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Antioxidant activity of *Withania somnifera* (L.) Dunal by different solvent extraction methods

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ABSTRACT

Withania somnifera (Solanaceae) Dunal commonly known as Ashwagandha, is an Indian medicinal plant that grows as an evergreen shrub in dry parts of India. *Withania somnifera* (L.) leaves, stem and root were screened for antioxidant activities by different solvent extraction methods. The extracts exhibited a good scavenging effect on DPPH, chelating activity and reducing power and the scavenging effect of the extracts was comparable with the standard ascorbic acid. The total phenolic content was determined as gallic acid equivalents. Among the different solvent extracts of each parts, methanol extract showed maximum antioxidant capacity than the other extracts like methanol > chloroform > acetone > ethyl acetate.

Key words:*Withania somnifera*; Solanaceae; DPPH; Chelating activity; Reducing Power.

INTRODUCTION

In the aerobic environment, the most dangerous by product are the species of reactive oxygen. The role of antioxidants is to detoxify reactive oxygen intermediates (ROI) in the body. Over the past several years, nutritional antioxidants have attracted considerable interest in the popular press as potential treatment for a wide variety of disease states, including cancer, atherosclerosis, chronic inflammatory diseases and aging. Plant phenolics are multifunctional antioxidants and they act at several ways in the oxidative sequence. It quenches the singlet oxygen and thus decreases the level of local oxygen concentration. The transition metal chelating action by phenolics has been described. ccumulating oxidative damage can then affect the efficiency of mitochondria and further increase the rate of ROS production [1] (Stadtman, 1992). Plant is a good source of natural antioxidant.

The use of medicinal plant as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in India, China and Near east, but it is doubtless an art as old as mankind. It is classified in the ancient Indian system of medicine (Ayurveda) as a rasayana, a group of plant derived drugs that improve overall physical and mental health and put off diseases by rejuvenating the body in incapacitated conditions. As an antioxidant, the plant has been proved to increase the levels of endogenous superoxide dismutase, catalase, and ascorbic acid, whole decreasing lipid peroxidation [2].

Kaur *et al*., (2004) reported antiproliferative and antioxidative activities of leaf extract of *Withania somnifera* [3]. It is known to contain 26 alkaloids and steroidal lactones called as withanolides, of which withaferin 'A' seems to be most bioactive and responsible for all the positive activities. Traditionally all parts of plants are used as medicine but root is most commonly used [4]. Several studies on this plant indicated that it possesses antiinflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic and rejuvenating properties besides positively influencing the endocrine, cardiopulmonary and central nervous systems [5]. Despite the availability of literature on the medicinal properties of *Withania somnifera* and its chemical constituents [6], only few reports exist on its antioxidant properties. We, therefore, have attempted to investigate the effect of *Withania somnifera* on antioxidant profiles.

MATERIALS AND METHODS

Plant Material

Withania somnifera (L.) Dunal plant materials were sourced from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Extraction

The plant material was washed, air dried and ground to a fine powder. The powder (40g) was exhaustively extracted with methanol (99.5%), acetone, ethyl acetate (99.7%) and chloroform (Qualigen) in using soxhlet apparatus for 4 h. The solvents were distilled off to concentrate the extract. Residual solution was concentrated in a rotary evaporator apparatus approximately at 40^0C.

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Evaluation of antioxidant activity

Determination of total phenolic content

Total phenolic concentrations were determined as Gallic acid equivalents (GAE) [7]. The dry extract (30mg) was diluted in 1 ml of methanol and 100µl of the solution was transferred to a 10 ml volumetric flask, to which 0.5 ml undiluted Folin-Ciocalteu reagent was added. After one minute, 1.5 ml of 20% (w/v) Na_2CO_3 was added and the volume made up to 10 ml with water. After one-hour incubation at 25° C, the absorbance was measured at 760 nm and compared to a pre-prepared Gallic acid calibration curve.

DPPH radical scavenging assay

The ability of the extracts to scavenge DPPH radical was assessed spectrophotometrically [8]. Briefly, 50 µl aliquot of the extract was mixed with 1.0 ml DPPH (0.1 mmol/l, in methanol), the resultant absorbance was recorded at 517 nm after 30 min incubation at 37 °C. The percentage of antioxidant activity was calculated using the following equation.

Percentage of inhibition = $[(A_{contrpl} - A_{sample}) / A_{control}]$ X 100%

Where $A_{control}$ was the absorbance of the control (blank, without extract) and A_{sample} was the absorbance in the presence of the extract.

Fe^{2+} chelating activity

The chelating activity of the extracts for ferrous ions Fe^{2+} was determined [9]. To 0.5 ml of extract, 1.6 ml of distilled water and 0.05 ml of $FeCl_2$ (2 mM) was added and after 30 sec, 0.1 ml ferrozine (5 mM) was added. Then the reaction mixture was incubated for 10 min at room temperature and the absorbance of the Fe^{2+} - Ferrozine complex was measured at 562 nm. A lower absorbance indicated a higher chelating power. The chelating activity of the extracts on Fe^{2+} was compared with that of EDTA (0.01 mM) and Citric acid (0.025 M).

Chelating activity was calculated according to the following equation

Chelating activity (%) = $[(A_{control} - A_{sample}) / A_{control}]$ X 100

where $A_{control}$ was the absorbance of the control (blank, without extract) and A_{sample} was the absorbance in the presence of the extract.

Reducing power

The reducing power of the methanol, acetone, ethyl acetate and chloroform extracts was measured according to the method of Oyaizu (1986)[10]. Various concentrations (0.10, 0.25, 0.50, 0.75 and 1.0 mg/ml) of the extracts (0.5 ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 500C for 20 min. TCA (10%: 2.5 ml) was added. The mixture was centrifuged at 650g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride and the absorbance was measured at 700 nm in a Hitachi U-1500 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of methanol, acetone, ethyl acetate and chloroform extracts was compared with that of L-ascorbic acid (0.2 mg/ml).

RESULTS AND DISCUSSION

Total phenolic content

In most of the antioxidant study the phenolic compounds was directly correlated with its antioxidant ability. Therefore, in the present study it was considered imperative to analyze the total phenolic content (Folin-Ciocalteu method) of different solvent (methanol, acetone, ethyl acetate, and chloroform) extracts of different parts of *Withania somnifera* (L.) methanol, acetone, ethyl acetate and chloroform is shown in Figure: 1. Among the different solvents extracts of each part, methanol extract showed maximum phenolic content than the other extracts. As per the analysis the total phenolic content of different

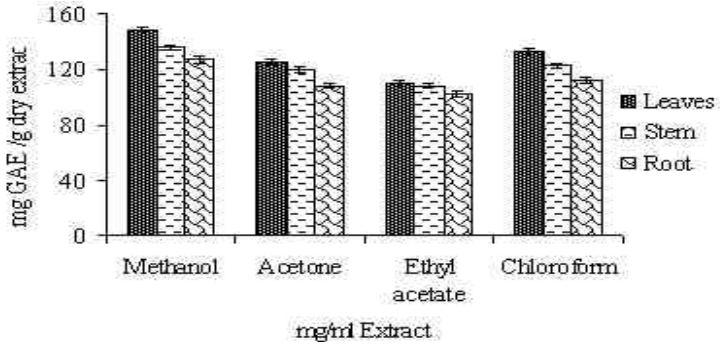

Figure 1 :Total phenolic content of different extracts of *Withania somnifera*(L.) Parts (Leaves, Stem and Root)

Solvent extracts of the different parts are rank in the order of methanol <chloroform <acetone <ethyl acetate. This may be due to polarity of the solvent. The extraction yields, nature of the compound, and the material from which the compounds were extracted strongly depend on the solvents due to the presence of different concentration of bioactive compounds with different polarities.

1,1-Diphenyl-2-Picrylhydrazyl Free Radical Scavenging Activity

It is well known that free radicals cause auto oxidation of unsaturated lipids in food [11]. In addition antioxidants are known to interrupt the free-radical chain of oxidation and to donate hydrogen from phenolic hydroxyl groups, thereby, forming stable free radicals, which do not initiate or propagate further oxidation of lipids [12]. The 1,1-Diphenyl-2-picrylhydrazyl radical has been widely used to evaluate the free radical scavenging capacity of antioxidants [13] . The determination of scavenging stable DPPH was a very fast method to evaluate the antioxidant activity of the extracts is shown in Figure: 2. With this method, it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule had an absorbance at 517 nm, which disappeared after acceptance of an electron or hydrogen radical from the antioxidant compound to become a stable diamagnetic molecule [14].

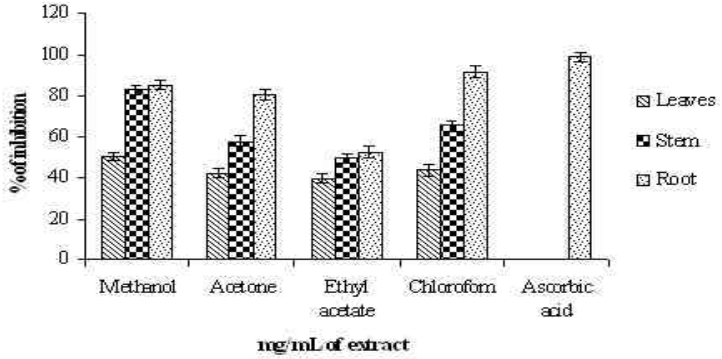

Figure 2: DPPH radical scavenging activity of different solvent extracts of W*ithania somnifera* organs (leaves, stem and root)

As can be seen in Figure: 2, at a dose of 1.0 mg/ml, the different solvent (methanol, acetone, ethyl acetate, and chloroform) extracts were capable of scavenging DPPH· free radicals. From the percentage scavenging values, it can be seen that the methanol extracts of leaves, stems, and root showed a maximum DPPH scavenging activity than the other solvent extracts. As per the antioxidant ability of different solvents and different parts of extracts are rank in the order of methanol <chloroform <acetone <ethyl acetate. The change in activity may be due to the efficient of extraction and solvent used for the extraction. None of the extracts were as effective DPPH· scavengers as that of positive control ascorbic acid (85%). These results indicated that the radical scavenging capacity of each extract might be related to the concentration of phenolics. The antiradical activity of phenolic compounds depends on their molecular structure, that is on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation [15].

Effect of extracts on Fe^{2+} chelating activity

The ability to chelate transition metals can be considered as an imperative antioxidant mode of action. Among the transition metals, iron is known as the most important pro-oxidant metal, which induces lipid oxidation due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxidase to reactive free radicals via the fenton type reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$). Fe^{3+} ion also produces radicals from peroxides, although the rate is tenfold less than of Fe^{2+} ion [16]. Fe^{2+} ion is the most powerful pro-oxidant among various species of metal ions [17]. Ferrozine a chelating reagent was used to indicate the presence of chelator in the reaction system. Ferrozine forms a complex with free Fe^{2+} but not with Fe^{2+} bound by extracts. In the presence of chelating agents, the complex formation of ferrous and ferrozine is disrupted, resulting in a decrease in red color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the coexisting chelator [18]. Therefore, in the present study, an attempt was made to determine the ability of different solvent (methanol, acetone, ethyl acetate and chloroform) extracts of leaves, stem and root of *Withania somnifera* (L.). The chelating activity of all the extracts of 1.0 mg/mL on Fe^{2+} was tested. All the extracts showed chelating activity on Fe^{2+} is shown in Figure: 3.

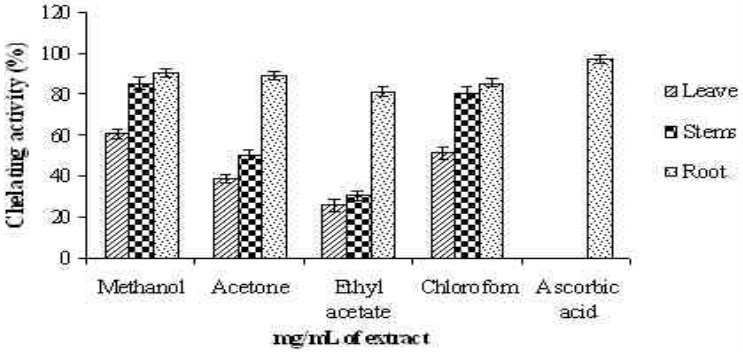

Figure 3: Fe^{2+} chelating activity of different extracts of W*ithania somnifera* (L) Organs (leaves, stem and root)

From the Fe^{2+} chelating activity, it can be seen that the methanol extracts of leaves, stem, and root showed a maximum chelating activity than the other solvent extracts of the different parts. It either was reported that chelating activity of a particular extract is mainly due to the presence of chelated metal ions or suppressed reactivity, by occupying all coordination sites of metal ion, by the antioxidants present in the extract [19]. Similarly, in the present study, the increased chelating activity of the callus extract could be due to the abundant presence of antioxidant active compounds. Therefore, it may be used as an effective agent in retarding Fe^{2+}- catalyzed lipid oxidation.

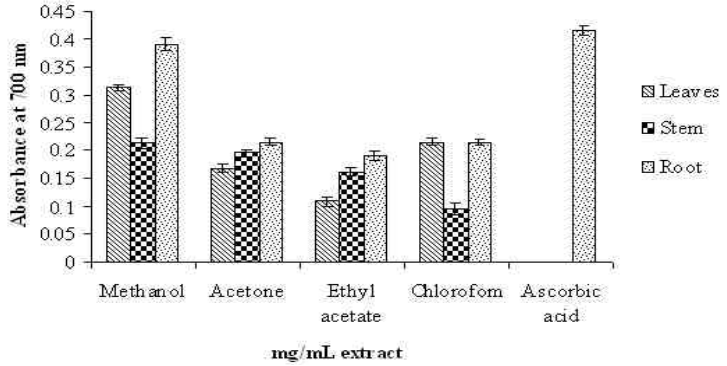

Figure 4: Reducing Power activity of different extracts of *Withania somnifera*(L) Organs (leaves, stem and root)

Reducing antioxidant power

In order to examine the reducing power of extracts, the reduction of Fe^{3+} to Fe^{2+} was investigated in the presence of extracts. The presence of reductant antioxidants in the tested samples would result in the reduction of Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The ferrous ion can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of different solvent (methanol, acetone, ethyl acetate, and chloroform) extracts of leaves, stem, and root of *Withania somnifera* (L.) has been recorded and shown in Figure:4. As per the reducing power ability of different solvents and different parts of extracts we can ranked in the order of methanol < chloroform <acetone <ethyl acetate. However, none of the extracts showed better activity than the ascorbic acid at 1 mg/mL. This might be due to polarity of solvent used and variation in the presence of antioxidant active principles in different parts extracts of *Withania somnifera* (L.). The reducing the ability of particular extracts indicates that inhibition of oxidation or lipid peroxidation in the preserved food stuffs. These activities are reminiscent to the activities of superoxide dismutase and catalase.

CONCLUSION

The results of the present study revealed that all the extracts of leaves, stem and root possessed antioxidant activity but the methanol extracts of *Withania somnifera* (L)

Dunal were the strongest radical scavengers in all the assays. In future by identification and isolation of antioxidative constituents from this plant will be promising application possibilities as natural antioxidants.

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