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ANTIOXIDANT POTENTIAL OF *Artemisia vulgaris, L.* LEAVES

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ABSTRACT

Aerobic organisms are dependent of oxygen, which plays an important role in energy production. Activated oxygen that functions as an xidant may be represented as a free radical. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are products of normal cellular metabolism which may cause damage to cellular lipids, DNA and proteins. The harmful effects of these reactive species are encountered by the presence of molecules called antioxidants and plants are found to be a richest source. With this, the candidate plant *Artemisia vulgaris, L.* was chosen to test their antioxidant potential. The result of the present study showed that the leaves of *Artemisia vulgaris, L.* possessed good enzymic and non-enzymic antioxidants.

Key words: Antioxidants, Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), Free Radical.

INTRODUCTION

Free radicals can be defined as molecules containing one or more unpaired electrons in atomic orbits. To this group belong reactive oxygen, nitrogen and chloride species which are all of the normal by-products of metabolism considered as endogenous source and also derived exogenously.[1] Oxidative stress occurs due to the imbalance between the production and the elimination of a variety of oxygen species (ROS) like superoxide, hydroxyl, alkoxyl radical and hydrogen peroxide. These ROS have the ability to degrade macromolecules such

as lipids, nucleic acids, proteins and pigments, finally leads to cell death.[2] Lipids are highly susceptible to free radical attack. The harmful effect of ROS on lipid membrane leads to production of malondialdehyde, and the process is known as lipid peroxidation (LPO).[3] For more than two decades, the free radical-mediated peroxidation of membrane lipids and oxidative damage of DNA have been thought to be associated with a variety of health problems, such as cancer, atherosclerosis, diabetes, stroke, neurodegenerative diseases and ageing.[4] The ROS-mediated cellular injury can be overcome by enhancing the endogenous defense capacity against oxidative stress through dietary or medicinal intake of antioxidants.[5] *Artemisia vulgaris* L. (mugwort) is a medicinal plant belonging to the family *Asteraceae* and is a tall aromatic perennial herb. In traditional medicine, this plant is used for the treatment of diabetes and the extracts of the whole plant are used for epilepsy and in combination of psychoneurosis, depression, irritability, insomnia, anxiety and stress.[6] This plant also showed antispasmodic, antiseptic, antibacterial, antimalarial, antitumour, antirheumatic and hepatoprotective properties.[7]

MATERIALS AND METHODS

Plant Material

The plant sampling was collected from Tamil Nadu Agricultural University, Coimbatore. The plant was grown as pot culture in Avinshilingam University campus. The plant was identified as Botanical Survey of India, Coimbatore by *Artemisia vulgairs*, L. (Voucher number BSI/SC/5/23/08-09/Tech-1711).

Sample Preparation

For both the enzymic and non-enzymic antioxidant analysis, the sample was prepared from the fresh leaves of *Artemisia vulgairs*, L. using standard methods.

Enzymic Antioxidants

The leaves of *Artemisia vulgaris* were tested for the activities of enzymic antioxidants namely superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione S-transferase and polyphenol oxidase. Superoxide dismutase activity was determined by the method proposed by Kakkar *et al.* (1984).[8] Catalase activity was assayed spectrophotometrically by the method proposed by Luck (1974).[9] Peroxidase activity was assayed by the method of Reddy *et al.* (1995)[10] in the fresh leaves of the plant. Glutathione reductase activity was determined by the method of David and Richard (1983).[11] The method proposed by Habig *et al.* (1974)[12] was employed for the assessment of glutathione

S-transferase. The method proposed by Esterbauer *et al.* (1977)[13] was used to simultaneously assay catechol oxidase and laccase spectrophotometrically, which was used to assay polyphenol oxidase in the leaf of the selected plant.

Non-Enzymic Antioxidants

The leaves of *Artemisia vulgaris* were tested for the levels of non-enzymic antioxidants such as ascorbic acid, tocopherol, reduced glutathione, total carotenoids, lycopene, total phenols, flavonoids and chlorophyll. The levels of ascorbic acid in *Artemisia vulgaris* leaves were quantified spectrophotometrically by the method of Roe and Keuther (1943).[14] The spectrophotometric method proposed by Rosenberg (1992)[15] was adopted to estimate the level of tocopherol. The amount of reduced glutathione present in the leaf sample was estimated by the method proposed by Moron *et al.* (1979).[16] The method proposed by Zakaria *et al.* (1979)[17] was used for the estimation of total carotenoids and lycopene. The method described by Mallick and Singh (1980)[18] was adopted to estimate total phenols. The levels of flavonoids were assayed by the method explained by Cameron *et al.* (1943).[19] The chlorophyll content of the leaves was estimated by the procedure explained by Witham *et al.* (1971).[20]

RESULTS AND DISCUSSION

The values presented in Table 1 showed that the leaves of *Artemisia vulgaris* possess considerable activities of all the enzymes analyzed. It is evident from the values that the leaf is a good source of enzymic antioxidants. The results presented in Table 2 revealed that the leaves of *Artemisia vulgaris* exhibited considerable amounts of all the non-enzymic antioxidants analysed. Metal treated *Bacopa monnieri* L. leaves exhibited increased superoxide dismutase and peroxidase activity. [21] The leaf extract of *Plumbago zeylanica* L. was reported to have good catalase activity. [22] The total antioxidant, free radical scavenging and reducing powers of *Amaranthus* species, *Centella asiatica*, *Murraya koenigii* and *Trigonella foenum graecum* were attributed to the presence of ascorbic acid, total and β-carotene and total polyphenol.[23] Both the fresh and dried form of *Pleurotus florida* and *Calocybe indica* possessed non-enzymic antioxidants namely vitamins C, E, A and GSH.[24] Ünver *et al.* (2009)[25] reported that the plant species like *Mentha piperita* L., *Rhus coriaria* L., *Thymbra spicata*, *Salvia officinalis*, *Rosmarinus officinalis* L., *Capparis ovata* L., *Origanum vulgare* L., *Laurus nobilis* L., and *Capsicumannum* L. were good sources of phenolics.[26] The study carried out by Adedapo *et al.* (2009)[27] revealed that the

proanthocyanidins, total phenols, flavonoids and flavonols were higher in the leaves of *Celtis africana* than its stem.[28] In the present study, it was evident that *Artemisia vulgaris* leaves proved to be a good source of antioxidants which can be used to combats the disease-related to oxidative stress.

TABLE 1: ENZYMIC ANTIOXIDANT ACTIVITIES IN *Artemisia vulgaris* LEAVES

ENZYMES	ACTIVITY
Superoxide dismutase (U#/g leaf)	33.97 ± 0.06
Catalase (U$/g leaf)	292.81±19.89
Peroxidase (U*/g leaf)	23.60 ± 0.09
Glutathione reductase (U+/g leaf)	2.22 ± 0.03
Glutathione S-transferase (U@/g leaf)	0.03 ± 0.001
PPO - Catechol oxidase (Units¢x10-3/g leaf)	0.46 ± 0.01
PPO - Laccase (Units¢x10-3/g leaf)	0.41 ± 0.003

The values are mean ± S.D of triplicates.

1 Unit = Amount of enzyme that causes 50% reduction in NBT oxidation

$ 1 Unit = Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units/minute

* 1 Unit = Changes in absorbance at 430 nm/minute

+ 1 Unit = mmoles of NADPH oxidized/minute

@ 1 Unit = nmoles of CDNB conjugated/minute

¢ 1 unit of catechol oxidase/laccase = Amount of enzyme which transforms 1 unit of dihydrophenol to quinine /minute

TABLE 2: NON-ENZYMIC ANTIOXIDANT LEVELS IN *Artemisia vulgaris* LEAVES

PARAMETERS	LEVELS
Ascorbic acid (mg/g leaf)	2.69 ± 0.03
Tocopherol (µg/g leaf)	6.68 ± 0.01
Reduced glutathione (nmoles/g leaf)	243.25 ± 13.42
Total carotenoids (mg/g leaf)	7.45 ± 0.34
Lycopene (mg/g leaf)	1.88 ± 0.17
Total phenols (mg/g leaf)	7.96 ± 0.33
Total flavonoids (mg/g leaf)	5.83 ± 0.07
Total chlorophyll (mg/g leaf)	2.01 ± 0.002

The values are mean ± S.D of triplicates.

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