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In Vitro Free Radical Scavenging Activity of Leaves Extracts of *Withania somnifera*

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Abstract

Free radical scavenging activity of ethanolic and aqueous extracts of *Withania somnifera* leaf was studied by *in-vitro* DPPH and NBT method. Addition of ethanolic and aqueous extracts of *Withania somnifera* leaf was found to scavenge the free radicals generated by α,α-Diphenyl β-Picryl Hydrazyl (DPPH) at (IC_{50} 1663μg/ml and 1455μg/ml) respectively and superoxide generated by photo reduction of riboflavin scavenged by Nitro-blue-tetrazolium (NBT) at (IC_{50} 1597μg/ml and 1352 μg/ml) respectively. These results indicates that leaf of *Withania somnifera* possess antioxidant property *in vitro*. Free radical scavenging activity of ethanolic and aqueous extracts of *Withania somnifera* leaf can be attributed to the presence of flavanoids and tannins in the extracts.

Key Words: Anti-oxidant, Free radicals, *Withania somnifera*, DPPH, NBT

Introduction

Free radicals, powerful oxidants are species that contain unpaired electrons. They are capable of randomly damaging all components of body (lipid, proteins, DNA and saccharides) and are involved in mutations. Radical reactions are also important in the development of chronic diseases that are life limiting like cancers, hypertension, cardiac infarction, atherosclerosis, rheumatism and and also in cataract [1]. Due to metabolic reactions in the body, oxidants or free radicals are generated. Reactive oxygen species and free radicals are formed in the body during mitochondrial electron transport chain, or by exposure to ionizing radiation or by influence or many xenobiotics. These free radicals are responsible for protein degradation i.e. cellular damage, finally leading to several pathological conditions [2]. Antioxidants are substances that protect cells from damage caused by free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals otherwise might cause. "Antioxidant" refers to a broad range of substances, each designed to handle one type of an equally broad range of hydroxyl free radicals. Vitamin A, vitamin C, vitamin E and beta carotene are some of the vitamin antioxidants. Certain minerals, e.g., selenium, copper, zinc and manganese empower these vitamin antioxidants. Vitamin C often is added to processed food as a preservative. It works by preventing oxidation [3]. Antioxidants mop up damaging chemicals in the body and guard against many chronic diseases. Heart disease, arthritis, cancer and many other chronic diseases derive from the same source: fortuitous mutations caused largely by free radicals. The cells are protected from decomposition by ensuring sufficient intake of antioxidants daily [4]. *Withania somnifera* Dunn. referred as Ashwagandha in Ayurveda, finds extensive use in this system of medicine, as a general tonic and in the treatment of various nervous disorders. Thus *Withania somnifera* is reputed to promote physical and mental health and can be classified in modern terminology as an adaptogen [5]. The leaves are applied locally to tumors and to tubercles glands. Extract of the leaves is used to cure sore eyes, ulcers and swellings. They are also used as a hypnotic and an anthelmintic [6]. The leaves contain steroidal lactones which are commonly called as 'Withanoloides' [7]. The qualitative chemical examination of leaves shows the presence of withanone, somnitol, glucose, inorganic salts, alkaloids, amino acids, flavanoids and tannins [8]. The likely active principles of *Withania somnifera* are glycowithanolides (WSG), consisting of sitoindosides VII to X and withaferin [9, 10]. Clinical investigations with the *W. somnifera* leaf extracts indicate that it exerts significant anti-tumor, anti-bacterial, anti-genotoxic, anthelmintic, anti-inflammatory and anti-ulcer [11]. The present study aimed to determine the *in vitro* antioxidant potential of *W. somnifera* leaf which may be one of the mechanism for its therapeutic efficacy.

Materials and Methods

Plant Material and chemicals: The leaves of *W. somnifera* were collected and identified by Dr. H. S. Chatree from Horticulture College, Mandsaur (M.P.). All the chemicals were purchased from sigma chemicals and High Media Chemicals.

Preparation of Plant extracts: Ethanolic and aqueous extracts of *W. somnifera* leaf were prepared by Soxhlet extraction method followed by defatting with petroleum ether. After complete extraction, all the extracts were filtered and the filtrates obtained were concentrated in vacuo using rotary evaporator at 30°C.

DPPH free radical scavenging activity

To determine the antioxidant activity a method based on the reduction of methanol solution of the colored free radical,α,α-Diphenyl β-Picryl Hydrazyl (DPPH) was used. The decrease in absorption maximum of 516 nm is proportional to the concentration of free radical scavenger added to the DPPH solution. This activity was expressed as the effective concentration at 50% reduction i.e. the concentration of the test solution required to give a 50% reduction in absorbance of the test solution as compared to that of blank solution. A stock solution of DPPH (1.3 mg/ml in ethanol) was prepared such that 75 μl of it in 3 ml ethanol gave an initial absorbance of 0.9. Decrease in the absorbance in presence of sample extracts (ethanolic and aqueous) at different concentration (100-500 μg) was noted after 15 minutes. The percentage reduction in absorbance was calculated from the initial and final absorbance of each solution. The IC_{50} values were calculated from the calibration curve of concentration of extract vs. percent reduction in absorbance [12, 13].

Super-oxide free radical scavenging activity

Super-oxide scavenging activity was determined by the NBT reduction method of McCord and Fridovich. The reaction mixture contained ethylene diamine tetra acetic acid [EDTA] 6.6 mM containing 3 μg sodium cyanide (NaCN), riboflavin (2 μm), nitroblue tetrazolium (NBT) 50 μM, various concentration of extract and phosphate buffer (67 mM, pH 6.8) in a final volume of 3 ml. The tubes were uniformly illuminated for 15 min. and optical density was measured at 530 nm before and after the illumination. The percentage inhibition of superoxide generation was measured by comparing the absorbance values of control and those of test compounds [14].

Results and Discussion

Due to metabolic reactions in body, oxidants or reactive oxygen species (ROS) are generated [15]. Free radicals are implicated in many disease conditions. Herbal drugs containing free radical scavengers like phenolics are well known for their therapeutic activity [16]. Preliminary phytochemical analysis of the tested extracts i.e. ethanolic and aqueous extract showed the presence of phenolics, tannins, flavanoids, alkaloids, glycosides and saponin. So, a study has been done to find out the *in-vitro* antioxidant activity of the extracts (ethanolic and aqueous) of *Withania somnifera* leaf. Both the extracts (ethanolic and aqueous) showed good *in vitro* antioxidant activity. Results are presented in Table: 1-2 and Figure: 1-4. IC_{50} value of aqueous extract in NBT and DPPH method is (1352 μg/ml and 1455 μg/ml, respectively) and the values for ethanolic extract in NBT and DPPH method is (1597 μg/ml and 1663 μg/ml, respectively). *W. somnifera* leaves are traditionally used in a variety of disorders, the probable mode of action can be through its free radical scavenging activity. The present study indicate that *W. somnifera* leaf extracts could inhibit the oxygen radicals as seen from scavenging super oxide and DPPH radicals, and it could reduce the oxygen radicals and subsequently reduce the harmful effects produced by the oxygen free radicals mediated injuries. The antioxidant activity of the samples can be attributed to the presence of various chemical components including phenolics [16]. The literature supports that phytoconstituents such as polyphenolic compounds in drugs are responsible for the antioxidant potential [17-19]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant [20]. The observed activity may be mainly due to flavonoids, tannins and phenolic content. Further *in vivo* studies are necessary to utilize the antioxidant potentials of the plant.

Table 1: Antioxidant activity of aqueous extract of *Withania somnifera*

S. No	Conc. μg/ml	Absorbance at 590nm (NBT)	Absorbance at 516nm (DPPH)	Percentage Reduction N.B.T	Percentage Reduction DPPH	IC50 (μg/ml) N.B.T	IC50 (μg/ml) DPPH
1	100	0.722	0.812	13.11	12.87		
2	200	0.706	0.796	15.04	14.59		
3	300	0.682	0.777	17.93	16.63	1352	1455
4	400	0.664	0.754	20.09	19.09		
5	500	0.639	0.736	23.10	21.03		
6	1000	0.498	0.577	40.07	38.09		

Absorbance of control = 0.831 (NBT) and 0.932 (DPPH). The percentage reduction was compared with respect to control.

Table 2: Antioxidant activity of ethanolic extract of *Withania somnifera*

S.No	Conc. μg/ml	Abs. at 590nm (NBT)	Abs. at 516nm (DPPH)	Percentage Reduction N.B.T	Percentage Reduction DPPH	IC50 (μg/ml) N.B.T	IC50 (μg/ml) DPPH
1	100	0.753	0.847	9.38	9.12		
2	200	0.740	0.832	10.95	10.72		
3	300	0.722	0.811	13.11	12.98	1597	1663
4	400	0.698	0.792	16.00	15.02		
5	500	0.681	0.773	18.05	17.06		
6	1000	0.548	0.624	34.05	33.04		

Absorbance of control = 0.831 (NBT) and 0.932 (DPPH). The percentage reduction was compared with respect to control.

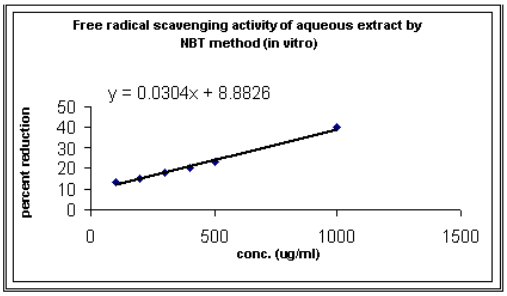

Figure 1: Free radical scavenging activity of aqueous extract *Withania somnifera* by NBT method

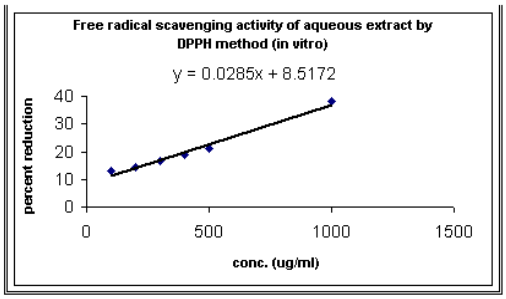

Figure 2: Free radical scavenging activity of aqueous extract *Withania somnifera* by DPPH method

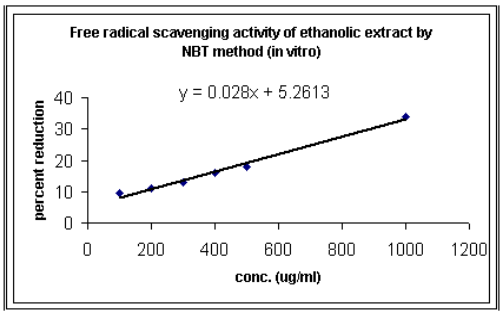

Figure 3: Free radical scavenging activity of ethanolic extract *Withania somnifera* by NBT method

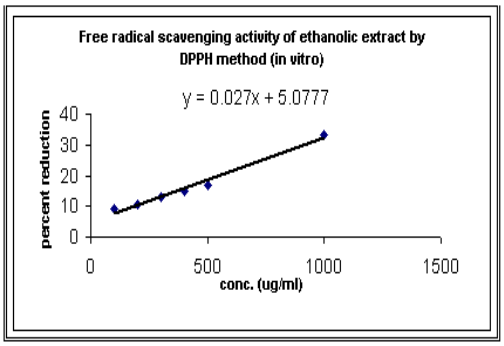

Figure 4: Free radical scavenging activity of ethanolic extract *Withania somnifera* by DPPH method

Conclusion

From the present investigation it can be concluded that *Withania sominifera* can be used as a potent antioxidant as depicted by the results of *in vitro* antioxidant activity. However further studies are needed to refine the technique and to study the *in vivo* antioxidant activity of the plant and to isolate the active principle (s) responsible for the antioxidant activity of the plant.

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