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In Vitro Study of Antioxidant Activity of *Withania Somnifera* (Ashwagandha) Root

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ABSTRACT

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. Total Phenolic content in the extracts measured as gallic equivalent in Ashwagandha (methanolic) 43.77 ± 1.7, aqueous = 42.52 ± 0.8, activity of Trolox by ABTS method IC_{50} value 428.38 ± 1.90 µg /ml, Total Antioxidant activity by ABTS method in Ashwagandha (methanolic) IC_{50} Value is 28.353 ± 0.269 mg/ml in aqueous 32.547 ± 0.532 mg/ml , Free radical scavenging capacity by DPPH in Ashwagandha (methanolic) IC_{50} Value 2.9 ± 0.09 mg/ml in aqueous 3.27 ± 0.05 mg/ml. 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*, known as 'Ashwagandha', has been an important herb in the Ayurvedic and indigenous medical systems for centuries in India.

Keywords: Ashwagandha, Antioxidant, Ayurveda, traditional medicine, decoction, extract.

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INTRODUCTION

Withania somnifera , Dunal (Solanaceae), also known as ashwagandha or winter cherry, is one of the most valuable plants in the traditional Indian systems of medicine. This plant is used in more than 100 formulations in Ayurveda, Unani and Siddha and is believed to be therapeutically equivalent to ginseng [11] The ethnopharmacological properties of the plant include adaptogenic, anti-sedative and anti-convulsion activities, and the plant is used to treat various neurological disorders, geriatric debilities, arthritis, stress and behavior-related problems[6]. W. somnifera is also used as a dietary supplement because it contains a variety of nutrients and phytochemicals. W. somnifera is traditionally used as a therapeutic agent for diarrhea, dyspepsia and gastrointestinal disorders[1]. It has been reported that the antioxidant activities in a plant are dependent on some phytoconstituents such as the phenolic compounds, the anthocyanin and ascorbic acids as well as many other important constituents [9].

MATERIALS AND METHODS-

Plant materials-

In January 2012, WSR, was collected from field-grown plants after six months of cultivation from Palampur (HP) These plants were identified with the help of the available literature and authentified by botanist. The roots of Ashwagandha were procured; and ground to obtain the fine powder.

Preparation of extracts

Ground dry plant materials (500 mg) were weighed in a test tube, followed by the addition of 10 ml of 80% aqueous methanol and in 10 ml water. The suspension was then gently stirred. The tubes were sonicated for 5 min (45°C) and centrifuged (25°C) for an additional 10 min at 1500 ×g. The resulting supernatants were collected. The extraction procedure was repeated three times, and the supernatants were combined before being evaporated using a rotary evaporator to a volume of approximately 1 ml. The concentrated extracts were then lyophilized and weighed. The extract was used for determining the *in vitro* antioxidant potential.

In vitro antioxidant potential

Determination of total phenolic content

The total phenolic content was estimated in the Ashwagandha extract using Folin-Ciocalteu reagent (FCR) based assay[2]. To a 50µl of plant extract, 950µl of water and 500µl of FCR and 2.5 ml of the 20% sodium carbonate solution were added. The mixture was then kept for 40 min at room temperature and absorbance was recorded at 725 nm. A standard curve of gallic acid was

prepared. A control solution was prepared using distilled water instead of extract and the absorbance was recorded against that control. Total phenolic contents (mg/g) in the Ashwagandha extract were expressed as gallic acid equivalent (GAE).

Total phenols = Optical density (O.D.) x 938

Measurement of total antioxidant activity by ABTS method

The total antioxidant activity of plant extracts was determined according to the method of Re *et al.* [10]. A 2mM ABTS solution was prepared in distilled water. ABTS radical cations (ABTS•+) were produced by mixing 50ml of the ABTS stock solution with 200µl of 70mM potassium persulfate. To ensure complete oxidation of ABTS, the mixture was held at room temperature in the dark for 16 to 20 h prior to analysis. The resulting ABTS•+ solution was diluted with phosphate buffer (about four fold) to give an absorbance reading of 0.5 ± 0.05 at 734 nm. The activity of Ashwagandha was evaluated in concentration ranging from 10 to 60 mg/ml and that of trolox from 1 to 3.5 mg/ml.Radical scavenging analysis was performed by mixing 30 µl of the sample solution into 3.0 ml of ABTS•+ solution and reading the absorbance at 734 nm after 3 minutes. A control solution of 30 µl distilled water in 3.0 ml of ABTS•+ solution was prepared and analyzed.

% ABTS•+ inhibition = [1 – (A_{734}nm test/ A_{734}nm control)] x 100

Free radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Hsu *et al.*[5]. A 100 µM solution of DPPH in methanol was prepared. The free radical scavenging action of Ashwagandha extract was seen in concentration ranges from 1 to 10 mg/ml. BHT was taken as standard antioxidant. The free radical scavenging action of BHT was evaluated in concentration ranges from 25 to 1000 µg/ml. A 0.5 ml sample solution was added to 2.0 ml of DPPH in a 20 ml test tube. A control solution was prepared by adding 0.5 ml of methanol to 2 ml DPPH solution. Samples were vertexed for 10 to 15 seconds and held at room temperature (22 ± 3°C) in the dark for 30 minutes. The absorbance of the sample and control solutions was determined at 517 nm and the % DPPH radical scavenging activity was calculated as follows:

% DPPH radical scavenging activity = [1– (A_{517}nm test / A_{517}nm control)] x 100

Superoxide anion radical scavenging assay

The superoxide anion radical-scavenging ability of extract was assessed by the method described by Gulcin *et al.* [3]. Superoxide anion radicals were generated in PMS–NADH system by oxidation of NADH and assayed by the reduction of NBT. In this test, superoxide anion radicals were generated in 2.5 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (300 µM)

solution, 0.5 ml of NADH (468 µM) solution and 1ml of plant extract sample in concentration ranging 10 to 60 mg/ml in case of Ashwagandha. The reaction was started by adding 0.5 ml of PMS (60 µM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm. The control solution was prepared using distilled water in place of extract. The decrease in absorbance of the reaction mixture indicated the superoxide anion scavenging activity. The superoxide anion radical scavenging activity was calculated using the following equation:

% Superoxide radical scavenging activity = $[(A_0 - A_1) / A_0] \times 100$ where,

* A_0 is the absorbance of the control (without samples)

* A_1 is the absorbance of the mixture containing samples.

RESULTS AND DISCUSSION-

Total Phenolic content in the extracts measured as gallic equivalent (mg of GAE/ gram of extract)

Ashwagandha (Methanolic) = 43.77 ± 1.7

Ashwagandha (aqueous) = 42.52 ± 0.8

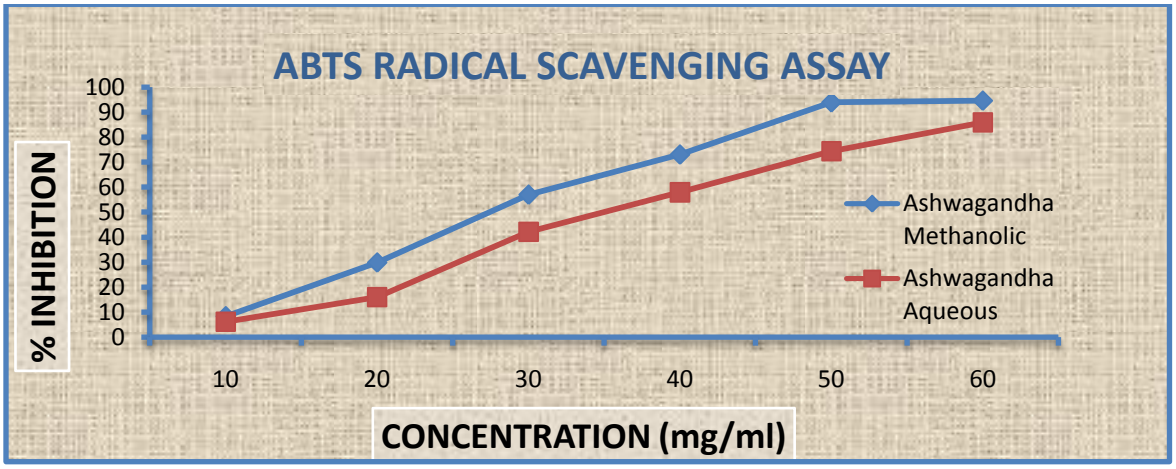

Figure 1. Total Antioxidant Activity of Ashwagandha by ABTS method

Measurement of total phenolic content

The total phenolic content in the extracts of Ashwagandha was determined in terms of gallic acid equivalent. The methanolic extracts of Ashwagandha were found to have more total phenolic content than aqueous, extracts.

Measurement of total antioxidant activity

The IC_{50} value for methanolic extract of Ashwagandha was 28.353 ± 0.269 mg/ml and that of aqueous extract was 32.547 ± 0.532 mg/ml (figure 2). The IC_{50} value of trolox, a standard antioxidant, was found to be 428.38 ± 1.90 µg /ml (figure 3). Hagerman *et al*[4] have reported that

the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{+}$) and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution than the specific functional groups.

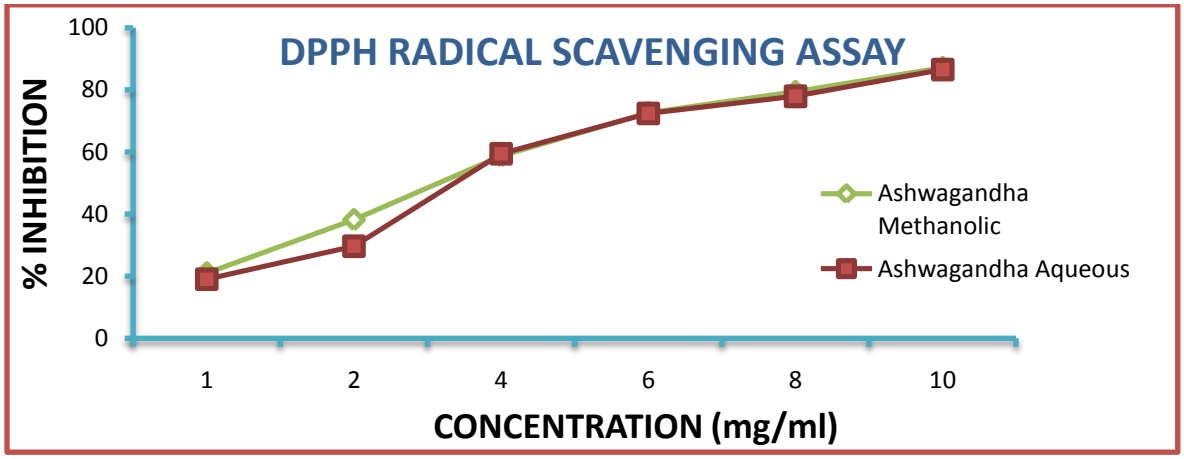

Figure 2.Total Antioxidant Activity of Ashwagandha by ABTS method

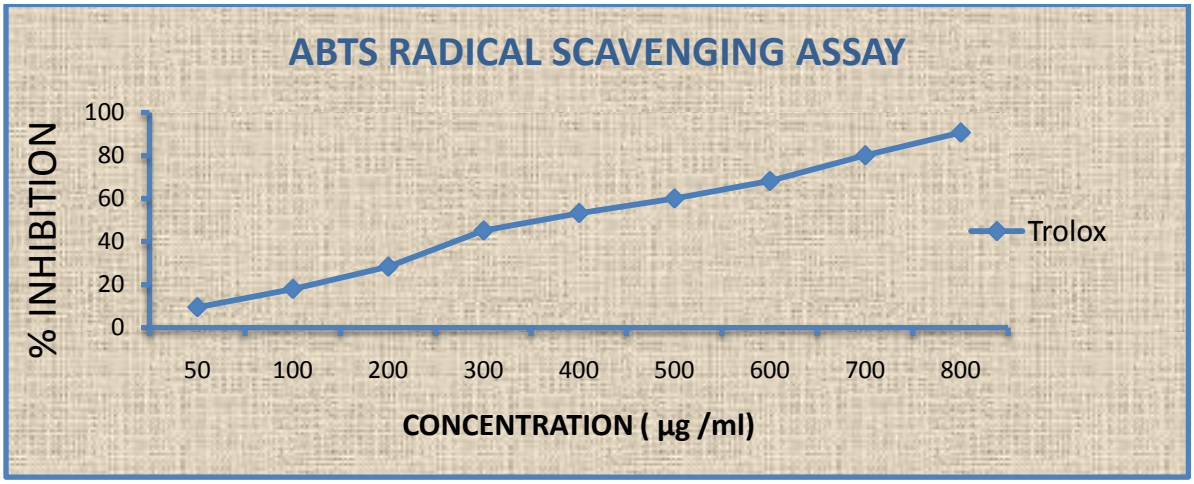

Figure 3. Total Antioxidant Activity of trolox by ABTS method

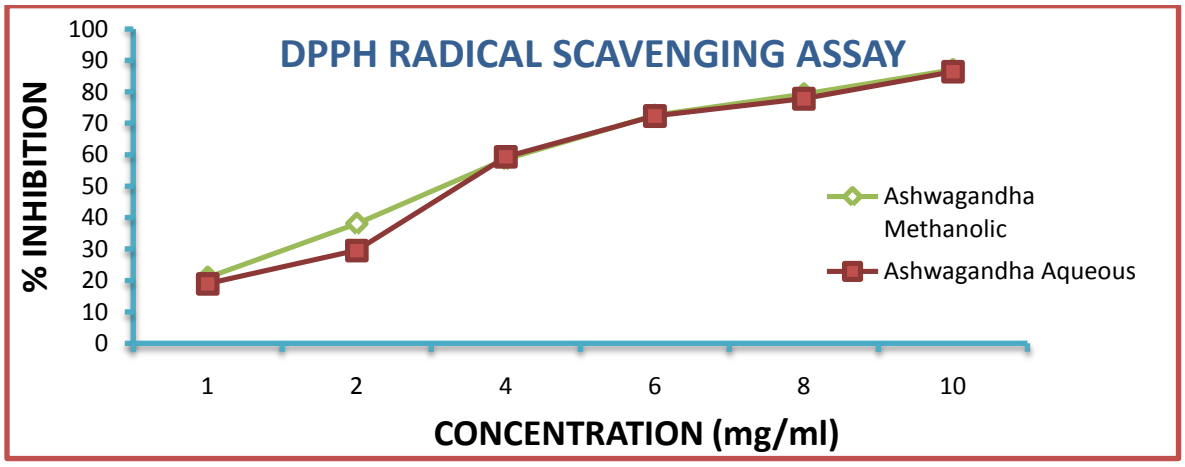

Figure 4. Free radical scavenging capacity by different concentrations of Ashwagandha by DPPH method:

Free radical scavenging assay

The IC_{50} value of methanolic extract of Ashwagandha was found to be 2.9 ± 0.09 mg/ml and that of aqueous extract was 3.27 ± 0.05 mg/ml BHT was taken as standard antioxidant and IC_{50} value of BHT was found to be 560.24 ± 17.07 µg /ml (figure.4). The effect of extract on DPPH radical scavenging is thought to be due to their hydrogen donating ability[7], visually noticeable as a change in color from purple to yellow.

superoxide radical anion scavenging assay

The IC_{50} value for methanolic extract of Ashwagandha was 34.260 ± 0.55 mg/ml and that of aqueous extract was 36.64 ± 0.85 mg/ml (figure 5) The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture [8].

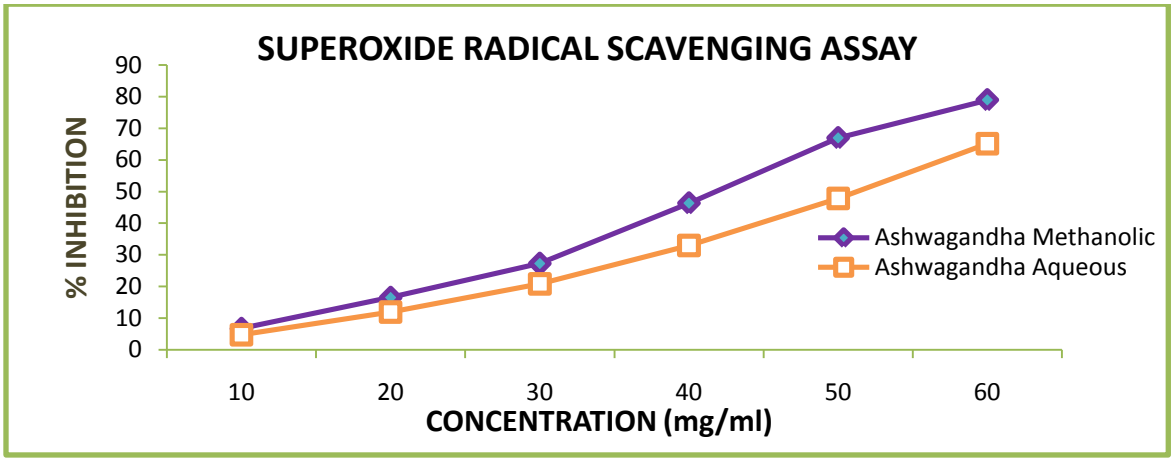

Figure 5. Superoxide radical scavenging capacity by different concentration of Ashwagandha extract.

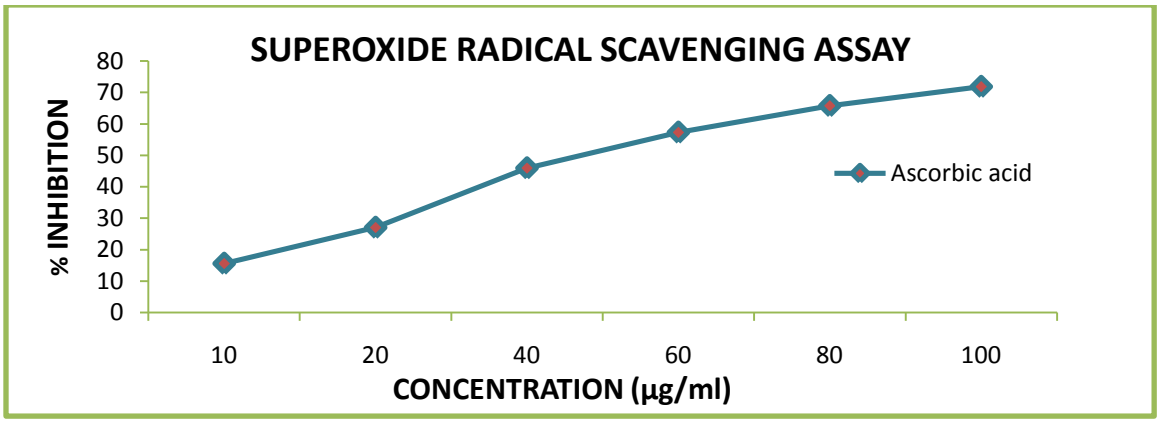

Figure 6. Superoxide radical scavenging capacity by different concentration of Ascorbic acid.

CONCLUSION

The antioxidant potentials vary in different parts (leaves and roots) of *Withania somnifera* plants.

The results showed 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*. Here from this study, it can be concluded that, the root of *Withania somnifera* are good source of nonenzymatic 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 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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