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Research Article

EVALUATION OF ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT POTENTIAL OF *EVOLVULUS ALSINOIDES* (L.)L.

D.GOMATHI, M.KALAISELVI, G.RAVIKUMAR AND C.UMA*

Department of Biochemistry, Karpagam University, Coimbatore – 641 021, Email: umaradhakrishnan29@gmail.com

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ABSTRACT

Introduction: Antioxidants are free radical scavengers which protect the human body against free radicals that causes various pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation and aging process. Some of the antioxidant enzymes and non-enzymatic molecules widely distributed in the biological system which are capable of scavenging free radicals. The study was conducted to evaluate the antioxidant potential of *Evolvulus alsinoides* by using enzymatic and non enzymatic parameters. **Methods:** The enzymatic antioxidants (Superoxide dismutase, Catalase, Glutathione-s-transferase, Glutathione peroxidase, Peroxidase, Ascorbate oxidase and Polyphenoloxidase) and non-enzymatic antioxidants (Ascorbic acid, Total reduced glutathione and α-Tocopherol) activities were determined. **Result:** The results obtained in the present study showed the significant levels of enzymatic and non-enzymatic antioxidants in *Evolvulus alsinoides* (L.)L. **Conclusion:** From above study it is concluded that, the *Evolvulus alsinoides* showed promising antioxidant activity which can be used as effective protecting agents against oxidative stress and various diseases.

Keywords: *Evolvulus alsinoides,* Enzymatic antioxidants, Ascorbic acid, Total reduced glutathione, 50% ethanol

INTRODUCTION

Environmental stress adversely affects plant performance and often results in significant reductions in crop yield and quality worldwide. The exposure of plants to environmental stresses such as drought stress, heat stress, chilling stress, salt stress and plant diseases can result in the production of reactive oxygen species (ROS) that contributes to diminished plant performance[1,2,3]. Reactive oxygen species (ROS) like peroxyl radicals, hydroxyl radicals, superoxide anions, singlet oxygen (species), the ferryl or perferryl ions, hydrogen peroxide ions, and peroxynitrates, are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism. Oxidative defense is provided by a number of enzymes and vitamins, including retinol, tocopherol, ascorbic acid and glutathione. When free radical levels increase, individuals may become deficient in these protective antioxidants resulting in oxidative stress[4]. In order to limit oxidative damage under stress condition plants developed a series of detoxification systems that breakdown the highly toxic ROS[5,6].

Antioxidants are free radical scavengers which protect the human body against free radicals that causes various pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation and aging process[7]. In nature, AOX are grouped as exogenous or endogenous. The endogenous group includes enzymes (and trace elements part-of) like superoxidase dismutase (Zn, Mn and Cu), glutathione peroxide (Se) and catalase, and proteins like albumin, transferrin, ceruloplasmin, metallothionein and haptoglobin. The most important exogenous AOX are dietary phytochemicals (such as polyphenols, quinones, flavonoids, catechins, coumarins, terpenoids) and the smaller molecules like ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin-E)[8].

Plants have developed an array of defense strategies (antioxidant system) to cope up with oxidative stress. The function of this antioxidant system is to scavenge the toxic radicals produced during oxidative stress and thus help the plants to survive through such conditions[9]. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reaction and pathogens [10]. These mechanisms employed ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)[11]. Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytocostituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power)[12,13].

Evolvulus alsinoides (L.)L. is a perennial herb belonging to the family *Convolvulaceae* with a small woody and branched root stock. [14] It is an important medicinal plant that grows in the open and grassy places almost throughout the India and subtropical countries of the world. Plant extracts have been used in traditional medicine for treatment of bronchitis, asthma and brain disorders like insanity, epilepsy, nervous disability, and scrofula. This extract also has exhibited antioxidant and immunomodulatory activities[15]. It has a known nootropic and anti-inflammatory activity[16,17]. The aim of the present work was to study the antioxidant properties of whole plant of *Evolvulus alsinoides (L.)L.*.

MATERIALS AND METHODS

Sample collection and extraction

The whole plant of *Evolvulus alsinoides* (L.)L. used for the investigation was obtained from Coimbatore District, Tamilnadu, India. The plant was authenticated by Dr.P.Satyanarayana, Botanical Survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2011-12/Tech.-514. Fresh plant material was washed under running tap water. The samples were prepared by grinding one gram each of whole plant material in 5 ml of 50% ethanol, separately, in a pre-chilled mortar and pestle and the extracts were centrifuged at 10,000 g at 4ºC for 10 minutes. The supernatants thus obtained were used within four hours for various enzymatic and non-enzymatic antioxidant assays.

Estimation of enzymatic and non-enzymatic antioxidants in *Evolvulus alsinoides*

The enzymatic antioxidant activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione s transferase, peroxidase, ascorbate oxidase and polyphenol oxidase were determined spectrophotometrically by using the methods of Das *et al*., 2000, Sinha, 1972 Rotruck *et al*., 1973, Habig *et al*., 1974, Addy and Goodman, 1972, Vines and Oberbacher, 1965[18-23].The non-enzymatic antioxidants like vitamin C, α-tocopherol were studied by the method of Sadasivam and Manickam, 1996[24] and the total reduced glutathione were estimated by Boyne and Ellman, 1972 method[25].

RESULT AND DISCUSSION

Free radicals can be scavenged through chemoprevention by utilizing natural antioxidant compounds present in foods and medicinal plants. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione. Some medicinal plants have been shown to have both chemopreventive and/or therapeutic effects on human diseases[26].

Figure 1 shows the activity of SOD and catalase of *Evolvulus alsinoides*. The levels were found to be 49.8±0.13 units/mg protein and 180.3±1.36 µmole of H_2O_2 consumed/min/mg proteins. SOD is one of the antioxidant enzymes that play a key role in cellular defense against ROS[27]. Similarly, CAT is also one of the principal antioxidant enzymes; it eliminates H_2O_2 by transforming it into H_2O and O_2. The stimulation of SOD activity along with CAT seemed to play a protective role against membrane damage as Cu is particularly toxic to membranes[28].

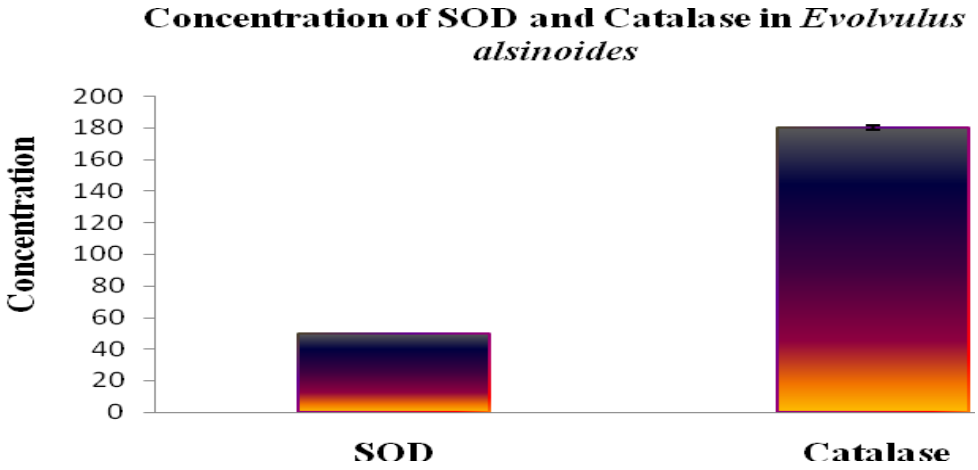

Figure 1
Values are expressed as mean±SD(n=3)
Units:SOD - Units/mg protein ; Catalase - µmole of H_2O_2 consumed/min/mg protein

Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organisms from oxidative damage. The enzyme plays an important role in peroxide detoxification. Glutathione peroxides utilize the reducing equivalents of glutathione to reduce hydrogen per oxide and it may be the main mechanism for protection against the deleterious effects of hydroperoxides. Glutathione reductase also known as GSR or GR is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH which is an important cellular antioxidant. Glutathione reductase plays an important role in protecting hemoglobin, red cell enzymes, and biological cell membranes against oxidative damage by increasing the level of reduced glutathione (GSSGR) in the process of aerobic glycolysis[10].

Glutathione peroxidase, Glutathione s transferase and Peroxidase levels in fresh plant were depicted in figure 2 which shows the high level of GPx, peroxidase activity (781.4 ± 1.46 µg of glutathione oxidized/min/mg protein and 467 ± 0.90 µmoles/g tissue) and 353.1 ± 1.03 µmoles of CDNB – GSH conjugate formed/min/mg protein. Peroxidases are referring to heme containing enzymes which are able to oxidise organic and inorganic compounds using hydrogen peroxide as co-substrate. The non-specificity of peroxidase makes the enzyme suitable to a broad range of electron donor substrates[29].

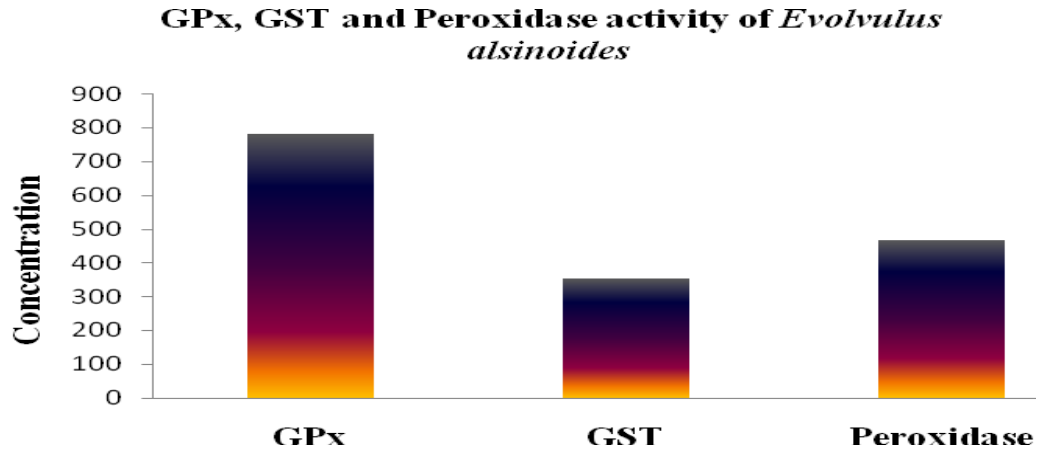

Figure 2
Values are expressed as mean±SD (n=3)
Units:GPx - µg of glutathione oxidized/min/mg protein ; GST - µmoles of CDNB – GSH conjugate formed/min/mg protein
Peroxidase - µmoles/g tissue

The polyphenol oxidase (PPO) comprising of catechol oxidase and laccase is an enzyme that catalyzes the aerobic oxidation of variety of phenolic substrates in the plant material[10] and ascorbate oxidase is an enzyme which catalyzes the one-electron oxidation of ascorbate with the concomitant four-electron reduction of dioxygen to water[30,31]. Our results for ascorbate oxidase, glucose-6-phosphate-dehydrogenase and polyphenol oxidase in fresh plant sample were represented in figure 3 which shows the high level of ascorbate oxidase and glucose-6-phosphate-dehydrogenase when compared to polyphenol oxidase. According to the results of Murata *et al.,* 2008[32], ascorbate oxidase is a member of the multicopper oxidase family and can be obtained from higher plants such as green zucchini squash and cucumber and fungal species.

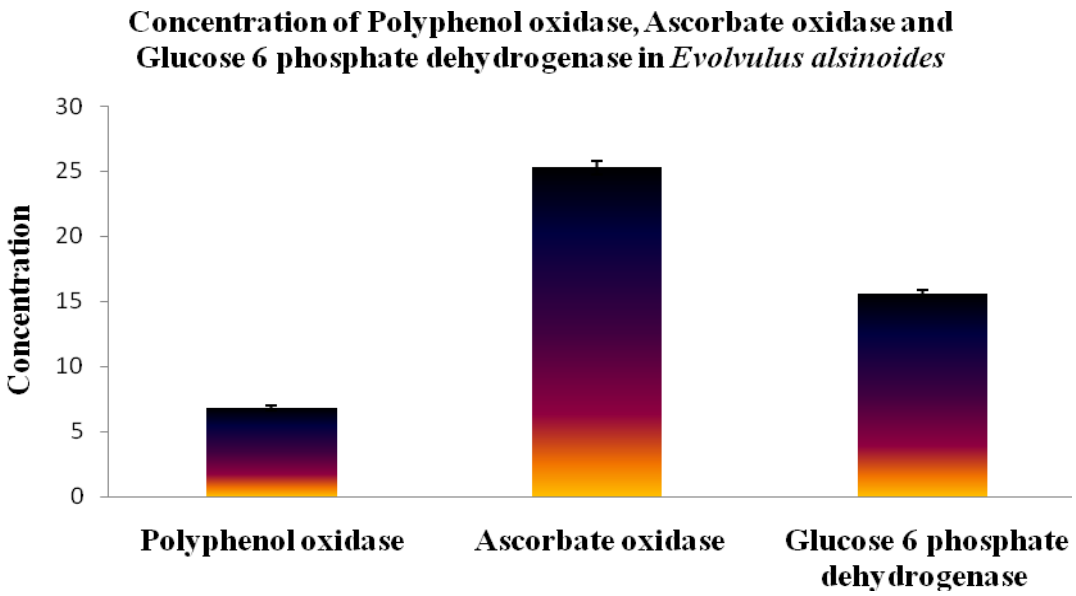

Figure 3
Values are expressed as mean±SD (n=3)
Units: Polyphenol Oxidase – µmoles/g tissue ; Ascorbate oxidase – Unit/g tissue ; Glucose 6 phosphate dehydrogenase Units/ mg protein

Reactive oxygen species (ROS) get special attention lately due to many factors such as drought, cold, heat, herbicides and heavy metals. All of these factors lead to increasing number and accumulation of ROS in plant cells[33]. Scientific research shows that ROS are harmful to the cell because they can raise the oxidative level through loss of cellular structure and function[34]. ROS detoxification agents in cells include antioxidative enzymes such as ascorbate oxidase, peroxidase, catalase and ascorbate peroxidase. Johnson *et al.,* 2003[35] said that ROS detoxification agents also includes non enzymatic antioxidants such as flavones, anthocyanins, carotenoids and ascorbic acid. The levels of total reduced glutathione and vitamin C of *Evolvulus alsinoides* were found to be 57.3 ± 0.51 µg/mg protein and 365.18 ± 0.94 µg/mg protein respectively (Figure 4). Ascorbic acid has been proposed to have roles on regulation of photosynthesis[36]. Ascorbic acid is readily oxidised to monodehydro ascorbic acid as part of its antioxidant function[37]. Ascorbic acid prevents or reducing oxidative damage was reported in ketoconazole treated drought stressed *C. roseus*[38].

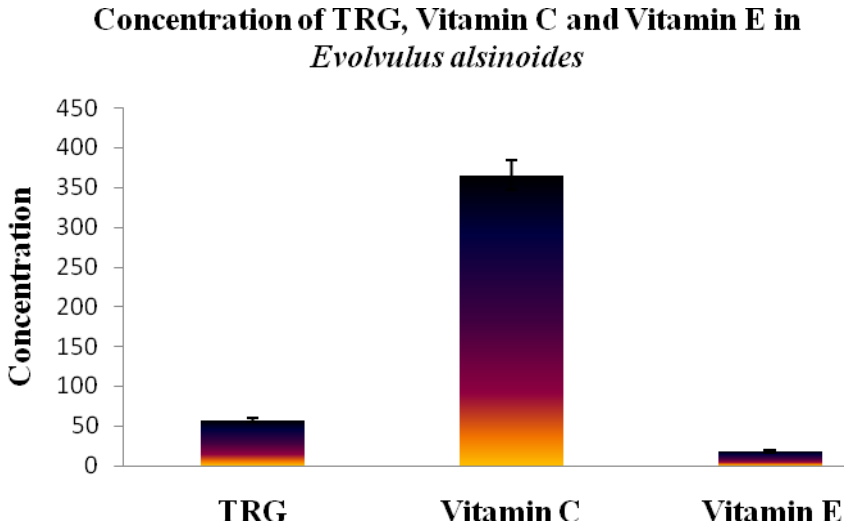

Figure 4
Values are expressed as mean±SD (n=3)
Units:Total reduced glutathione - µg/mg protein ; Vitamin C - µg/mg protein ; Vitamin E - µg/mg protein

Vitamin E (α-tocopherol) is probably the most important lipid-soluble antioxidant protecting membranes, lipids and lipoproteins[39]. Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels have been shown to enhance immune response and resistance to diseases[40]. Many studies have suggested that high intake of Vitamin E may slow down the development and progression of atherosclerosis. Some clinical trials also reported beneficial effects of Vitamin E supplementation in the secondary prevention of cardiovascular events[41,42].

CONCLUSION

The results of the above study clearly indicated that the presence of significant level of enzymatic and non-enzymatic antioxidant activity in *Evolvulus alsinoides* that could protect against oxidant and free

radical injuries. Thus, the effective source of *Evolvulus alsinoides* (L.)L. could be employed in all medicinal preparations to combat diseases associated with oxidative stress including cancer, diabetes and related disorders.

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