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

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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 induce 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 salinity 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 (H₂O₂). The concentration of H₂O₂ reduced per min per mg protein was calculated using the Extinction coefficient of H₂O₂ (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 abilit y 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 \(\mu\) 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 Spectrophotometer (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. Under treatment 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 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, a 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 | Dehydrofukinone | C15H22O | 23.7 | 1.74 |
| 1 | Neophytadiene | C20H38 | 30.05 | 3.09 |
| 2 | Podocarp-7-en-3-one, 13α-methyl-13-vinyl- | C20H30O | 31.89 | 3.03 |
| 3 | á-Amyrin | C30H50O | 33.62 | 0.52 |
| 4 | Phenol | C14H22O | 25.37 | 0.2 |
| 5 | i) Hexadecane | C16H34 | 27.75 | 0.07 |
| 6 | Hydrocarbons | C35H70 | 31.34 | 7.81 |
| 7 | 17-Pentatriacontene | C35H70 | 31.34 | 7.81 |
| 8 | Tetrapentacontane, 1,5-di-bromo- | C54H108Br2 | 32.64 | 0.79 |
| 9 | Tetratriacontane | C34H70 | 33.12 | 2.89 |
| 10 | Octatriacontyl pentafluoropropionate | C33H61F5O2 | 34.25 | 1.9 |
| 11 | Triacontyl pentafluoropropionate | C33H61F5O2 | 34.25 | 1.9 |
| 12 | Iv) Steroids | C33H54O3 | 28.86 | 0.11 |
| 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 | Heptadecyl 3-chloropropanoate | C20H39ClO2 | 28.97 | 1.55 |
| 15 | Arachidyl palmitoleate | C36H70O2 | 29.57 | 0.93 |
| 16 | Dodecyl 3-mercaptopropionate | C15H30O2S | 30.94 | 1.35 |
| 17 | Phthahlic acid, di(2-propylpentyl) ester | C24H38O4 | 33.4 | 26.68 |
| 18 | v) Terpene alcohols | C20H40O | 30.29 | 1.13 |
| 19 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C20H40O | 30.29 | 1.13 |
| vii) Fatty acids | n-Hexadecanoic acid | C16H32O2 | 30.75 | 3.45 |
| 20 | Eruic acid | C22H42O2 | 34.41 | 0.87 |
| 21 | Oleic acid, eicosyl ester | C38H74O2 | 34.85 | 1.2 |
| 22 | viii) Alcoholic compounds | C20H42O2 | 34.54 | 1.62 |
| 23 | 2-Octadecynoic acid | 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). Pthalic acid, di (2-propylenyl) ester (32.48%) 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, 13α-methyl-13-vinyl-(3.03%), Æ-Amyrin (0.52%), Hexadecane (0.007%), 17-Pentatriacontene (7.81%). Tetrapentacanthone,1,54-dibromo- (3.03%) Tetraatriaconte (2.89%). Octaocatronic pentfluoropropionate (0.73%). Triacyctol pentafluoropropionate (1.9%). Trietraconate (4.81%). Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (0.11%). Stigmast-3-5-diene (0.74%). Heptadecyl 3-chloropropionate (1.55%). Arachidyl palmitate (0.93%). Dodecyl 3-mercaptopropionate (1.35%). 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.13%). n-Hexadecanoic acid (3.45%). Ergic acid (0.87%). Oleic acid, eicosyl ester (1.2%) and 2-Octadecxyethanol (1.62%).

The treatment contained 3 terpenes, 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 (1.96%), 10-Heneicoseone (ct) (4.15%). Pentacos-1-ene (1.79%), Heptacos-1-ene (1.06%), 17-Pentatriacontane (0.45%). 2,4-Di-tert-butyphenol (1.26%). Tris(2,4-dimethyl-butylyphenyl) phosphate (0.83%), Dibutyl phthalate (2.09%), Campesterol (0.70%). Stigmasterol (0.25%). Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (0.72%). Æ-Siosterol (14.85%). Ethyl iso-alochoolate (15.6%), Phytol (3.66%), 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 conditions was studied by Parida [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 conditions was studied by Parida [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 conditions was studied by Parida [17]. 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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.

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