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EFFECTS OF SALINITY ON GROWTH, ANTIOXIDANT CONTENTS AND PROXIMATE COMPOSITIONS OF SABAH SNAKE GRASS (*CLINACANTHUS NUTANS* (BURM. F.) LINDAU)

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Abstract

This study was carried out to determine the effect of salinity on growth, antioxidant contents and proximate compositions of Sabah snake grass (*Clinacanthus nutans* (Burm. f.) Lindau). Six salinity levels were used, namely 0 (control), 4, 8, 12, 16 and 20 dS/m. Highest salinity level, 20 dS/m, significantly increased the phenolic content (1.95 mg GAE/g), flavonoids content (3.84 mg QE/g), and proximate compositions such as ash content (19.83%), crude protein content (16.43%), crude fat content (18.45%) and crude fiber content (10.73%) of *C. nutans* although the plant growth and leaf relative water content were reduced. Therefore, salt-stressed *C. nutans* can be considered as excellent sources of antioxidant contents and protein for human consumption.

Introduction

Plants are good source of minerals, vitamins and phytochemicals. Two groups of phyto-chemical, namely phenolic and flavonoid compounds, are known as antioxidant agents (Landrum and Bone 2001). These two phytochemical compounds had also been identified in *Clinacanthus nutans* (Burm. f.) Lindau (Sathisha 2013). Several researches have shown that many medicinal plants have therapeutic potentials as natural antioxidants due to their phenolic components (Cook and Samman 1996). The presence of phenolic and flavonoids plays an important role in reducing free radical induced tissue damage (Mimica-Dukic *et al.* 2004) by activated oxygen species (Rice-Evans and Packer 1998) and in the maintenance of health and protection from some age-related degenerative disorders such as cancer and coronary heart diseases (Hill 1952).

Salinity affects plant growth because the high concentrations of soluble salts through their high osmotic pressures restrict the uptake of water by the roots and interferes with balanced absorption of essential nutritional ions by plants (Tester and Devenport 2003). Besides, salinity affects both the primary and secondary metabolism of the plant and hence gives different bioactivity of the plants (Hong *et al*. 2008). Salinity causes water deficit, resulting in the generation of oxidative stress in plant tissues by impairing the cellular electron transport within different subcellular compartments, leading to the generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radicals (Sreenivasulu *et al*. 2000). Plants respond to oxidative stress by ROS scavenging through activation of the antioxidant system, which includes both enzymatic and non-enzymatic defence mechanisms. The non-enzymatic system involves the synthesis of several secondary metabolites of the phenylpropanoid pathway, such as flavonoids, phenolic acids, tannins and phenolic diterpenes.

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In this case, phenolic compounds play an important role in adsorbing and neutralizing free radicals, quenching Singlet Oxygenor decomposing Peroxide (Ksouri *et al*. 2007; Oueslati *et al*. 2010). In short, salinity or salt stress is closely related to the accumulation of polyphenol constituents such as flavonoids and phenolic acids. Hence, it is very important to evaluate the effect of salinity on growth and antioxidant contents of *C. nutans. Clinacanthus nutans* is a popular medicinal plant in south-east Asia with reported bioactivities, but the effects of salinity on *C. nutans* physiology have been little studied. Considering the increase of salinization on arable lands, this study was carried out to test the effect of salinity on growth, antioxidant content, and proximate compositions of *C. nutans*.

Materials and Methods

The experiment was carried out at Faculty of Sustainable Agriculture, Universiti Malaysia Sabah during June to November, 2015. Field experiment was conducted under a rain shelter that allows full sunlight for plants establishment and experimental analysis was conducted in laboratory.

The soil was filled into 30 polythene bags of size 46 cm height × 39 cm wide. *Clinacanthus nutans* was planted and grown for eight weeks with non-saline irrigation water in order to achieve full establishment prior to treatment. The required quantity of sea water was collected from Sulu Sea, Mile 0, Sandakan, Sabah (8.500437, 120.845047). The EC of sea water was determined and showed 40.4 dS/m. Six salinity treatments, namely $T_1 = 0$, $T_2 = 4$, $T_3 = 8$, $T_4 = 12$, $T_5 = 16$ and $T_6 = 20$ dS/m were evaluated in this study. The control plants were irrigated with non-saline water. To avoid osmotic shock, salinity levels were gradually increased by daily increments of 4 dS/m in all treatments until the final salinity levels were achieved. After five weeks, when the targeted salinity levels were achieved, 200 mL of the respective treatment solutions were applied to each polybag on a daily basis for a period of four weeks at morning time. At evening time, all plants were irrigated with non-saline water to avoid accumulation of salts in the plants.

The plant height, leaf length and leaf width of each plant was measured in cm from the replications of each treatment and then averaged to get the mean.

Relative water content (RWC) was determined as described by González and González-Vilar (2001). The leaf relative water content was determined by using the formula:

$$\text{RWC (\%)} = \frac{\text{(Fresh weight - Dry weight)}}{\text{(Fully turgid weight - Dry weight)}} \times 100\%$$

Ethanolic extraction of *C. nutans* samples was done according to the method described by Crozier *et al.* (1997) with slight modification. The phenolic content of plant extracts was determined by the Folin-Ciocalteu's reagent based on the method described by Singleton and Rossi (1965). Phenolic content of *C. nutans* extracts was determined by using the following formula and expressed in mg GAE/g dry sample.

$$C = \frac{GAE \times V \times DF}{M}$$

where, C = Phenolic content in mg GAE/g dry sample, GAE = Concentration of gallic acid from the calibration curve in mg/ml, V = Volume of the sample in ml, DF = Dilution factor, and M = Weight of dry sample extract in g.

The flavonoids content of plant extracts was determined by using aluminium chloride calorimetric method described by Chang *et al.* (2002) with a little modification. The proximate compositions of dried *C. nutans* leaves including ash content, crude protein content, crude fat content, and crude fiber content, were determined using standard analytical methods (AOAC

2003). All measurements were done in three replicates and values presented in percentage. Crude protein content was determined using combustion method by LECO CHN628 elemental determinator. Crude protein was estimated as % N × 6.25. Crude fat content was determined by semi continuous solvent extraction methods (Soxhlet 1879) with a little modification. The crude fat in the initial sample was calculated as:

$$\text{Crude fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100\%$$

The recorded data were analysed statistically following CRD using SAS statistical software package version 9.4 (2012). The treatment means were compared by LSD test at 5% significant level.

Results and Discussion

The results showed that plant height of *Clinacanthus nutans* significantly decreased with increasing salinity levels (Table 1). From the results, it is clear that control plants showed the highest plant height (90.08 cm), closely followed by plants treated with 4 dS/m salinity level (85.64 cm). Plant height was statistically similar in plants grown at 8, 12 and 16 dS/m salinity level, and significantly reduced to 73.59 cm at the highest salinity level (20 dS/m). Maximum plant height reduction was found at 20 dS/m treatment (18.30%) compared to control treatment.

The results showed that increased salinity level caused insignificant reduction in leaf length of *C. nutans* (Table 1). The highest leaf length was found in control plants (15.06 cm), followed by plants treated with 4 dS/m salinity level (14.70 cm). At 20 dS/m treatment, plants showed the lowest leaf length (14.20 cm) with a maximum leaf length reduction of 5.71% compared to the control treatment.

Generally the results showed a decrease in leaf width of *C. nutans* with increasing salinity although statistical analysis did not show significant difference among the treatments (Table 1). From the results, control plants had the highest leaf width (4.00 cm), followed by plants treated with 4 dS/m salinity level (3.94 cm). As compared to control treatment, leaf width of *C. nutans* had reduced 4.40% to 3.82 cm in plants that were treated with the maximum concentration, 20 dS/m.

Table 1. Effect of salinity on morphological growth of *C. nutans.*

Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)
T_1 = 0 dS/m	90.08a	15.06a	4.00a
T_2 = 4 dS/m	85.64ab	14.70a	3.94a
T_3 = 8 dS/m	79.54bc	14.46a	3.88a
T_4 = 12 dS/m	80.71bc	14.42a	3.88a
T_5 = 16 dS/m	78.06bc	14.28a	3.83a
T_6 = 20 dS/m	73.59c	14.20a	3.82a

Means followed by the same letter in column are not significantly different (LSD test, $p < 0.05$).

From the results, we found that increasing salinity tended to increase the phenolic content of *C. nutans* (Table 2). The phenolic content of *C. nutans* was augmented from 1.07 to 1.95 mg GAE/g in control plants and plants treated with 20 dS/m, respectively. The phenolic content of untreated *C. nutans* was corresponded with work reported by Raya *et al.* (2015) which ranged between 0.93 and 1.17 mg GAE/g in matured and young leaves of *C. nutans*, respectively. It was also reported in studies conducted with barley (Ghafoor *et al.* 2015), European searocket (Ksouri

et al. 2007), and red pepper (Navarro *et al.* 2006) that phenolic content rose with increasing salinity levels.

Table 2. Effect of salinity on phenolic content and flavonoids content of *C. nutans.*

Treatment	Phenolic content (mg GAE/g)	Flavonoids content (mg QE/g)
T_1 = 0 dS/m	1.07d	1.76e
T_2 = 4 "	1.51c	2.15d
T_3 = 8 "	1.70b	2.45c
T_4 = 12 "	1.78b	3.68b
T_5 = 16 "	1.83ab	4.01a
T_6 = 20 "	1.95a	3.84ab

*Means followed by the same letter in column are not significantly different (LSD test, $p < 0.05$).

In this case, phenolic compounds play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxide (Ksouri *et al.* 2007, Ouselati *et al.* 2010). Therefore, the phenolic content of *C. nutans* was increased under salinity stress.

Flavonoids content of *C. nutans* increased alongside an increasing salinity level (Table 2). From the results, the lowest flavonoids content was observed on control plants (1.76 mg QE/g), then significantly increased at higher salinity levels. At 16 dS/m, treated plants produced the highest flavonoids content (4.01 mg QE/g), then slightly dropped to 3.84 mg QE/g at 20 dS/m salinity level. Maximum flavonoids content was found at 16 dS/m treatment (127.84%) compared to control treatment. However, at the highest salinity level, 20 dS/m, flavonoids content in *C. nutans* was slightly declined to 3.84 mg QE/g. The reduction of flavonoids content at the highest salinity level may due to the disturbance of enzymatic activities in high salinity, which in turns caused photosynthesis and production of polyphenols to be declined (Wong *et al.* 2006). Our results were in agreement with the findings of Borgognone *et al.* (2014) on artichoke and cardoon leaves, Taarit (2012) on sage (*Salvia officinalis* L.) and Lopez-Berenguer *et al.* (2009) on broccoli (*Brassica oleracea* L.), suggesting that accumulation of phenolic and flavonoids contents was significantly increased after treatment of salinity.

The effect of salinity on proximate compositions of *C. nutans* such as ash content, crude protein content, crude fat content and crude fiber content was shown in Table 3. The ash content of *C. nutans* was significantly affected by salinity (Table 3). Results showed that increased salinity tended to increase the ash content of *C. nutans*. Plants treated with high salinity levels (16 and 20 dS/m) produced the highest ash content (19.83%), with a maximum increment of 15.49% compared to control plants. The ash content was significantly low in control plants (17.17%) and plants treated with low salinity level, 4 dS/m (18.00%).

Crude protein content was lower in plants grow at 4 dS/m as compared to control plants, but increased in plants treated with salinity level higher than 8 dS/m (Table 3). The highest crude protein content was found in plants treated with the 20 dS/m (16.43%) while the lowest crude protein content was observed at 4 dS/m salinity level (11.90%).

There was a significant difference among treatments on crude fat content of *C. nutans* (Table 3). Increasing salinity level significantly increased the crude fat content of *C. nutans*. The highest crude fat content, about 37.28% more than the control, was observed at 20 dS/m (18.45%). In contrast, the lowest crude fat content was found in control plants (13.44%), which showed significant differences with all other treatments.

Proximate analysis was carried out to know the nutritional significance of *C. nutans* under salinity treatments. From the results, increasing salinity increased ash content, crude protein content, crude fat content and crude fiber content of *C. nutans*. As described by McClements (2003), the ash content is a measure of the total amount of minerals present within a plant, whereas the mineral content is a measure of the amount of specific inorganic components present within a plant, such as calcium (Ca), sodium (Na), potassium (K) and chloride (Cl). The ash content was significantly increased in plants treated with high salinity level compared to untreated control plants due to the accumulation of minerals such as Na and Cl in treated plants. The results of this study are also in conformity with Uzun *et al.* (2013) and Amouei (2013) where they observed increasing ash content with increasing salinity levels on bitter vetch and *Atriplex leucoclada* L, respectively. As shown in the results, we found that *C. nutans* had ash content ranged between 17.17% in control plants and 19.83% in plants treated with 16 and 20 dS/m salinity level. Our data were in agreement with Kharnngan (1991) who stated that the total ash content of *C. nutans* is less than 21%.

Table 3. Effect of salinity on proximate compositions of *C. nutans* leaves.

Treatment	Ash (%)	Crude protein (%)	Crude fat (%)
T1 = 0 dS/m	17.17b	12.28c	13.44d
T2 = 4 dS/m	18.00b	11.90d	15.17cd
T3 = 8 dS/m	19.33a	12.28c	16.41bc
T4 = 12 dS/m	19.67a	12.91b	16.87abc
T5 = 16 dS/m	19.83a	12.92b	17.74ab
T6 = 20 dS/m	19.83a	16.43a	18.45a

*Means followed by the same letter in column are not significantly different (LSD test, $p < 0.05$).

Results of a recent study by Ali *et al.* (2014) on *Portulaca oleracea*, *Hibiscus sabdariffa* and *Sorghum bicolor* support the previous results. Uzun *et al.* (2013) and Kapoor and Srivastava (2010) also reported that increasing salinity level tended to enhance the crude protein synthesis on bitter vetch and *Vigna mungo* L., respectively.

As for crude fat content in *C. nutans*, results proved that increasing salinity level significantly increased the crude fat content of *C. nutans*. The highest crude fat content was observed in plants treated with the maximum salinity treatment, 20 dS/m (18.45%), which was about 37.28% more than the control plants (13.44%). The significant increase in crude fat content of *C. nutans* in respond to salinity may because plants have some tolerance level at these salinity levels. These results fit with those previously reported on *P. oleracea* by Teixeira and Carvalho (2009) and *Oenothera biennis* seeds by Heuer *et al.* (2002). They observed an increase in the total fat content of plants there were exposed to moderate saline environment, however in contrast, the crude fat content was compromised at higher levels of salinity.

Clinacanthus nutans could be an important option for edible landscape and potential nutrition source especially for certain countries with large areas of saline soils. Because of its high potential values, there appears a need for a proper cultivation of *C. nutans* on a commercial scale where the present findings will be useful. Future research is also needed to understand and expand the knowledge of *C. nutans* as a salt-tolerant medicinal plant such as the evaluation of its relative salt tolerance at different growth stages.

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