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ANTIOXIDANT AND ANTIMICROBIAL EFFICACIES OF WITHANIA COAGULANS SEED EXTRACT AGAINST PATHOGENIC BACTERIA AND FUNGI

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ABSTRACT

The present study was conducted to evaluate the nutritional profile, phytochemical screening, antimicrobial and antioxidant activities of aqueous and acetonic extract of Withania coagulans by standard chemical analysis methods. Proximate analysis revealed that aqueous extract of W. coagulans is a rich source of soluble solids (16.1 ± 1.5 %), fiber (15.5 ± 0.1 %), pH (11.5 ± 0.19 %), ash (11.2 ± 0.2 %), sugar (9.7 ± 0.1 %) and ascorbic acid (9.7 ± 0.1 mg/100ml). Phytochemicals detected were flavonoids, glycosides, carbohydrate & sugar, phenolic compounds, proteins & amino acids, tannins, gum and mucilage while phytosterols were absent. The antifungal and antibacterial activities of plant extract was determined against ten pathogenic bacterial strains (Escherichia coli,Bacilluscereus, Staphylococcus aureus,Clostridium, Escherichia coli (Human), Bacillus subtilius, Xanthomonas, Salmonella typhi,Salmonella heidelberg and Klebsiella pneumonia) and five fungal strains (Penicillium, Aspergillus niger, Alternaria alternata, Entomola and Aspergillus flavus) using the agar well diffusion method. The acetonic extract of W. coagulans indicated highest antibacterial activity (12 mm) against Clostridium and Salmonella heidelberg and lowest action (5 mm) against E. coli (Human.). Highest antifungal activity (10 mm) was recorded against Alternaria alternata and Penicillium while Aspergillus niger showed lowest zone of inhibition (6 mm). Streptomycin was used as a control. The acetonic extract of W. coagulans exhibited highest free radical [(1, 1-diphenyl-2-picryl hydrazyl (DPPH)]) scavenging activity (72 %) at concentration of 30 mg/ml, with an IC50 value (18µg/ml) as compared to control (4µg/ml). The presence of bioactive compounds, nutrients and biochemical screening indicated that the seeds of W. coagulans can serve as a potential source of useful drugs for the treatment of various pathologies.

Keywords: Nutritional, Phytochemical, Antimicrobial, Antioxidant, *Withania coagulans.*

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1. INTRODUCTION

Phytomedicines are the oldest known health-care products. Their significance is still rising although it varies with the medical, historical and ethnological background of each state. These plants are also essential for pharmacological exploration and drug development, not only when plant components are used directly as therapeutic mediators, but also when they are used as basic materials for the production of drugs or as models for pharmacologically active compounds. The use of herbal medicines is increased in the developing countries due to the fact that modern lives saving drugs are beyond their reach. However, expert administration after appropriate identification is essential for their usage (Eisenberg *et al.*, 1993). In recent time due to the increased resistivity of antibiotics for several infections the influence of antimicrobial activity has increased (Davis, 1994). Similarly the search of new antioxidants in the traditional medicinal plants antioxidant components attracts many researchers

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towards itself. The biological oxidation by reactive oxygen species (ROS) causes the breakdown of cell membrane, damages the membrane protein and may cause DNA mutation which results in the propagation of several severe diseases like cancer, cardiovascular diseases and liver injury (Liao and Yin, 2000). The antioxidants or free radical scavengers prevent the body from several severe diseases. Polyphenolic compounds such as alkaloids, tannins, glycosides, flavonoids and phenolic acids are present in the medicinal plants which have been concluded to have several biological effects along with antioxidant action (Rice-Evans *et al*., 1995).

Withania coagulans (*W. coagulans*)is a medicinal plant, belongs to family Solanaceae and well-known as Indian cheese maker. The plant has shownpromising results in the treatment of several diseases in animals and humans models of pathological situations. The fruit of *W. coagulans* is diuretic, hypoglycemic (Hemalatha *et al*., 2004) and hypolipidemic (Hemalatha *et al*., 2006). Literature reported that hypoglycemic and antidiabetic potentials of fruits of *W. coagulans* are due to the presence of magnesium and calcium**.** *W. Coagulans* has also shown anti-depressant, immuno modulate, anti-microbial, antitumor, cytotoxic, free radical scavenging activities and experimental models of diabetes (Mohammad and Reza, 2012).

The impact of herbal medicines among folks of developing countries upsurges since these are benign, easy to administer and non-toxic (Eisenberg *et al*., 1993). Due to the pharmacological importance ofmedicinal plants *W. coagulans* is selected for evaluation of nutritional profile, phytochemical, antimicrobial and antioxidant activities.

2. MATERIAL AND METHODS

2.1. Collection of Sample

Theseeds of *W.coagulans* were collected from the local market of Peshawar, brought to the PCSIR Laboratory, kept in polyethylene bags till analysis.

2.2. Proximate Analysis

Proximate analyses (pH, ash, acidity, moisture, crude fiber, total soluble solids, protein, total sugar and ascorbic acid) of *W. coagulans* were evaluated using the standard procedures of AOAC (2000).

2.3. Phytochemical Screening

For the qualitative assessment of phytochemical compounds chemical tests were carried out on the aqueous extract using standard procedures as described by AOAC (2005).

2.4. Antimicrobial Bioassays

The antibacterial and antifungal activities of *W. coagulans* were explored by agar well diffusion method (Bauer *et al*., 1966; Murray *et al*., 1995; Olurinola, 1996).

2.5. Antioxidant Activity (DPPH Free Radical Scavenging Activity)

The free radical scavenging activity of acetonic extract of *W. coagulans*was analyzed by DPPH. The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. IC_{50} values were obtained by probity analysis (Aqil *et al*., 2006).

3. RESULTS

The results of proximate analysis of aqueous extract of *W. coagulans* are shown in Table 1. The percentage of crude fat was (2.5 ± 0.1 %) and fiber was (15.5 ± 0.1 %). The calculated percentage for protein (%) was (1.57 ± 0.2). The moisture content was (7.8 ± 0.15 %) while the ash content was (11.2 ± 0.2 %). Similarly total soluble solids,

acidity and pH calculated were (16.1 ± 1.5), (2.3 ± 0.1) and (11.5 ± 0.19) % respectively. Vitamin C was (9.7 ± 0.1/100ml).

Table-1. Proximate values of aqueous extract of *W. coagulans*

S#	Constituents	*W. Coagulans* (%)
1	Crude Fats	2.5 ± 0.1
2	Crude Fiber	15.5 ± 0.1
3	Crude Protein	10.9 ± 0.2
4	Moisture	7.8 ± 0.15
5	Ash	11.2 ± 0.2
6	Total Soluble Solids	16.1 ± 1.5
7	Total Acidity	2.3 ± 0.1
8	pH	11.5 ± 0.19
9	Vitamin C (mg/100ml)	35.1 ± 2.0
10	Total Sugar	9.7 ± 0.1

The phytochemicals of *W. coagulans* extract detectedare presented in Table 2. Results revealed that flavonoids, glycosides, carbohydrate & sugar, phenolic compound, protein & amino acid, tannins and gum & mucilage were present in *W. coagulans* extract while phytosterols were absent.

Table-2. Qualitative analysis of the photochemical of aqueous extract of *W. coagulans*

S#	Phytoconstituents	*W. coagulans*
1	Alkaloid	+
2	Saponins	+
3	Carbohydrate & Sugar	+
4	Glycosides	+
5	Phenolic Compound	+
6	Flavonoids	+
7	Protein & Amino acid	+
8	Phytosterols	–
9	Tannins	+
10	Gum and Mucilage	+

Besides, the zone of inhibition of plant extract as compare to standard streptomycin against different bacteria and fungi are summarized in Figure 1. The antibacterial activities ofacetonic extract of *W. coagulans* were tested against ten pathogenic bacteria i.e., *Escherichia coli,Bacilluscereus, Staphylococcus aureus,Clostridium, Escherichia coli (Human), Bacillus subtilius, Xanthomonas, Salmonella typhi,Salmonella heidelberg and Klebsiella pneumonia.* Plant extract showed the highest antibacterial activity against *Clostridium* and *S. heidelberg*with the zone of inhibition of 12 mm. In addition, lowest zone of inhibition of acetonic seed extract were exhibited against *E. coli (Human)* with a zone diameter of 5 mm but minimal activity was recorded against *E. coli* and *B. cereus* with a zone diameter of 7 mm and 6 mm respectively, Furthermore, acetonic extract of *W. coagulans* t indicated the same activity (9 mm) against *B. subtilius, S. typhi, K. pneumonia* and *S. aureus* and 8 mm zone of inhibition against *Xanthomonas.*

The antifungal activity of extract was evaluated against five fungal strains (*Penicillium, Aspergillus niger, Alternaria alternata, Entomola and Aspergillus flavus*)) Figure 1. Results displayed that *W. coagulans* inhibited highest zone of inhibition against *A. alternata* and *Penicillium,*while their growth inhibition zone measured was 10 mm. Moreover, lowest activity was recorded against *A .niger* with its zone diameter of 6 mm, but modest inhibition (7 mm) was measured against *Etymology.* On the other hand the seed extract was not active against *A. flavus.* Streptomycin was used as a control.

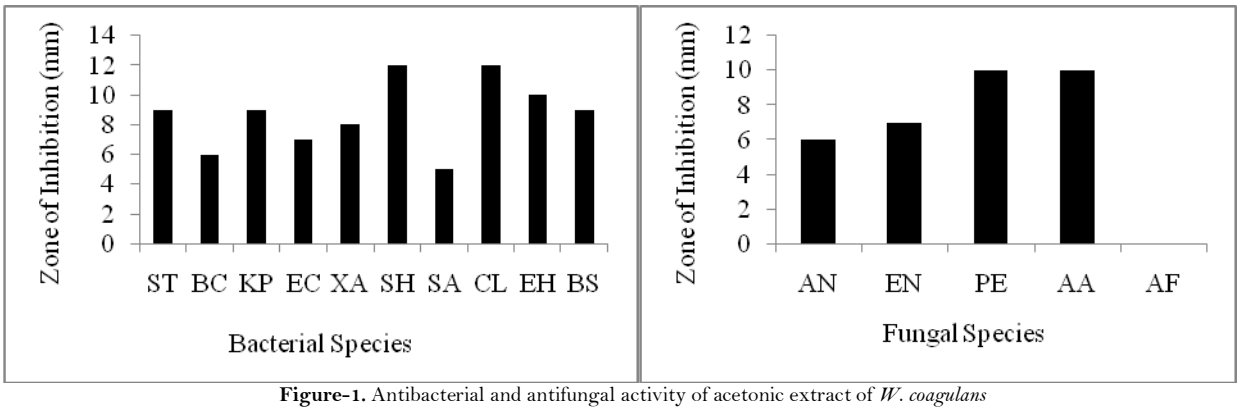

Figure-1. Antibacterial and antifungal activity of acetonic extract of *W. coagulans*

The scavenging activity of acetonic extract of *W. coagulans* is summarized in Table 3&4. The lowest scavenging activity was 8.92 % at concentration of 1 mg/ml and highest scavenging activity was 72 % at concentration of 30 mg/ml. Ascorbic acid was used as a control.

Table-3. DPPH radical scavenging activity in percent (%) of acetonic extract of *W. coagulans*

S#	Concentration (mg/ml)	Scavenging Activity Percent (%)	Vitamin C(Standard)
1	1	8.92±0.9	38.08±1.18
2	5	22.17±1.4	53.58±1.16
3	10	33.01±1.7	68.37±1.15
4	15	42.34±2.0	81.28±2.45
5	20	54.37± 1.19	83.12±2.78
6	25	64.26±2.13	84.21±2.54
7	30	72.0±2.45	85.0±2.75

Mean ± S.D (n=3)

Table-4. DPPH radical scavenging activity of acetonic extract of *W. coagulans* (IC_{50} in mg/ml)

S #	Acetonic Extracts	IC_{50} Value (mg/ml)
1	*W. Coagulans*	18
2	Vitamin C	04

4. DISCUSSION

Proximate analysis of the extract observed is given in Table 1. In proximate analysis different concentration of biochemicals and other contents are studied. Highest percentage of ascorbic acid (35.1 ± 2.0 %) shows that it is a good source of vitamins. Ash, protein, fat, moisture, total soluble solids, pH and total sugar show valuable and reasonable correlation with their parameters. Furthermore the plant extract is the best source of energy, protein, minerals, dietary fiber and flavor thus contributing to the delectableness and supplementation of food in animal and humans diet (Okeke *et al.*, 2001).

The phytochemical screening of chemical constituent of extract of *W. coagulans* showed that flavonoids, glycosides, carbohydrate & sugar, phenolic compounds, protein & amino acid, tannins and gum & mucilage are present in the extract while phytosterols are absent [Table 2]. The presences of these phytochemicals in the seeds of *W. coagulans* are medicinally valuable and play a very important role in various biological activities. Among the phytochemicals flavonoids exhibits antibacterial, anti-inflammatory and anti-allergic activities. It also has hydroxyl radicals, lipid peroxy radicals and super oxide anion scavenging abilities (Okwu and Okwu, 2004). Similarly, alkaloids are advantageous chemicals as these show receptivity against predators and parasites in plants. These also have cholesterol lowering effect. Literature has also been reported that alkaloids in some cases are carcinogenic

(Okwu and Josiah, 2006). Moreover, glycosides show hypoglycemic activity. Mostly glycosides are toxic as it decreases the heart rate, sympathetic activity and systemic vascular resistance, while tannins have the ability to scavenge free radicals and also displays spasmolytic action in smooth muscle cells (Koleva *et al*., 2002).

Furthermore, the antibacterial results revealed that acetonic extract of *W. coagulans* showed highest activity against *clostridium* and *S. heidelberg* when compared with the standard streptomycin. *Clostridium* cause diarrhea in children because of the circulation of virus in community (Bartlet, 2002). Additionally, plant extract showed lowest activity against *E. coli (Human)* with zone of growth of 5 mm compare with streptomycin (15 mm). Literature reported that this is a most common bacterium and cause the pulmonary tract diseases, urinary tract infections (UTI), wounds, burns, dysentery-like diarrhea and other blood diseases (Logan and Berkeley, 1981). Besides, seeds of *W. coagulans* showed moderate activity (9 mm) against *B. subtilius*, *S. typhi*, *K. pneumonia* and *S. aureus*. Researches demonstrated that *B. subtilius* is a gram-positive bacterium and causes food poisoning, nausea, vomiting and abdominal cramps by contamination of food (Jeffrey, 2004). *K. pneumonia* is a gram-negative bacterium and causes pneumonia in organisms, while *S. aureus* is a gram-positive bacterium and causes severe infectious diseases like meningitis and pneumonia in humans. On the other hand, extract of *W. coagulans* exhibited 8mm zone diameter against *Xanthomonas* as compared to control (13 mm). The *Xanthomonas* is a gram-negative rod shape bacterium and causes systemic infections and typhoid fever in humans (Den *et al*., 2003).

Similarly, the antifungal activity of *W. coagulans* was evaluated against five fungal strains. Results [Figure 1] revealed that extract showed highest activity against *A. alternata* and *Penicillium* (10 mm) compared with standard streptomycin 10 mm and 14 mm for *A. alternata* and *Penicillium* respectively. These microorganisms belong to the family Ascomycota causes the cough, chest problem, asthma, dyspnea and fever (Schuster *et al*., 2002). Furthermore, acetonic extract of *W. coagulans*showed minimum activity against *Entomola* (7mm) compared to streptomycin, while lowest activity was recorded against *A. niger* with its zone of inhibition of 6 mm compared with control (9 mm). Studies explored that *A. niger* is a filamentous fungi, causes the lung diseases and weakened the immune system (Chen *et al*., 2002). Lastly, the plant was inactive against *A. flavus* compared with streptomycin (15 mm).

There are different techniques accessible to evaluate the antioxidant activity of plants. The most convenient and easy technique for the determination of antioxidant capacity of phytomedicines is free radical scavenging assay by 1,1 diphenyl-2-picryl hydrazyl (DPPH) stable radical using spectrophotometer. DPPH radical get one electron in the presence of an antioxidant and decreases the absorbance (Rahman and Moon, 2007). DPPH show strong absorption at 517 nm in visible region.

Ascorbic acid has higher DPPH radical scavenging capacity than extract. It was also found that antioxidant ability of the acetonic extract of *W. coagulans* depend on the concentration as shown in Table 3, i.e., scavenging activity was increased with increase of concentration (Greenwald, 2002). Concentration of extract at which the inhibition percentage approaches to 50 % is its IC_{50} value. IC_{50} value is negatively correlated to the scavenging action, as it expresses the amount of antioxidant required to reduce its radical concentration by 50 %. The lesser the IC_{50} value, the greater is the antioxidant action of the extract. The antioxidant action of the sample increases with decrease of the IC_{50} value. Beside this it has been observed that in plants antioxidant capacity is due to the presence of phenolic compounds like polyphenol, flavonoids, phenolic terpenes and terpenoids etc. (Mehta and Pezzut, 2002; Tsao *et al*., 2004).

The IC_{50} was 18 mg/ml for *W. coagulans*, while the IC_{50} for ascorbic acid standard was 4 mg/ml which is lowest in all the values [Table 4]. IC_{50} value described as the extract concentration leading to the 50 % decrease in the initial concentration of the DPPH was calculated from linear regression of the extract concentration against the antioxidant activity mean percentage.

5. CONCLUSION

In conclusion, seeds of *W. coagulans* contained appreciable amounts of nutrients and bioactive compounds which are vital nutritional requirements of organisms and could be useful as feed supplement to improve health and growth performance in humans. Furthermore, the results confirmed the high free radical scavenging capacity of the *W. coagulans* which exceed 70% of DPPH inhibition. In addition, the extract also showed potent antimicrobial activity against all the pathogenic microbial strains with maximum inhibition against *S.heidelberg*, *Clostridium*, *Penicillium*and *A. alternata*. Finding of the present study constitute supportive evidence to validate folkloric use of this plant as a remedy for innumerable health disorders related to oxidative stress including cardiovascular diseases, neurodegenerative disordersand cancer. It is expected that using natural products as therapeutic agents will probably not cause resistance in microorganisms. Therefore, the outcomessuggests that the seeds of *W. coagulans*could probably be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made through a simple process.

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