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Research Article

EVALUATION OF ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT PROPERTIES OF *AERVA LANATA* (L) – AN *INVITRO* STUDY

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ABSTRACT

The present paper focuses on assessing the level of enzymatic and non-enzymatic antioxidants of *Aerva lanata*. It is a medicinal plant is used to treat against various disorders. This study has revealed that the quantification of enzymatic antioxidants such as Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione S transferase, Ascorbate oxidase, peroxidase, polyphenol oxidase and non-enzymatic antioxidants such as Vitamin C, reduced glutathione. Based on the finding of this study *Aerva lanata* has a good source of enzymatic and non-enzymatic antioxidants. It has ability scavenge the free radicals and protect against oxidative stress causing diseases. In future, *Aerva lanata* may serve as a good pharmacotherapeutic agent.

Keywords: *Aerva lanata*, Enzymatic antioxidants, Non-enzymatic antioxidants, Oxidative stress, Free radicals

INTRODUCTION

Oxidative stress associated with the production of ROS is believed to be involved not only in the toxicity of xenobiotics but also pathophysiology in various tissue damages[1]. The toxicity of organs such as liver, heart, kidney, brain etc., are associated with the metabolic activation of foreign compounds to form free radicals or with the production of reactive oxygen species (ROS) such as superoxide anion, hydroxy radicals, hydrogen peroxide radicals etc,. These are responsible for tissue damaging effects such as lipid peroxidation and DNA damage.

ROS mediated oxidative stress to macromolecules namely lipids, proteins and DNA have been implicated in pathogenicity of major diseases such as cancer, rheumatoid arthritis, degeneration process of aging, myocardial infraction[2]. Several forms of toxins produced from allopathic drugs, these toxins have been claimed to involve free radical mechanism. Among which are those produced by paracetamol, adriamycin, paraguat etc., cause oxidative stress[3].

But plant based drugs produced very less side effects than allopathic drugs. Plant contains enzymatic antioxidants (SOD, Catalase, Glutathione peroxidase, Glutathione S transferase), Non-enzymatic antioxidants (Vitamin C, Reduced glutathione) scavenge free radicals produced by ROS and protect organ from ROS induced oxidative stress[4]. The network of antioxidants explained in Figure 1. Among plant materials, fruits, vegetables are reported to be rich in antioxidants[5].

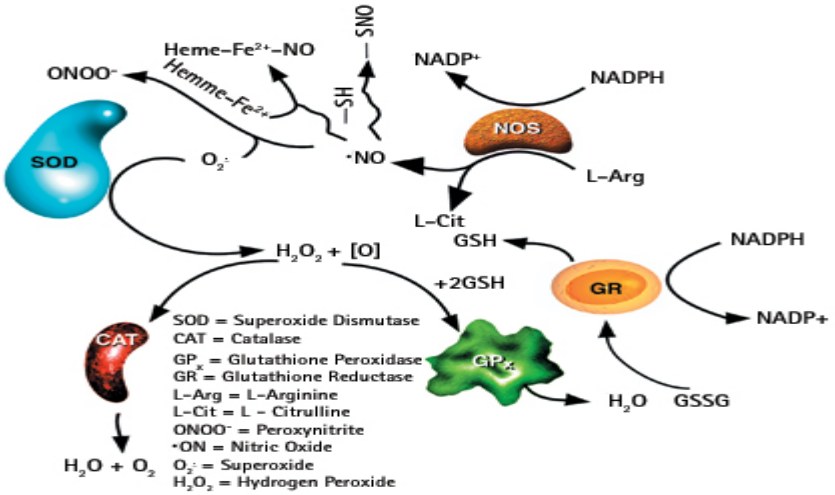


Fig. 1: Antioxidant network [http://blog.targethealth.com/wp-content/uploads/2008/01/image010.jpg]

Figure 1: The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are: super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx). SOD, the first line of defense against free radicals, catalyzes the dismutation of super oxide anion radical (O2 •¯) into hydrogen peroxide (H2O2) by reduction. The oxidant formed (H2O2) is transformed into water and oxygen (O2) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein GPx enzyme removes

H2O2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH).

Aerva lanata known as polpala is used to treatment for renal disease. Sometimes erect herb found throughout tropical India as a common weed in fields and wasteland. The plant is useful for curing diabetes. It is anthelimintic, demulcent and is helpful in lithiasis, cough, sore throat and wounds[6]. The plant has been reported to possess anti inflammatory and nephroprotective in rats[7].

The main objective of this study is, to evaluate the level of enzymatic, non enzymatic antioxidants and lipid peroxidation of *Aerva lanata.*

MATERIALS AND METHODS

Plant Collection

Fresh plants parts were collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical survey of India, TNAU Campus, Coimbatore. The voucher No. BSI/SC/5/23/10-11/Tech/22. Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

Plant Sample Extraction

The samples were prepared by grinding one gram of *Aerva lanata* in 2 ml of 50% ethanol, separately, in a pre-chilled mortar and pestle and the extracts were centrifuged at 10,000g at 4ºC for 10 minutes. The supernatants thus obtained were used within four hours for various enzymatic and non-enzymatic antioxidants assays.

Assay of Superoxide Dismutase (SOD)

The assay of superoxide dismutase was done according to the method of Das et al.[8]. In this method, 1.4ml aliquots of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer of pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM Hydroxylamine hydrochloride and 0.1ml of 50 mM EDTA) was added to 100µl of the sample extract and incubated at 30ºC for 5 minutes. 80 µl of 50 µM riboflavin was then added and the tubes were exposed for 10 min to 200 W-philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

Assay of Catalase (CAT)

Catalase activity was assayed by the method of Sinha[9]. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of µmoles of H_2O_2 consumed/min/mg protein.

Assay of Glutathione Peroxidase (GPX)

Glutathione peroxidase was assayed according to the method of Rotruck et al.[10] with some modifications. The reaction mixture consisting of 0.4 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H2O2, 0.2 ml of water and 0.5 ml of enzyme was incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5 ml of 10% TCA and after centrifugation, 2 ml of the supernatant was added to 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). The color developed was read at 412 nm and the enzyme activity is expressed in terms of µg of glutathione utilized/min/mg protein.

Assay of Glutathione S Transferase (GST)

Glutathione transferase activity using chlorodinitrobenzene or dichloronitrobenzene as substrates was assayed spectrophotometrically essentially as described by Habig et al. [11]. The cuvettes (final volume of 3.0 ml) contained 0.1 M phosphate buffer (pH 6.5), 1 mM GSH and 1 mM of chlorodinitrobenzene and 20 µl of appropriately diluted enzyme from the different sources. Change in absorbance at 340 nm was followed against a blank containing all reactants excepting enzyme protein, Specific activity was expressed as µmol conjugate formed/min/mg protein

Assay of Peroxidase

The assay was carried out by the method of Addy and Goodman[12]. The reaction mixture consisted of 3ml of buffered pyrogallol (0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)) and 0.5 ml of 1% H_2O_2. To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol).

Assay of Ascorbate Oxidase

Assay of ascorbate oxidase activity was carried out according to the procedure of Vines and Oberbacher[13]. The sample was homogenized [1: 5 (w/v)] with phosphate buffer (0.1 M/ pH 6.5) and centrifuged at 3000 g for 15 min at 50ºC. The supernatant obtained was used as enzyme source. To 3.0 ml of the substrate solution (8.8 mg ascorbic acid in 300 ml phosphate buffer, pH 5.6), 0.1 ml of the enzyme extract was added and the absorbance change at 265 nm was measured for every 30 seconds for a period of 5 minutes. One enzyme unit is equivalent to 0.01 OD change per min.

Assay of Polyphenol Oxidase (PPO)

Assay of Polyphenol oxidase activity was carried out according to the procedure of sadasivam and Manickam[14]. To 2.0ml of enzyme extract and 3.0ml of distilled water added and mixed together. 1.0ml of cathecol solution (0.4mg/ml) added to the above solution and the reactants were quickly mixed. The enzyme activity was measured as change in absorbance/min at 490nm.

Estimation of Reduced Glutathione (TRG)

The amount of reduced glutathione in the samples was estimated by the method of Boyne and Ellman[15]. 1ml of the sample extracts were treated with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl dissolved in 100ml water). After centrifugation, 2.0 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na_2HPO_4 and 1.0 ml of DTNB reagent (40 mg DTNB in 100 ml of aqueous 1% tri sodium citrate). Absorbance was read at 412 nm within 2 minutes. GSH concentration was expressed as nmol/mg protein.

Estimation of Vitamin C

The determination of ascorbic acid was carried out by the procedure given by Sadasivam and Manickam[16]. The assay mixture for vitamin C consisted of 0.1 ml of brominated sample extract, 2.9 ml of distilled water, 1 ml of 2% DNPH reagent and 1-2 drops of thiourea. After incubation at 37ºC for 3 h, the orange-red osazone crystals formed

were dissolved by the addition of 7 ml of 80% sulphuric acid and absorbance was read at 540 nm after 30 minutes. Vitamin C concentration was expressed in terms of mg/g tissue.

Assay of Malondialdehyde (MDA)

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of Buege and Aust[17]. 1.0 ml of the sample extract was added with 2.0 ml of the TCA-TBA-HCl reagent (15% (w/v) TCA, 0.375% (w/v) TBA and 0.25N HCl). The contents were boiled for 15 minutes, cooled and centrifuged at 10,000 g to remove the precipitate. The absorbance was read at 535 nm and the malondialdehyde concentration of the sample was calculated using extinction coefficient of 1.56 x 105 M^{-1} Cm^{-1}.

Statistical Analysis

The results obtained were expressed as mean ±SD. The Statistical comparison among the groups were performed a statistical package program (SPSS 10.0).

RESULTS

Estimation of Enzymatic Antioxidants

The level of enzymatic antioxidants such as SOD and CAT showed in Figure 2. The concentration SOD is 20.88±1.65 Units/mg protein and catalase is 34.57±0.44 µmole of H_2O_2 consumed/min/mg protein.

Figure 3 shows the level of GPx and GST. The concentration Glutathione peroxidase is 246.35±6.83 µg of glutathione oxidized/min/mg protein and Glutathione S transferase is 252.67±3.73 µmoles of CDNB – GSH conjugate formed/min/mg protein.

Figure 4 shows Ascorbate oxidase and peroxidase. The concentration Ascorbate oxidase is 32.33±2.52 Unit/g tissue and Peroxidase is 346±6.0 µmoles/g tissue.

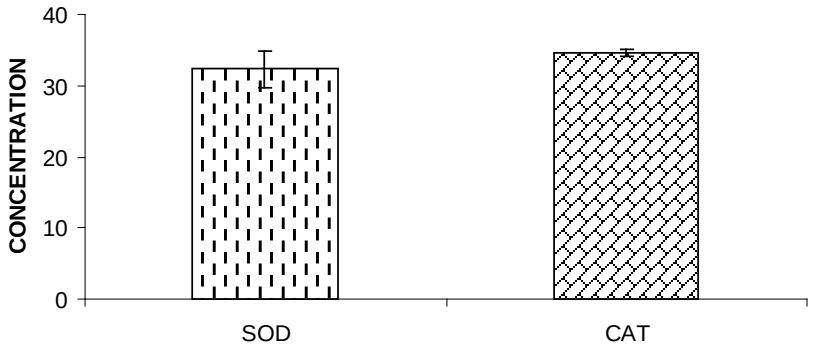
Estimation of Non-Enzymatic Antioxidants

The level of non-enzymatic antioxidants such as Vitamin C and reduced glutathione showed in Figure 5. The concentration vitamin C is 274.31±5.88 µg/mg protein and reduced glutathione is 57.54±2.58 µg/mg protein.

Estimation of Polyphenol Oxidase and LPO

The level of non-enzymatic antioxidants such as Vitamin C and reduced glutathione showed in Figure 6. The concentration peroxidase is 1.64±0.171 µmoles/g tissue and LPO is 4.153±0.311.

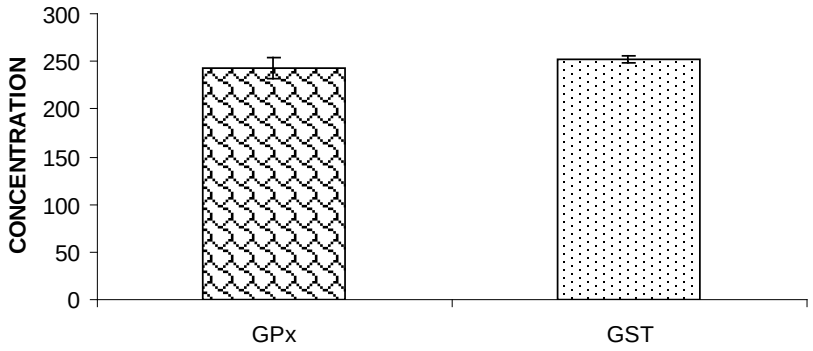
FIG 2 : CONCENTRATION OF SOD,CAT IN *Aerva lanata*



Values are expressed as mean±SD (n=3)

Units: SOD: Units/mg protein, Catalase: µmole of H_2O_2 consumed/min/mg protein

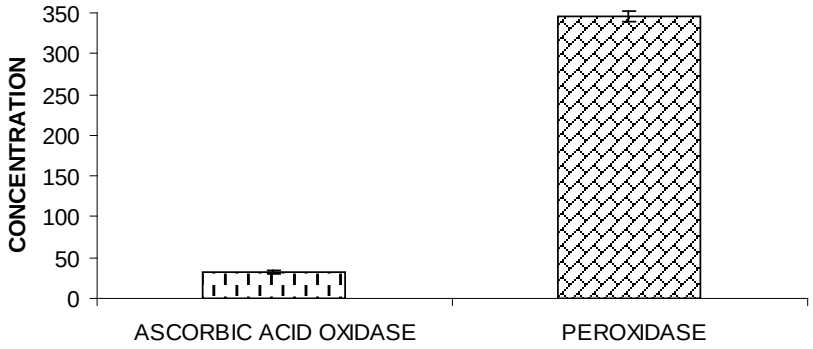
FIG 3 : CONCENTRATION OF GPx, GST IN *Aerva lanata*



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.

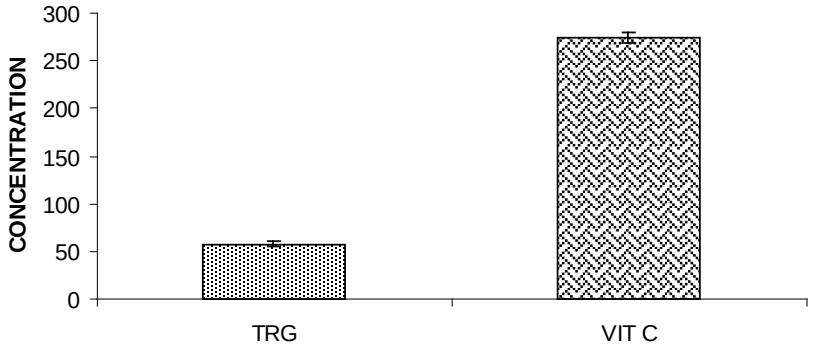
FIG 4 : CONCENTRATION OF ASCORBIC ACID OXIDASE, PEROXIDASE IN *Aerva lanata*



Values are expressed as mean±SD (n=3).

Units: Ascorbate oxidase – Unit/g tissue; Peroxidase - µmoles/g tissue

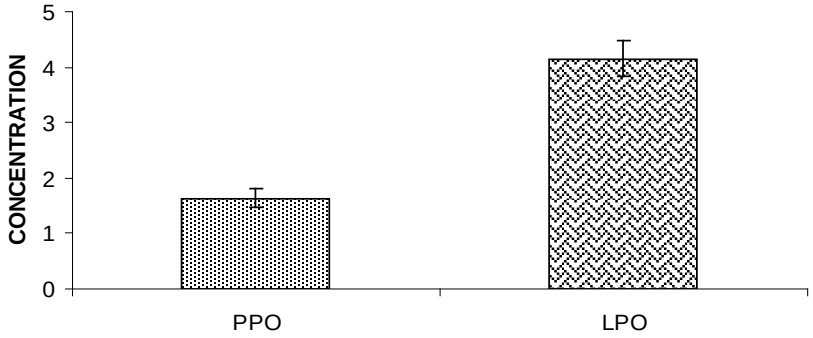
FIG 5 : CONCENTRATION OF TRG, VITAMIN C IN *Aerva lanata*



Values are expressed as mean±SD (n=3).

Units: Total reduced glutathione - µg/mg protein; Vitamin C - µg/mg protein

FIG 6 : CONCENTRATION OF PPO, LPO IN *Aerva lanata*



Values are expressed as mean±SD (n=3).

Units: Polyphenol Oxidase – µmoles/g tissue, LPO – nM of MDA formed / min/ mg protein.

DISCUSSION

Oxidative damage has been suggested to occur as a consequence of ROS produced as a by product of ETC in mitochondria. A number of studies have been suggested that ROS can affect critical events associated with many disorders. The body relies on several endogenous defence mechanisms to help protection against free radical induced cell damage that include the antioxidant enzymes such as SOD, CAT, GPx and GST. SOD and CAT are the two major scavenging enzymes that remove toxic radicals.

SOD is key defense enzymes in liver injury caused by ROS and oxidative stress. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. CAT appears to be most effective defence against hydrogen peroxide radical. It is present in peroxisomes of nearly all aerobic cells serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition without the production of free radicals[20]. GPx plays a significant role in peroxyl scavenging mechanism and it maintaining functional integration of the cell[18].GST is a group of multi functional enzyme, which plays a central role in detoxification of electrophillic chemicals[19].

Ascorbic acid oxidase is widespread in plant tissues. The role of this enzyme is to regulate the levels of oxidised and reduced glutathione and NADPH. Phenol oxidases are copper proteins catalyse the aerobic oxidation of certain phenolic compounds to quinones. The poly phenol oxidase comprises catechol oxidase and laccase. The activities these enzymes are important with regard to defence mechanism against diseases[21].

Vitamin C is a water soluble antioxidant and most effective scavenger of oxygen free radicals and other oxygen derived species. It can act an oxidant and pro-oxidant and protect DNA from free radical damage[22]. Glutathione antioxidant systems play a fundamental role in cellular defence against free radical and their oxidant species. It function by reaction with superoxide radical, peroxy radical and singlet oxygen followed by the formation of oxidised glutathione and other disulphides[23].

LPO happened by the oxidative degradation of polyunsaturated fatty acid in membrane. Free radicals generated by reactive metabolite of drugs and chemical agents induce the production of MDA. This MDA damage the membrane bilayer. The enzymatic and non enzymatic antioxidants are scavenging these free radicals to prevent the production of MDA.

CONCLUSION

Based on all these findings *Aerva lanata* is the very good source of enzymatic and non-enzymatic antioxidant. In our previous study suggested *Aerva lanata* does not contain a toxic substance. In future, this plant may serve as very powerful therapeutic agents.

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