International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 12, Issue 2, 2020

Original Article

EVALUATION OF BIOACTIVE COMPONENTS AND ANTIOXIDANT ACTIVITY OF *VERNONIA ELAEAGNIFOLIA* DC. (ASTERACEAE) IN GLYCOPHYTIC AND HALOPHYTIC CONDITIONS

JATHINA M.[1*], ABDUS SALAM A. K.[2]

[1,2]Department of Post Graduate Studies and Research in Botany, Sir Syed College, Taliparamba, Kannur, Kerala, India 670142
Email: jathina2163@gmail.com

Received: 12 Nov 2019, Revised and Accepted: 14 Jan 2020

ABSTRACT

Objective: The investigate some of the antioxidant activities and bioactive potentials of *Vernonia elaeagnifolia* grown hydroponically in glycophytic and halophytic conditions.

Methods: Plants were grown in Hoagland solution and subjected to (Sodium chloride (NaCl) treatments. Plants cultivated without salt stress served as the control. The phytochemical analysis was done with the help of Gas Chromatography-Mass Spectrometry (GC-MS) and antioxidants were analyzed following standard procedures using UV-Visible Spectrophotometer.

Results: Significant changes were observed in the presence of antioxidants like superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), total polyphenols, proline and ascorbic acid. GPX and proline proved to be the major components in imparting salinity stress tolerance. The control showed the presence of 23 bioactive components while the treatment contained 19 components.

Conclusion: The study thus provides information regarding the place of collection of the particular plant. Glycophytic conditions prove to be the best choice in terms of bioactive components and fatty acid contents. The investigations reveal that the plant contains several bioactive components and antioxidants, which prove it as a promising plant for developing potential drugs.

Keywords: *Vernonia elaeagnifolia,* Asteraceae, GC-MS, Antioxidants, Glycophytic, Halophytic

DOI: http://dx.doi.org/10.22159/ijcpr.2020v12i2.37490. Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

India has one of the richest gene pool of medicinal plants in the world [1]. Medicinal plants form an important part of treatment in the indigenous medicine systems such as Ayurveda, Unani, Siddha, Traditional Chinese Medicine, Julu etc. [2]. Many traditional healing herbs and their parts have proved its medicinal value and used to prevent, alleviate or cure many human diseases [3]. Environmental stresses impose a severe threat to the growth and distribution of medicinal plants. Abiotic stresses like salinity leads to several changes in the concentration of phytochemicals and antioxidants, which in turn affect the bioactive potential of plants. Even though the effect of salinity stress has been investigated in a number of plants, the effect of the stress on plants in general as well as medicinally important plants, in particular, have not yet been elucidated. *Vernonia elaeagnifolia* (Family-Asteraceae) is a quick-growing evergreen climber, able to thrive in both glycophytic and halophytic conditions. Its leaves are made into paste form with goat's milk and consumed in the morning by local people of Pachalur and Periyur tribes to get relief from sprain [4]. The leaves are also used by local people as leech repellent [5]. Salinity stress cause alterations in the phytochemical composition of plants. Hence, detailed information regarding the place of collection of medicinal plants is essential to extract out the complete medicinal potential of plants. Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable tool to analyze the volatile and semi-volatile molecules present in biological samples. It also serves as the paramount choice to investigate the tolerance mechanisms of plants under conditions of nutrient deficiencies, abiotic stresses and mineral toxicities. The present study investigates some of the antioxidant activities and bioactive potentials of *Vernonia elaeagnifolia* grown hydroponically in glycophytic and halophytic conditions.

MATERIALS AND METHODS

Plant material

Plant cuttings were collected from the coastal regions, washed with water and then grown in Hoagland solution for cultivation. Modified Hoagland solution [6] prepared as described by Taiz and Zeiger [7] was used for the hydroponic study. Continuous propagation of plants was done throughout the period of experimentation.

Treatment with NaCl

Screening tests on the effect of sodium chloride (NaCl) treatments on *Vernonia elaeagnifolia* showed that tolerance of the plant varied and hence the concentrations in which the propagules survived but exhibited approximately 50% growth retardation was selected as the treatment. The treatment used for the present study is 250 millimolar (mM). Plants cultivated in Hoagland solution without any salt stress served as the control.

Sampling

Samples of treatments and control were collected at a comparable interval of four days up to 20 d of growth.

Antioxidant analysis

Enzymatic antioxidants

Enzyme extracts preparation and assay of enzyme activity

Fresh plant tissues (0.5 g) were weighed and homogenized in 5 ml of ice-cold 50 mmol potassium phosphate buffer (pH 7.0) using a pre-chilled mortar and pestle. The homogenized extract was filtered using muslin cloth and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatants were collected and used for the enzyme assay [8].

Catalase (CAT, EC: 1.11.1.6)

Activity was determined by the method of Aebi [9]. The activity of CAT was determined as a decrease in absorbance recorded at 240 nm for 1 min following the decomposition of Hydrogen peroxide (H_2O_2). The concentration of H_2O_2 reduced per min per mg protein was calculated using the Extinction coefficient of H_2O_2 (39.4 $mmol^{-1}$ cm^{-1}).

Superoxide dismutase (SOD, EC: 1.15.1.1)

Activity was assayed by the method of Giannopolitis and Ries [10]. SOD activity was monitored for determining the ability of SOD to

inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The formazan accumulation was quantified using Shimadzu UV–VIS spectrophotometer, by recording the absorbance at 560 nm against the blank. Results were expressed as units SOD mg^{-1} $protein^{-1}$.

Guaiacol peroxidase (GPX, EC: 1.11.1.7)

Guaiacol peroxidise activity was measured according to Chance and Maehly [11]. The increase in absorbance due to oxidation of guaiacol was measured at 420 nm using Shimadzu UV–VIS spectrophotometer for 3 min at intervals of 30 s.

Ascorbate peroxidase (APX, EC: 1.11.1.11)

Activity was assayed as described by Nakano and Asada [12]. The absorbance was read at 290 nm at an interval of 15 sec up to 60 seconds. One unit of the enzyme was defined as µ moles of ascorbate oxidized per minute per mg protein.

Non-enzymatic antioxidants

Ascorbic acid estimation

Ascorbic acid content was measured by the method of Mukherjee and Choudhari [13]. The estimation was based on the reduction of dinitrophenylhydrazine to phenyl hydrazone. The sample was extracted using 6% Trichloroacetic acid (TCA) and the concentration of ascorbic acid in the sample was calculated from a standard curve of known concentration of ascorbic acid in 6% TCA.

Proline estimation

Free proline content was extracted from fresh plant samples using 3% sulphosalicylic acid and estimated following the method of Bates *et al.* [14] using L-proline as standard.

Total phenolic content estimation: The total phenolics of the plant extract was determined by the method described by Makkar [15] using Folin–Ciocalteau Phenol reagent and the results were expressed in terms of Gallic acid equivalents (GAE).

GC-MS analysis

The GC-MS analysis of leaf extracts of the selected plants was performed using Thermo Scientific Trace 1300 Gas chromatograph with TG-5MS Column (30m x 0.25 mm ID x 0.25µM) interfaced to an ISQ–QD Mass Specrophotometer (Perkin-Elmer GC Clarus 500 system) at R. D., Sir Syed College, Taliparamba, Kannur. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 1 µl was employed. The injection port temperature was set at 280 ° C and ion source temperature at 200 ° C. The oven temperature was programmed from 60 ° C for 3 min with an increase of 5 ° C/min to 240 ° C with a hold time of 5 min. The scan interval was programmed for 0.2 sec with a mass range of 40–550 amu. The total GC running time was 35 min. The components were identified based on the comparison of their relative retention time and mass spectra with those of the Wiley NIST 7N Library data. The results were also confirmed by comparing the compounds of elution and their respective indices on non-polar phases with other available literatures.

Statistical analysis

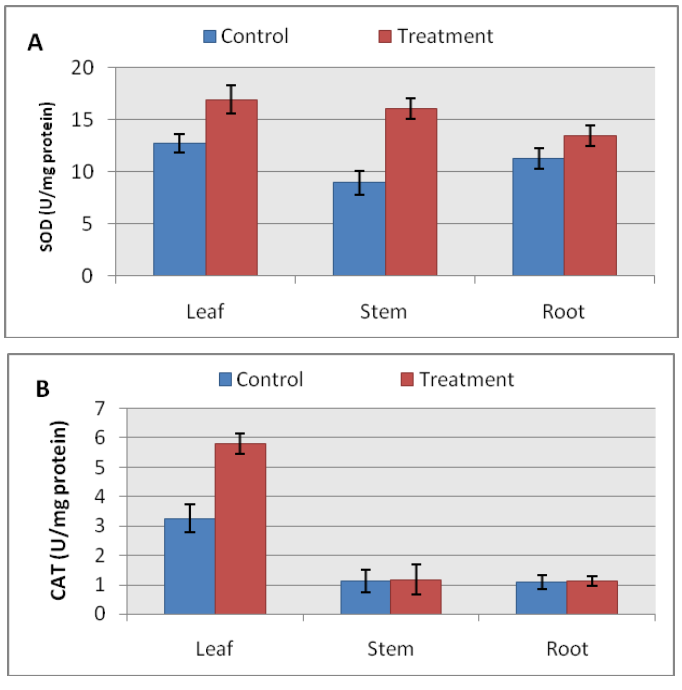
The statistical analysis was done using Microsoft excel. Each set of data is an average of triplicates. The data represents a mean±standard error.

RESULTS

Reactive oxygen species (ROS) are routinely produced during various physiological processes of the plants. However, under stress conditions such as abiotic or biotic stresses, there is an increased production of reactive oxygen species which causes unrestricted oxidative damage to the cells. Significant changes were observed in the concentration of antioxidants in *Vernonia elaeagnifolia* when grown in saline and non-saline conditions. Both enzymatic and non-enzymatic antioxidants showed an increasing trend upon exposure to saline conditions. Fig. 1A shows the amount of SOD in the case of both control and treatment. The leaves exhibited more activity than stem and root tissues.

The catalase activity increased about two-fold in case of treatment in the leaf tissues. The stem and root tissues showed only a slight increase in catalase activity (fig. 1B). The fig. 1C shows the ascorbate peroxidase activity. Undertreatment conditions, APX activity was higher in the leaf and stem tissues. The root tissues showed a gradual reduction in the amount of ascorbate peroxidase activity in case of the control.

Out of the analyzed enzymatic antioxidants, guaiacol peroxidase proved to be the major antioxidant that provides salinity stress tolerance. Fig. 1D shows the GPX activity of *Vernonia elaeagnifolia.* The leaf tissues exhibited a 3fold increase in the amount of guaiacol peroxidase activity. The root tissues showed a two-fold increase in the GPX activity in case of treatment when compared to the control.



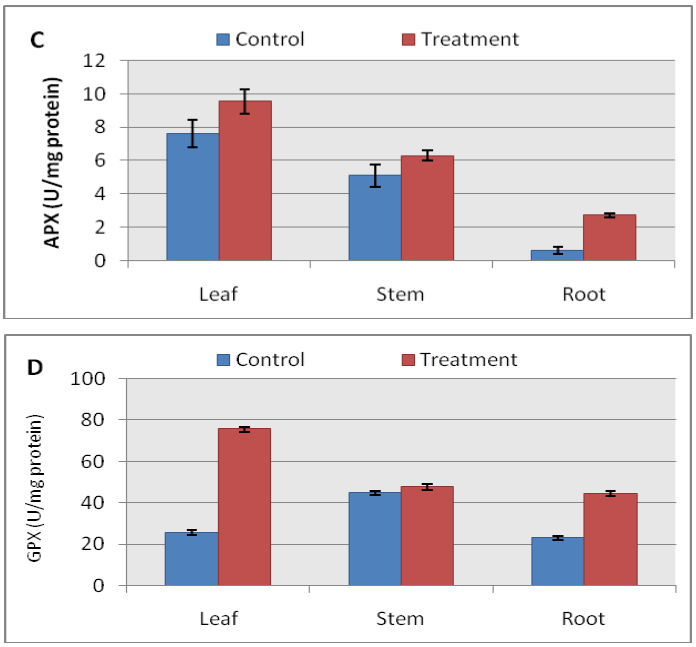


Fig. 1: Enzymatic antioxidants: A) Superoxide dismutase (U/mg protein). B) Catalase (U/mg protein). C) Ascorbate peroxidase (U/mg protein). D) Guaiacol peroxidase (U/mg protein)

The non-enzymatic antioxidants also showed a significant increase in their activity upon exposure to saline conditions. All the tissues (leaf, stem and root) exhibited a significantly higher concentration of ascorbic acid and total phenol content (fig. 2A and fig. 2B). Ascorbic acid content was higher in root tissues when compared to leaf and stem tissues. The proline content exhibited a 2 fold increase in the leaf tissues, 6 fold increase in stem tissues and a 4 fold increase in root tissues (fig. 2C).

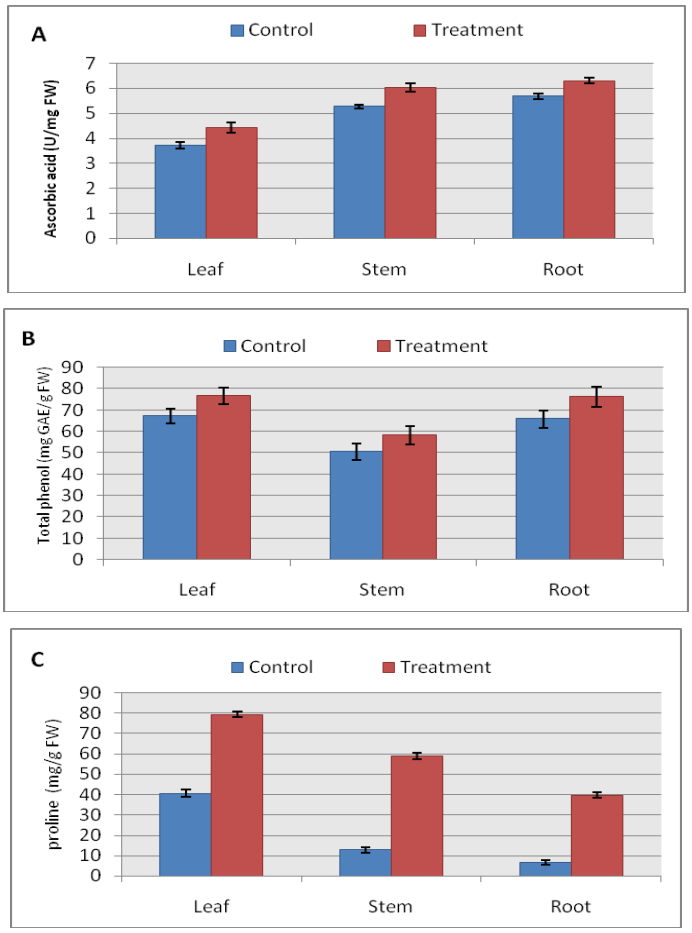


Fig. 2: Non-enzymatic antioxidants: A) Ascorbic acid (mg/g fresh weight). B) Total Phenol [mg gallic acid equivalents (GAE)/g fresh weight]. C) Proline (mg/g fresh weight)

The GC-MS analysis was done using chloroform extract of leaf tissues of *Vernonia elaeagnifolia.* The chromatogram revealed the presence of numerous peaks corresponding to different bioactive components (fig. 3A and fig. 3B).

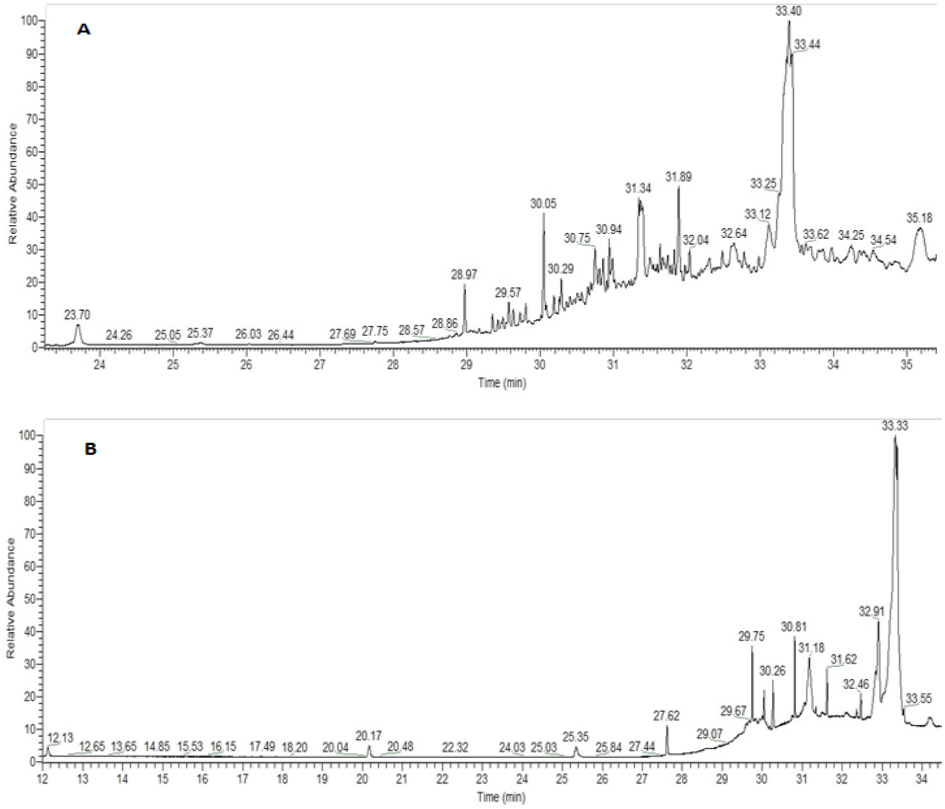


Fig. 3: GC-MS chromatogram of chloroform leaf extract: A) Control. B) Treatment

Table 1: List of bioactive compounds in chloroform extract of *V. elaeagnifolia*: control

S. No.	Name of the compound	Molecular formula	Retention time	Peak area %
i) Terpenes				
1	Dehydrofukinone	C15H22O	23.7	1.74
2	Neophytadiene	C20H38	30.05	3.09
3	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	C20H30O	31.89	3.03
4	á-Amyrin	C30H50O	33.62	0.52
ii) Phenol				
5	2,4-Di-tert-butylphenol	C14H22O	25.37	0.2
iii) Hydrocarbons				
6	Hexadecane	C16H34	27.75	0.07
7	17-Pentatriacontene	C35H70	31.34	7.81
8	Tetrapentacontane, 1,54-dibromo-	C54H108Br2	32.64	3.03
9	Tetratriacontane	C34H70	33.12	2.89
10	Octatriacontyl pentafluoropropionate	C41H77F5O2	33.25	0.73
11	Triacontyl pentafluoropropionate	C33H61F5O2	34.25	1.9
12	Tritetracontane	C43H88	35.18	4.81
iv) Steroids				
13	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	C33H54O3	28.86	0.11
14	Stigmastan-3,5-diene	C29H48	33.69	0.74
v) Esters				
15	Heptadecyl 3-chloropropanoate	C20H39ClO2	28.97	1.55
16	Arachidyl palmitoleate	C36H70O2	29.57	0.93
17	Dodecyl 3-mercaptopropionate	C15H30O2S	30.94	1.35
18	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	33.4	26.68
vi) Terpene alcohols				
19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	30.29	1.13
vii) Fatty acids				
20	n-Hexadecanoic acid	C16H32O2	30.75	3.45
21	Erucic acid	C22H42O2	34.41	0.87
22	Oleic acid, eicosyl ester	C38H74O2	34.85	1.2
viii) Alcoholic compounds				
23	2-Octadecoxyethanol	C20H42O2	34.54	1.62

The chloroform leaf extract of the control contained 23 compounds while the treatment contained 19 bioactive components. The bioactive components in the control included 4 terpenes, 1 phenol, 7 hydrocarbons, 1 diterpene alcohol, 2 steroids, 4 esters, 3 fatty acids and 1 alcoholic compound (table 1). Phthalic acid, di (2-propylpentyl) ester (26.68%) was the major compound present in the control. It had antimicrobial and antifouling properties. The other bioactive components in the control include Dehydrofukinone (1.74%), Neophytadiene (3.09%), Podocarp-7-en-3-one, 13á-methyl-13-vinyl-(3.03%), á-Amyrin (0.52%), Hexadecane (0.007%), 17-Pentatriacontene (7.81%), Tetrapentacontane,1,54-dibromo-(3.03%), Tetratriacontane (2.89%), Octatriacontyl penta-fluoropropionate (0.73%), Triacontyl pentafluoropropionate (1.9%), Tritetracontane (4.81%), Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (0.11%), Stigmastan-3,5-diene (0.74%), Heptadecyl 3-chloropropanoate (1.55%), Arachidyl palmitoleate (0.93%), Dodecyl 3-mercaptopropionate (1.35%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.13%), n-Hexadecanoic acid (3.45%), Erucic acid (0.87%), Oleic acid, eicosyl ester (1.2%) and 2-Octadecoxyethanol (1.62%).

Table 2: List of bioactive compounds in chloroform extract of *V. elaeagnifolia*: treatment

S. No.	Name of the compound	Molecular formula	Retention time	Peak Area %
i) Hydrocarbons				
1	3-Dodecene, (E)-	C12H24	12.13	0.82
2	Cetene	C16H32	27.62	1.98
3	10-Heneicosene (c,t)	C21H42	29.75	4.15
4	Pentacos-1-ene	C25H50	31.62	1.79
5	Heptacos-1-ene	C27H54	32.46	1.06
6	17-Pentatriacontene	C35H70	33.55	0.45
ii) Phenols				
7	2,4-Di-tert-butylphenol	C14H22O	25.35	1.26
iii) Esters				
8	Tris(2,4-ditert-butylphenyl) phosphite	C42H63O3P	29.61	0.83
9	Dibutyl phthalate	C16H22O4	30.26	2.09
iv) Steroids				
10	Campesterol	C28H48O	29.67	0.78
11	Stigmasterol	C29H48O	31.18	8.25
12	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	C33H54O3	31.51	0.72
13	ç-Sitosterol	C29H50O	32.91	14.85
14	Rubrosterone	C19H26O5	33.33	29.15
15	Ethyl iso-allocholate	C26H44O5	33.38	15.6
v) Terpene alcohols				
16	Phytol	C20H40O	31.34	0.24
vi) Terpenes				
17	Neophytadiene	C20H38	30.19	0.11
18	Spinasterone	C29H46O	35.22	0.28
19	Squalene	C30H50	35.52	0.93

The treatment contained 3 tepenes, 1 phenol, 6 hydrocarbons, 2 esters, 6 steroids and 1 terpene alcohol (table 2). Out of the 19 compounds, Rubrosterone (29.15%) was the major compound present in the treatment. The other bioactive components in the treatment include 3-Dodecene, (E)-(0.82%), Cetene (1.98%), 10-Heneicosene (c,t) (4.15%), Pentacos-1-ene (1.79%), Heptacos-1-ene (1.06%), 17-Pentatriacontene (0.45%), 2,4-Di-tert-butylphenol (1.26%), Tris(2,4-ditert-butylphenyl) phosphite (0.83%), Dibutyl phthalate (2.09%), Campesterol (0.78%), Stigmasterol (8.25%), Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (0.72%), ç-Sitosterol (14.85%), Ethyl iso-allocholate (15.6%), Phytol (3.36%), Neophytadiene (0.11%), Spinasterone (0.28%) and Squalene (0.93%).

DISCUSSION

Tolerance to NaCl stress is strongly associated with the efficiency of antioxidant enzymes, SOD, CAT and GPX in scavenging reactive oxygen species [16]. Sudden increase in SOD activity was recorded in two mangroves, *B. gymnorrhiza* and *B. parviflora* during NaCl stress [17]. The increased production of antioxidants during stress conditions was studied by Parida *et al.* [18]. The enhancement in phenol content is supposed to be an adaptive mechanism, towards various kinds of stresses [19]. Non-enzymatic antioxidants like ascorbate is a ubiquitous soluble antioxidant in plant cells which directly helps to scavenge ROS and act as a reducing substrate for APX and GPX to detoxify H_2O_2 [20]. The increased level of antioxidants in *V. elaeagnifolia* thus reveals the ability of the plant to withstand saline conditions. The variations in phytochemical composition when grown in saline and non-saline conditions also highlight the stress adaptive changes.

Most of the compounds detected using GC-MS were promising therapeutic agents. Fatty acids and alcoholic compounds were present in the control while it was absent in case of the treatment. Erucic acid, n-Hexadecanoic acid and Oleic acid eicosyl ester were the fatty acids found in the control. n-Hexadecanoic acid was reported to exhibit antimicrobial, anti-inflammatory and antioxidant activity in Vitex negundo [21]. Ogunlesi et al. [22] reported the anti-inflammatory property of Oleic acid and its derivatives in Sesamum raditum. Studies in Tinospora smilacina reported the potential of fatty acid esters and free fatty acids to act as anti-inflammatory agents [23]. In the present study, the treatment showed an increase in the number of steroids. Phytosterols play a major role in reducing the damages caused due to the production of reactive oxygen species during stress conditions [24] and also possess anti-inflammatory properties [25].

CONCLUSION

Salinization has become a serious issue in the present scenario. High salt concentration enhanced the activities of various antioxidant enzymes and clearly exhibited a differential extent of alterations in GPX, APX, SOD and CAT activities on exposure to NaCl. Phytochemical investigations revealed that the plant contains several bioactive components which prove it as a promising plant for developing potential drugs. Also, it was evident that the number of bioactive components faced retrogression on exposure to salinity stress. The increasing demand for medicinal plants has resulted in a proclivity to implement hysterical ways for the collection of drug material from plants. The present study highlights the abundance of bioactive components in glycophytic conditions when compared to the halophytic conditions. The anti-inflammatory and insect repellent properties mentioned by local people in traditional medicine can be well explored when *Vernonia elaeagnifolia* is collected from glycophytic conditions. The accurate information concerning the chemical composition of natural extracts is thus essential to explicate the potentialities of medicinal plants.

ACKNOWLEDGMENT

The authors are extremely thankful to Council of Scientific and Industrial Research (CSIR) for providing financial assistance.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the above article.

REFERENCES

1. Kamboji K. Genetic engineering of the unsaturation of fatty acids in membrane lipids alters the tolerance of *Synechocystis to* salt stress. Proc Natl Acad Sci USA 2000;96:5862-967.
2. Rao PM, Alberico GL, Cramer GR. Is the salt tolerance of maize related to sodium exclusion? Preliminary screening of seven cultivars. J Plant Nutr 2012;16:2289-303.
3. Al-Khanjari S, Al-Kathiri A, Esechie HA. Variation in chlorophyll meter readings, nodulation and dry matter yields of alfalfa (*Medicago sativa* L.) cultivars differing in salt tolerance. Crop Res 2002;24:350-6.
4. Samuel JK, Andrews B. Traditional medicinal plant wealth of pachalur and periyur hamlets, Dindigul District, Tamilnadu. Indian J Traditional Knowledge 2010;9:264-70.
5. Vongsombath C, de Boer HJ, Palsson K. Keeping leeches at bay: field evaluation of plant-derived extracts against terrestrial bloodsucking leeches (Haemadipsidae) in Lao PDR. Acta Trop 2011;119:178-82.
6. Epstein E. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, New York; 1972.
7. Taiz L, Zeiger E. Plant physiology: (III rd Edition) Sinauer Associates. Inc. Publishers, Sunderland, Massachussetts; 2002.
8. Yin D, Chen S, Chen F. Morphological and physiological responses of two *Chrysanthemum* cultivars differing in their tolerance to waterlogging. Environ Exp Bot 2009;67:87–93.
9. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
10. Giannopolitis CN, Reis SK. Superoxide dismutase I. Occurrence in higher plants. Plant Physiol 1997;59:309–14.
11. Chance B, Maehly AC. Assay of catalase and peroxidase. Methods Enzymol 1955;2:764-5.
12. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol 1981;22:867-80.
13. Mukherjee SP, Choudhari MA. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol Plant 1983;58:166-70.
14. Bates LS, Waldern RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205-8.
15. Makkar HPS. Measurement of total phenolics and tannins using the folin-ciocalteu method. Quantification of Tannins in Tree and Shrub Foliage. Springer, Dordrecht; 2003.
16. Thatoi N, Patra JK, Das SK. Free radical scavenging and antioxidant potential of mangrove plants: a review. Acta Physiol Plant 2014;36:31-42.
17. Takemura T, Hanagata N, Sugihara K, Baba S, Karube I, Dubinsky Z. Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. Aquat Bot 2000;68:15–28.
18. Parida AK, Das AB, Mohanty P. Invesigations on the antioxidative defence response to NaCl stress in a mangrove, *Bruguiera parviflora*: differential regulations of isoforms of some antioxidative enzymes. Plant Growth Regul 2004;42:213–26.
19. Agastian P, Kingsley SJ, Vivekanandan M. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica 2000;38:287–90.
20. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 2002;7:405-10.
21. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res 2010;4:191-5.
22. Ogunlesi M, Okiei W, Osibote EA. Analysis of the essential oil from the leaves of *Sesamum raditum*, a potential medication for male infertility factor, by gas chromatography-mass spectrometry. Afr J Biotech 2010;9:1060-7.
23. Li RW, Leach DN, Myers P, Leach GJ, Lin GD, Brushett DJ, *et al*. Anti-inflammatory activity, cytotoxicity and active compounds of *Tinospora smilacina*. Benth Phytother Res 2004;18:78-83.
24. Chen L, Su J, Li L, Li B, Li W. A new source of natural D-borneol and its characteristic. J Med Plants Res 2011;5:3440-7.
25. Patel SS, Savjani JK. Systematic review of plant steroids as potential anti-inflammatory agents: current status and future perspectives. J Phytopharmacol 2015;4:121-5.