

Antioxidant profile changes in leaf and root tissues of *Withania somnifera* Dunal

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Abstract

An important herbal plant *Withania somnifera* was evaluated for its antioxidant potential changes in leaves and roots. The main antioxidant constituents Non-enzymatic (ascorbic acid, α -tocopherol and reduced glutathione) and enzymatic (superoxide dismutase, ascorbate peroxidase, catalase, peroxidase and polyphenol oxidase) antioxidants were estimated from both leaves and roots. The analyses were carried out in the field-collected samples. It was found that plant contain a significant quantity of non-enzymatic and enzymatic antioxidants in these organs. But, the quantity varies in both organs. The roots contain more amounts of these antioxidants than leaves. Here from this study, it can be concluded that, the root of *Withania somnifera* are good source of non-enzymatic and enzymatic antioxidant components.

Keywords: antioxidant enzymes; medicinal herb; non-enzymatic antioxidants; traditional medicine; *Withania somnifera*.

Abbreviations: ROS_reactive oxygen species; SOD_superoxide dismutase; CAT_catalase; POX_peroxidase; APX_ascorbate peroxidase; GR_glutathione reductase; DHAR_dehydro-ascorbate reductase; H₂O₂_hydrogen peroxide; TCA_trichloro acetic acid.

Introduction

Globally a positive trend has blossomed in favours of traditional and integrative health sciences both in research and practices. Although synthetic pharmaceuticals now dominate the drug scene, medicinal plants continue to hold a place in international health care (Jaleel and Panneerselvam, 2007). Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries to promote primary health care

with better cultural acceptability, human compatibility and lesser side effects (Jaleel et al., 2006; Remya et al., 2009).

Drought, temperature extremes, heavy metals and salinity in soil are the most common abiotic constraints (stresses) that plants encounter. Abiotic stress adversely affects growth and productivity and triggers a series of morphological, physiological, biochemical and molecular changes in plants.

Currently the multiple stress factors are the potential threat to plant production globally (Jaleel et al., 2007a-c). Exposure to abiotic stresses leads to the generation of reactive oxygen species (ROS), including singlet oxygen, perhydroxyl radical, hydroxyl radicals, hydrogen peroxide and alkoxy radical (Jaleel et al., 2006, 2009a). The ROS may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cells. The ROS in plants are removed by a variety of antioxidant enzymes and/or lipid-soluble and water-soluble scavenging molecules (Jaleel et al., 2007d).

Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Jaleel et al., 2007e). The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) function in detoxification of super oxide and hydrogen peroxide (H_2O_2) (Jaleel et al., 2007f). The primary scavenger is SOD, which converts O_2^- to H_2O_2 . This toxic product of SOD reaction is eliminated by APX in association with dehydro-ascorbate reductase (DHAR) and GR, the DHAR and GR are the key enzymes in regeneration of ascorbic acid (Blokhina et al., 2003; Manivannan et al., 2008). H_2O_2 is also scavenged by CAT though the enzyme is less efficient than APOX-GR system (Zhao et al., 2008). Protective roles of the antioxidant enzymes in stress have been reported for a number of plants (Jaleel et al., 2007g). Non-enzymatic components contain cystein, reduced glutathione and ascorbic acid (Jaleel et al., 2008a). In environmental stress tolerance, such as drought, high activities of antioxidant enzymes and high contents of non-enzymatic constituents are important. Apart from CAT, various POX and peroxiredoxins, four enzymes are involved in the ascorbate-glutathione cycle, a pathway that allows the scavenging of superoxide radicals and H_2O_2 . These include APX, DHAR, MDHAR and GR (Blokhina et al., 2003; Jaleel et al., 2008b). Most of the ascorbate glutathione cycle enzymes are located in the cytosol, stroma of chloroplasts, mitochondria and peroxisomes. APX is a key antioxidant enzyme in activity is known to confer oxidative stress tolerance (Jaleel et al., 2008c). Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant systems. These include β -carotenes, ascorbic acid, α -tocopherol, GSH and

enzymes including SOD, POX, APX, CAT, PPO and GR (Blokhina et al., 2003; Jaleel et al., 2008d-f).

Antioxidants such as ascorbate and glutathione are involved in scavenging H_2O_2 in conjunction with MDAR and GR, which regenerate ascorbate (Jaleel et al., 2009a). Tocopherols, collectively known as vitamin E, are a class of lipid-soluble antioxidants synthesized exclusively by photosynthetic organisms (Jaleel et al., 2009b). Tocopherols are essential components of the human diet because they perform numerous critical functions including quenching and scavenging various reactive oxygen species (ROS) and free radicals and protecting polyunsaturated fatty acids from lipid peroxidation (Gopi et al., 2007). ROS scavengers GSH and ascorbic, which accumulate in response to oxidative stress, are part of a well-established ascorbate cycle. Remarkable increase in GSH and ascorbic acid levels under various abiotic stresses (Jaleel et al. 2007a, 2008d) indicated the induction of antioxidant mechanism such as GSH-APX cycles in plants (Kishorekumar et al., 2008). Free radical-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Sankar et al., 2007).

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and standardized. The medicinal plant examined in this study has long been used in Indian traditional medicine, Ayurveda as well as many other traditional drug practices of the rest of the world. *Withania somnifera* Dunal, known as 'ashwagandha', has been an important herb in the Ayurvedic and indigenous medical systems for centuries in India (Jaleel et al. 2008c). In view of its varied therapeutic potential, it is the subject of considerable modern scientific attention (Jaleel et al. 2008d). A perusal of the literature showed that there is a lack of information on the content variations of antioxidants of this plant in its different organs. The present investigation was therefore undertaken to study the variations of antioxidant profiles in leaves and roots of *Withania somnifera*.

Materials and methods

Sample collection

The fresh leaves and roots of *Withania somnifera* were collected from the Botanical Garden of

Annamalai University, washed and used for analyzing non-enzymatic and enzymatic antioxidant potentials.

Antioxidant content estimations

Ascorbic acid content was assayed as described by Omaye et al. (1979). The extract was prepared by grinding 1 g of fresh material with 5 mL of 10% trichloro acetic acid (TCA), centrifuged at 3,500 rpm for 20 min, re-extracted twice and supernatant made upto 10 mL and used for assay. To 0.5 mL of extract, 1 mL of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-CuSO₄ reagent) was added and incubated at 37 °C for 3 h. Then, 0.75 mL of ice-cold 65% H₂SO₄ was added, allowed to stand at 30 °C for 30 min, and the resulting colour was read at 520 nm in a spectrophotometer (U-2001-Hitachi). The AA content was determined using a standard curve prepared with AA. The reduced glutathione content was assayed as described by Griffith and Meister (1979). Two hundred mg fresh material was ground with 2 mL of 2% metaphosphoric acid and centrifuged at 17,000 rpm for 10 min. Adding 0.6 mL 10% sodium citrate neutralized the supernatant. One ml of assay mixture was prepared by adding 100 µL extract, 100 µL distilled water, 100 µL 5, 5-dithio-bis-(2-nitrobenzoic acid) and 700 µL NADPH. The mixture was stabilized at 25 °C for 3-4 min. Then, 10 µL of glutathione reductase was added and the absorbance was read at 412 nm.

The α -Toc content was assayed as described by Backer et al. (1980). Five hundred mg of fresh tissue was homogenized with 10 mL of a mixture of petroleum ether and ethanol (2:1.6 v/v). The extract was then centrifuged at 10,000 rpm for 20 min and the supernatant used for estimation of α -toc. To one mL of extract, 0.2 mL of 2% 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in the dark for 5 min. The resulting red colour was diluted with 4 mL of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α -toc content was calculated using a standard graph made with known amount of α -toc.

Enzyme extractions and assays

The activity of APX was determined by the method of Asada and Takahashi (1987). The reaction mixture (1 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H₂O₂ and 200 µL of enzyme extract. The absorbance was read as

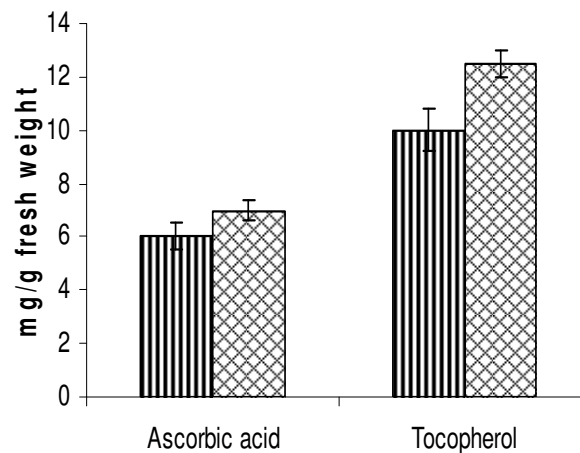


Fig 1. Variations in the ascorbic acid (AA) and α -tocopherol (α -toc) contents in leaf and root of *Withania somnifera*. Values are given as mean $\pm$ S.D. of seven replicates in each group.

decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H₂O₂ (extinction coefficient 2.9 mM⁻¹ cm⁻¹). The enzyme activity was expressed in units mg⁻¹ protein (U = change in 0.1 absorbance min⁻¹ mg⁻¹ protein).

Superoxide dismutase (EC 1.15.1.1) activity was assayed as described by Beauchamp and Fridovich (1971). The reaction mixture contained 1.17 $\times$ 10⁻⁶ M riboflavin, 0.1 M methionine, 2 $\times$ 10⁻⁵ M KCN and 5.6 $\times$ 10⁻⁵ M nitroblue tetrazolium salt (NBT) dissolved in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8). 3 mL of the reaction medium was added to 1 mL of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30 °C for 1 h. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 hr⁻¹ mg⁻¹ protein.

Catalase (EC 1.11.1.6) was measured according the method of Chandlee and Scandalios (1984) with slight modification. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.4 mL of 15 mM H₂O₂ and 0.04 mL of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm.

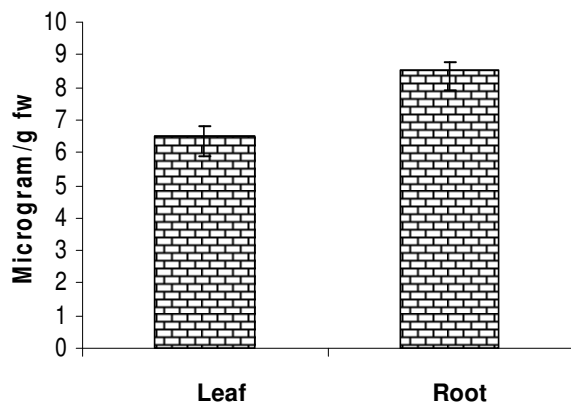


Fig 2. Variations in the reduced glutathione (GSH) contents in leaf and root of *Withania somnifera*. Values are given as mean±S.D. of seven replicates in each group.

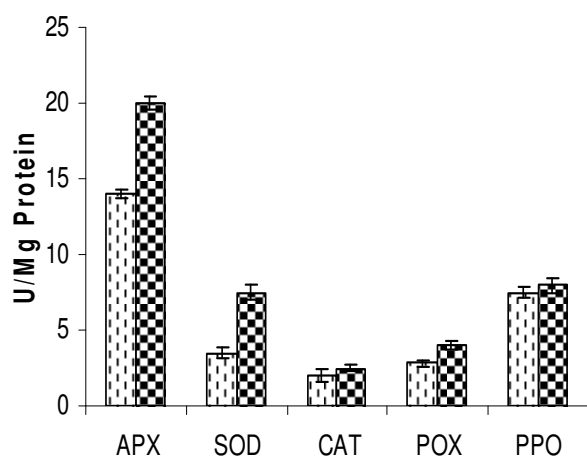


Fig 3. Variations in the Ascorbate peroxidase (APX), Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POX), Polyphenol oxidase (PPO) activities in leaf and root of *Withania somnifera*. Values are given as mean±S.D. of seven replicates in each group.

The enzyme activity was expressed in units mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Polyphenol oxidase (PPO; EC 1.10.3.1) activity was assayed by the method of Kumar and Khan

(1982). Assay mixture for PPO contained 2 mL of 0.1 M phosphate buffer (pH 6.0), 1 mL of 0.1 M catechol and 0.5 mL of enzyme extract. This was incubated for 5 min at 25 °C, after which the reaction was stopped by adding 1 mL of 2.5 N H_2SO_4 . The absorbancy of the benzoquinone formed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time of the same assay mixture. PPO activity is expressed in U mg^{-1} protein (U = Change in 0.1 absorbance min^{-1} mg^{-1} protein). Peroxidase (EC 1.11.1.7) was assayed by the method of Kumar and Khan (1982). Assay mixture of POX contained 2 mL of 0.1 M phosphate buffer (pH 6.8), 1 mL of 0.01 M pyrogallol, 1 mL of 0.005 M H_2O_2 and 0.5 mL of enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 mL of 2.5 N H_2SO_4 . The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at time zero. Protein content was estimated by the method of Bradford (1976) to express all enzyme activities. The activity was expressed in units mg^{-1} protein. One unit (U) is defined as the change in the absorbance by 0.1 min^{-1} mg^{-1} protein.

Results and discussion

The antioxidant potentials vary in different parts (leaves and roots) of *Withania somnifera* plants. The results showed (Fig. 1,2) that, the non-enzymatic potentials (ascorbic acid, reduced glutathione and tocopherol) were more in roots than leaves of *Withania somnifera*. Similarly the activities of antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, catalase, polyphenol oxidase and peroxidase were also have their maximum activities in root tissues of *Withania somnifera* (Fig. 3). The transcript of some of the antioxidant genes such as glutathione reductase or ascorbate peroxidase was higher during recovery from a stress period and appeared to play a role in the protection of cellular machinery against damage by reactive oxygen species (Shao et al., 2008). Previous investigations showed the antioxidant profile variations in tender and mature leaves of *Strychnos Nux-vomica* (Remya et al., 2009) and different plant parts like root, stem, leaves and flowers of *Catharanthus roseus* (Jaleel and Panneerselvam, 2007). In a study by Ksouri et al. (2008) the variations of antioxidant and polyphenol contents are reported in halophyte species. Natural

antioxidant contents and biological activities of traditional medicinal plants like *Cyanara cardunculus* are previously reported (Falleh et al., 2008), all these works are highly significant because the scientific validation of traditional medicinal plants are important.

Here from this study, it can be concluded that, the root of *Withania somnifera* are good source of non-enzymatic and enzymatic antioxidant components. Our results points out the significance of *Withania somnifera* as an important medicinal plant, which have good antioxidant potentials throughout its leaf as well as root. Further studies to isolate individual active principles and antioxidant activity of individual extracts of leaf as well as root through radical scavenging assay and their pharmacological validation in terms of modern medicine will be of great pharmacological importance in future and that is underway in our lab.

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