Research Article

Antioxidant Potential and Phytochemical Study of *Withania coagulans* Dunal; Investigating the Herbal Medicine of Pakistan

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Abstract | *W. coagulans* Dunal, belongs to Solanaceae family is a useful plant holding an important place in Ayurvedic medicines. Presence of biomedical compounds polyphenols, carotenoids, vitamins, and trace elements as secondary metabolites imparts antidiabetic, hepatoprotective, hypolipidemic, antidepressant, immunosuppressive, anti-microbial properties to the *W. coagulans* Dunal. Plant samples from Mianwalli, Kohat, and Sargodha city were gathered for antioxidant potential determination. Free radical scavenging activity and total antioxidant activity experiments were performed on the samples using the DPPH and Phosphomolybdenum methods. Results shows leaves extract from Mianwali has the highest total antioxidant capacity while stems collected form the Sargodha has highest free radicals scavenging ability with values of 47.60± 3.00 Vit C equiv mg/g DW and 50.00± 3.00 IC50 value.

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1. Introduction

Reactive oxygen species (ROS) damage living organisms. Hydrogen peroxide (H2O2), free radicals like the hydroxyl radical (OH) and superoxide anion (O2) are among the reactive oxygen species produced in cells (Madkour, 2019). Oxidants initiate chemical chain reactions that damage DNA or proteins (Gupta, 2015). Imperfect DNA repair mechanisms damage the DNA and ultimately causes cancer (Valko *et al.*, 2016), whereas protein damage causes enzyme inhibition, denaturation, and degradation (Berlett *et al.*, 1997).

Presence of antioxidant molecules in food systems decreases the harmful effects produced by (ROS) in the human body (Cakmakci et al., 2015; Gocer *et al.*, 2013). Antioxidants Molecules are defined as molecules that inhibit the oxidation of other molecules or they can scavenge the ROS directly or indirectly (Lü *et al.*, 2010) (Figure 1). Superoxide dismutase enzymes (SODs) glutathione peroxidase (GPx), and glutathione Reductase (GRd, catalase (CAT) are the antioxidant enzymes that exhibit the highest antioxidant Defense effectiveness (Gupta and Sharma, 2011).

Humans have tried to cure ailments using nature since ancient times (Balick and Cox, 2020). Medicinal plants have been used to combat ailments since antiquity (Gopinath *et al.*, 2017). Evidence confirms that approximately 60,000 years ago plants were cultivated for getting the drugs (Jamshidi *et al.*, 2018).

Plant containing compounds that have been proven to have a therapeutic effect or have compounds that can act as the precursor for the synthesis of other therapeutically important chemicals is termed as medical plant (Sofowora *et al.*, 2013). Globally medicinal herbs trade is worth over USD 100 billion per year (Sofowora *et al.*, 2013). *W. coagulans* is a significant medicinal plant (Khan *et al.*, 2021) found in Pakistan (Gilani et al., 2009), Iran (Lateef and Qureshi, 2020), Nepal, Afghanistan, and dried parts of India (Youn *et al.*, 2013).

Withania coagulans are generally found as a straight shrub 60-120cm in height. The leaves of the plant are covered by the tomentum on both sides. The flowers appear in axillary clusters and are dioecious. Ovoid shape ovary is present while plant lack style and stigma in the male part. Stamens reach halfway up the corolla tube in female flowers. Style and ovary are glabrous while mushroom shape stigma is also present the seeds have a diameter of 2.5–3 mm (Gupta, 2012).

The ripe fruits are utilized for Wound healing (Melguizo-Rodríguez *et al.*, 2021) while dry fruits of *W. coagulans* are traditionally used as anti-diabetic (Ram *et al.*, 2021) Anti-bacterial (Qasim *et al.*, 2020) anti-microbial (Peerzade et al, 2002), hepatoprotective (Qureshi *et al.*, 2019), hypolipidemic (Lateef and Qureshi, 2020), anti-oxidant (Keshari *et al.*, 2018), anti-tumor (Ahmad *et al.*, 2017), anti-depressant (Gosavi *et al.*, 2020) immunosuppressive (Reddy *et al.*, 2016; Mirakzehi *et al.*, 2017), anti-inflammatory agent (Qureshi *et al.*, 2019) diabetes treatment (Lateef and Qureshi, 2020). Seeds have anti-inflammatory, diuretic, and ophthalmia-curing properties, while flower buds have anthelmintic properties (Maurya and Jyendra, 2010; Mudassir *et al.*, 2018; Saratha *et al.*, 2019).

1.1 Objectives

The objective of the study was to estimate the Antioxidant potential of *Withania coagulans* Dunal plants found at various geographical locations in Pakistan (Table 2). Plants from different geographical locations including Sargodha, Kohat, and Mianwali, Punjab, Pakistan were collected. Present research work was performed at the Biochemistry Lab, University of Okara.

- Determination of DPPH free radical scavenging activity
- Determination of Total antioxidant activity

2. Material and Methods

2.1 Laboratory plastic ware, glassware

High Precision Balance, Test tubes, micro-titer plate, Volumetric flask, Vortex mixer, Aluminum foil, Pipettes, Cuvettes, micro-pipette, water bath, beakers, Eppendorf tubes, Uv-vis spectrophotometer were the glassware's used for testing.

2.2 Sampling and processing of Withania coagulans

W. coagulans plants were collected from the 3 Different geographical locations in Pakistan. Kohat City, University Rd. (Kc); Mianwali Musa khel (Mi); Sargodha city (Sc) were the cities where the process of plant collection was performed.

2.3 Plant processing

During the performance of our assays, sterile conditions were maintained to reduce the risk of contamination and to ensure personal safety. After collection plants were separated into different parts. Leaves were air-dried before further processing. The dried leaves were completely dried using vacuum drying. For milling, the leaves were grounded to a fine powder (100 meshes) in a lab-scale grinder at a controlled temperature. Air tight bags were then used to store the ground samples at 25°C for the later use. Isolation of extracts from *W. coagulans*.

Table 1: *W. coagulans* collection sites throughout Pakistan, their coordinates and elevations.

Location (abbreviation)	Coordinates	Elevation (m)
	71° 03′ 08.84″ E	
Kohat City, University Rd. (Kc)	33° 35′ 05.29″ N	1001
	71° 26′ 34.96″ E	
Mianwali Musa Khel (Mi)	32° 38′ 04.83″ N	271
	71° 45′ 02.98″ E	
Sargodha City (Sc)	32° 04′ 44.86″ N	189
	72° 40′ 18.31″ E	

2.4.1 Plant material

In the laboratory, leaves samples isolated from *W. coagulans* Dunal plants were air dried, before being powdered with a pestle and mortar for metabolites extraction using methanolic extraction.

Table 2: Chemical composition of reagents used in the antioxidant assays.

Reagents	Composition
DPPH	96 mg of DPPH/1000 ml of methanol.
20% Sodium Carbonate	200 g of Na_2CO_3/1000 ml of dH_2O.
Folin-Ciocalteau	Commercially Available.
1 M Potassium Acetate	98.14232 g of KCH3COO/1000 ml of dH_2O.
10% Aluminum Chloride	100 g of $AlCl_3$/1000 ml of dH_2O.
0.2 M Phosphate Buffer	1.42 g of disodium hydrogen phosphate (Na_2HPO_4) and 1gram of sodium dihydrogen phosphate (NaH_2PO_4) in 50 ml dH_2O.
1% Potassium ferricyanide	100 g of $K_3[Fe(CN)_6]$ in 1000 ml dH_2O.
10% Trichloracetic acid	100 g of $C_2HCl_3O_2$ in 1000 ml dH_2O.
0.1% Ferric chloride	10 g of $FeCl_3$ in 1000 ml of dH_2O.
Total antioxidant capacity reagent	1.63 ml of H_2SO_4, 1.679 g of NaH_2PO_4 and 0.247 g of $(NH_4)_2MoO_4$ in 50 ml of dH_2O.
200 mM Ascorbic acid	35.2 g of $C_6H_8O_6$ in 1000 ml of dH_2O.
0.25 N HCl	9.12 g of HCl in 1000 ml dH_2O.
1.5% Trichloroacetic acid	15 g of $C_2HCl_3O_2$ in 1000 ml dH_2O.
0.375% Thiobarbituric acid	3.75 g of $C_4H_4N_2O_2S$ in 1000 ml of dH_2O.
Stopping solution	0.25 N HCl, 1.5% Trichloroacetic acidand 0.375% Thiobarbituric acid were mixed to make stopping solution.

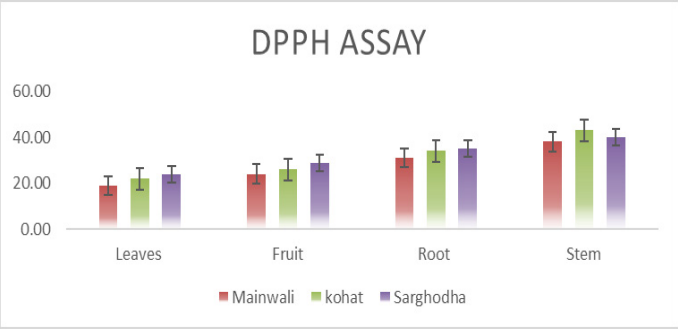

Figure 1: IC50 values for the free radical scavenging capacity of *W. coagulans*.

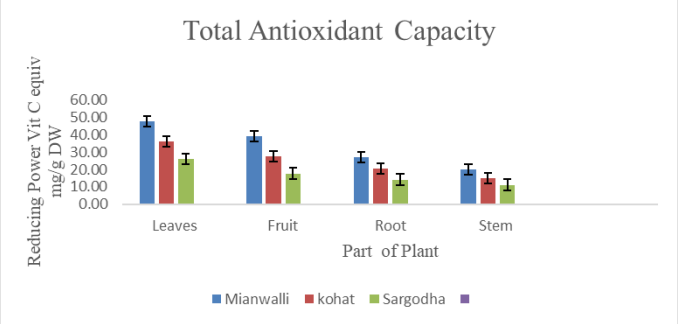

Figure 2: Comparison of the total antioxidant capacity of different parts of *W. coagulans*.

2.4.2 Methanolic extraction procedure

Each sample yielded 250 mg of powdered material, which was collected in pre-weighed Eppendorf tubes. Each sample received 500 ml of a 1:1 mixture of chloroform and methanol. After that, the samples were sonicated for 5 minutes before being vortexed for one minute. Three times the sonication vertexing cycle was performed. Then samples were centrifuged for 5 minutes at maximum speed. The green supernatant was separated into separate preweighed Eppendorf tubes (wi). Same procedure was repeated with the debris twice. At room temperature, Eppendorfs were allowed to dry. After that, the dried Eppendorfs were weighed once more (wf).

wf - wi = weight of the isolated extracts

Dried material was dissolved in 100mg/ml DMSO; to remove any lumps for 5 minutes all samples were sonicated.

Samples were then subjected to the following antioxidant assays with reagents given in the Table 2:

- DPPH free radical scavenging activity
- Total antioxidant capacity

2.4.3 DPPH free radical scavenging activity

Free radicals scavenging activity of samples was measured by the DPPH assay as reported by (Marinova et al., 2011).

2.5 Sample preparation

The 100ppm sample solution was prepared. For negative and positive control Ascorbic acid and DMSO were used, respectively. 200 µl was the total volume of the reaction mixture. Structure of Ascorbic acid is given in Figure 1.

2.6 Procedure

A reaction mixture of 200 µl was made by adding 20 µl of the sample. For a 30-minute reaction period, the plate was incubated at 37°C in incubator. A 96-well microplate reader was utilized for measuring absorbance at 517 nm. The change of color from deep violet to light yellow indicated antioxidant activity.

2.7 Total antioxidant capacity

TAC of *W. coagulans* was measured by Phosphomolybdenum method (Imran et al., 2011).

2.8 Sample preparation

The 100ppm sample solution was used as compared to 200 µl of total reaction mixture. The total antioxidant capacity reagent volume was kept constant at 198 µl.

2.9 Procedure

Initially, samples were placed in the wells in various concentrations, 198 mL of antioxidant capacity reagent was added and then the whole reaction mixture was placed in the water bath for 90 minutes for incubation. Chemical reactions inside the reaction mixture changed the color of the mixture to dark blue; then the reaction mixture was cooled down and using a microplate reader absorbance was measured at 630 nm.

3. Results and Discussion

3.1 DPPH free radical scavenging capacity

This analysis is utilized to determine the antioxidant capacity of extract toward free radicals. Samples were evaluated inside the test tube using DPPH and the absorbance of the sample was measured at 517 nm. Scavenging ability was found to be concentration dependent. Higher concentrations showed sudden and clear change while the lower concentration does not show a significant color change. A higher IC50 value lowers the free radicals scavenging capacity.

3.2 Total antioxidant capacity

Antioxidant capacity was determined using the Phosphomolybdenum method and expressed as an equivalence of ascorbic acid (mg/g of the extract. The absorbance of the sample was measured at 630 nm and every sample showed a minimum antioxidant capacity based on the presence of antioxidants.

Systematic research and analytical investigation of the contents and function of plant and vegetable remedies began to replace empiricism only in the last century. As a result, there is a growing interest in plants as a good source of therapeutic agents in pharmaceutical research that is returning to nature after a period of pure chemistry**.** Globally interest in the traditional medicine is increasing (Patwardhan et al., 2005).

3.3 DPPH free radical scavenging capacity assay

Results were found to be 38.00±3, 31.00±3, 24.00 ±3, and 19.00±3 in the leaves, fruits, roots, and stem respectively in Mianwali city. The extract obtained from Kohat city has the highest IC50 of 43.00±3 in Leaves while lowest in Stem with a value of 22.00±3. Leaves obtained from the Sargodha city have 40.00 ±3 while the stem has the lowest value of IC50 of 24.00 ± 3. Results confirm that leaves have the highest ability to scavenge the DPPH Free radical while stem has the least. Leaves obtained from the Mianwali 19.00 ± 3 have the highest value of DPPH free radical scavenging capacity while leaves of Sargodha have the lesser potential of scavenging the DPPH radical comparatively. Our finding supported by (Ilahi *et al.*, 2013; Deepika *et al.*, 2011) who evaluated the fruits of *Withania coagulans*.

3.4 Total antioxidant capacity assay

Results confirmed that leaves extract obtained from the Mianwali has the highest value of total antioxidant capacity at 47.60 ±3.00 and lowest in the stem extract obtained from the Sargodha 11.00 ± 3.00 Vit C equiv mg/g DW. Leaves of *W. coagulans* have the highest value of total antioxidant capacity 47.60 ± 3.00 while stem extract obtained from the Sargodha has the lowest antioxidant capacity 11.00 ± 3.00 Vit C equiv mg/g DW.

Our findings support the results of Akhter *et al* (2018) who studied the leaves and stem of *W. coagulans*. Raj et al. (2017) results also support our finding about the root's antioxidant ability and free radical scavenging capacity.

Conclusions and Recommendations

Anti-oxidant content in the *Withania coagulans* changes according to the location as the environmental conditions vary. With values of 47.60±3.00 Vit C equiv mg/g DW and 50.00±3.00, the Mianwali leaf extract has the highest total antioxidant capacity and free radical scavenging activity, demonstrated by results.

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Novelty Statement

We undertook this research to assess and compare the total antioxidant capacity and free radical scavenging ability of different sections of Withania coagulans Dunal, which had previously been lacking.

Author's Contribution

First author's conducted and documented the research while second and third author's edited and formatted the manuscript

Abbreviations

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate); DNA (Deoxyribonucleic acid); IC50 (The half-maximal inhibitory concentration); ROS (Reactive oxygen species); DMSO (Dimethyl sulfoxide); TAC (Total Anti-oxidant capacity); Vit C equiv mg/g DW (Vitamin C equivalent mg/g Dry weight).

Conflict of interest

The authors have declared no conflict of interest.

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