
Effect of *Withnia coagulans* fruits extract on antioxidant activity using Fenton Reaction

Yogita Soni, Wasim Raja

Central Laboratory Facility, Chhattisgarh Council of Science and Technology,
Raipur, Chhattisgarh, India

***Corresponding Author**

Email: drwasimraja84@gmail.com

ABSTRACT

The importance of traditional medicinal plants is increasing now a day because of various advantages over the synthetic drugs. *Withania coagulans* Dunal was studied for its antioxidant activity. Various physicochemical parameters were studied such as ash value, total ash, acid insoluble ash, water soluble ash, sulphated ash alcohol soluble extractive value etc. Various qualitative phytochemical tests were done for the presence of alkaloids, glycosides, carbohydrates, flavonoids etc. It was observed that 50% ethanol extract of *Withania coagulans* contains carbohydrates, proteins, glycosides, steroids and sterols, anthraquinones and triterpenoids. In the present study the methanol extract *Withania coagulans* was evaluated for antioxidant activity by the OH free radical scavenging activity using Fenton reaction. This study was conducted to investigate the effect of *Withania coagulans* fruit extract using Fenton reaction. The dried fruit of *Withania coagulans* was extracted with methanol using a Soxhlet extractor. The total phenolics content of bark as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of plant extract was carried put with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. The *Withania coagulans* fruit extract, there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. The result suggests that the *Withania coagulans* fruit extract can be used as food antioxidant together with the improvement of food palatability. Further studies are in processing of analyzing the synergic association of extract with synthetic antioxidant and to identify compounds with antioxidant activity in cinnamon extracts. In conclusions the present study indicated that *Withania coagulans* fruit extract may be a potential source of a natural antioxidants.

Keywords: *Withania coagulans*; fruit; extraction; methanol; phenolic content; flavonoid content; antioxidant activity.

INTRODUCTION

Withania coagulans Dunal belongs to family *Solanaceae* and is a well-known medicinal plant in indigenous system of medicine mainly distributed in the east of the Mediterranean region extending to South Asia. It is found in many parts of India (Kiritikar and Basu, 1999). The drug has been reported to possess anti-inflammatory (Budhiraja, et al., 1977), cardiotonic activities (Budhiraja, 1983), hepatoprotective (Budhiraja, 1986), antifungal (Choudhary hypoglycemic, free radical scavenging activity (Hemalatha, et al., 2004), hypolipidemic (Hemalatha, 2006), wound healing activity (Hemalatha, 2008), and diabetic nephropathy (Ojha, et al., 2014). Pharmacognostical standardization of *W. coagulans* fruits has also been reported in our previous paper (Prasad, et al., 2010). *W. coagulans* has a wide range of active phytoconstituents mainly withanolides, withaferin A, and coagulins (Valizadeh, et al., 2015;

Hemlata, 20090). Traditionally, the ripen and dried fruits of *W. coagulans* were used for anti-inflammatory, antioxidant, and as strangury and also reported to possess nephroprotective activity in rats against diabetes-induced nephropathy (Khare, 2008).

The use of medicinal plant products to manage or arrest the carcinogenic process provides an alternative to the use of conventional medicine for treatment of the disease. One such plant with this kind of therapeutic potential is *Withania coagulans*. Some of the naturally occurring compounds present in *Withania coagulans* possess anti-cancer activity against the range of cancers including breast, skin, colon, blood and colorectal cancer (Desai et al., 2008Rai et al., 2016).

Another challenge faced by medical practitioners today is antibiotic resistance. The indiscriminate and irrational use of antibiotics these days has led to the evolution of new resistant strains of bacteria that are somewhat more lethal compared to the parent strain (Chandra et al., 2017). Therefore, there is a pressing need for some novel antimicrobial molecules which have a broad spectrum of activity against both gram-negative and gram-positive bacteria without having any side effects. Plant sources are best suited to meet this requirement (Bassetti et al., 2011).

In this regard, *Withania coagulans* contains carbohydrates, proteins, glycosides, flavonoids, alkaloids, steroids and sterols, anthraquinones and triterpenoids (Salwaan et al., 2012). These bioactive components are responsible for anti-inflammatory, hepatoprotective, anti-fungal, hypoglycemic, free radical scavenging, hypolipidemic and wound healing activity. Recent investigation has shown that withanolides isolated from the aqueous extract of fruits possess a good antihyperglycemic and antidyslipidemic activity (Mathur et al., 2011Prasad et al., 2010).

The bioactive principles in this fruit may find scope in being used as a natural, plant based, toxin free, cost effective alternative in pharmacology industry. Besides its therapeutic applications, this fruit may also be used effectively in combating resistant food borne and nosocomial pathogens.

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MATERIALS AND METHODS

Plant material – *Withania coagulans* fruit was collected from Local Market, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of *Withania coagulans* fruit (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 °C temperature. The final extract was kept in air tight box.

Deoxyribose assay to assess OH⁻ radical scavenging activity

The OH⁻ radical scavenging activity of *Withania coagulans* fruit extract (10–100 µg/ml) was determined according to the deoxyribose method reported of Halliwell, *et al.*, (1987). In the protocol the presence of 100 µM EDTA, FeCl₃, H₂O and ascorbic acid were prepared in degassed H₂O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 µM EDTA, 1 mM H₂O₂, 100 µM L- ascorbic acid, 100 µM FeCl₃, H₂O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples were kept in incubation at 38°C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples were heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC₅₀ value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs: } 532 \text{ nm Control Abs.} - 532 \text{ nm sample Abs.} \times 100}{532 \text{ nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC₅₀, the concentration necessary for 50% inhibition concentration of TBARS.

RESULT

The results of the effects of the examined *Withania coagulans* fruit extract as well as control solutions on OH- radical production. They show that all extract of *Withania coagulans* fruit extract and control solutions as a DMSO inhibited the production of OH- radicals. The % of free radical scavenging activity of hydro-methanolic extract of *Withania coagulans* presented in Table 1 have reducing power, the free radical OH- scavenging activity of the extract increases with increasing the concentration.

Table 1–Antioxidant activities of *Withania coagulans* fruit extract using Fenton reaction

Constrictions (in µl)	% of inhibition	
	Ascorbic Acid	<i>Withania coagulans</i> fruit extract
10	22.71	17.21
20	38.13	23.99
30	46.12	30.45
40	52.30	41.40
50	61.35	52.15
60	73.10	63.60
70	79.52	67.12
80	82.28	72.55
90	86.17	78.55
100	90.42	83.25

Blank: 0.4320

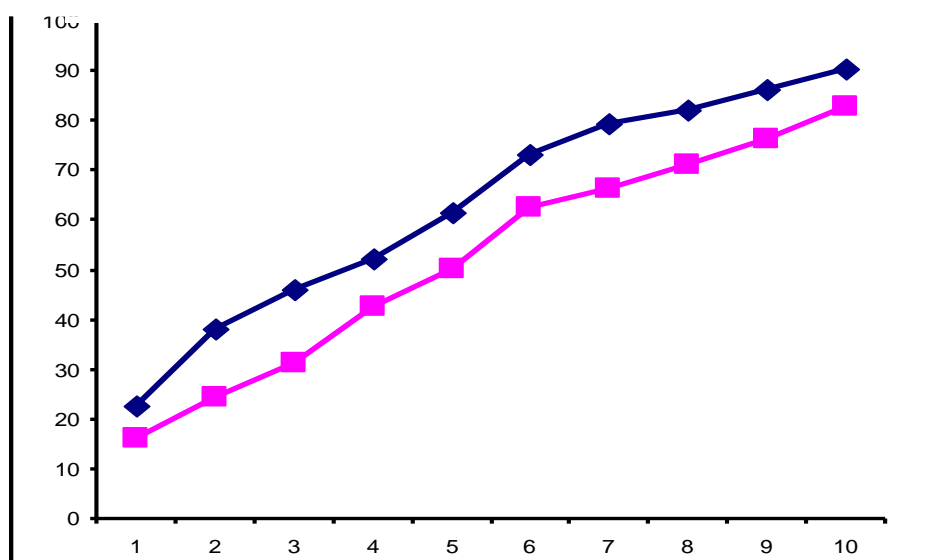


Fig. 1 Antioxidant Activity of Withania coagulans fruit extract

DISCUSSION

The body's innate mechanism has many enzymes and nonprotein compounds that protect it from the free radicals and reactive oxygen species produced inside the body during normal metabolism and also due to external stimuli. Major compounds include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione, which also play a major role in detoxification and coordinate the body's antioxidant defense processes. The superoxide dismutase is a metalloprotein that scavenges superoxide anions. Catalase is a heme protein, localized in the peroxisome or the microperoxisome, which catalyzes the decomposition of H₂O₂ to water and oxygen and thus protects the cell from oxidative damage produced by H₂O₂. The glutathione peroxidase catalyzes the reaction of hydroperoxides, which reduces glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxide. Glutathione reductase is involved in the regeneration of glutathione that has been converted to GSSG by oxidation and thiol transfer reactions. Glutathione, a major nonprotein thiol, is mainly involved in detoxification (Halliwell & Gutteridge, 1985).

The current study indicates that *Withania coagulans* fruit extract effectively scavenged hydroxyl radicals in vitro. This types of free radicals are generated inside the body during the normal metabolism or in presence of xenobiotics. The stable free radicals OH were also scavenged by *Withania coagulans* fruit extract. These results show that *Calendula officinalis* has a profound effect on the antioxidant defense system in vitro.

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Bioactive components present in *Withania Coagulans* was determined using the GCMS method. The spectral analysis of the sample by the GCMS showed several peaks however compounds of interest possessing antioxidant, antibacterial and anti-carcinogenic properties in *Withania coagulans* were identified to be Withanolide related compounds such as squalene (terpenoid), flavonoid, alkaloid and phenols. Withanolides are synthesized via the mevalonate pathway of terpenoids formation and arise from the initial cyclization of 3S-squalene- 2, 3-epoxide (Khodaei et al., 2012; Evans et al., 1995; Manach et al., 2004). The *Withania coagulans* extract was found antioxidant potential and having the ability to trigger cellular antioxidants, can be exploited for its use against a number of disorders including cardiovascular diseases, inflammation, and cancer.

CONCLUSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc. Experimental data revealed that there might be correlation between total phenolic and antioxidant capacity of different extracts of lemon grass. Some literature

on *Withania coagulans* fruit extract are demonstrated that antioxidant was not solely dependent on phenolic content but it may be due to other phytoconstituents as tannins, triterpenoid or combine effect of them. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as in vivo and in vitro assays will be necessary in discovering new biological antioxidants.

In the present study, the phenolic content of *Withania coagulans* fruit extract was found to be high which might have responsible for its antioxidant and free radical scavenging activity in the in vitro study models. Thus our results were congruent with the findings of others. Further studies can be designed to prove the antioxidant activity of *Withania coagulans* fruit extract in experimental animal models and also an attempt can be made to analyze the phenolic antioxidants present in it.

Pharmacognostical studies of the finding therein will enable the identification of the plant to the future investigation. This will provide a basis for the pharmacognostical standardization of the plant drug. Preliminary Phytochemical study of methanol extract of the fruit parts is found to contain carbohydrates, protein, some steroids, anthraquinone, flavonoids, tannin, phenolic compounds and triterpenoids are present. The antioxidant activity was determined and the plant extract showed free radical inhibition method and moderate activity by fenton reaction. The activity was compared with rutin and ascorbic acid.

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