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In Vitro Study on total Phenols, Flavonoids Content and DPPH Activity of *Withania* Species

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Abstract— *The escalating interest in appraisal of antioxidant power of herbal plant as medicine, the current study was carried out to explore the antioxidant potential of aqueous extracts of Withania somnifera root and Withania coagulan fruit in-vitro. Antioxidant activity; total phenol,total flavonoids and DPPH free radical scavenging assay of Withania somnifera root and Withania coagulans fruit aqueous extracts were determined by using reference standards gallic acid, quercetin and ascorbic acid, respectively. The highest total phenols content (mgGAE/g) and total flavonoids content (mgQE/g) was found to be 33.1±0.82 and 1.86±0.01 respectively in aqueous somnifera root extracts as compared to coagulans fruit extract . The DPPH radical scavenging activity of the both extracts was increased with the increasing concentration and was observed high in aqueous extract insomniferaroot (IC_{50}= 54) than coagulans fruit (69µg/ml) aqueous extract.Thus,Withania somnifera root* has potent antioxidant activity *and may serve as a good pharmacotherapeutic agent which could be explored to provide affordable medicines to masses.*

Keywords— DPPH (2, 2-diphenyl-1-picrylhydrazyl), Total Flavonoids Content, Total Phenol Content, Withania somnifera, Withania coagulans.

I. INTRODUCTION

The use of medicinal plants are increasing day by day in developing and developed countries due to their no side effects[1]. Traditional herbal medicines are new therapeutic contender because of their structural complexity, chemical diversity and wide variety of antimicrobial activity[2].The genus *Withania* (Family: Solanaceae) is a highly-acclaimedremedial plants in the Indian Ayurvedic system of medicine because of its valuable pharmaceutical and nutraceutical properties. It is a small group of herbs distributed from the Northern Africa to the South-west of Asia. Among the twenty-three-known species of *Withania*, only two (*Withania somnifera* and *Withania coagulans*) are economically significant medicinal plant[3].

Withania somnifera commonly known as "Ashwangandha" or "Indian Ginseng" and is an important plant in Indian traditional Ayurvedic system of medicines[4]. Ashwagandha improves energy and also memory by enhancing the brain and nervous function; shows anxiolytic effects, has hepatoprotective property, raises haemoglobin level and red blood cell count, improve energy level, improve the cell-mediated immunity; promotes vigour and vitality along with cheerful sexual life and reproductive equilibrium and act as powerful adaptogen[5].*Withania coagulans* also known as 'Indian cheese maker' or 'vegetable rennet'[6]. It is traditionally used as digestant, anti-flatulent, sedative, antihyperglycemic antimicrobial, anti-inflammatory, antitumor, hepatoprotective, cardiovascular, immune-suppressive, free radical scavenging and central nervous system depressant activities[7].

There are several plants used in Ayurvedic medicinal systems origin with potential therapeutic activity, which are widely used as Ayurvedic medicine[8]. Therefore, an endeavour has been made to investigate the antioxidant properties of *Withania somnifera* root and *Withania coagulans*fruit.

II. MATERIALS AND METHODS

Sample Collection

Withania somnifera roots and *Withania coagulan* fruits were collected from local market of Delhi and authenticated by scientist of National Institute of Ayurveda, Jaipur. Rajasthan. Roots and fruits were washed and dried in open air for 2-3 weeks at 35-40º C and then dried material was pulverized in an electric grinder and stored in plastic containers in refrigerator (5^0 C), until further analysis.

Aqueous Extract Preparation

20 g of powdered plant material was kept in 200ml conical flask and add 100 ml of distilled water. The mouth of the conical flask was covered with the aluminum foil and kept in a reciprocating shaker for 25 mins for continuous agitation at 150 rev/min for thorough mixing. Then extracts was filtered by using muslin cloth followed by Whatmann

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filter paper No. 42 (125mm). The content was filtered by using rotatoryvacuum evaporator with the water bath temperature of 65º C and finally the residue were collected and used for the analysis[9].

Withania coagulan

Withania somnifera

Determination of Total Phenols Content:

Total phenols were determined by Folin-Ciocalteu Reagent. A dilute extract of root and fruit (0.5 ml of 1:10g/ml) or Gallic acid (standard phenol compound) was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and 4 ml of aqueous sodium carbonate (1M). The mixtures were kept at dark ambient condition for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250mg/l solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound[10].

Determination of total flavonoids content:

Aluminum chloride colorimetric method was used for flavonoids determination. Root and fruit extracts (0.5 ml of 1:10 g/ ml) in aqueous were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 g/ml in aqueous[11]

DPPH radical scavenging activity:

The ability of the aqueous extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined Spectrometric method. Aliquots of the sample extract at different concentrations 20-200 µg/ml were added to 1 mm aqueous solutions of DPPH. Each mixture was vortexes vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage. IC_{50} value was also determined by graph[12]. DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \text{ X } 100$$

Statistical Analysis:

The results obtained were expressed as mean ±SD and student t-test of three determinations and also statistically analyzed to ascertain its significance. The significance was estimated at ($p \leq 0.05$level).

III. RESULTS AND DISCUSSION

Table.1: Antioxidant potential of Withania somnifera root and Withania Coagulans fruit aqueous extract

Antioxidants	*Withania Somnifera root*	*Withania Coagulans fruit*
TPC (mgGAE/ml)	33.1±0.82	14.5±0.78*
TFC (mgQE/ml)	1.86 ±0.01	1.08±1.7ns

(n=3) Values are expressed as means±SD.* significant, ns-non-significant when compared with *W.coagulan* aqueous extract at P ≤0.05. TPC-,Total Phenol Content TFC-Total Flavonoids Content

It has been documented that phenols as well as flavonoids show antioxidant activity and their effects on human nutrition and health through scavenging or chelating process[13]. In the present study, total phenols content of *Withania somnifera and Withania coagulans* aqueous

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extractexpressed in Gallic equivalents (GAE) were found to be 33.1±0.82 and 14.53±0.78 GAE mg/g respectively as shown in Table 1.The data showed that *somnifera* root powder was significantly increased by 56.19% from *coagulans* fruit aqueous extract at P ≤0.05 levels. The flavonoids content was measured by aluminum chloride technique in terms of quercetin equivalent and found low value in both extracts of *Withania somnifera* and *Withania coagulan* had 1.86.±0.01 and 1.08 ±1.7 (mgQE/ml) respectively. *W. somnifera* aqueous extract was insignificantly increased by at 22.54 % at p≤0.05 when compared to *W. coagualan.* According to study based on phytochemical analysis showed that *W. somnifera* had 180.80±0.01 mg/100mg gallic acid equivalent phenolic content whereas flavonoid content was 136.97 ±0.01 mg/100 mg quercetin equivalent in*Withania somnifera* root methanolic extract[14].

The medicinal effects described in the ayurveda that traditional plants have excellent phenolic acids and flavonoids content that are important ingredients to prevent against oxidative stress related disorders[15]. The results acquired in this study thus suggest the identified bioactive constituents in both *Withania* species is proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit.

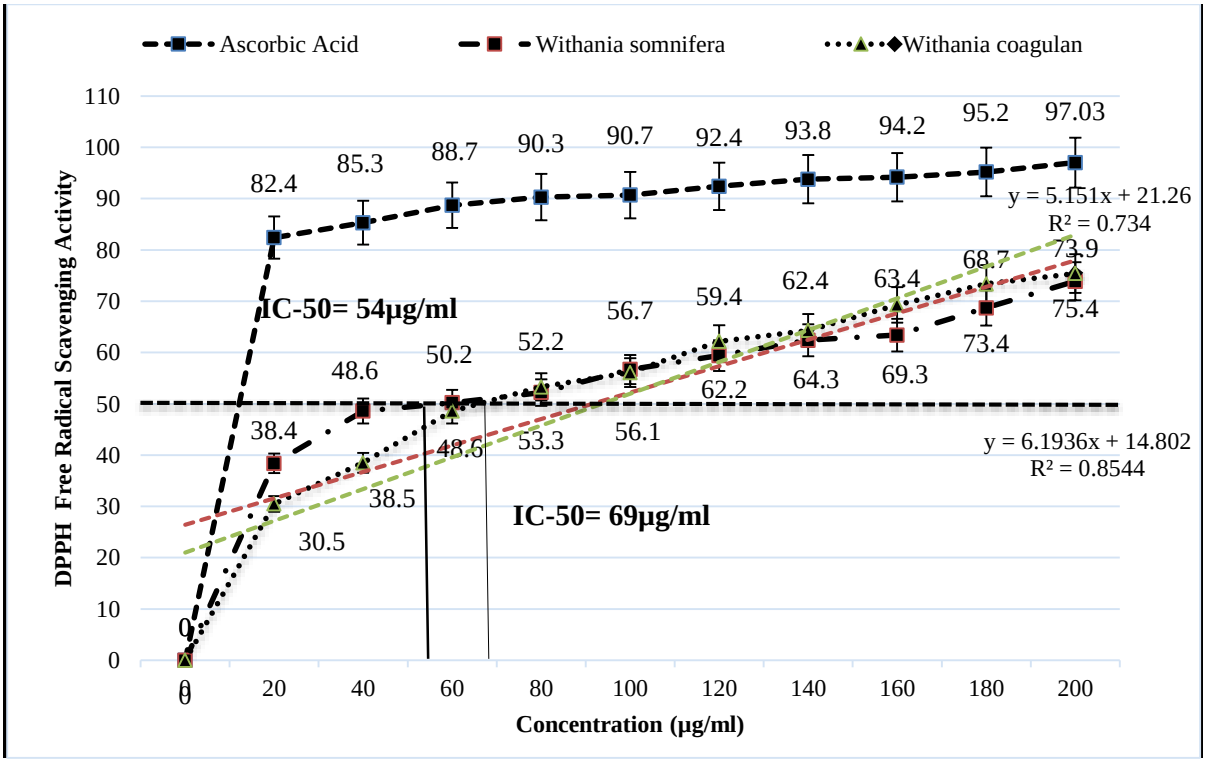

Fig.1: Scavenging effects of the aqueous extract of Withania somnifera and Withania Coagulans on DPPH at Different concentration.

DPPH free radical scavenging method is a sensitive way to determine the antioxidant activity of plant extracts. The reduction capability of free radicals was determined by decrease in its absorbance at 517nm[16]. Several concentrations ranging from 20–200 µg/ml of the aqueous extract of *Withania somnifera* and *Withania coagulan*were tested for their antioxidant activity in different in vitro models.IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds[17]. The percentage scavenging at IC_{50} values were intended for both extracts were illustrated in Fig 1. It shows that there was a decrease in the concentration of DPPH radicals due to the scavenging ability of the soluble constituents in the aqueous extract of *W.somnifera and W.coagulans* and the standard ascorbic acid as a reference compound. As the concentration increases the free radical scavenging activity of plant extract also increases. The data depicts that IC_{50}value was 54µg/ml *for W. somnifera* and 69µg/ml *for W. coagulans* which was lower value according to data shown by Shariar[18] that methanol extract of *Withania somnifera* root showed IC_{50} of 267.818 µg/ml. Similar data stated by Nadia

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Alam[19] that the *Withania somnifera* extracts was 101.73-801.93 µg/ml at IC_{50} which indicated higher value from present data.

IV. CONCLUSION

Although both *Withania* species had potent antioxidant activity but from the study data indicated that *Withania somnifera* aqueous root extract showed higher bioactive compounds as well as DPPH radical scavenging activity when compared to *Withania coagulans* fruit aqueous extract. Thus,*W. somnife*ra root will definitely serve as a high-quality phytotherapeutic agent in various metabolic and degenerative diseases.

V. FUTURE PROSPECTIVE

The phytochemistry and pharmacology characteristics of both *Withania* species have been widely investigated but the studies on toxicology of the extracts in different solvents are very few. So,further exploration is needed for identification and isolation of the particular compound responsible for the specific activity.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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