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Investigation of the Pharmacognostical, Phytochemical and Antioxidant Studies of Plant *Withania coagulans* Dunal

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The importance of traditional medicinal plants is increasing now a days because of various advantages over the synthetic drugs. *Withania coagulans* Dunal was studied for its antioxidant activity. Various physicochemical parameters were studied such as ash value, total ash, acid insoluble ash, water soluble ash, sulphated ash alcohol soluble extractive value etc. Various qualitative phytochemical tests were done for the presence of alkaloids, glycosides, carbohydrates, flavonoids etc. It was observed that 50% ethanol extract of *Withania coagulans* contains carbohydrates, proteins, glycosides, steroids and sterols, anthraquinones and triterpenoids. The antioxidant activity of *Withania coagulans* Dunal was studied by DPPH and Nitric oxide method and it was observed that it has antioxidant activity. It showed more activity in DPPH method than Nitric oxide method. Preliminary Phytochemical study of 50% ethanolic extract of the root parts is found to contain carbohydrates, protein, some steroids, anthraquinone, flavonoids, tannin, phenolic compounds and triterpenoids are present. The antioxidant activity was determined and the plant extract showed low activity nitric oxide free radical inhibition method and moderate activity by DPPH method. The activity was compared with rutin and ascorbic acid.

Keyword: *Withania coagulans* Dunal, Phytochemical and Antioxidant Studies

1. Introduction

Now a days there is a growing focus on the importance of medicinal plants in the traditional health care system *viz*, Ayurveda, Unani, Homeopathy in solving health care problems ^[1]. The active principles may be carbohydrates, glycosides, tannins, lipids, alkaloids etc. These active principles are manufactured chemically to produce the synthetic drugs ^[2]. The vast use of Traditional medicine may be due to the following reasons:

Problems with Modern drugs

- High cost and long time taken in development of new drug.
- Toxicity.
- Non-renewable source of basic raw materials
- Inadequate, specially in management of certain chronic diseases.
- Renewable source.
- Cultivation and Processing environment friendly.
- Plant constitute to be a major source of new lead generations.

Herbal preparations called “Phytopharmaceuticals” are preparations made from different parts of herbs or plants. They come in different formulations and dosage forms including tablets, capsules, elixir powder, extract, tincture, cream and parenteral preparations [3].

To ensure reproducible quality of a herbal remedy, the following aspects need to be considered.

- Authenticated and reproducibility of herbal ingredients
- Inter / intra species variation in plants environmental factors
- Plant parts used
- Time of harvesting
- Post harvesting factors
- Contaminants of herbal ingredients
- Pesticides, fumigants and other toxic metals[4].

Oxidation is the chemical process by which an atom, molecule or ion robs another of one or more of its electrons. Chemicals exhibiting this tendency for stealing electrons are referred to as oxidizing agents. The most familiar oxidizing agent is oxygen itself. Oxidation reactions may involve highly reactive molecules called free radicals.

Free radicals can be defined as chemical species possessing an unpaired electron, which is formed by homolytic cleavage of a covalent bond of a molecule, by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. In simple words, free radicals are molecules that have lost an electron and try to replace it by reacting with other molecules.

However, when the presence of free radicals increases to an abnormal level the danger begins. The danger exists in the potential for genetic material in the form of code structure to be altered in a manner that is destructive to the related cell. They bounce around and attack healthy cells, tearing the cell membranes and

spilling cytoplasm, and subjecting the cells to infection, genetic damage and mutation. Some disorders in which free radicals are implicated are Alzheimer's disease, arthritis, hemorrhoids, Parkinsonism, rheumatism, heart attack, AIDS, cataract, stroke, cancer and a long list of degenerative diseases including aging.

The exogenous sources of ROS include electromagnetic radiation, cosmic radiation, cigarette smoke, car exhaust, UV light, ozone and low wave length. Electromagnetic radiation. Similarly, the endogenous sources of ROS are mitochondria electron transport chain, respiratory burst by phagocytes, beta-oxidation of fat in peroxisome, auto-oxidation of amino acids, catecholamines, haemoglobin and ischaemia injury.

Phytoconstituents with antioxidant activity

In the present era natural products have a crucial role in the therapeutics and human clinical trials. A number of plants constituents possessing antioxidant potential have been reported. Here we will be dealing with phenols, flavonoids, tannins and vitamins[5].

2. Materials and Processing

2.1 Treatment:-

The leaves and stem of the plant were separated. The foreign earthy matter and residual materials were removed carefully and then subjected for washing and stored. The fresh leaves and roots were used for pharmacognostical studies. For phyto chemical studies the leaves were dried under shade, while the root portion was dried in oven at temperature between 40 to 60 °C. After drying, the material was grinded to coarse powder by grinder and used for study.

2.2 Chemical Used:

50% ethanol, DPPH, DMS, nitric oxide solution, toluene, ethyl acetate, formic acid and various reagents were used in preliminary phytochemical test.

***Withania coagulans* DUNAL**

Family : Solanaceae.
Syn : Hindi - Akri, Punir.
Bengali - Ashwagandha
English - Indian cheese maker
Punjabi - Spin bajja, panir.

Botanical description: (Macroscopical) A rigid, grey under shrub, 60-120 cm high.

Leaves: Lanceolate - oblong, clothed with a persistent, greyish Mentumon on both sides, base narrowed into a stout.

Flowers: Yellow in axillary cymose clusters, berries globose, red or Brownish, smooth, enclosed in leathery calyx.

Seeds: Dark brown, ear shaped, glabrous, pulp brown.

2.3 Habitat:

It grows as short shrub (35-75 cm) with central stem. This shrub is common Afghanistan and East Indian. It has milk coagulating property. It is also found in North West India, in Punjab and in Pakistan. It is also known as Pakistani herb.

2.4 Chemical Constituents:

Berries contain milk coagulating enzyme esterase, free amino acids, fatty oil, essential oil and alkaloids. The essential oil was active against *Micrococcus pyogenes* var. aureus and also shows anthelmintic activity.

The withanolides, withacoagin, coagulan and withasomidienone have been isolated from plant along with other withanolides and withaferin. 3- β -hydroxy-2,3-dihydrwithanolide E isolated from plant showed significant hepatoprotective activity and anti-inflammatory activity equal to hydrocortisone. The ethanolic extract shows anti-fungal activity.

2.5 Uses:

It is used as emetic, diuretic. Ripe fruits used as sedative, CNS depressant, anti-inflammatory. Also used in chronic liver trouble. Dried fruits used as carminative, dyspepsia, flatulence. Leaves used as alterative, febrifuge.

2.6 Determination of Physiochemical Parameters

2.6.1 Ash Values

Ash values are helpful in determining the quality and purity of crude drugs in powder form. Ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash values were determined. For determination of different ash values the shade dried leafs and stem of *Withania coagulans* Dunal were powdered and the powder was passed through sieve No. 120. Only fine powder was used for the determination of ash values^[6].

2.7 Determination of Total Ash

About 3 gm of the powdered drug was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing temperature to make it dull red until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get a constant weight. The percentage of the total ash was calculated with reference to the air-dried drug. The total ash values are recorded in Table No.1 and 2.

2.8 Determination of Acid Insoluble Ash

The resultant ash obtained in the determination of total ash, was boiled with 25 ml of 2N hydrochloric acid for five minutes, and filtered. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred to pre-weighed silica crucible, which was previously ignited, incinerated, cooled and weighed. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug. The acid insoluble ash values are recorded in Table No. 1 and 2.

2.9 Determination of Water Soluble Ash

The resultant ash obtained in the determination of the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble ash was collected on an ashless filter paper and washed with hot water.

The insoluble ash was transferred into a silica crucible and ignited for 15 minutes and weighed. The procedure was repeated to get the constant weight. The percentage of water-soluble ash are calculated with reference to the air-dried drug and recorded in Table No.1 and 2.

2.10 Determination of Sulphated Ash

A silica crucible was heated to redness for 20 minutes allowed to cool in a desiccators and weighed. 1 gm of powdered drug materials were being examined transferred to the crucible and weighed the crucible and its contents accurately. Ignited gently at first until the substance is thoroughly charred, cooled and moistened with 1ml of concentrated sulphuric acid heated gently until white fumes are no longer evolved and ignited at 800 °C until all black particles have disappeared. Allowed the crucible to cool, added few drops of sulphuric acid and ignited as before. Allowed to cool and weighed. Repeated the operation until two successive weighing did not differ by more than 0.5 mg. The sulphated ash value are recorded in Table No 1 and 2.

Table 1: Data showing the different ash values of *Withania coagulans* Dunal (Aerial Parts).

S. No.	Types of Ash	Ash Value in %w/w
1.	Total ash	11.376
2.	Acid insoluble ash	3.21
3.	Water soluble ash	4.37
4.	Sulphated ash	10.39

Table 2: Data showing the different ash values of *Withania coagulans* Dunal (root)

S. No.	Types of Ash	Ash Value in %w/w
1.	Total ash	12.961
2.	Acid insoluble ash	0.26
3.	Water soluble ash	4.637
4.	Sulphated ash	7.39

2.11 Extractive Values

These help in evaluating the constituents of crude drug, which cannot be determined by any other means. It also indicates the nature of the constituent present in the drug. There are different types of extractive values according to the Indian Pharmacopoeia, Indian herbal pharmacopoeia and British herbal pharmacopoeia. The selected plant was subjected for following extractive values^[6].

2.12 Alcohol Soluble Extractive Value

About 5 gm of powdered materials was macerated with 100 ml of 90% ethanol in a stoppered conical flask for 24 hours with occasional shaking during first 6 hours and the first 5 ml was discarded. Then 25 ml of the filtrate was evaporated on a tarred evaporating dish and the residue was dried at 105 °C, until a constant weight of residue was obtained. The percentage of alcohol soluble extractive was calculated with respect to the air-dried material. The alcohol soluble extractive values are recorded in Table No 3.

2.13 Water Soluble Extractive Value

About 5 gm of powdered material was macerated with 100 ml of chloroform water in a stoppered conical flask for 24 hours, with occasional shaking during the first 6 hours and the first 5ml was discarded. The 25 ml of the filtrate was evaporated on a tarred evaporating dish; the resultant residue was dried at 105 °C, until a constant weight of residue was obtained. The percentage of water-soluble extractive was calculated with respect to the air-dried material. The water-soluble extractive values are recorded in Table No 3.

Table 3: Data showing the different Extractive values for the aerial parts and root of *Withania coagulans* Dunal

S.No.	Plant Material	Extractive Values (% w/v)	
		Alcohol soluble	Water soluble
1	Aerial parts of <i>Withania coagulans</i>	11.81	21.35
2	Roots of <i>Withania coagulans</i>	4.946	7.95

2.14 Phytochemical Screening of *Withania coagulans* Dunal.

The plant extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and proving the identity of a substance. The active ingredients, after isolation, can be incorporated into the modern medicine system for the development of newer formulation for therapeutic ailments.

Systematic investigation of the plant material for its phytochemical behavior involves four different stages.

- Procurement of raw material and quality control.
- Extraction, isolation, purification and characterization of the constituents of interest.
- Investigation of biosynthetic pathways of the particular compound.
- Quantitative evaluation.

2.15 Qualitative Phytochemical Analysis

The 50% ethanol extracts of root of *Withania coagulans* were subjected to the following chemical tests separately for the identification of various active constituents^[7].

2.16 Tests for Alkaloids

- Dragendorff's Test:** To 1 ml of the extract, added 1 ml Dragendorff's reagent, and orange red precipitate indicated the presence 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.
- Mayer's Test:** To 1 ml of the extract, added 2 ml of Mayer's reagent, a dull white precipitate revealed the presence of alkaloids.
- Hager's Test:** To 1 ml of the extract, added 3 ml of Hager's reagent, the formation of yellow precipitate confirmed the presence of alkaloids.

2.17 Tests for 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.
- Fehling's Test:** To 1 ml of the extract, added equal quantities of Fehling's solution A and B, upon heating formation of a brick red precipitate indicated the presence of carbohydrates.
- Benedict's Test:** To 5 ml of Benedict's reagent, added 1 ml of extract solution and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

2.18 Tests for Proteins and Amino Acids

- Biuret Test:** To 1 ml of the extract added 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulfate solution. Formation of violet color indicated the presence of proteins.
- Xanthoprotein Test:** To 1 ml of the extract added 1 ml of concentrated nitric acid. A white precipitate was formed, it was boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange color indicated the presence of aromatic amino acids.
- Lead Acetate Test:** To the extract, 1 ml of lead acetate solution was added. Formation,

of a white precipitate indicated the presence of proteins.

2.19 Tests for Steroids

- Liebermann Burchard Test:** Dissolved the extract in 2 ml of chloroform in a dry test tube. Added 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. The solution became red, then blue and finally bluish green, indicated the presence 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 chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.
- Ninhydrin Test:** Added two drops of freshly prepared 0.2% ninhydrin reagent to the extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

2.20 Tests of Glycosides

- Legal's Test:** Dissolved the extract in pyridine and added sodium nitroprusside solution to make it alkaline. The formation of pink red to red color showed the presence of glycosides.
- Baljet Test:** To 1 ml of the test extract added 1 ml sodium picrate solution and the yellow to orange color revealed the presence of glycosides.
- Borntrager's Test:** Added a few ml of dilute sulfuric acid to 1 ml of the extract solution. Boiled, filtered and extracted the filtrate with chloroform. The chloroform layer was treated with 1 ml of ammonia. The formation of red color showed the presence of anthraquinone glycosides.
- Keller Kiliani Test:** Dissolved the extract in acetic acid containing traces of ferric chloride and transferred to a test tube containing sulfuric acid. At the junction, formation of a reddish brown color, which gradually became blue, confirmed the presence of glycosides.

2.21 Test for Flavonoids

Shinoda Test: To 1 ml of the extract, added magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of red color showed the presence of flavonoids.

Table 5: Data showing results of chemical test for 50% ethanol extract, root of *Withania coagulans Dunal*.

S.No.	Tests	50% Ethanol extract
1.	Alkaloids a) Dragondorff's Test b) Wagner's Test c) Mayer's Test d) Hager's Test	-ve -ve -ve +ve
2.	Carbohydrates a) Molisch Test b) Fehling's c) Benedict's Test	+ve +ve +ve
3.	Protein a) Biuret Test b) Xanthoprotein Test c) Lead Acetate Test	+ve +ve -ve
4.	Amino Acid a) Ninhydrin Test	-ve
5.	Glycoside a) Legal Test b) Baljet Test	-ve -ve
6.	Steroids and Sterols a) Liebermann Burchard Test b) Salkowsky Test	+ve -ve
7.	Anthraquinones a) Borntrager's Test	+ve
8.	Flavonoids a) Extract + Tin + HCl	+ve
9.	Tannins and Phenol compounds	+ve
10.	Terpenoids	+ve
11.	Saponin Test a) Foam Test	-ve
12.	Fixed oils a) Spot Test	-ve

2.22 Fluorescence Analysis of Root Powder Of *Withania coagulans DUNAL*

The fluorescence character of root powder of *Withania coagulans Dunal* was studied both in daylight and UV light. The fluorescence analysis was also studied by treating root powder with chemical reagents. The observations are recorded in Table 4.

Table 4: Data Showing Fluorescence Analysis Of root Powder of *Withania coagulans* Dunal

S.No	Drug	UV Light	Visible Light
1	In 1(N) NaOH	Green	Light Greenish brown
2	In C ₂ H ₅ OH in	Green	Dark Greenish brown
3	In 1(N) HCl	Green	Yellowish
4	In 50% HNO ₃	Green	Orange
5	Only powder	Green	Yellowish brown

Anti-Oxidant activity :**3. Materials and Methods**

Nitric.: oxide radical inhibition activity:

Chemicals and Reagents Used

- Sodium nitroprusside solution: Weighed accurately 0.2998 of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).
- Naphthyl ethylenediamine dihydrochloride (NEDD): (0.1%) Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 1:1 or 50% glacial acetic acid by heating and made up the volume to 100ml in a volumetric flask with distilled water.
- Sulphanilic acid (0.33%w/v) reagent: Weighed accurately 0.33 g of Sulphanilic acid and dissolved in 20% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask.
- Phosphate buffer saline (BS) (pH 7.4)
- Dimethyl Sulfoxide (DMSO)

3.1 Preparation of sample solution

21 mg of each of the 50 ethanolic extracts of root of *Withania coagulans* Dunal was dissolved in 1 ml of distilled Dimethyl sulfoxide (DMSO) to obtain a solution of 21 mg/ml. Each of these

solutions were serially diluted to obtain, lower concentrations.

3.2 Preparation of standard solution:

Weighed iazotizat 10 mg of Ascorbic acid and Rutin and dissolved in 1 ml of Dimethyl Sulfoxide separately. From this solution serial dilutions were made to obtain lower concentrations using DMSO.

4. Method:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions which were measured by using a modified Griess-Ilosvay method. ⁽⁸⁾

The reaction mixture (6 ml) containing sodium nitro prusside (10 mm, 4 ml), Phosphate buffer saline (PBS, 1 ml) and extract in DMSO was incubated at 25 °C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed, 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of Naphthyl ethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 minutes in diffused light. A pink colored chromophore was formed. The absorbance of these solutions, was measured at 540 nm against corresponding blank solution. IC₅₀ value is the-concentration of the sample required to inhibit 50% nitric oxide radical.

5. Result and Discussion

The use of the plants for healing, care and reinvigoration is as old as humanity. But only in last century did empiricism begin to be replaced by systematic research and analytical investigation of contents and function of the plant and vegetable remedies. Thus at present a received interest in plants as an excellent source of therapeutic agent pharmaceutical research looking back to nature after a phase of pure chemistry.

Our present study has been taken with a view to determine the pharmacognostical constants, phytochemical studies and Antioxidant evaluation of the 50% ethanol extract of *Withania coagulans* Dunal. From the literature review we found that *Withania coagulans* Dunal has been used traditionally for anthelmintic activity, in chronic complaints of liver and as blood purifier. So, *Withania coagulans* was selected for pharmacognostical and phytochemical and antioxidant studies.

5. 1 Phytochemical Evaluation

The 50% ethanol extract root of *Withania coagulans* was subjected to preliminary phytochemical studies. It has been observed that 50% ethanol extract of *Withania coagulans* contains carbohydrates, proteins, glycosides, steroids and sterols, anthraquinones and triterpenoids.

5.2 Antioxidant Activity

The anti-oxidant activity of *Withania coagulans* Dunal was studied by DPPH and Nitric oxide method and it was observed that it has anti-oxidant activity. IC₅₀ values are shown in table 7 and 8. It showed more activity in DPPH method than Nitric oxide method.

6. Conclusion

From over present study entitled "Pharmacognostical, Preliminary Phytochemical and Anti-Oxidant Study of *Withania coagulans* (Dunal)" aerial parts and root parts of *Withania coagulans* Dunal, the following conclusion could be drawn.

Pharmacognostical studies of the finding therein will enable the identification of the plant to the future investigation. This will provide a basis for the pharmacognostical standardization of the plant drug.

Preliminary Phytochemical study of 50% ethanolic extract of the root parts is found to contain carbohydrates, protein, some steroids, anthraquinone, flavonoids, tannin, phenolic compounds and triterpenoids are present.

The antioxidant activity was determined and the plant extract showed low activity nitric oxide free

radical inhibition method and moderate activity by DPPH method. The activity was compared with rutin and ascorbic acid.

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