and extracted with rate of ethanol: water (60:40, v/v) for 60 minutes at a material:solvent ratio (1:20, w/v). Then it was filtered and evaluated for its antioxidant capacity and bioactive compounds. To determine the content of chlorophyll and carotenoids, fresh ice plants was extracted in acetone solvent.

The bioactive compounds were analyzed including the content of polyphenol was analyzed following method described by Hossain et al. (2013), based on standard curve (y=0.0082x + 0.0595, r^2=0.9996) to determine and the unit was used as milligram of gallic acid equivalents (mgGAE/100 gram of fresh ice plant weight-FW). The content of flavonoid was analyzed following the method described by Eswari et al. (2013), based on the standard curve (y=0.0054x + 0.0026, r^2=0.9995) to determine and the unit was used as milligram of quercetin equivalent (mgQE/100 g FW). The content of tannin was analyzed

Fig. 1: Ice plant grown at experimental practice area of An Giang University

Fig. 2: Ice plant samples are used to analyze in present study
Note: M_1=0, M_2=10, M_3=30, M_4=50, M_5=70, M_6=100mM NaCl
In while M1-M6 are denoted for the samples and 0-100 are shown for salt concentration

following the method described by Laitonjam et al. (2013), based on the standard curve (y=0.0098x + 0.0478, r^2=0.9996) to determine and the unit was used as milligram of tannic acid equivalents (mgTAE/100 g FW). The content of saponin was analyzed following the method described by Adewole et al. (2013), based on formulation (y=OD*200*V*F/3.097*W) to determine and the unit was used as milligrams saponin equivalent (mgSE/100g FW). The content of alkaloid was analyzed following the method described by Dutta (2014), based on the standard curve (y=0.0035x + 0.7552, $r^{2=}$0.9983) to determine and the unit was used as milligram colchicine equivalent (mgCE/100g FW).

The chlorophylsl and carotenoids was analyzed following the method described by Singh et al. (2014), the unit was used asmg/g FW

The ice plant extract was analyzed for antioxidant activity through DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reduction activity potential) assays. The FRAP evaluation of extract was analyzed according to the method described by Adedapo et al. (2009), based on the standard curve (y=0.5177x + 0.0855, r^2=0.9981) to determine and the unit was used as millimol ferric sulfate (mM $FeSO_4$/100g FW). The DPPH evaluation of extract was determined following to the method described by Tola et al. (2014), based on formulation DPPH (%)=[(OD control – OD sample)/OD control] x 100, where, OD control was the absorbance of control blank, OD sample was the absorbance of extract sample.

Statistical Analysis

Data were collected and statistically processed using Excel and SPSS 16.0 software. Use F test (ANOVA) and Duncan test to compare differences between treatments.

RESULTS & DISCUSSION

Effect of Salt Concentration on the Content of Bioactive Compounds in Ice Plant

The ability to synthesize phytochemicals was affected by various process during plant growth. Each different plant will conduct various metabolism and synthesis processes. The levels of primary and secondary metabolites accumulated in plants depend on genetic and environmental factors and they affect the growth and synthesis of phytochemicals in plants (Bazargani et al., 2021).

Among biocomponents, Polyphenols, found abundantly in plants, encompass a wide range of active phytochemicals. Virtually all polyphenols exhibit antioxidant properties, which are linked to their potential health benefits in some diseases such as cancer, cardiovascular, neurodegenerative, and aging. They also considered as antidiabetic agent, promoting of the immune system, protecting again the UV radiation (Koleva et al., 2021). Tannins, a prominent group of antioxidants in plants, are found in cereals, cacao, some leafy and green vegetables, coffee, tea, and nuts. They have gained considerable interest in last years by multifunctional roles and have contribution positively to human health and food flavors. Recent studies have shown that the consumption of tannins can have health benefits (Ojo, 2022; Oluwole et al., 2022). The effects of tannin on human and animal biology vary considerably and depend on the composition of the diet (Ojo, 2022; Ikusika et al., 2023). Flavonoids are another group of antioxidant compounds found in many different plants, and they have diverse biological activities (Kang & Joo, 2023).

Alkaloids can be derived from both natural (plants and animals) and synthetic sources and are typically bitter in taste (Sailaja et al., 2017; Olofinsan et al., 2023). Alkaloids affects the nervous system and have potent sedative and tranquilizing properties, and can be found in seeds, roots, stems and leaves of higher plants. Alkaloids have been used in various applications in plant and human disease treatments (Sailaja et al., 2017; Olofinsan et al., 2023).

The level of polyphenol, flavonoid, tannin, saponin and alkaloid in ice plant with various supplied salt concentrations was showed in Fig. 3. These compounds had an increase with increasing added salt concentration (from 10 to 100mM NaCl). Polyphenol ranged from 86.05 to 205.22mg GAE/100g FW (Fig. 3A), flavonoid ranged from 17.81 to 32.02mg QE/100g FW (Fig. 3B), tannin ranged from 33.72 to 62.74mg TAE/100g FW (Fig. 3C), saponin ranged from 58.92 to 162.34mg SE/100g FW (Fig. 3D), and alkaloid ranged from 111.82 to 234.02mg CE/100g FW (Fig. 3E). However, between samples of M_4, M_5 and M_6 did not have a statistical difference at the significant level $P \leq 0.05$. Results found that there was an increase from 45.4 to 138.6% in polyphenol content, from 18.8 to 79.8% in flavonoid content, from 10.1 to 86.1% in tannin content, from 3.5 to 175.5% in saponin content, from 27.4 to 109.3% in alkaloid content compared to control sample. This result was due to salt stress are considered to increase the production of metabolites in plants such as phenolic compounds, in agreement with Zhang et al. (2022). The increased photosynthetic activity at high saline concentrations may be attributed to a general biochemical stimulation in plants, rather than the induction of CAM (Crassulacean Acid Metabolism) activity (Loconsole et al., 2019). The high NaCl level influences the metabolism (metabolic rate) of plant and can cause the accumulation of phenolic compounds and the antioxidant activity of ice plants increased (Agarie et al., 2009). He et al. (2023) showed that there was increased total phenolic content when ice plants were treated to artificial seawater with different concentration. However, Mndi et al. (2023) reported that when ice plant was treated with salt concentration from 0-800ppm of NaCl, polyphenol and flavonols content of it decreased. Bayat et al. (2022) found that there was an increase bioactive compounds content,

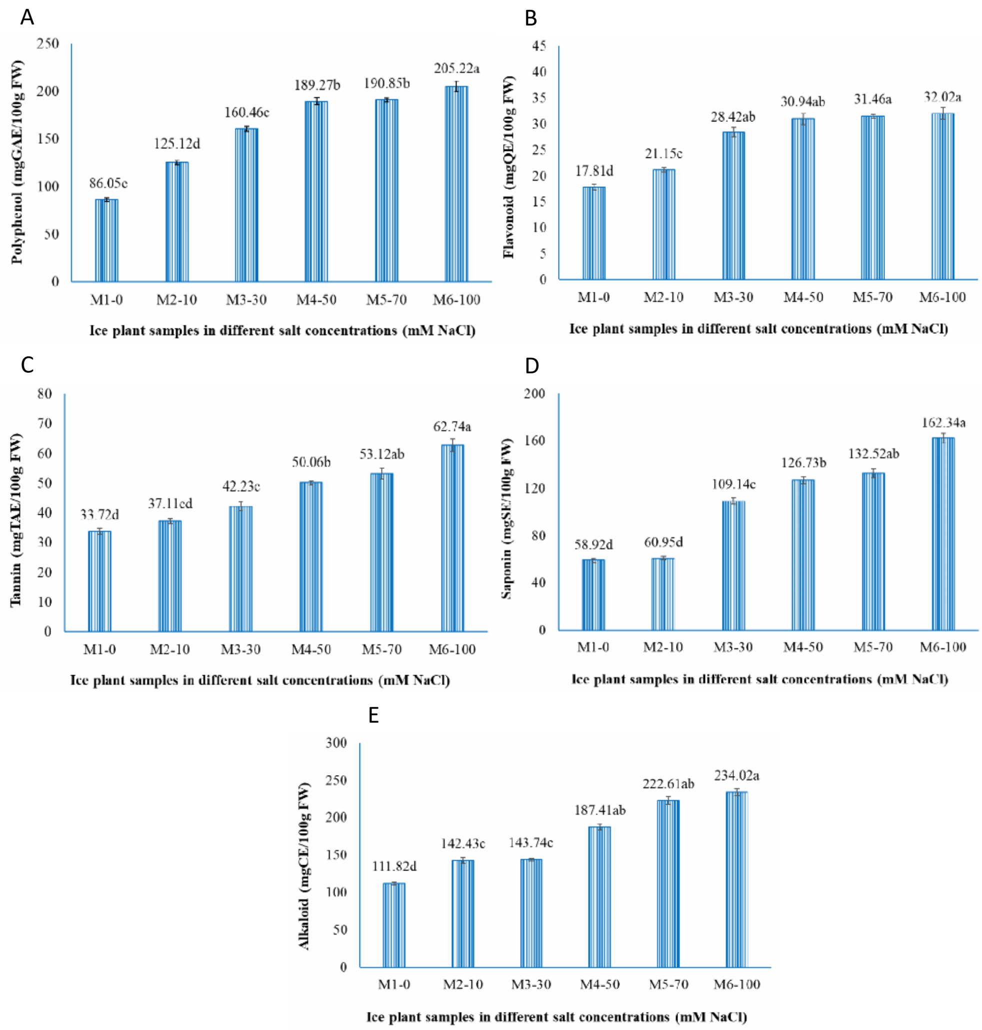

Fig. 3: Graphs show the content of polyphenol (a), flavonoid (b), tannin (c), saponin (d) and alkaloid (e) in ice plant samples
*Note: Mean bars with different letters (a, b, c, d) are statistically difference by LSD analysis at the significance level ($p \leq 0.05$)

when the plant was treated at the appropriate salt concentration. In particular, the total phenolic content in *Salvia lavandulifolia* plant increased at all tested salt levels. The content of flavonoids, anthocyanins and lycopene in the brinjal plant increased at treated salt concentrations from 100-300mM of NaCl (Jameel et al., 2024). According to Gugliuzza et al. (2023), the total phenolic level was an increase as increasing treated salt concentration from 0-150mM NaCl on the *Quercus ilex* plant. Lungoci et al. (2023) showed that phenolic and flavonoid content was an increase as increasing the appropriate salt concentration for *Nepeta cataria* plant. Šamec et al. (2021) found that the content of total phenols, total phenolic acids, total flavonoids, total flavonols was an increase in Chinese cabbage and white cabbage leaves which treated with salt at a concentration of 0-100mM NaCl and decreased when treated at a concentration of 200mM NaCl. Similarly, the total phenolic content was an increase at treated salt level from 0-150mM of NaCl and a decrease at salt concentration from 200-250mM NaCl. Total flavonoid content increased at salt concentration from 0-200mM of NaCl and decreased at a salt concentration of 250mM NaCl. Saponin content increased when increasing salt concentration from 0-200mM NaCl and decreased when at 250mM NaCl in *Hibiscus cannabinus* plant (Birhanie et al., 2022). Soheilikhah et al. (2021) reported that it had a notable increase in the level of phenolic, flavonoids, anthocyanins and saponins in *Hyssopus officinalis* plants which treated with salt at a concentration of 50-200mM NaCl. Azeem et al. (2023) reported that salt stress from 0-100mM of NaCl on *Moringa oleifera* plant increased phenolic and flavonoid content.

Effect of Salt Concentration on the Content of Color Compounds of Ice Plant

Chlorophyll is a photosynthetic pigment responsible to giving plants green color. In human body, chlorophyll was also considered as an antioxidant and created more attractive colors for products when consumed. Besides, chlorophyll was ability to against cancer disease due to can prevent the absorption of carcinogenic compounds in the digestive system (Viera et al. 2019; Hulkko et al. 2022). In high plants, chlorophyll has two common forms: chlorophyll a and b with an approximate ratio of 3:1 and chlorophyll a shown a blue green and chlorophyll b shows a yellow-green (Cvitkovic et al., 2021). However, it has been identified that this ratio of chlorophyll a to chlorophyll b varies depending on genetic and environmental conditions (Subham et al., 2018, Ana et al., 2020). Carotenoids considered as pro-vitamin A, against cancer disease, improving cognitive ability of brain and vision of eye. As well as, carotenoids also participate in immune regulation activities and prevent degenerative diseases (Eggersdorfer & Wyss, 2018).

In the current study, the level of chlorophyll a, chlorophyll b, and total chlorophyll in the samples all tended to increase with increasing added salt level. The content of chlorophyll a ranged from 5.02 to 7.41mg/g FW (Fig. 4A), chlorophyll b ranged from 1.91 to 4.32mg/g FW (Fig. 4B), and total chlorophyll ranged from 6.93 to 11.74mg/g FW (Fig. 4C), reaching the highest content in samples with an added salt concentration of 100mM NaCl (M_6 sample). In particular, for samples with added salt concentrations of 30-50mM NaCl (M_3, M_4, and M_5 samples), the level of chlorophyll a, chlorophyll b, and total chlorophyll did not show a statistical difference at the significant level $P \leq 0.05$. The total carotenoid content in ice plants also tended to increase with increasing added salt concentration from 0-30mM NaCl, and reaching the highest content of 0.63mg/g FW (M_3 sample), and was statistically different from the remaining samples. When the added salt concentration increased from 30-100mM NaCl, the carotenoid content tended to decrease slightly, and there was no statistical difference between M_4, M_5, and M_6 samples at the significance level $P \leq 0.05$ (Fig. 4D). The chlorophyll a/chlorophyll b ratio ranged from 1.72 to 3.61, with the lowest chlorophyll a/b ratio in the M6 sample and the highest in the M_2 sample. The remaining samples did not show statistical difference at the significant level $P \leq 0.05$ (Fig. 4E). The chlorophylls/carotenoids ratio ranged from 14.05 to 20.52 and tended to increase with increasing salt concentration (Fig. 4F). The chlorophylls/carotenoids ratio is considered an index of environmental stress on plants and damage to the photosynthetic system, impacting the aging of plants (Filimon et al., 2016). Acosta-Motos et al. (2017) showed that salt-loved plants adapted to salt treatment by keeping or rising chlorophyll content as a way to protect photosynthesis. However, Rodríguez-Hernández and Garmendia (2022) observed there was high chlorophylls and total carotenoids levels in ice plant samples treated with salt. He et al. (2023) reported there was an increase total chlorophyll content and chlorophylls/carotenoids ratio in ice plants treated with different salt concentrations, but carotenoid content and the chlorophyll a/chlorophyll b ratio did not differ significantly.

Moreover, Xu and Mou (2016) showed that under controlled nutritional conditions, salt stress (10-40mM NaCl) significantly increased the chlorophyll content in spinach, although the carotenoid content increased only slightly. Sanpapao et al. (2023) found that when culturing the microalga *Dunaliella salina*, chlorophyll and carotenoid content increased at salt concentrations from 0.00-0.07 g NaCl/L, and with treated salt levels from 0.5-2.5 M NaCl, and chlorophyll content tended to increase, while carotenoid content tended to decrease. Additionally, it has been reported that an increase in carotenoid content is often accompanied by high levels of reactive oxygen species in plant cells exposed to abiotic stresses, such as high or low salinity beyond the optimal level (Sanpapao et al., 2023; Zafar et al., 2023).

Another study showed that most plants have a large accumulation of carotenoids under different treated conditions including light, salinity, and nutrient (Moslemipetroudi et al., 2021). Some reports also noted an increase in total carotenoid content with increasing salt concentration (Zafar et al., 2022; Mola et al., 2023). Jameel et al. (2024) found that brinjal plants treated with salt concentrations from 100-300mM of NaCl showed decreased chlorophyll but increased carotenoids.

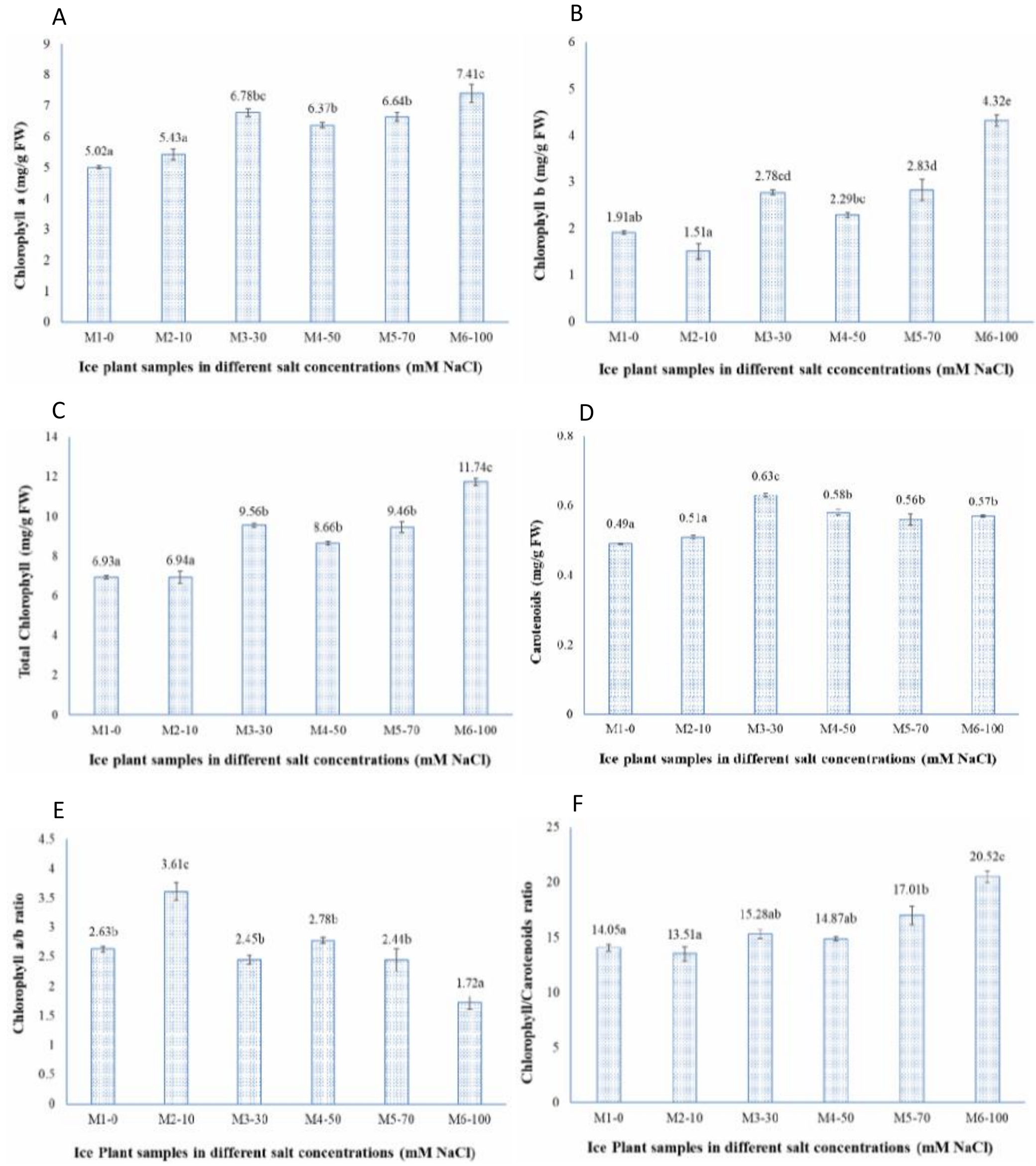

Fig. 4: Graphs show the content of chlorophyll a (a), chloropyll b (b), tottal chlorophyll (c), carotenoids (d), chlorophyll a/b ratio (e), and chloropyll/carotenoids ratio (f) in; ice plant samples; Note: Mean bars with different letters (a, b, c, d) are statistically difference by LSD analysis at the significance level (p≤0.05)

Šamec et al. (2021) showed that it had an increase in the concentration of color compounds (chlorophylls and carotenoids) in Chinese cabbage and white cabbage leaves treated with salt concentrations from 0-100mM of NaCl, with a decrease observed when treated at a salt concentration of 200mM NaCl.

Effect of Salt Concentration on the Antioxidant Activity of Ice Plant Extract

The natural antioxidants (polyphenols, flavonoids, carotenoids, vitamin C and other secondary metabolites) present a lot of plants can stop or put off cell damage due to oxidants including oxygen, nitrogen, and free radicals. In addition, antioxidants have the ability to prevent the development of various chronic and degenerative sickness (Munteanu and Apetrei, 2021; Nwachukwu et al., 2021). The DPPH and FRAP assay are popular methods for evaluating antioxidant activity of antioxidant components in both foods and isolated extracts, due to their simplicity, affordability, and rapid execution make them ideal for efficiently screening numerous samples (da Rosa et al., 2024).

In the present research, the antioxidant level of ethanol extract from ice plant was measured by the DPPH and FRAP assays. Results showed that salt stress affected scavenging of free radicals (DPPH) and ferrous reducing activities (FRAP) with $P \leq 0.05$. The samples had a tendency to increase antioxidant level with increasing supplied salt concentration from 10-100mM NaCl. Specifically, the percent of scavenging free radicals DPPH of samples were 39.31% (M_1-control), 70.92% (M_2-10mM NaCl), and 75.74% (M_6-100mM NaCl). These treated samples exhibited higher antioxidant activity than the control sample by 80.4 to 92.7% (Fig. 5A). Similarly, the ferrous reducing ability of samples also increased from 46.41 to 73.32mM $FeSO_4$/100g FW, expressed higher iron reduction ability than the control sample by 14.7 to 57.8% (Fig. 5B). The increase in antioxidant activity was considered to be linked to the level of antioxidant components accumulation in ice plants when exposed to salt stress.

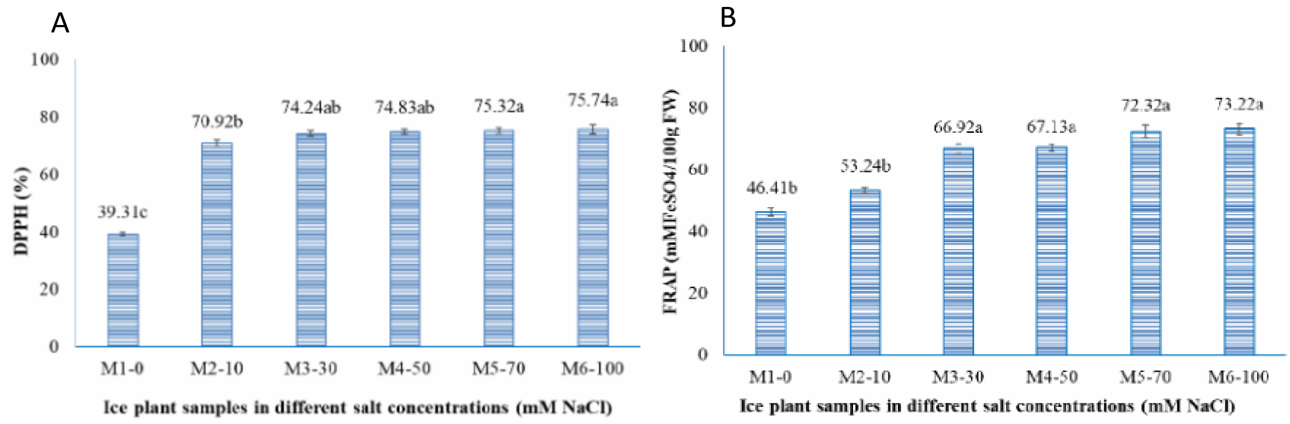

Fig. 5: Graphs show the antioxidant activity as DPPH (a) and FRAP (b) of ice plant rude extracts; Note: Mean bars with different letters (a, b, c, d) are statistically difference by LSD analysis at the significance level (p≤0.05)

This result was also similar to the findings of other researchers. Gugliuzza et al. (2023) found an increase in total antioxidant activity in *Quercus ilex* plants when increasing the treatment salt concentration from 0-150mM NaCl. Lungoci et al. (2023) showed there was an increase in free radical scavenging ability (DPPH) with appropriate salt concentration for *Nepeta cataria* plant. Similarly, Azeem et al. (2023) reported that salt stress from 0-100mM of NaCl in *Moringa oleifera* plant increased scavenging of free radicals DPPH and iron reducing ability by FRAP assay. However, Birhanie et al. (2022) showed there was an increase in DPPH value with treated salt concentration from 0-150mM of NaCl, followed by a decrease with salt concentration from 200-250mM of NaCl. Similarly, for the iron reduction ability (FRAP assay), there was an increase with the treated salt concentration from 0-100mM of NaCl, and a decrease with salt concentration from 150-250mM of NaCl. Mndi et al. (2023) showed that salt stress from 0-800 ppm NaCl did not affect the iron reduction ability (FRAP assay) of ice plant extract, while the DPPH radical scavenging ability was slightly reduced. According to Sanpapao et al. (2023), it had a decrease in DPPH value when *Dunaliella salina* plant was treated in salt levels from 0.5-2.5 M of NaCl. Uzlasir et al. (2023) reported there was an increase in DPPH value when the microalgae *Phaeodactylum tricornutum* was treated with salt concentrations from 15-30 ‰ and a decrease with salt level at 35 ‰.

Conclusion

Research findings showed that treating salt at concentrations from 10-100mM NaCl had a significant effect on the antioxidant components of ice plants. At salt concentrations of 30-70mM NaCl, the plants grew well in terms of biomass, and the accumulation of bioactive compounds, color compounds, and antioxidant activity was also quite high. At a salt concentration of 100mM NaCl, ice plants experienced a decrease in biomass, but the accumulation of bioactive compounds, color compounds, and antioxidant activity was higher (Figs. 1-4). These results suggest that, depending on the purpose of using the ice plant (such as using it as a food vegetable or for producing pharmaceuticals and cosmetics), farmers can choose the appropriate salt concentration during the cultivation process to harvest ice plants that meet their target.

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