



IN- VITRO ANTIOXIDANT, ANTI-CARCINOGENIC AND ANTIBACTERIAL POTENTIAL OF *WITHANIA COAGULANS* FRUIT (PANEER POO)

Home Science

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ABSTRACT

The antioxidant activity of *Withania coagulans* (Paneer Poo) fruit was studied using three methods: DPPH, FRAP and Phosphomolybdenum assays. The *invitro* anti carcinogenic potential on MCF-7 breast cancer cell line was assessed by the MTT assay. The antibacterial activity of aqueous extract of *Withania coagulans* fruit was assessed on two gram positive and two gram negative bacteria. DPPH assay revealed that *Withania coagulans* fruit has potent free radical scavenging activity that increased with increase in concentration. From the FRAP and Phosphomolybdenum assays it was evident that *Withania coagulans* fruit exhibited good reducing capacity that increased with increasing concentration. *Withania coagulans* fruit exhibited antibacterial activity against all the tested pathogenic microbial strains but was more effective in inhibiting the growth of *S. pneumoniae*. The MTT assay on MCF-7 breast cancer cell line revealed that the cell viability decreased with increase in concentration of the sample.

KEYWORDS

Antioxidant, Antibacterial, In-vitro, Anticarcinogenic, *Withania coagulans*

INTRODUCTION

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., 2008; Rai 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., 2011; Prasad 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.

AIM OF THE STUDY

The present study was carried out to assess the antioxidant, antibacterial and anticarcinogenic effect of *Withania coagulans* fruit.

OBJECTIVES OF THE STUDY

- To identify specific bioactive components that are responsible for antioxidant, antibacterial and anticarcinogenic properties of *Withania coagulans* fruit by the GCMS. (Gas Chromatography – Mass Spectroscopy) method.
- To assess the antioxidant activity of *Withania coagulans* fruit by the DPPH, FRAP and Phosphomolybdenum assays.
- To determine the antibacterial activity of *Withania coagulans* fruit on selected gram positive and gram negative bacteria.
- To determine the *in vitro* anticarcinogenic activity of the *Withania coagulans* fruit by the MTT assay on MCF-7 breast cancer cell line.

MATERIALS AND METHODS

Design of the study

The design of the study is experimental in nature. This study was conducted after obtaining approval from the Institutional Ethics committee.

Gas chromatography–Mass Spectrometry (GC–MS)

The *Withania coagulans* fruit extract was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation of mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Antioxidant activity of *Withania coagulans*

The antioxidant activity of *Withania coagulans* was assessed by the following methods:

- DPPH ((2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay
- Fe³⁺ reducing antioxidant power (FRAP)
- Phosphomolybdenum reduction assay

Assessment of antibacterial activity

The antibacterial activity was analyzed by the well diffusion method. 25 mL of Muller Hinton agar was prepared according to the standard procedure and poured into the plates and was allowed to solidify. The antibacterial activity of aqueous extract of *Withania coagulans* was assessed on two gram positive and two gram negative bacteria [*Staphylococcus aureus* (ATCC 27661), *Streptococcus pneumoniae* (ATCC 6305), *Enterobacter cloacae* (ATCC 13048), *Escherichia coli* (ATCC 25922)]. The standard inoculum suspension was streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organism and the plates were allowed to dry for 5 minutes. After drying, the different concentrations (0.31, 0.62, 1.25, 2.5, 5 mg/mL) of the extract were poured into the wells. Antibiotics such as Gentamicin, ampicillin and tetracycline were used as standards. Finally the inoculated plates were incubated for 24 hours at 37°C for bacteria. The zone of inhibition was measured and noted.

Assessment of Anti-carcinogenic activity

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is based on the ability of a mitochondrial

dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilisation of the cells by the addition of a detergent results in the liberation of the crystals which are solubilised. The number of surviving cells is directly proportional to the level of the formazan product created. The color can then be quantified using a simple colorimetric assay. The results can be read on a multiwell scanning spectrophotometer (ELISA reader) (Cauley et al., 2013; Jadeja et al., 2009; Zhang et al., 2015; Riss, 2016).

RESULTS AND DISCUSSION

Assessment of bioactive components in *Withania coagulans* by the GCMS (Gas Chromatography – Mass Spectroscopy) method.

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).

Assessment of antioxidant activity of *Withania coagulans* DPPH assay

The antioxidant activity of *Withania coagulans* using DPPH assay is presented in figure 1.

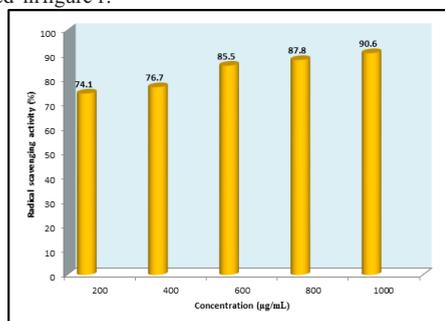


Figure 1: Antioxidant activity using DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable nitrogen centred free radical that can be effectively scavenged by antioxidants. On accepting a hydrogen atom from a donor, DPPH which is dark purple in colour changes to yellow colour and gets converted into a non-radical form known as 2,2- diphenyl-1-picrylhydrazine. Ethanolic extract of *Withania coagulans* exhibited potent free radical scavenging activity that increased with increase in concentration. IC50 value indicates the concentration of the test sample required to inhibit 50 % of free radicals. The IC₅₀ value was 134.9 µg/mL.

The presence of antioxidant compounds such as withanolides, phenols and flavonoids in *Withania coagulans* fruit makes it a potential plant based natural source of antioxidants that can find application in the pharmaceutical and food industry.

Phosphomolybdenum assay

The total antioxidant activity of *Withania coagulans* fruit was assessed using the Phosphomolybdenum assay. The results are presented in table 1.

Table 1: Antioxidant activity using Phosphomolybdenum assay

concentration (mg/mL)	Absorbance at 695 nm
0.31	0.14
0.62	0.24
1.25	0.49
2.5	1.92
5	2.80

The results of the present study indicate that the extract of *Withania coagulans* fruit exhibited greater reducing power that increased with increase in concentration. Maximum absorbance was observed at 2.80 at a concentration of 5 mg /mL. The antioxidant activity might be attributed to the presence of high amounts of polyphenols (45.14 µg/mg GAE) in the extract that was quantified.

FRAP assay

Reducing Antioxidant power is also used in evaluating the antioxidant activity as the reducing capacity of the compound serves as a notable indicator of its potential antioxidant activity. In the present study, reducing power of *Withania coagulans* fruit extract was determined using FRAP assay. Antioxidant activity using FRAP assay is given in table 2.

Table 2: Antioxidant activity using DPPH assay

Concentration(µg/mL)	Percentage inhibition of free radicals
200	74.1
400	76.7
600	85.5
800	87.8
1000	90.6

Compounds with reducing power indicates that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary or secondary antioxidants. In this assay the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of compounds present in the extract. Presence of reducers causes the conversion of Fe³⁺ ferri cyanide complex to Fe²⁺ ferrous form. From the results obtained, it was evident that *Withania coagulans* exhibited good reducing capacity that increased with increasing concentration.

Antibacterial activity of *Withania coagulans* fruit

Interpretation of inhibition zones of test culture was adopted from Johnson and Case (1995). A diameter of the zone of inhibition of 10 or less indicates the test product having antimicrobial activity against the test organisms (pathogens), diameter zone of inhibition of 11 to 15 indicates the test product having an intermediate antimicrobial activity against the test organisms. A diameter with a zone of inhibition of 16 or more indicates a high antimicrobial activity against the test organisms. A zone of inhibition is an area around the point in the media where the antibiotic is introduced and where no test organisms (pathogenic bacterial strains) are found to be growing.

The observations of the antibacterial activity of *Withania coagulans* on *S. aureus*, *S.pneumoniae*, *E.cloacae* and *E.coli* is tabulated in table 3.

Table 3: Antibacterial activity of *Withania coagulans*

Concentration mg/mL	Zone of Inhibition				
	Gram Positive			Gram Negative	
	S. aureus	S.pneumoniae	E.cloacae (Enterobacter family)	E.coli (Enterobacter Family)	
	(Antibiotic – Gentamicin) 29	(Antibiotic – Ampicillin) 22	(Antibiotic Tetracycline) 18	(Antibiotic - Tetracycline) 22	
0.31	7	7	4	4	
0.62	8	6		5	
1.25	11	6		7	
2.5	12	10		10	
5	16	10		15	

The zone of inhibition against *S. aureus* showed that as the concentration of sample increased, the zone of inhibition also increased. However, the zone of inhibition produced by Gentamicin which was used as a reference standard had the highest zone of

inhibition. Similar observations were made with *S.pneumoniae*, *E.cloacae* and *E. coli*. The reference antibiotics used for *S. pneumoniae* was Ampicillin, and for *E.cloacae* and *E. coli* was Tetracycline. The highest Zone of inhibition was produced by the highest concentration

of the sample (5mg/ ml). Thus the zone of inhibition was directly proportional to the concentration of sample used. Though *Withania coagulans* fruit extract exhibited good antibacterial properties, its activity was lower than the antibiotics used (table 3).

It is clear from these findings that *Withania coagulans* exhibited antibacterial activity against all the tested pathogenic microbial strains. It is evident that Paneer poo (*Withania coagulans*) was more effective in inhibiting the growth of *S.pneumoniae* when compared to other pathogenic strains. The results of the study thereby indicate that *Withania coagulans* acts as a successful antibacterial agent and that its use as an antimicrobial agent in future is promising. *Withania coagulans* fruit extracts, therefore can be used as antimicrobial agents in new drugs for therapy of infectious diseases.

Determination of *in vitro* anticarcinogenic activity of the *Withania coagulans* fruit on MCF-7 breast cancer cell lines

The *in vitro* anticarcinogenic potential of *Withania coagulans* fruit on MCF-7 (ECACC catalogue no. 86012803) cancer cell line was assessed by the MTT assay. The absorbance values were noted in duplicates using the ELISA reader at 570 nm once the purple colour developed after the incubation time of 24 hours. The results are presented in table 4 and figures 2.

Table 4: Cell viability and per cent growth inhibition of *Withania coagulans* on MCF-7 breast cancer cells

Concentration ($\mu\text{g/ml}$)	%inhibition	cell viability
40	35.92	64.08
60	63.25	36.75
80	75.06	24.94
100	83.17	16.83
200	86.63	13.37

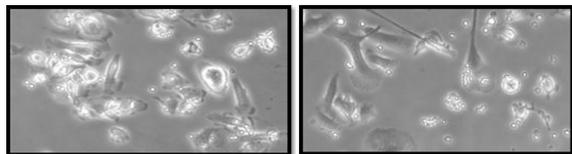


Figure 2 : MCF 7 cells (control)MCF 7 (treated) after 24 hours Incubation – showing cancer cell

It can be inferred that the cell viability decreased with increase in concentration of the sample. This explains the dose dependent activity of *Withania coagulans* fruit in inhibiting cancer cell proliferation. *Withania coagulans* showed a potent cytotoxic activity against human breast cancer cell line (MCF-7) by showing an inhibition rate of 83.17 % at 100 $\mu\text{g/mL}$ and 86.63 % at 200 $\mu\text{g/mL}$ respectively.

IC50 represents the concentration of a drug that is required for 50 per cent inhibition of viral replication *in vitro*. In this study, IC50 value on cytotoxic activity of breast cancer cell line was 52.71 $\mu\text{g/mL}$.

CONCLUSION

The results of this study strongly support the fact that *Withania coagulans* fruit (Paneer poo) is a natural, cost effective, easily available antioxidant, antibacterial and anticarcinogenic agent that could find good scope in the pharmaceutical industry as a potent therapeutic agent. It may also find its application in combating the challenge of antibiotic resistant food borne and nosocomial pathogens.

Conflict of interest

The authors declare that no conflict of interest is associated with this work.

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