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Pharmacological Activities of Withania Coagulan

Tuba Altaf* and Habib-ur-Rehman

Department of Chemistry, Mirpur University of Science and Technology (MUST), New Campus, Mirpur-10250 (AJK), Pakistan

ABSTRACT

Withania coagulans has long been used in the indigenous system of medicine for the treatment of various ailments including diabetes, cancer, cardiovascular diseases, antimicrobial, inflammation and wound healing. The plant is also used as a diuretic agent. Keeping in view the medicinal importance of the plant and biological activities of its extracts and chemical constituents, it was selected for carrying out further studies. Phytochemical screening showed the presence of several classes of chemical constituents, including alkaloids, flavonoids, glycosides, saponins and phenolic compounds. The methanol extract of the fruits of the plant has also been subjected to different bioassays to determine its biological profile. The extract has shown significant antioxidant, antiglycation and metal chelating activities in the ABTS and DPPH, Bovine Serum Albumin (BSA) and Ferrozine assays, respectively. The antimicrobial activity of the extract in n-hexane, ethyl acetate, chloroform and aqueous solvents was also determined by the agar diffusion method. All the extracts showed significant activity except the chloroform extract. Our investigation also revealed the presence of polyphenols and flavonoids in the extract. The presence of these important classes of compounds showed importance of the plant in terms of its chemical constituents and a crucial role it may play in development of lead compounds for their application in treating human sufferings.

***Corresponding author**

Tuba Altaf, Department of Chemistry, Mirpur University of Science and Technology (MUST), New Campus, Mirpur-10250 (AJK), Pakistan.

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Introduction

The man used plants as food, shelter and cope with the deceases, as they possess medicinal properties and used to treat human sufferings. Because of its pharmacological and neutraceutical activities, Withania coagulans is one of the most significant pharmaceutical plants in the Withania genus find important medicinal uses in the Ayurvedic medicine system [1-6].

Figure 1: Withania Coagulans- a Plant of the Family, Solanaceae

Various parts of the plant- leaves, fruits, barks, flowers, and even the entire plant may be used to treat diseases. Certain substances that have physiological effects are formed and contained in the bodies of the plants [7]. The secondary metabolites present in the plant possess biological activities and are considered crucial for protecting the species from diseases and shielding them from biotic and abiotic stress through different processes [8]. The animals, plants, fungi, bacteria, and algae afforded divers classes of natural products and structures possessing wide spectrum of biological activities. They may be classified on the basis of their biosynthetic pathways [9]. Three primary classes- flavonoids and related phenolic and polyphenolic chemicals, terpenoids, sulphur and nitrogen containing compounds such as alkaloids can be distinguished among secondary metabolites [10]. For thousands of years, humans have utilized a variety of plants that contain substances known as stimulants, flavors, scents, colors, pesticides, poisons, and most importantly as medicinal agents [11]. Gaining a grasp of the chemicals makeup of the plants might help one determine their potential medical usefulness [12]. Plant based phytomedicines have demonstrated significant potential in the management of incurable infections, disorders, such as opportunistic AIDS infections [13]. Most phytochemicals, including curcumin, resveratrol, and anthocyanins, have shown to have antioxidant, antimicrobial, antiviral and anti- inflammatory properties. The polyphenols present in the plant could protect the cardiovascular system in human [14]. The natural products with anticancer activities fall into several categories of chemical constituents including lactone sesquiterpenes, alkaloids and macrocyclic poly-ethers [15]. The nanoparticles and nanomedicines are examples of new technologies that are being developed and effort are being made to improve the anticancer properties of drugs by controlling the release of molecules with unique mechanism of action [16].

W. coagulans is a tiny, greyish, whitish shrub that grows over the eastern mediterranean regions and into south Asia [17]. It mostly inhabits in the warm, temperatures and tropic regions which are widely found in drier part of India and Pakistan [18]. This plant is related to the family of 23 famous species of the Withania. Only two (W. coagulans and W. somnifera) are economically significant

[19]. It is commonly referred to as an Indian cheese make; its berries are used to coagulate milk [20]. The high withanin contents of the plant are responsible for its coagulating properties [21]. Moreover, the withanolides extracted from the aqueous extract of the fruits of the plant exhibit strong anti-hyperglycemic and anti-dyslipidamic properties [22].

Withania coagulans, grayish shrub with densely packed branches that are either yellow or gray [23-24]. The ripe berries are globose, yellowish to dark brown in color [25]. The seeds are ear shaped, glabrous, dark brown and with fruity fragrance. The plant produces fruit in January through May and flowers from November to April [26]. The plant is primarily found in the southeast of the provinces of Sistan and Balochistan and in India, the plant is commonly found in drier parts of the Punjab, Haryana, Gujrat, and Rajasthan [27-28]. It is thought to be of oriental origin. It can be found in the wild, Mandsaur and Bastar forests in Madhya Pradesh and Punjabi foothills [29]. It grows in warmer, drier desert-like climates with 150 mm of annual rain fall on average. It is a drought resistant plant [30]. The six species of the Solanaceae family including the Withania coagulans been studied extensively for their medicinal uses and chemical constituents and [31].

Many phytochemicals, including alkaloids, steroids, esters, phenolic mixtures, saponins, tannins, proteins, starches, free amino acids, essential oils, and natural acids are present in the plant [32]. The plant is enriched with withanolides, such as withaferin-A, withanolide-A, withacoagulin and coagulanolide [33]. Over and above 13 alkaloids, 138 withanolides and several sitoinsides (a withanolides containing a glucose moiety at the 22nd carbon) have been separated from the leaves, berries, and roots [34]. The withanolides skeleton consists of 22 hydroxyergostan-26-acid- 26, 22-lectone. Numerous new structural variants are produced by altering the side chains or the carbocyclic backbone [35]. Several important phytochemicals have been isolated from various parts of the plant. The important chemical constituents of the plant are given in Table-1.1.

Pharamcological Potential of Withania Coagulans

Withania coagulans contains 40 distinct kinds of withanolides and more than 12 alkaloids, which formed the basis of its pharmacological properties. The plant is extensively used as an antihyperglycemic, antihypercholestetilemic and as remedy to cure anticancer [45]. All parts of the plant possess therapeutic properties because biologically active substances like phenols, flavonoids, tannins, saponins and alkaloids are found in the plant [46]. The concentration of one of the major constituents of the plant, "withanolides" varies in different seasons and parts of the plant, which is responsible for many biological activities like anti-inflammatory, anticancer, hepatoptotective, antihyperglycemic, cardiovascular and free radical scavenging properties [6].

Table 1: The Chemical Constituents Reported from Withania Coagulans

Chemical Constituents	References
Coagulin, Coagulin-A	[36]
Chlorogenic acid, 3β-Hydroxy-2, 3-dihydrowithanolide-F, 3β, 14α, 20αF, 27-tetrahydroxy-1-oxo- 20R, 22R-witha-5, 4-dienolide	[37]
Ajugin-A, β-Sitosterol, β-Sitosterol glycoside	[38]
Ajugin E	[39]
Withaferin	[40]
Withaferin A	[41]
Ergosta-5, 25-diene-3β, 24-ε -diol	[42]
Coagulin H: 5α, 6β, 14α, 15α, 17, 20-Hexahydroxy-1-Oxo-witha-2, 24-dienolide, Coagulin-I, Coagulin-J, Coagulin-K, Coagulin-L	[43]
14, 15β- Epoxywithanolide-I, 17β-Hydroxywithanolide-K, 17β, 20β-Dihydroxy-1-Oxo-witha- 2, 5, 24-trienolide	[44]

Withania coagulans has shown promising antidiabetic activity in various studies. The presence of primary bioactive compounds including alkaloids and withanolides like coagulan C, coagulan L and 17β-hydroxy withanolide K (structure 15 and 18) are believed to contribute to its hypoglycemic effect [47]. The reports indicate that the extracts the plant help in lowering blood sugar levels, boosts sensitivity to insulin and balance pancreatic beta cell function. [48]. The plant also showed antioxidant properties that protect against oxidative stress which is often elevated in diabetic patients [49].

In both carved and open wound models, the aqueous methanol extract of W. coagulans exhibited a significant amount of wound healing activity. Moreover, it promoted the formation of collagen, DNA and proteins [50]. 3β-hydroxy-2, 3 dihydrowithanolide F, Withanolide coagulin H (structure 4, 12) exhibited anti-inflammatory effect by encouraging the healing process and preventing the synthesis of proinflammatory cytokines [51].

Withania coagulans has shown potent anti-cancer activity, primarily attributed to its bioactive compounds known as withanolides, demonstrating cytotoxic effects on a range of cancer cell lines by preventing tumor development and triggering apoptosis (programmed cell death) [52]. Some specific withanolides, such as withaferin A, withacoagulin D and cogulin L (structure 8 and 16) have been studied for their ability to disrupt cancer cell proliferation and metastasis. They can also modulate signaling pathways involved in cancer progression, such as the NF-kB and STAT3 pathways, leading to reduced inflammation and tumor growth [50-53].

As a part of a therapy plan, diuretics are used to control fluid excess. Withania coagulans is used in the traditional system of medicines for treating various diseases [54]. It has been reported that the plant possesses diuretic activity. According to Lipschitz test, the aqueous extract of the fruit of the plant has increased the urine volume and concentration of many salt ions [55].

Flash chromatography was used to separate the withanolides coagulin-C and withacoagulin from the extract of the Withania coagulans. When it comes to managing a change in the lipid profile, withacoagulin works better than coagulin-C, hence a promising medicine for cardiovascular complications [56]. Its further effectiveness in the isoprenaline-induced myocardial injury in rats, as results proved significant [57].

Experimental Methods

Collection of Plant Material: The dried berries of the plant were acquired from a local herbal store in Mirpur (AJK), Pakistan. The stalks were removed, and leaves were taken out before making a coarser powder using a commercial blender. The powdered material was stored in a polythene bag for its further use.

Extraction Process: The maceration process was used for extraction of the plant. The powdered material (227 g) was soaked in 500 mL methanol for 15 days. During the period, the extract was shaken gently on daily basis and the extract was filtered out by using a filter paper. The process of maceration was repeated thrice to assure maximum extraction. The filtrate was concentrated by rotary evaporator under reduced pressure to afford a dark brown, waxy crude extract.

The extract (0.05 g) was added in 50 mL methanol and dissolved. For the preparation of stock solution, the same solvent was used in which extraction was carried out. This extract was stored for the planned phytochemical analysis.

Qualitative Phytochemical Screening

Several qualitative tests were carried out as part of the preliminary phytochemical screening process to identify classes of different chemical constituents of the plant, including proteins, terpenoids, alkaloids, flavonoids including proteins, terpenoids, alkaloids, flavonoids, and tannins. The Deagendorff's, Mayer's and Wagner's tests indicated the presence of alkaloids in the palnt while the Molisch, Benedict, Fehling and Iodine tests indicated the presence of carbohydrates. The Frothing test indicated the presence of saponins. The Salkowski's test showed the presence of phytosterols while the Emulsion test indicated the presence of lipids. The Biuret and Xanthoproteic tests indicated the presence of proteins. The Borntrager's and Baljet's tests indicated the presence of glycosides. The Vanillin-HCl and Alkaline tests indicated the presence of flavonoids while the Spot test indicated the presence of fixed oils.

Quantitative Phytochemical Screening

Estimation of Total Phenolic Content (TPC): The Folin-Ciocalteu method was employed to find out the actual phenolic concentration in methanol extract of the fruit of the plant using gallic acid as standard. Plant extract and standard was prepared in mg/ mL concentration for this analysis. The mixture was formed by dissolving 100 µL of the extract and solution was diluted with 450 µL distilled water and 150 µL of Folin-Ciocalteu reagent (1:1). Reaction mixture was left for 5-10 minutes at room temperature. Bluish color was developed. After that 500 µL of 20 % sodium carbonate was added and mixture was 18 placed in dark for 1 hour away. The absorbance was determined at 765 nm. Using the different concentration of standard solution of gallic acid were used to plot a calibration curve. Total phenol contents were shown in terms of gallic acid equivalent (mg of GA/g of extract)

Estimation of Total Flavonoid Content (TFC): Total flavonoid contents were ascertained by

Aluminum Chloride procedure. The plant extract (100 µL) was diluted with methanol and 10% solution of aluminum chloride 100 µL prepared in distilled water was added. After that 100 µL of 1M CH3COONa was added in the reaction solution. The solution was incubated for 1 hour in dark at room temperature. The absorbance of the solution was observed at 540 nm. Quercetin is a well-known plant flavonol and was taken as standard compound. A standard calibration graph was plotted between different concentrations of the quercetin (10 µL to 50 µL) on x-axis and their corresponding absorbance. Total concentration of flavonoid in the extract was calculated and described as quercetin equivalent (mg of Q/g of extract).

Evaluation of Pharmacological Activities

Antioxidant Activity: The ability of a material to stop or reduce cell damage caused by reactive oxidative species is known as antioxidant activity. Unstable free radicals induce oxidative stress, which damages cells and causes aging, cancer, and other illnesses. The antioxidant activity of the extract of fruits was assessed using the DPPH (2, 2-diphenyl 1-picrylhydrazyl) and ABTS (2, 2'-azino-bis (3ethylbenzothiazoline-6-sulfonic acid) assays.

The stock solution of the extract (50 mg/mL) was prepared. Different concentration (equivalent to 100, 200, 300, 400 and 500 uL) of the fruit extract and standard were taken in separate test tubes. Ascorbic acid was used as a standard for this assay. Using methanol solutions were diluted to total volume of 0.3 mL. After 19 that 4 % solution of DPPH reagent was made in methanol. From this 4 % solution about 1 mL of the DPPH solution was added to the test tubes containing extract and standard. These tubes were kept for incubation for 30 minutes in the dark at room temperature. Absorbance of each concentration was measured at 517 nm against a blank (reaction mixture without the extract) using a UV-visible spectrophotometer. The percentage free radical scavenging activity (RSA) was calculated using a standard formula.

Potassium persulfate was used to oxidize the ABTS. In a 1:1 ratio, 2.45 mM potassium persulfate was combined with 7 mM ABTS and placed in the dark. The ABTS solution was diluted with methanol and absorbance of the solution was recorded at 734 nm. The plant extract (100 µL) was added into 3 ml of the diluted solution. The mixture was incubated for 10 minutes and the absorbance for different concentrations ofextract and standard (rutin) was measured. The inhibition percentage was calculated by using a standard formula.

Metal Chelating Activity

The reagent-metal ion binding affinity, such as Fe2+, was taken into consideration when developing spectroscopic techniques. The Dinis method was used to assess ferrozin's Fe2+ chelation. A 0.005 mL solution of FeSO4 (2 mM) was mixed with a varied amount of sample and standard chemicals. This provides the interaction between the sample and Fe2+, which is sample chelating the Fe2+ ions, 0.2 mL of ferrozine reagent (5 mM) was added to initiate the reaction. The solution was left for 20 minutes. The absorbance of the solution was recorded at 562 nm. Percentage chelating activity of the samples and standard were determined by using the standard formula.

Antiglycation Activity

Antiglycation activity was evaluated by assessing the ability of a substance to prevent or reduce the production of harmful advanced glycation end products (AGEs) formed through the reaction between sugars and protein. One common method to measure antiglycation activity is the Bovine Serum Albumin (BSA) assay.

The fixed quantities of NaCl (8 g), KCl (0.22 g), NaHPO4 (1.44 g) and 0.2 g of KH2PO4 (0.2 g) were dissolved in 100 mL of distilled water, and the mixture was diluted to 1000 mL. This phosphate buffer solution was kept at a pH 7.45. The Bovine Serum Albumin was prepared by mixing 0.085 g of BSA in 1

mL of PBS (85 mg/mL). The glucose solution (1.5 mM) was prepared by adding 0.027 g of glucose mixed in 100 mL of PBS. One milliliter of amino guanidine (1 mg/mL) was diluted with 1 milliliter of distilled water.

The 300 µL of BSA, 300 µL of glucose solution and 200 µL of various concentrations of 0.02-1 mg/ mL of the aqueous plant extracts were taken in ependroph tubes for this glycation assay. Amino guanidine was taken as reference in hydrazine type substance with the same quantity in separate ependroph. The BSA (300 µL) and glucose were used as a control. The incubation period was 10- 14 days at 60 oC. The samples were centrifuged at 2000 rpm after their treatment with trichloroacetylene for 20 minutes to afford pallets. After dissolving these pallets once more in appropriate solvent, absorbance was measured at 370 nm. A decreasing trend in absorbance value was noted. The % age inhibition of glycation was calculated by using a standard formula.

Antimicrobial Activity

The following methodology was used to determine the antimicrobial activity by utilizing the traditional agar diffusion method against several gram positive and negative bacterial strains. The extract of the fruit was mixed in different solvents (chloroform, n hexane, ethyl acetate and distilled water separately) in mg/mL for this assay. The clinical bacteria like Escherichia coli, Pseudomonas aeruginosa, and Salmonella enteric and non-clinical spore Bacillus subtilis were used as gram positive strains. Media of these cultures was prepared by using nutrient agar. According to manufacturer's instructions, a measured mass (7 g) of the agar powder was dissolved in 250 mL of distilled water in a suitable container. To emasculate the combination, it was autoclaved at 121oC. The agar was autoclaved and allowed to cool at about 50 oC.

The agar media was poured into sterile petri dishes, filling them to a depth of about 4-5 mm. For the agar diffusion assay petri dish was inoculated with each bacterial culture using a sterile cotton swab. After inoculation, wells were created and loaded with each of the extract (50 µL) along with respective solvents as negative control. For positive control, 5 µL of an RNA inhibitor drug, rifampicin (0.1 %) was used to compare the bactericidal potential of samples. Discs were placed making sure to space them evenly to avoid overlapping zones of inhibition. Plates were incubated at appropriate temperature (35-37 oC) for 18-24 hours. The zones of inhibition of the samples were measured after incubation to determine the antibacterial activity of the extract.

Results and Discussions

The findings of the investigations are presented in the form of a tables and graphs. The results are interpreted, and their significances are discussed highlighting the importance of phytochemical analysis in understanding the biological properties of the plant and possibility of their potential uses in the health care and as medicine. The powdered material (227.64 g) afforded 72.75 g of the extract and hence percentage yield was found to be as 31.94%. The extract 100 mg was dissolved in 100 mL of methanol. This stock solution was used for further analysis and for the identification of the chemical constituents present in the plant.

Phytochemical Screening

The bioactivity of the plant is due to the presence of active secondary metabolites in it. The medicinal importance of the phytochemicals depends on varied amounts of these phytochemicals in different seasons and parts of the plant. A number of secondary metabolites such as alkaloids, flavonoids, fixed oils, glycosides, and phenolic compounds as well as primary constituents like proteins, carbs, amino acids, lipids, were found in the alcoholic extract of the fruits of the plant. The extracts were subjected to different qualitative test and bioassays to determine the biological activities of the plant. The results of the phytochemical screening are presented in the following tables.

Table 2: Results of the Qualitative Analysis Performed on the Extracts of the Plant

S #	Phytochemicals	Tests	Results
1.	Detection of Alkaloids	Dragendorff's Test	
		Mayer's Test	
		Wagner's Test	
2.	Detection of Reducing Sugars	Molisch's Test	
		Benedict's Test	
		Fehling's Test	
3.	Detection of starch	Iodine Test	
4.	Detection of saponins	Foam Test	
5.	Detection of phytosterols	Salkowski's Test	
6.	Detection of lipids	Emulsion Test	
7.	Detection of proteins	Biuret Test	
8.	Detection of glycosides	Xanthoprotic Test (Aromatic Amino Acids)	
		Borntrager's Test (Anthraquinone Glycosides)	
9.	Detection of flavonoids	Baljet's test (Cardiac Glycosides)	
		Vanillin-HCl Test	
		Alkaline Test	
10.	Detection of fixed oils	Spot Test	

Total Phenolic Content

The Folin-Ciocalteu (FC) method was used to determine the extract's total phenolic content. The process is based on an electron transfer reaction between the FC reagent and the antioxidant species in the extract. The phenolic chemicals serve as electron donors, while the FC reagent acts as the oxidant. The end product of this redox reaction is a blue solution. The intensity of the blue color is directly related to the sample's phenolic content. The total phenolic content of the extract was estimated applying gallic acid as a standard. A representative aph was produced by plotting the different concentrations of gallic acid (50–250 µg/mL) on the horizontal axis and its corresponding absorbance on the y-axis, respectively. The extract's ability to reduce phenols was measured in gallic acid equivalents (GAE). The results showed that the methanol extract of the fruits of the plant has a total phenolic content of 172.2709 µg/mL.

Total Flavonoid Content

For this assay Aluminum chloride method was used talking quercetin as the flavonoid standard. Different concentrations of quercetin ranging from 0 to 50 µg/ mL were prepared to plot a standard curve with concentration on horizontal axis against

absorbance value on vertical-axis. The equation y = mx + c, which was derived from the standard curve, was used to determine the total flavonoid concentration in the plant extract. The results showed that the plant has a total flavonoid content as 30.96932 µg/ mL.

Evaluation of Pharmacological Activities

Antioxidant Assays: A well-known radical trike or scavenger is DPPH. When the antioxidant plant extract reacts with the free radical DPPH, DPPH solution turns from dark lavender to The antioxidant activity of the plant was compared with the ascorbic acid and a comparison is presented in the Figure-2.

When assessing the antioxidant capability of any dosage of medication or even plant, DPPH is the most useful molecule. Antioxidant potential of the fruit extract was determined by preparing various concentration of the extract and the standard using dilution series method, ascorbic acid in this case, diluted and treated with DPPH solution. Absorbance was observed at 517 nm. It is seen that as the concentration of extract increase the percentage inhibition also increase and the absorbance value decrease accordingly. The antioxidant activity of plant are shown in Table-3. The antioxidant activity of the plant was compared with that of ascorbic acid and a comparison of both activities is presented in the Figure-2.

Table 3: The Antioxidant Potential of Winthana Coagulans

S #	Concentration of Extract (µL)	Absorbance (nm) at 517	% Scavenging Activity
1.	100	0.067	54.4
	200	0.048	67.3
	300	0.038	74.1
2.	400	0.024	83.6
	500	0.016	89.1

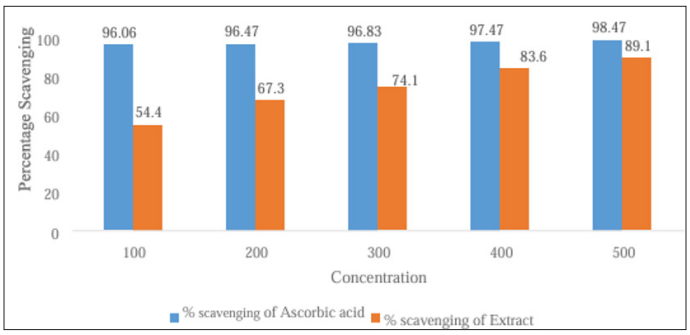

Figure 2: Comparison of Antioxidant Activities of Plant and Ascorbic Acid by DPPH Assay

Like the DPPH, ABTS can also be used to assess the efficacy of the extracts of the plant. ABTS and potassium persulphate were used in this procedure. The absorbance was determined after 12 hours intervals at 734 nm. The antioxidant activity of the extract of the plant determined by using the ABTS assay is given in Table-4. A comparison of the antioxidant activity of the plant with that of rutin is presented in the Figure-3.

Table 4: Antioxidant Activity of the Extracts of the Plant by the ABTS Assay

S #	Concentration Extract in µL	Absorbance (nm) at 734	% Scavenging Activity
01	100	0.306	68.0
02	200	0.197	79.5
03	300	0.101	89.5
04	400	0.035	96.3
05	500	0.020	98.0

Metal Chelating Assay

The metal chelating activity of the extracts of the plant was evaluated by using ferric sulphate and Ferrozine. Ferozine is a particular reagent that combines with ferrous ions (Fe^{2+}) present in the solution, which affords a stable pink colored complex.

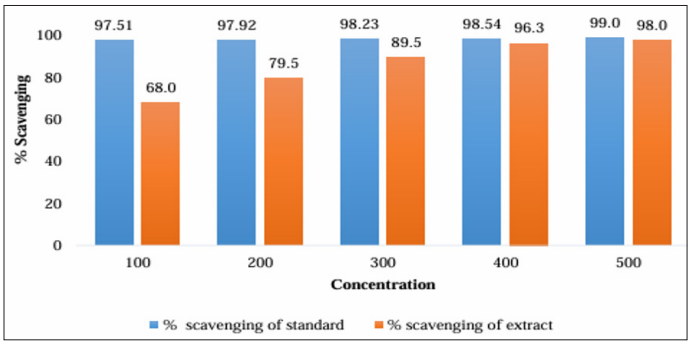

Figure 3: Comparison of the Scavenging Activity of the Plant and Rutin by ABTS Assay

The absorbance value of this complex was measured spectrophotometrically. The chelators in extract of the plant were allowed to react with a known concentration of ferrous ions. On addition of ferrozine to the mixture, chelating agent in the extract will bind ferrous ions, reducing the amount of free Fe2+ ions available to react with ferrozine. The absorbance of the solution was measured at 562 nm. The quantity of free ions is inversely correlated with the intensity of the pink color. As a result, a lower absorbance indicates that the sample has a stronger chelating capacity. The metal chelating activity of the extracts of plant is given in the Table-5. A comparison of the metal chelating activity of plant with ascorbic acid is presented in Figure-4.

Table 5: Metal Chelating Activity of Withana Coagulans

S #	Concentration of Extract (µL)	Absorbance (nm) at 562	% Scavenging Activity
01	100	0.076	37.0
02	200	0.060	40.0
03	300	0.048	67.0
04	400	0.033	77.5
05	500	0.023	84.0

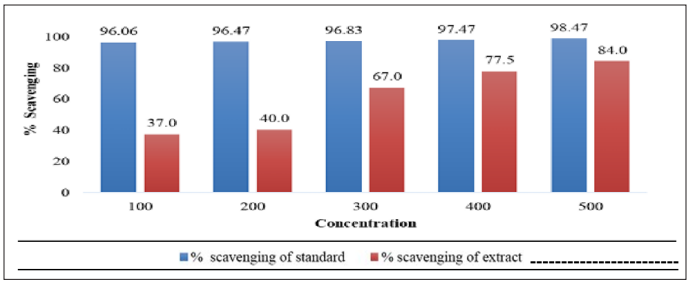

Figure 4: Comparison of Scavenging Activity of Plant and Ascorbic Acid by Metal Chelating Assay

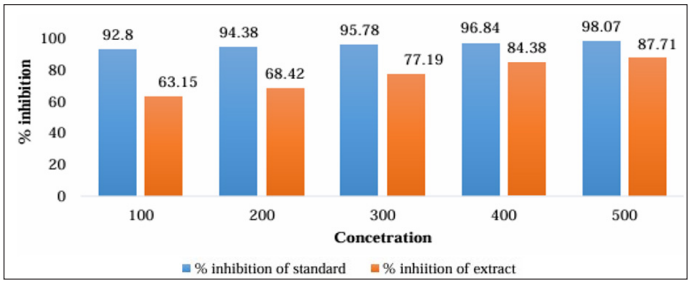

Figure 5: Comparison of Antiglycation Activities of Plant and Amino Guanidine by BSA Assay

Antiglycation Assay (BSA)

The formation of the non-enzymatic adducts between amino groups of protein and reducing sugar's carbonyl groups is known as glycation. A sugar and a biological amine's free amino group combine to produce an unstable substance called a Schiff base. This complex is subsequently rearranged to create more stable amadori products, also known as early glycation products.

Advanced glycation end products (AGEs) are irreversible molecules that are formed during the later stages of glycation. As a result of oxidation, cyclization, and dehydration reactions take place. Tissue proteins undergo structural and functional changes because of the buildup of protein glycation products in living things, plant extracts have long been used to treat diabetes, by utilizing active compounds in it to lower blood glucose levels. Another method of treating diabetes that is independent of blood glucose regulation is the suppression of advanced glycation end products (AGEs), which may help avoid or lessen some of the problems associated with the disease. Due to inadequate safety profiles, many synthetic inhibitors of AGE production are not currently used in clinical settings. Nonetheless, in addition to being potent antioxidants and glycation inhibitors, the phenolic phytochemicals found in fruits, vegetables, and herbs are often safe for ingestion by humans. It is recognized that significant levels of possibly very active polyphenols are present in the extract of the fruit of Withania coagulans. The ability of the plant extract to inhibit the formation of advance glycation end products (AGEs) was evaluated by using Bovine Serum Albumin method (BSA). The BSA solution in buffer (PBS) was prepared and treated with glucose solution to induce glycation of the BSA. Different concentrations of the extract were mixed with a solution, and this mixture was incubated at 60-80 degree centigrade for 10-15 days. After incubation, the glycation was ascertained by measuring the absorbance at 370 nm, which corresponds to the formation of the AGEs. The antiglycation activities of the plant are given in the Table-6 while a comparison of the antiglycation activities of the plant with that of amino guanidine by the BSA assay is presented in Figure-5.

Table 6: The Antiglycation Activities of the Plant by the Antiglycation Assay

S #	Concentration of Extract (µL)	Absorbance (nm) at 370	% Inhibition
1	100	0.21	63.15
2	200	0.18	68.42
3	300	0.13	77.19
4	400	0.089	84.38
5	500	0.070	87.71

Antimicrobial Activity

The agar diffusion method was applied to check the antimicrobial activity of the extract against four bacterial strains, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica and Bacillus subtilis. The methanol extract was further dissolved in four different solvents, distilled water, n- hexane, ethyl acetate, and chloroform. Their activity was compared to the standard antibiotic rifampicin (0.1%, 5 µL /well) as positive control. Each well received 50 µL of extract (prepared at 50 mg in 1.5 mL of solvent), and zone of inhibition was recorded after incubation. The results are summarized in the Table-7.

Table 7: A Comparison of the Antimicrobial Activities of the plant with Rifampicin

Zones of Inhibition zone in mm					
Microorganism	**Rifampicin**	**Distilled Water**	**Ethyl Acetate**	**Chloroform**	**n-hexane**
E. coli	23	03	16	15	00
P. aeruginosa	30	20	10	13	00
S. enterica	35	18	15	13	15
B. subtilis	25	14	16	10	00

The antimicrobial screening of W. coagulans extract revealed that the activity significantly depends on the solvent used and bacterial strain tested. Ethyl acetate and aqueous extracts generally demonstrated greater antimicrobial potential compared to chloroform and n-hexane extracts. Among all extracts, ethyl acetate exhibited the strongest inhibition against E. coli (16 mm) and S. enterica (15 mm) highlighting its efficacy in extracting semi polar bioactive compounds like flavonoids and phenolic. Similarly, the n-hexane extract showed promising activity against E. coli (15 mm), which is noteworthy given that non-polar solvents typically exhibit weak antibacterial properties, this suggest the presence of lipophilic bioactive constituents such as steroids or terpenoids in the n-hexane extract of the plant. The chloroform extract showed minimal to no activity. This suggests that the active antimicrobial constituents of Withania coagulans are more likely to be extracted in polar or semi-polar solvents. These findings also underscore the importance of solvent polarity and bacterial susceptibility in determining antimicrobial efficacy.

Conclusions

The antioxidant, antiglycation, antimicrobial and metal chelation properties of Withania coagulans studied. The extracts of the plant were subjected to various bioassays and test experiments. The studies showed that the extracts of the plant possess biological activities. As extracts of the plant possess significant antioxidant and metal chelation properties, they may be used in antioxidant therapies and metal chelation strategies. The ability of the extracts to effectively store metal ions make the plant a strong candidate for further investigations in terms of its effectiveness and possible

natural therapy. This research enhances the understanding of W. coagulans and its beneficial roles in health care and possible use as medicine.

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