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STUDY OF INVITRO ANTI OXIDANT ACTIVITY OF *WITHANIA SOMNIFERA* FRUIT EXTRACTS

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ABSTRACT: *Withania somnifera* commonly known as Ashwagandha, grown in Indian Territory and Mediterranean, which is a vital medicinal plant from ancient times. In the current study we have used the extracts of *Withania somnifera* fruit for the study of Invitro anti Oxidant activity. The activity is analyzed by using DPPH (1,1-Diphenyl-2-picrylhydrazyl) method as well as Hydrogen peroxide(H_2O_2) method with Ethyl Acetate, Alcoholic extract and Ascorbic acid. In first method the radical form of DPPH is scavenged by an antioxidant through the donation of hydrogen to form a stable DPPH molecule which leads to a colour change from purple to yellow and a decrease in absorbance was measured at 517nm and the effect of extracts is compared with the standard drug ascorbic acid and plant extract. In another method we have used hydrogen Peroxide(H_2O_2) radical scavenging activity was performed with the different concentrations of plant extract andstandard Ascorbic acid solutionand Ethanolic plant extracts and Absorbance of hydrogen peroxide at 230nm was determinedagainst a blank solution containing phosphate buffer without hydrogen peroxide, the effect of extracts is compared with the standard drug ascorbic acid. The result clearly indicates that the alcoholic extract shows less percentage of inhibition when compared to Ascorbic acid as well as Ethyl acetate. The study indicates that Ashwagandha could prove to be a good natural source of a potent and relatively safe antioxidative agent.

KEYWORDS: *Withania somnifera*, Anti Oxidant activity, DPHP, H_2O_2.

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1. INTRODUCTION

Withania somnifera commonly known as Ashwagandha, Winter cherry, Indian Ginseng, Poison Goose berry, usually grown in Indian Territory as well can be found growing in Africa, and Mediterranean. An erect, evergreen, tomentose shrub, 30-150 cm high, found throughout the drier parts of India. Roots are stout fleshy, whitish brown, leaves simple ovate, glabrous, those in the floral region smaller and opposite, flowers inconspicuous, greenish or lubrid-yellow, in axillary, umbellate cymes; berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow, reniform. The roots are the main portions of the plant used therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring. The name somnifera is derived from latin which means Sleep Inducing, and the name Ashwagandha is found in ancient Ayurveda which means the "smell of the horse". Due to its high medicinal values it is widely used in Ayurvedic medicinal preparations. Some previous studies also proved that *W.somnifera* is having a capability to treat rheumatism and neurodegenerative disorders with its antioxidant property [1-2]. Free radicals cause auto oxidation of unsaturated lipids in food[3]. The antioxidants are used to interrupt the free radical chain of oxidation and to donate free radical from phenolic hydroxyl groups and forming stable free radicals which do not initiate further oxidation of lipids[4].

Taxonomic Hierarchy

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliphyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	Withania
Species	*W.somnifera*

Herbal Uses

Historically, the plant has been used as an antioxidant[5], adaptogen[6], antiinflammatory agent[7]more recently to treat ulcers[8], bacterial infection[9], and venom toxins[10]. Clinical trials and animal research support the use of WS for anxiety[11-12], cognitive and neurological disorders, hyperlipidemia and Parkinson's disease. *Withania somnifera* is having chemopreventive properties which make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. Recently it is also used to inhibit the development of tolerance and dependence on chronic use of various psychotropic drugs[13].

Chemical constituents

The main important biological as well as chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds, including ergostane type steroidallactones, withaferin-A, withanolides-A-Y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone etc. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X)[14-15].It also constitutes main amino acids such as tyrosine, cystine, aspartic acid, proline, glutamic acid, alanine, glycine, tryptophan and high volume of Iron. Many biochemically heterogeneous alkaloids have been reported in the roots. Basic alkaloids include cuscohygrine, anahygrine, tropine, pseudotropine, anaferine, isopelletierine, withananine, withananinine, pseudo-withanine, somnine, somniferine, somniferinine. Neutral alkaloids include 3tropyltigloate and an unidentified alkaloid. Other alkaloids include withanine, withasomnine, and visamine. Withanine is sedative and hypnotic[16]. The plant leaves (Indian chemotype) are reported to contain withanolides 12, unidentified alkaloids 5 (yield, 0.09%), many free amino acids, chlorogenic acid, glycosides, glucose, condensed tannins, and flavonoids. The green berries contain amino acids, a proteolytic enzyme, condensed tannins, and flavonoids. They contain a high proportion of free amino acids which include proline, valine, tyrosine, alanine, glycine, hydroxyproline, aspartic acid, glutamic acid, cystine and cysteine.

Importance of *Withania somnifera*

In Ayurveda it is known as 'Rasayana' because it promotes health and longevity, arrest ageing process, increase capability of individual to resist adverse environmental conditions[17].Ashwagandha improves energy and also memory by enhancing the brain and nervous function; shows anxiolytic effects, has hepatoprotective property, raises hemoglobin level and red blood cell count, improve energy level; has potent antioxidant activity, improve the cell-mediated immunity; promotes vigor and vitality along with cheerful sexual life and reproductive equilibrium and act as powerful adaptogen[18-23]. The plant also include some ethnopharmacological properties such as adaptogenic, anti-sedative and anti-convulsion activities, and the plant is used to treat several neurological disorders, geriatric debilities, arthritis, stress and behavior-related problems[24]. The experiments also proved that antioxidant activities in a plant are dependent on some phyto-constituents such as the anthocyanin, phenolic compounds and ascorbic acids as well as many other important constituents[25].The anti-stress activity in rats using alcoholic extracts of dry *Withania somnifera* roots is explained by Singh (1987) and anti stress and anti inflammatory effects. Experiments also revealed that Withania plant extracts also effective in treatment of hyperthyroidism [26]. It is also proven that extracts are effective and potential on suppressing the tumor growth [27].

2.MATERIALS AND METHODS

Collection of plant samples

Withania somnifera fruits were procured from locally available medicinal market.

Extract Preparation

The collected fruits of *Withania somnifera* were shade dried for 24hrs and 100gms of dried fruits dissolved in 100ml of ethanol by using solvent extract method. The antioxidant activity of the *W. somnifera* extracts are studied by evaluating their free radical scavenging effects on the DPPH radical, which was based on the method proposed by Ferreira et al[28]. Radical scavenging activity (RSA) was calculated as the percentage of DPPH discoloration using the following equation: % RSA=$[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Invitro Antioxidant Activity

DPPH radical scavenging activity method

The study of antioxidant activity in the DPPH radical scavenging activity is by scavenging the radical form of DPPH. When the radical form of DPPH is scavenged by an antioxidant through the donation of hydrogen to form a stable DPPH molecule, this leads to a colour change from purple to yellow and a decrease in absorbance was measured at 517nm, and the effect of extracts will be compared with the standard drug ascorbic acid and plant extract of 10,20,40,60,80,100,120,140,180,200 µg/ml in methanol (1 ml).

H_2O_2 Method radical scavenging activity method

The hydrogen peroxide radical scavenging activity was performed with the different concentrations of plant extract andstandard Ascorbic acid solutionand Ethanolic plant extracts viz. 10,20,40,60,80,100,120,140,180,200 µg/ml in methanol (1 ml) where added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.and the effect of extracts will be compared with the standard drug ascorbic acid.

3. RESULTS AND DISCUSSION

DPPH Method

Table1: Representation of experimental results of anti oxidant activity with DPPH method

S.No.	Conc. Of the Extract (µg/ml)	% Inhibition		
		Ethyl Acetate	Alcoholic Extract	Ascorbic Acid
1.	10	32.030±0.648	29.795±1.777	32.330±0.848
2.	20	34.480±0.921	32.599±0.243	33.193±0961
3.	40	43.365±0.468	33.473±0.193	40.405±0.264
4.	60	46.020±0.632	32.430±0.484	47.044±0.896
5.	80	52.692±0.863	34.759±0.956	52.991±0.864

6.	100	65.086±0.762	38.828±1.762	63.085±1.772
7.	120	71.811±0.263	57.079±0.286	72.112±0.643
8.	140	84.126±0.869	62.879±1.434	84.133±0.964
9.	180	87.618±0.783	62.304±1.151	91.610±0.826
10	200	92.412±0.694	63.258±1.878	93.305±0.69
11.	IC_{50}	67.180±0.462	106.281±0.624	106.100±0.268

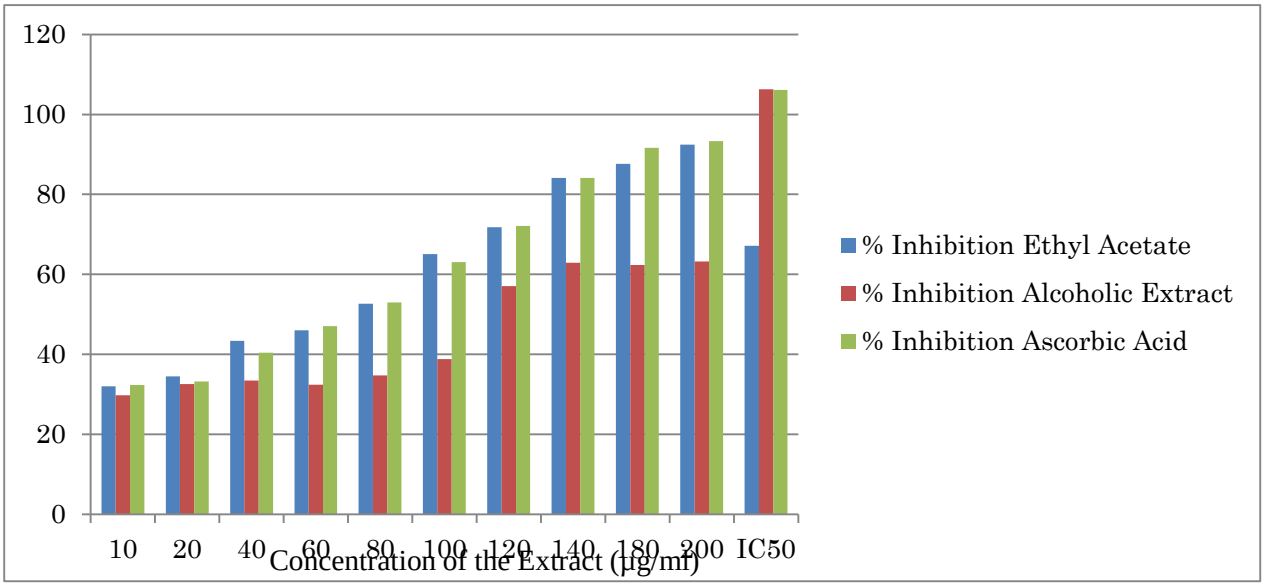

Figure 1: Graphical representation of results of DPPH method between Concentration of *Withania somnifera* fruit extract and % of Inhibition with Ethyl Acetate, Alcoholic extract and Ascorbic acid

H_2O_2 Method

Table 2: Representation of experimental results of anti oxidant activity withH_2O_2method

S.No.	Conc. Of the Extract (µg/ml)	% Inhibition		
		Ethyl Acetate	Alcoholic Extract	Ascorbic Acid
1.	10	1.369±0.641	0.706±0.115	1.486±0.824
2.	20	15.326±1.023	2.572±1.079	16.510±1.059
3.	40	16.062±0.489	9.525±1.683	20.031±0.889
4.	60	22.201±0.968	13.850±1.772	24.007±1.075
5.	80	24.762±0.846	16.378±1.446	26.837±0.930
6.	100	28.528±0.926	21.551±3.769	31.505±0.920
7.	120	46.280±0.286	26.248±0.977	48.905±1.731
8.	140	51.686±0.962	36.172±1.580	52.678±1.182
9.	180	66.082±0.982	53.578±1.182	65.524±0.990
10	200	76.106±0.561	65.707±0.995	77.012±1.059
11.	IC_{50}	124.160±0.368	175.010±0.462	121.120±0.264

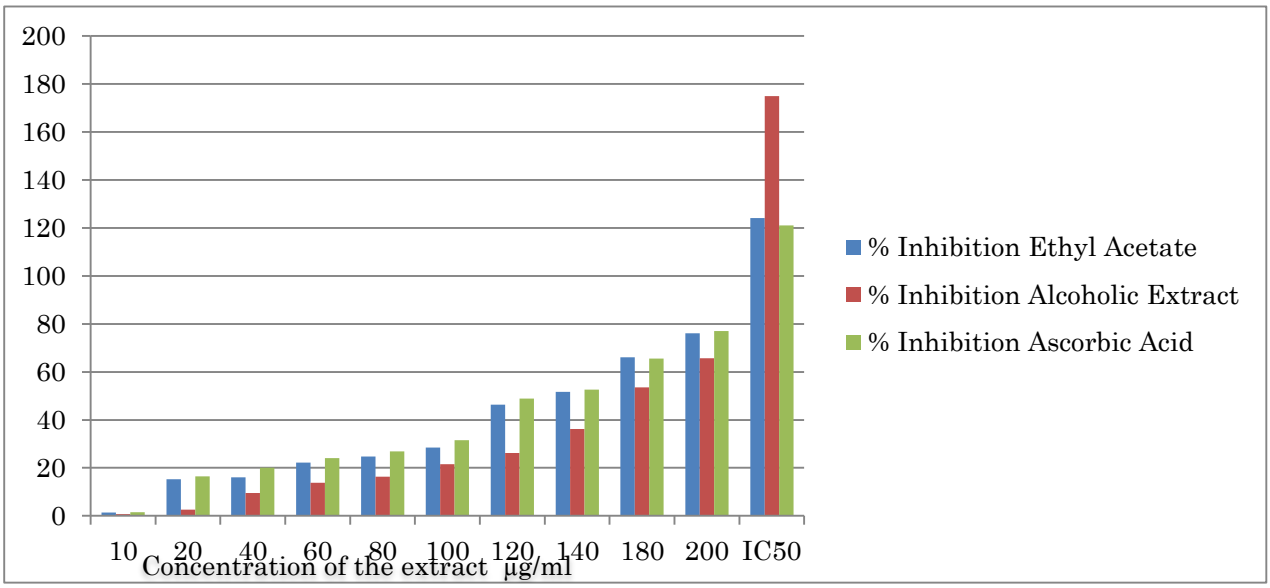

Figure 2: Graphical representation of results of H_2O_2 method between Concentration of *Withania somnifera* fruit extract and % of Inhibition with Ethyl Acetate, Alcoholic extract and Ascorbic acid

4. CONCLUSION

Antioxidants are naturally present in foods or they can be added during food processing. Hence the antioxidants for foods should be reasonable in cost, stable, nontoxic, effective at low concentration, have carry□through, and should not change flavor, color, and texture of the food matrix[29]. By using DPPH method the color change from purple to yellow is caused due to the decreased absorbance, when the DPPH was scavenged by an antioxidant by donating hydrogen to form a stable DPPH molecule. When the molecule is in radical form it had an absorbance at 517nm, but it was disappeared after accepting a hydrogen radical from antioxidant compound and became a stable diamagnetic molecule[30]. The fruit extracts of Withania somnifera has shown significant property as antioxidant and it can be further utilized in food processing.

CONFLICT OF INTEREST

Authors have no any conflict of interest.

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