FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT PARTS OF *WITHANIA SOMNIFERA*

SUMATHI, S., PADMA, P.R., GATHAMPARI, S. AND VIDHYA, S.
Department of Biochemistry and Biotechnology
Avinashilingam University for Women, Coimbatore – 641 043, Tamil Nadu

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ABSTRACT :

Antioxidants are the essential defense mechanism to protect the body against free radical damage. The objective of the study was to investigate the *in vitro* antioxidant activity of different parts of *Withania somnifera* (leaves, fresh tubers and dry tubers) towards free radical DPPH and the extent of inhibition of lipid peroxidation using hydrogen peroxide as prooxidant. The plant extracts exhibited significant antioxidant effect in the order as follows: leaves>fresh tubers>dry tubers. The results suggested that *Withania somnifera* could be a potential source of antioxidants and may be used in preparations to combat free radical mediated damage.

Keywords: Free radicals, Antioxidants, Lipid Peroxidation and Medicinal Plants.

INTRODUCTION

Oxygen is essential for the survival of all living beings on this earth. During the process of oxygen utilization approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals[1].

Free radicals are very unstable and react quickly with other compounds to capture the needed electron to gain stability. Generally free radicals attack the nearest stable molecules and steal its electron. When the attacked molecule loses its electron it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. All these radicals are known as reactive oxygen (ROS) species. The production of this ROS is a usual phenomenon that continuously takes place in cells as a consequence of metabolism[2].

To prevent the free radical damage, the cellular system has been endowed with a number of defense mechanisms of antioxidants. An antioxidant is a chemical or molecule that halts or slows oxidation by safely interacting with free radicals and terminates the chain reaction before

vital molecules are damaged [3].

Medicinal plants and herbs continue to be a source of proven medicaments and of new and revolutionary drugs. They contain substances known to modern and ancient civilizations for their healing properties. Herbal medicine is a major component in all traditional medical practices all over the globe. The medicinal plants and herbs come to our rescue by providing sources of antioxidants. To determine the rich source of antioxidants among the plants and herbs that is widely distributed in our environment, we conducted systematic studies on plants to identify the mechanism of antioxidant action [4].

Withania somnifera (Solanaceae), commonly known, as 'Ashwagandha' is a subtropical undershrub used as an Ayurvedic herb. The plant is attributed with curative properties against a number of diseases including cancer. It is categorized as an adaptogen, used to promote health and longevity by augmenting defense against diseases, arresting the aging process, revitalizing the body in debilitated conditions, increasing the capability of the body to resist adverse environmental factors and creating a sense of mental well being. The active constituents in ashwagandha extract include steroidal lactones called withanolides along with other various other alkaloids. The extract acts as rejuvenating tonic and it is useful for sexual strength, cures sterility in women if taken for a few days soon after the menstrual cycle [5].

Previous studies have proved the antioxidant potential of different parts of *Withania somnifera* and leaves are found to possess better antioxidant properties.

In the present study, the free radical scavenging activity and extent of inhibition of lipid peroxidation by different parts of *Withania somnifera* namely leaves, fresh and dry tubers was analyzed using single cell suspensions.

Materials and Methods

The different parts of *Withania somnifera* viz. fresh tubers, dry tubers and leaves were investigated for this study. The leaves and fresh tubers were collected from the plants grown in pot cultures. The dry tubers were purchased commercially from local market.

Determination of antioxidant activity using DPPH assay [6]

Aqueous extract of different parts of Withania somnifera were prepared. To 20ì l of the extracts, 500 ì l of DPPH and 480ì l of methanol was added and incubated at 37^0 C for 30 minutes. The percentage of free radical scavenging activity was measured at 518 nm using spectrophotometer.

Determining percentage inhibition of lipid peroxidation *in vitro*

The percentage inhibition of

lipid peroxidation by *Withania somnifera* was done using single cell suspension from liver cells. The single cell suspension was prepared from goat liver. The fatty layer on the liver was removed and chopped in to pieces using sterile blade by placing it on ice. Homogenate was prepared in PBS and the filtrate filtered through sieves of reduced pore size to get single cells.

Aqueous extract of the samples was prepared. The oxidant used to induce lipid peroxidation was 1% H_2O_2. The extent of inhibition of lipid peroxidation was determined according to the method of Okhawa *et al.*, (2001)[7].

Results and Discussion

The total antioxidant activity of different parts of plant extract was measured on the basis of their ability to scavenge stable free radical-DPPH. The three different plant extracts were screened for their antioxidant activity in the DPPH antioxidant assay and the results are depicted in the table below

Table-1 Percentage of free radical scavenging activity of different parts of *Withania somnifera*

Samples	Percentage free radical scavenging activity
Fresh tubers	68.8
Dry tubers	23.98
Leaves	79.4

The DPPH assay showed that among the three different plant parts, leaves possessed highest scavenging activity as compared with fresh tubers. The percentage scavenging activity was found to be the least in dry tubers as compared with the other two.

Literature review confirms that various plant extracts possess radical scavenging potential. The aqueous and methanol extracts of the leaves of *Warburgia salutaris* displayed promising antioxidant activities as evidenced by DPPH assay[8].

The aqueous extract of leaves and flower and ethanolic extract of stem bark from the Siamese neem tree exhibited higher free radical scavenging effect on DPPH [9] .

Similarly total extracts of *Euryale ferox* showed relatively high level of radical scavenging activity towards DPPH [10]. Vaentoval *et al.*,

(2002) have reported that leaf extracts of *Smallanthus sonchifolius* showed potent antioxidant activity. Similarly the results show that the percentage free radical scavenging activity of *leaves of Withania somnifera* was found to be more potent than the fresh and dry tubers.

The primary target of free radicals are the lipids in the membranes. In the present study, the lipid system employed was single cell suspensions to determine the differential response of the plant extracts in inhibiting the lipid peroxidation, which is a measure of its antioxidant potential.

The extent of inhibition of lipid peroxidation on the lipid system was analyzed and the results are tabulated below

Table-2
Percentage inhibition of lipid peroxidation observed in different parts of *Withania somnifera*

Samples	Percentage inhibition
Fresh tubers	28.6
Dry tubers	27.57
Leaves	41.63

The ability of the leaves to protect intact cells against free radical attack was greater in leaves than in fresh tubers and dry tubers. The fresh tubers had slightly greater activity than dry tubers but it was lesser than the leaves.

The study also reveals that some component in the plant tested was capable of entering into the intact cell and render protection against oxidative stress.

Several studies support our results. *In vitro* evaluation of antioxidant activities of *Auricularia auricula* leaves showed significant inhibition to lipid peroxidation and potent hydroxyl radical scavenging activity in rat hepatocytes [12].

The leaf extracts of *Gongronema latifolium* showed significant decrease in the malondialdehyde levels in the rat hepatocytes [13].

The aqueous and ethanolic extracts of selenium enriched rice showed significantly higher antioxidant activity against lipid peroxidation in rat hepatocytes [14].

In the present study, the leaves of *Withania somnifera* showed higher percentage inhibition of lipid peroxidation in the liver single cell suspensions than fresh and dry tubers.

Conclusion

The results suggest that the extract of different parts of *Withania somnifera* are potential scavengers of radicals and protect membrane lipids in the order: leaves > fresh tubers > dry tubers. The antioxidant activity of the sample may be attributed to the presence of various active principles like withanolides, glycowithanolides, sitoindosides vii-x. The study indicates that Ashwagandha could prove to be a good natural source of a potent and relatively safe antioxidative agent.

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