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Evaluation of antioxidant, antidiabetic potential contain from fruits of *Withania Coagulans*

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ABSTRACT

The current investigation examined the phytochemical screening, antioxidant capacity, and antidiabetic effect of *Withania coagulans* fruit extract in both aqueous and methanolic forms. Withanolides, which may be used as a natural diabetic therapy, were screened using Fourier Transform Infrared Spectroscopy analysis. In order to examine the antidiabetic potential of *Withania coagulans* fruits, the study also attempted to evaluate the alpha amylase inhibitory action. By delaying and lengthening the duration of total carbohydrate digestion, the enzyme inhibition lowers the rate of glucose absorption and, as a result, attenuates the rise in postprandial plasma glucose. Various withanolides are in charge of distinct medicinal effects. It is extensively used to treat a variety of conditions, including cancer, diabetes mellitus, nervous weariness, handicap, sleeplessness, wasting illnesses, and stunted growth in children. Fresh extract underwent phytochemical screening, which identified the presence of secondary metabolites as flavonoids, alkaloids, and phenols. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the antioxidant activity of the crude methanolic and crude aqueous extracts of W. Coagulans was examined. Using ascorbic acid as a reference, it illustrates the extract's ability to scavenge DPPH radicals. The fruit extract's bioactive ingredients are characterised using FTIR spectroscopy. Researchers in the field will greatly benefit from the current data and fresh insights from this study, which will help them better understand the plant's therapeutic qualities, put conservation measures into place, and investigate biotechnological approaches for long-term sustainable use.

Key words: *Withania coagulans,* Phytochemicals, Antioxidant, FTIR , antidiabetic.

INTRODUCTION

A significant portion of the global population uses plant-based pharmaceutical chemicals as their primary means of treating a variety of illnesses, according to the World Health Organization. Due to their antidiabetic effects in animal models and human pilot trials, the whole plants, fruit, and roots of *Withania coagulans* (family: Solanaceae) have garnered attention in the last few decades.

In South Asia, *Withania coagulans* is regarded as an underappreciated plant that grows in small, dispersed populations. It can be found in Nepal, East India, Pakistan, Afghanistan, and Iran. The drier regions of Rajasthan, Punjab, Gujrat, Simla, and Kumaon are home to the plant in India. It is dispersed throughout stony, dry, hot locations up to 1700 metres in altitude ((Khodaei et al 2012, Pezeshki et al 2011, Gilani et al 2009). Berries can help treat diabetes since they have hypoglycemic qualities. In addition to chronic liver problems, dyspepsia, and digestive tract infections, they also help with impotence, insomnia, and excessive salivation Because it contains an active ingredient called a cyclic enzyme, it is known to have anti-inflammatory, diuretic, anti-bacterial, cardioprotective, anti-fungal, hypoglycemic, hepatoprotective, anti-oxidative, and anti-mutagenic qualities. *W. coagulans* includes a variety of phytochemicals, including tannins, flavonoids, and -sterols, in addition to withanoids. According to some research, *W. coagulans* and its active components offer a wide range of pharmacological and therapeutic qualities, making them potential novel medications for the treatment of a variety of illnesses.

This plant has been reported to possess antimicrobial,anti-inflammatory, antitumor, hepatoprotective, anti-hyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities so the

intension of present study is to evaluate the said plant for phytochemical analysis and antioxidant properties with assessment of antidiabetic property.

MATERIALS AND METHODS

Collection of sample: Plant materials of *Withania coagulans* were collected from the local market of Bhiwandi Thane (India) and authenticated from Botany Department of college.

Preparation of Crude Extract: The fruits of *Withania coagulans* L. dunal was collected and cleaned. It was dried in hot air oven at 55°C for 48 hrs.The dried fruits were grinded into coarse powder using a electric grinder.and passed through sieve no 80. This powder was stored in an air-tight container and used for extraction and further studies.

Extraction: The plant material was ground into powder, and the extract was prepared. 20 g of dried powdered *Withania coagulans* was taken in a 250ml conical flask and extract was prepared using three solvent system in 100ml solvent. Methanol, aqueous and petroleum ether and kept at rotary shaker for 24 hrs. It was then filtered using muslin cloth. The final extract was obtained and used for the assay.

Phytochemical analysis: Active ingredients such as tannins, saponins, flavonoids,phenol, alkaloids,and glycosides were checked using fresh extract of *withania coagulan*s fruit.the following accepted practices were applied. A series of qualitative tests were carried out as per the standard protocols described by Tiwari P. et al., 2011; Saxena M, et al., 2012 and Salwaan C. et al., 2012

a. Test for Detection of Alkaloids:
Wagner's Test: To 1 ml of the extract, added 2 ml of Wagner's reagent. The formation of a reddish brown precipitate indicated the presence of alkaloids.(Nazneen peerzade,et al;2017).

b. Test for Detection of Saponins :
Foam test: 0.5 ml of the extract was taken and mixed with 2 ml of water. The solution was shaken for a few minutes. The presence of saponins is indicated if the foam persists for more than 10 minutes.

c. Test for Detection of Reducing Sugar:
Benedict’s Test: Five drops of filtrate was added to 2 ml of Benedict’s reagent and then the solution was heated in water bath for few minutes. The formation of orange/red precipitate indicates the presence of reducing sugar.(Nazneen peerzade et al.,2017).

d. Test for Detection of Phenols:
Lead Acetate Test: A few drops of lead acetate solution were added to the extract. The presence of phenols is indicated by the formation of yellow precipitate. (Nazneen peerzade et al., 2017).

e. Tests for Detection of Steroids:
Salkowski Test: Dissolved the extract in chloroform and added equal volumes of concentrated sulfuric acid. Formation of bluish red to cherry red color in lower chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.(Salwan et.al.,2012).

f. Tests for Detection of Carbohydrates:
Molisch Test: To 2 ml of the extract, added 1 ml of α -naphthol solution, and concentrated sulfuric acid through the sides of test tube. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.(Salwan et. al.,2012).

g. Tests for Detection of flavonoid:
Alkaline reagent test: Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour ap- peared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present. (Nagaraju kancherla et.al.,2019)

h. Test for Detection of tannin:
Ferric chloride test: 2 ml of peel extract was taken and three drops of FeCI, diluted solution was added, production of a blue or greenish-black color clearly indicates the presence of tannins.

Quantitative Analysis: The estimation of total contents of certain metabolites such as Flavonoids, Alkaloids, Phenols and Proteins in methanolic extract of *W. coagulans* fruit was carried using standard protocols described as follows.

a) Total Flavonoid Estimation: The total flavonoid content in methanolic extract was determined by following the modified spectrometric method. Varying concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml) of Quercetin (standard) were prepared from 1 mg/ml stock solution. For testing the concentration of Flavonoids in the extract, 0.5 ml of extract was taken from 1mg/ml of individual stock solutions. After making volume of solution to 2.5 ml, 0.15 ml of 5% sodium nitrate solution was added and incubated at room temperature for 6 minutes. 0.15 ml of 10% aluminum chloride was added and incubated at room temperature for 6 minute. 2 ml of 4% sodium hydroxide was added and mixed thoroughly. Final volume of solution was made to 5 ml with distilled water and after mixing, incubated at room temperature for 15 minutes. The absorbance was taken at 510 nm. The concentration of flavonoids in the extract was calculated using the standard curve. The total flavonoid content in the extract was expressed as mg of flavonoid / g of extract.

b) Determination of Antioxidant activity: Antioxidant activity was measurement by DPPH (2, 2-diphenyl-1-picryl-hydrazyl- hydrate) method by using ascorbic acid as standard. A stock solution was prepared by adding analytical grade methanol in weighted amount of the methanolic cru-de extracts of all parts of both plants. Both plant samples of root, stem, leaves in different concentrations (200, 400, 600, 800, 1000 ug/ml) were prepared in methanol from stock solution. Similarly, ascorbic acid samples with same con-centrations were prepared. Methanol as solvent was used to prepare DPPH(0.002%) solution. 2 ml of DPPH solution was added and dissolved separately in standard solution (ascorbic acid) and2ml concentrated sample. This resulted solution was incubated for half an hour for measuring optical density at 517 nm. The control contains methanol only. The % scavenging activity (inhibition percentage) was measured by the formula given (Senguttuvan et al., 2014).

c) Alpha-amylase inhibitory assay of *Withania coagulans* extracts: A total of 250 µl of *W.coagulans* extract samples was placed in tubes, and to each tube, 125 µl of 0.02 M sodium phosphate buffer (pH 6.9) with a-amylase (5 mg/ml) was added. Sodium phosphate buffer (pH 6.9) (500 µl, 0.02 M) was then added to each tube, and the mixtures were incubated at 25°C for 20 min. Next, 600 µl of starch solution (2%) in 0.03 M phosphate buffer (pH 6.9) was added and incubated further at 25°C for 10 min. Dinitrosalicyclic acid (500 µl) was added to stop the re- action. The reaction tubes were incubated in a water bath at 100°C for 10 min. Subsequently, 6 ml of water was added, and optical density (OD) was measured at 540 nm.The α-amylase inhibitory activity was calculated as follows:

$$\text{Inhibition [\%]} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

For control, the sample was replaced with water.(Arun Dev sharma.*et.al*.,2022)

d) FTIR

A significant amount of compositional and structural data about plants can be analysed using Fourier Transform Infrared (FTIR) spectroscopy. Additionally, FTIR spectroscopy is a well-known approach for identifying and characterizing functional groups that saves time. Based on the peak values in the IR radiation band, the FTIR spectrum was used to identify the functional groups of the active components present in the extract.

RESULTS AND DISCUSSION

1. Phytochemical analysis:The qualitative test of phytochemicals was initially Done by biochemical tests. The preliminary phytochemical tests result indicates the presence of flavonoids , phenol and alkaloids.

Table : 1. Qualitative phytochemical analysis

Solvent				
Sr.no	**Tests**	Methanol	Pt.ether	D/W
1.	Alkaloids (Wagner's test)	-	+	+
2.	Carbohydrates (Molish test)	+	+	+
3.	Steroid (salkowski test)	-	+	+

4.	Phenol ($FeCl_3$ test)	+	+	+
5.	Tannin (FeCl3 test)	+	-	+
6.	Flavonoids	+	+	+

+Presence of the compounds
-Absence of the compounds

2. Determination of total Flavonoids .

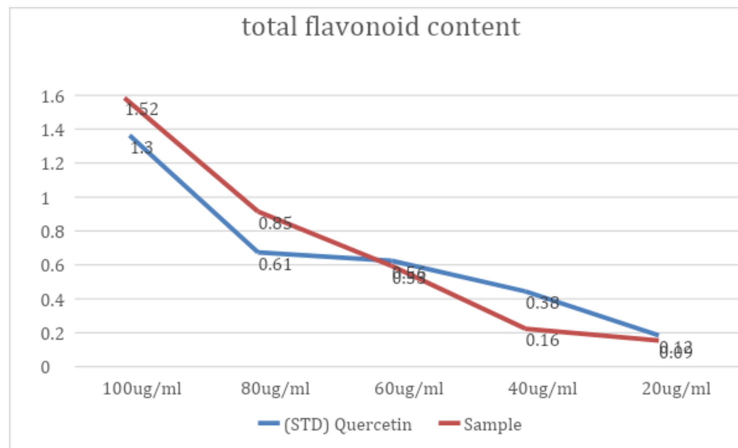

Fig 1.Graphical presentation of total Flavonoid content of extract .

Table 2: Total flavonoids content :

Concetration standards	**(STD) Quercetin**	**Sample**
100ug/ml	1.30	1.52
80ug/ml	0.61	0.85
60ug/ml	0.56	0.53
40ug/ml	0.38	0.16
20ug/ml	0.12	0.09

3. Alpha-amylase inhibitory assay:

Formula

$$\textbf{Inhibition [\%]} = \frac{\textbf{Abs of control - Abs of sample}}{\textbf{Abs of control}} \times 100$$

Table3 : Alpha-amylase inhibitory assay

Solvent	**Control(O.D at 530nm)**	**Sample(O.D at 530nm)**	**Inhibition %**
D/W	0.43	0.17	60.46

Pt.ether	0.43	0.28	34.88
Methanol	0.43	0.25	60.46

4. Antioxidant (DPPH) Assay:
Formula:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Abs (control)} - \text{Abs(sample)}}{\text{Abs (Control)}} \times 100$$

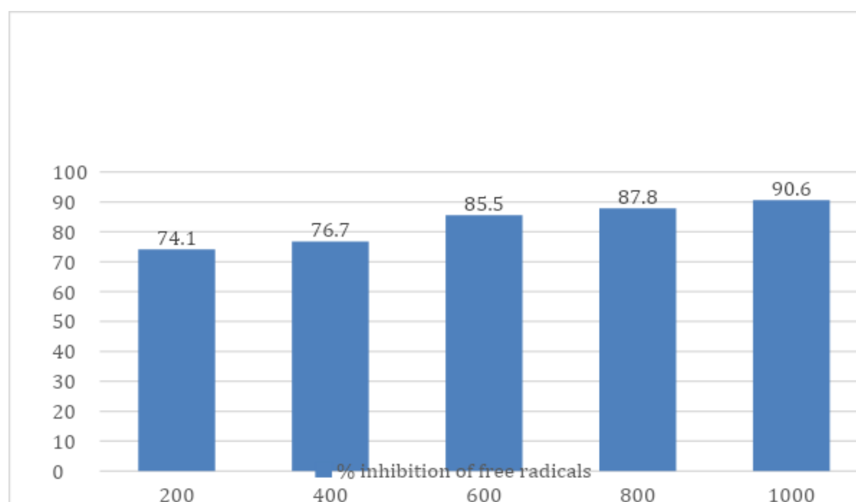

Fig-2 Antioxidant (DPPH) Assay

5. Fourier-transform infrared spectroscopy (FTIR)

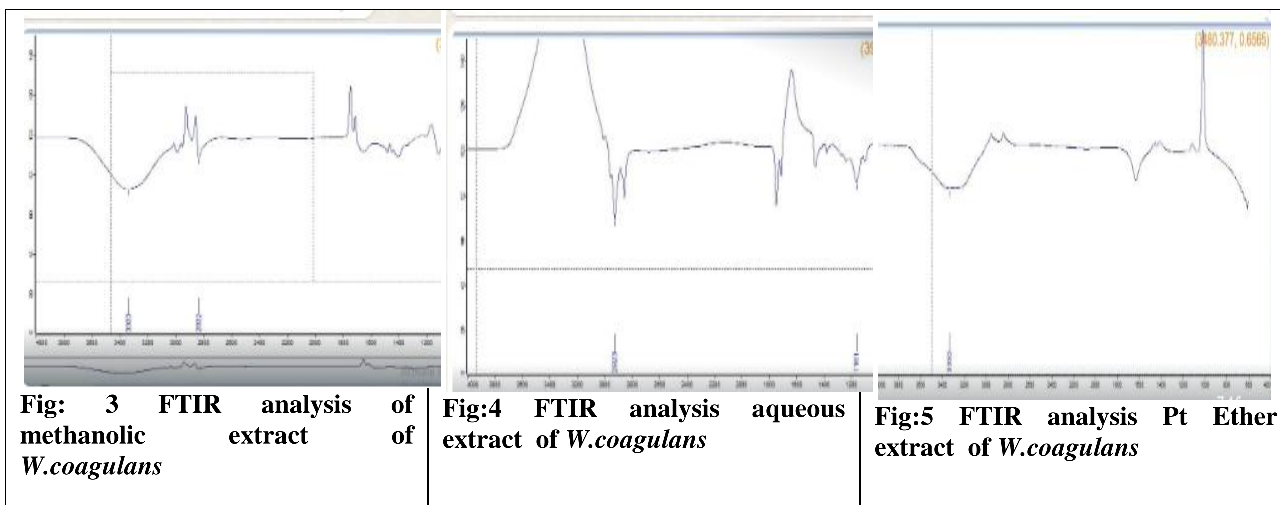

Fig: 3 FTIR analysis of methanolic extract of *W.coagulans*	**Fig:4 FTIR analysis aqueous extract of *W.coagulans***	**Fig:5 FTIR analysis Pt Ether extract of *W.coagulans***

Qualitative phytochemical analysis of all three solvent extract is indicated in (Table 1). Result shows presence of Alkoaloids, Flavonoids, Carbohydrates, steroids, tannins, proteins and absence of glycosides in all plant extracts. (Table 2) and fig 1 shows the flavanoid content in comarision to standard quercetin while Table3 depicts the antidiabetic charecteristic of plant extract throughs alpha-amylase inhibitory assay.

The Phytochemical screening of fresh Withania Coagulans extract revealed the presence of secondary metabolites such as phenols,flavonoids and Alkaloids. Antioxidant activity of crude methanolic and crude aqueous extract was investigated by 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay (fig 2). On the basis of the results of the study, extract have significant antioxidant activity. The exact components responsible for the antioxidant activity of both extracts are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of these

antioxidant components. The antioxidants have potential for application in food, pharmaceuticals and cosmetics. investigation of alpha amylase inhibitory activity reveals the inhibition. This study also attempted to evaluate the alpha amylase inhibitory activity to analyse antidiabetic potential of *Withenia coagulans* The inhibition of the enzyme delay carbohydrate digestion and protract overall carbohydrate digestion time, resulting in the reduction in glucose absorption rate and consequently dulling the postprandial plasma glucose rise.The functinal group studies reveals following results as dipicted in graph above.

FTIR spectra of the methanolic extract showed strong bands at Absorbance 3466.79cm-1.The FTIR Spectrum of sample Show the presence of hydroxyl compound.s andmethyl group (Fig 3)FTIR spectra of the aqueous extract showed strong bands at frequency range 39381.46 cm-1.(fig-4) The FTIR Spectrum of sample Show the presence of carboxylic acid and amide compounds.FTIR spectra of the petroleum ether extract showed strong bands at frequency range 3480.377 cm-1.The FTIR Spectrum of sample Show the presence of hydroxyl compound.(fig-5)

As Withana coagulans Dunal also contain alkaloids, Steroids, amino acids, flavonoids, proteins, carbohydrates and tannins.(Gupta V., 2013;Prasad S. K, 2010) Hence this plant was selected for screening antidiabetic activity and extracted by using Methanol and water solvent system (Salwaan C, 2012; Srivastava S. K, 2015).

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