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In vitro estimation of total phenolics and DPPH radical scavenging activity of *Withania somnifera* extract

K Prashanth Kumar, V Ravinder Reddy, M Gnana Prakash and Pranay Kumar K

Abstract
Withania somnifera commonly known as Ashwagandha, is an important plant in Indian traditional ayurvedic medicine and is believed to increase longevity and vitality. *W. somnifera* contains a variety of phytochemicals i. e flavonoids and plant polyphenols. *Withania somnifera* extract was analysed for its reducing ability as an antioxidant using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay and for total phenolics using the Folin-Ciocalteu method. The analysed DPPH radical scavenging activity of *W. somnifera* was 77.3% and total phenolic content of *W. somnifera* extract was 308 mg gallic acid equivalents/g extract (standard curve equation: y=0.0058x + 0.0966, R2=0.9906). The results revealed that *Withania somnifera* extract was a better source of antioxidants.

Keywords: *Ashwagandha*, DPPH radical scavenging activity, total phenolics, *Withania somnifera*

1. Introduction
Plants are an important source of bioactive molecules for drug discovery. *Withania somnifera* (L.) Dunal, (*Solanaceae*) commonly known as Ashwagandha or Indian ginseng, is a green shrub found in drier parts of India, Pakistan, Afghanistan, Sri Lanka. In India, it is cultivated widely in Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat and Haryana [1]. It is categorized as a rasayana in Ayurveda and traditional Indian systems of medicine. The rasayanas, apart from their use for promoting physical and mental health also provide defence against diseases and arrest ageing process [2, 3]. The extract of W. somnifera is a complex mixture of a large number of phytochemicals including phenolic compounds and flavonoids. However, the pharmacological effect of the roots of *W. somnifera* is attributed to withanolides [4]. Polyphenols are the biggest group of phytochemicals, and many of them have been found in plant-based foods. Polyphenols are strong antioxidants that complement and add to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress caused by excess reactive oxygen species (ROS) [5, 6]. Modulation of cell signalling pathways by polyphenols may help significantly to explain the mechanisms of the actions of polyphenol-rich diets. Dietary polyphenols have been shown to play important roles in human health. High intake of fruits, vegetables and whole grains, which are rich in polyphenols, has been linked to lowered risks of many chronic diseases including cancer, cardiovascular disease, chronic inflammation and many degenerative diseases [7, 8]. Polyphenols have been found to be strong antioxidants that can neutralize free radicals by donating an electron or hydrogen atom [9]. In addition to radical scavenging, polyphenols chelates metal ions such as Fe^{+2} directly and reduce the rate of Fenton reaction, thus preventing oxidation caused by highly reactive hydroxyl radicals [10, 11]. Other mechanisms such as inhibition of xanthine oxidase and elevation of endogenous antioxidants such as glutathione peroxidase, catalase and superoxide dismutase that decompose hydroperoxides, hydrogen peroxide and superoxide anions, respectively, and inhibit the expression of enzymes such as xanthine oxidase [12].
In India indigenous remedies have been used in treatments of diseases such as neurologic degeneration, diabetes etc. *Withania somnifera* is used as rejuvenator, anti-ageing and adoptogenic (antistress) agent.
The aim of present study was to determine total phenolic content by Folin-Ciocalteu method and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (%). These methods were used to find out antioxidant potential of *W. somnifera* extract.

2. Materials and Methods

2.1 Plant material and preparation of extracts

Finely ground dry plant material (50 mg) is taken in a test tube of 5 ml capacity. To remove pigments and fats that interfere with absorbance, plant material was extracted with diethyl ether containing 1% acetic acid before estimating the phenols. Add 10 ml of aqueous acetone (70%) to plant material and keep it in incubator at 39 ºC for 30 minutes. The contents of beaker then transferred to centrifugation tubes, vortexed and centrifuged for 10 minutes at 3500 rpm at 4 ºC. Collect the supernatant and use it for estimation of total phenol content [13].

2.2 Determination of total phenolic content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method with some modification. In a 10 ml of different clean test tubes 500 µL of different concentrations of extracts in water was mixed with 0.5 mL of 10 fold diluted Folin-Ciocalteu reagent. After 5 min 0.5 ml of 7.5% (w/v) Sodium carbonate solution, 4.5 ml of DW were added, vortexed and incubated in a dark place for 120 min, the optical density was measured at 760 nm against a blank using Metstar MUV-61PCS spectrophotometer. The total phenolic contents were calculated on the basis of the calibration curve of gallic acid standards (10 ppm-100 ppm) and expressed as gallic acid equivalents (GAE), in milligrams per gram of the sample [14].

2.3 DPPH (2, 2-diphenyl-1-picrylhydrazy) radical scavenging activity

DPPH radical scavenging activity determined according to the method described by Hatami *et al*. (2014) with slight modifications. The samples were extracted in cold solution of 3% oxalic acid in 8% glacial acetic acid and centrifuged at 15000 rpm at 4 ºC and supernatant collected and used for assay. 1.5 ml of extract of different concentrations are taken in different test tubes to the equal volume of DPPH solution (0.2 mM) in methanol and shaken vigorously. The tubes were maintained in dark for 30 mins and absorbance was measured at 517 nm against blank using Metstar MUV-61PCS spectrophotometer. Ascorbic acid is used as control. The radical scavenging activity was calculated by using following formula and expressed in percent of radical scavenging activity. The determination of DPPH radical scavenging activity was carried out in triplicate and results were averaged.

$$RSA\ (\%) = \frac{(\text{Abs of control}) - (\text{Abs of test sample})}{(\text{Abs of control})}$$

3. Results

3.1 Total phenolic content (TPC)

The amount of TPC was determined by the Folin-Ciocalteu method. Gallic acid was used as a standard and total phenols were expressed as mg/g GAE using standard curve equation: $y=0.0058x + 0.0966$, $R^2=0.9906$, where y is absorbance at 760 nm and x is TPC in *Withania somnifera* extract. The total phenolic content measured in *Withania somnifera* extract was 308 mg gallic acid equivalents/g extract. Total phenolic contents measured by Folin-Ciocalteu method shown in Table.1.

3.2 DPPH radical scavenging activity

The measured radical scavenging activity (RSA) % of *Withania somnifera* extract was 77.3. DPPH radical scavenging activity of *Withania somnifera* extract was shown in Table.1.

Table 1. Total phenolic content (mg GAE/g) and DPPH radical scavenging activity (%) of *Withania somnifera* extract

Contents	Total phenolic content (TPC) mg GAE/g	DPPH radical scavenging activity in %
Withania somnifera extract	308	77.3

4. Discussion

Total phenolic content was determined by Folin-Ciocalteu method, it relies on the transfer of electrons / hydrogen ions in alkaline medium from phenolic compounds to form a blue chromophore complex (phosphomolybdic-phosphotungstic-phenol complex) where the maximum absorption occurs and reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer [14]. Total phenol content of *Withania somnifera* extract in the present study are in consistent with the earlier findings of [15, 16]. This TPC in *Withania somnifera* extract could be due to presence of polyphenols i. e. hydrolysable tannins include ellagitannin, gallotannins, ellagic acid, punicalgin, anthocyanins.

DPPH is a stable free radical that easily accepts an electron or hydrogen, when an antioxidant/reducing agent reacts with the DPPH which converts it to α, α-diphenyl-*β* picryl hydrazine and solution losses its colour depending upon the number of electrons taken up [17]. The radical scavenging activity of *Withania somnifera* in the present study was similar to the reports of [16-18, 19]. The content of total phenolics was well correlated with the antioxidant capacity [19]. Radical scavenging activity in present study could be due to content of phenols in the extracts. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors and singlet oxygen quenchers and also may have a metallic chelating potential [20].

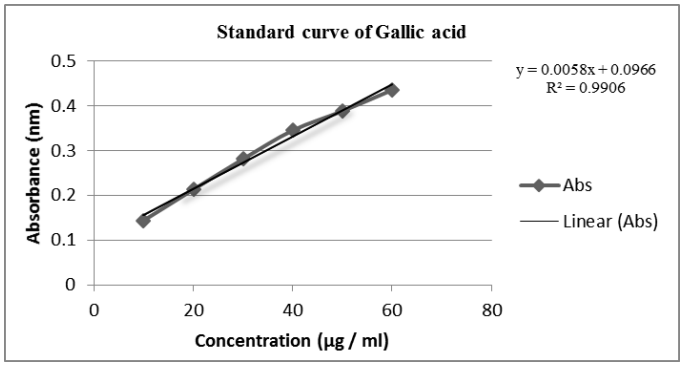

Fig 1: Standard curve of Gallic acid

5. Conclusion

The amount of total phenolic content was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalents. The total phenolic content and DPPH radical scavenging activity (%) of *Withania somnifera* extract was 308 mg GAE/g extract and 77.3% respectively. Result of present study indicated that *W. somnifera* extract

contain high amount of phenolic compounds which exhibited greatest antioxidant activity. The free radical scavenging activity of *W. somnifera* extract may be due to presence of hydroxyl group in their structure.

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