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Physicochemical and phytochemical evaluation of different extracts of *withania somnifera*

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Abstract
Withania somnifera is the best rejuvenating agent. *Withania somnifera* commonly known as "Ashwagandha", and is belongs to family Solanaceae. The present study comprises physico-chemical and phytochemical evaluation of different extracts of *Withania somnifera* by using different standard methods. The evaluation of physico chemical parameters was carried out by the determination of ash values, extractive values and moisture content. Whereas phytochemical investigation was carried out to estimate the presence of carbohydrates, glycosides, flavonoids, tannins, phytosterols and phenolic compounds in different extracts of *Withania somnifera*. Results revealed the presence of carbohydrates, alkaloids, saponons, phytosterols, flavonoids, fats and oils. The present investigation will helpful in Assessing the quality and purity of a crude drug and laying down pharmacopoieal standards for *Withania somnifera*.

Keywords: *withania somnifera*, ashwagandha, phytochemical evaluation, winter cherry

1. Introduction
Withania somnifera belonging to family Solanaceae is commonly known as Winter cherry [1]. In Sanskrit, it is well known as Ashwagandha. It is also known as Indian ginseng and Poison gooseberry [2]. It is mostly cultivated in many regions of India like Madhya pradesh, Punjab, Gujarat and Rajasthan [3]. It is also cultivated in Nepal. The plant is errect grayish herb with stout roots. The roots are tuberous, fleshy with good aroma. The colour of the root is brownish white. Flowers are greenish yellow and the fruit has orange red berry in nature.
The *Withania somnifera* is act as a good rejuvenating agent as it is used to increase energy, strength, health, muscle fat, blood, semen and cell production [4, 5, 6, 7]. The plant is also has immunomodulatory activity as this is involved in enhancing the WBC count [8]. It is also used as antiaging drug, as it improves the RBC count, hemoglobin count and hair melanin [9]. It is also has capability to change plasma corticosterone levels [10]. The plant also decreases the triglycerides and low density lipoproteins and also having hypoglycemic effect and is used as antidiabetic agent [11]. The plant also having thyrotropic activity, which may increases serum T4 levels [12, 13]. It is also has anxiolytac and antidepressive activities [14]. Previously reported chemical constituents of *Withania somnifera* are withanolides, withaferin A and withanolide D.

2. Materials and Methods
2.1 Collection of plant material
The crude drug of *Withania somnifera* was collected from local market of Tirupati. They were identified, verified taxonomically and authenticated in the Department of Botany, S.V.University, Tirupati. The plant material was coarsely powdered with the help of rotary grinder and the powder is stored in airtight plastic containers. The prepared powder was used for all phytochemical analysis.

2.2 Preparation of extracts
The collected plant material was washed in water and dried at room temperature for 15-20 days and it was subjected for size reduction. The prepared powder was used for extract preparation. The 100 g of plant material was extracted with the help of Soxhlet apparatus by using 400 ml petroleum ether for about 48 h. After defatting, the marc was dried in hot air oven at 50°C and it is packed in Soxhlet apparatus for further extraction with 400 ml of 95% ethanol until the absence of residue on evaporation. The aqueous extract was prepared by cold maceration with the help of 3% methanol-water for seven days with frequent shaking. Solvents were removed from the extracts by using the rotary vacuum evaporator.

2.3 Physicochemical evaluations
i) Moisture content
Weighed quantity (3 g) of the shade dried powder of *Withania somnifera* was taken in a tared glass bottle and initial weight was taken. The powder was heated in an oven at a temperature of 105°C and is weighed. This procedure was repeated till the constant weight was obtained. The moisture content of the sample was calculated in the percentage with reference to shade dried plant powder by using formula [15].

$$\% \text{ Moisture content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

ii) Ash values [16]
a) Determination of total ash
An accurately weighed quantity (2 g) of the shade dried powder of *Withania somnifera* was incinerated in a crucible at a temperature of 450°C in a muffle furnace untill carbon free ash was obtained and then cooled, weighed. The percentage of total ash was calculated with reference to the

shade dried powder by using the following formula.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of the crude drug taken}} \times 100$$

b) Determination of acid insoluble ash

The ash obtained was boiled with 2 M HCl (25 ml) for five minutes and it was filtered using an ash less filter paper. Insoluble matter retains on the filter paper and it was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

c) Determination of water soluble ash

The ash above obtained, was boiled with 25 ml of water for 5min, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited at a temperature not exceeding 450°C, for 15min in a muffle furnace. The difference in the weight of ash and the weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash-Weight of water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

iii) Extractive values [17]

Extractive values of shade-dried powder of *Withania somnifera* were determined using following methods.

a) Determination of alcohol soluble extractive

An accurately weighed quantity of the shade dried powder of *Withania somnifera* (5 g) was macerated with ethanol (100 ml) in a closed flask for 24 h, with occasional shaking during the first 6 h. Then it was allowed to stand for 18 h and then filtered rapidly to prevent any loss during evaporation. Evaporate approximately 25 ml of the filtrate in a porcelain dish and dried at 105°C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the shade dried plant powder.

b) Determination of water soluble extractive

Weighed quantity of the shade dried powder of *Withania somnifera* (5 g) was macerated with water (100 ml) in a closed flask with frequent shaking for the first 6 hrs and allowed to stand for 18 hrs. After that, it was filtered taking precaution against loss of water. Evaporate 25 ml of filtrate in a tared flat bottom shallow dish and it was dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the shade dried plant powder.

c) Determination of petroleum ether soluble extractive

Weighed quantity of the shade dried powder of *Withania somnifera* (5 g) was macerated with 100 ml petroleum ether in a closed flask for about 24 h, with frequent shaking for the first 6 hrs and allowed to stand for 18 hrs. After that, it was filtered rapidly taking precaution against loss of petroleum ether due to its volatility. Evaporate 25 ml of filtrate in a porcelain dish and dried at a temperature of 105°C and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to the shade dried plant powder.

2.4 Phytochemical Evaluation

The freshly prepared petroleum ether, methanolic and aqueous extracts of *Withania somnifera* were qualitatively analyzed for the presence of major phytochemical constituents using the following standard procedures.

i) Detection of Carbohydrates [18]

100 mg of extract was dissolved in 10 ml of water and filtered. The filtrate prepared was used to test the presence of proteins and amino acids.

a) Molisch's Test

To the 1 ml of filtrate add 2 drops of Molisch's reagent in a test tube and add 2 ml of concentrated sulphuric acid carefully along the sides of the test tube. Formation of violet color at the interface of two liquids indicates the presence of carbohydrates.

b) Fehling's Test

To the 1 ml of filtrate add 4 ml of Fehling's reagent (2 ml Fehling A and 2 ml Fehling B solutions) in a test tube and heated for about 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

c) Barfoed's Test

1 ml of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on water bath. Formation of a brick-red precipitate within 5 minutes indicates the presence of monosaccharides. Disaccharides generally don't give any reaction even for ten minutes.

ii) Detection of Proteins and Amino acid [19]

100 mg of extracts were dissolved in water (10 ml) and then it was filtered. The filtrate was used to test the presence of proteins and amino acids.

a) Millon's Test

2 ml of filtrate was treated with 2 ml of Millon's reagent in a test tube and it was heated in a water bath for about 5 min, cooled and few drops of NaNO2 solution were added to the test tube. Formation of white precipitate and it turns to red upon heating indicates the presence of proteins and amino acids.

b) Ninhydrin Test

To the 2 ml of filtrate add 2-3 drops of Ninhydrin reagent in a test tube and boiled for about 2 min. Formation of deep blue colour indicates the presence of amino acids.

c) Biuret Test

To the 2 ml of filtrate add 2 ml of 10% sodium hydroxide solution in a test tube and heated for about 10 min, to the above solution, add a drop of 7% of copper sulphate. Formation of violet colour confirms the presence of proteins.

iii) Detection of Glycosides [20]
0.5 g of extract was hydrolyzed with 20 ml of dilute 0.1 N HCL and then filtered. The filtrate obtained was used to test the presence of glycosides.

a) Legal Test
To 1 ml of filtrate add three ml of sodium nitropruside in pyridine and methanolic alkali (KOH) in a test tube. Appearance of blue colour in alkaline layer indicates the presence of glycosides.

b) Keller-killiani Test
1 ml of filtrate was shaken with 1 ml of glacial acetic acid which contains traces of ferric chloride. Add 1 ml of concentrated H_2SO_4 slowly along the sides of the test tubes. Appearance of blue colour in acetic acid layer and red colour at the junction of the two liquids indicates the presence of glycosides

c) Modified Borntrager Test
To the 1ml of filtrate add 2 ml of 1% ferric chloride solution in a test tube and heated for about 10 min in boiling water bath. The mixture was cooled and was shaken with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Appearance of pink colour in the ammonical layer indicates the presence of glycosides.

iv) Detection of Alkaloids [21]
0.5 g. of extract was taken and it was dissolved in 10 ml of dilute 0.1 N HCL and then filtered. The filtrate was used to test the presence of alkaloids.

a) Dragendorff's Test
To the 2 ml of filtrate, Dragendorff's reagent (2-3 drops) was added. Appearance of reddish brown colored precipitate indicates the presence of alkaloids.

b) Hager's Test
To the 2 ml of filtrate add Hager's reagent which gives yellow colored precipitate indicates the presence of alkaloids.

c) Mayer's Test
To the 2 ml of filtrate, 2-3 drops of Mayer's reagent were added, this leads to formation of cream colored precipitate indicates the presence of alkaloids.

d) Wagner's Test
To the 1 ml of the extract, add 2 ml of Wagner's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

v) Detection of Flavonoids [22]
a) Shinoda Test
To the extract (100 mg) in a test tube add few fragments of magnesium metal. To the test tube add 3 to 4 drops of conc HCL. Formation of magenta colour or light pink colour indicates the presence of flavonoids.

b) Alkaline Reagent Test
To the 100 mg of extract in a test tube add few drops of NaOH solution. Intense yellow colour is formed to which add few drops of dilute hydrochloric acid, and then the yellow colour becomes colourless which indicates the presence of flavonoids.

c) Fluroscence test
To the 100 mg of extract add 0.3 ml boric acid solution (3 %w/v) and to that add 1 ml oxalic acid solution (10 %w/ v) and evaporated to dryness. The residue obtained was dissolved in 10 ml of ether. Under UV light the ethereal layer shows greenish fluorescence which indicates presence of flavanoids.

vi) Detection of Phenolic Compounds and Tannins [23]
100 mg of extract mixed with 1 ml of water and then it was boiled and filtered. The filtrate was used for the following test.

a) Ferric Chloride Test
Take 2 ml of filtrate in a test tube to that add 2 ml of ferric chloride solution (1%). Formation of bluish to black colour indicates the presence of phenolic nucleus.

b) Lead Acetate Test
To the 2 ml of filtrate in a test tube add 2 to 3 drops of lead acetate solution. Appearance of yellowish precipitate indicates the presence of tannins.

vii) Detection of Fats and Oils [24]
Oily Spot Test
One drop of the extract was placed on the filter paper and then the solvent was allowed to evaporate. Appearance of oily stain on the filter paper indicates the presence of fixed oil.

viii) Detection of Saponins [25]
Foam Test
To 1 ml of extract add 20 ml of distilled water and it was shaken in a graduated cylinder for about 15 min. Formation of 1 cm layer of the foam in test tube indicates the presence of saponins.

ix) Detection of Phytosterols [26]
To 0.5 g of extract add 10 ml of chloroform and it was filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

a) Libermann's Test
To the 2 ml of filtrate in hot alcohol in a test tube to that add few drops of acetic anhydride. Formation of brown precipitate indicates the presence of sterols.

b) Salkowski Test
To the 2 ml of extract add few drops of concentrate sulfuric acid and then it was shaken and then allowed to stand. Appearance of red colour in lower layer indicates the presence of sterols.

3. Results and Discussion
Withania somnifera was subjected to systematic physicochemical and phytochemical screening by extracting with various organic solvents in the order of increasing polarity to determine the soluble constituents in a given amount of plant material. The present work is helpful in

analysing the quality and purity of the crude drug. In this study the parameters used for the evaluation of *Withania somnifera* were moisture content, extractive values by different solvents (includes petroleum ether, methanol and water), ash values (total ash, water soluble and acid insoluble ash) (Table 1). On incineration, drugs leave an ash and it consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful to detect the exhausted drugs, low-grade products and excess of sandy matter which is applicable to powdered drugs.

Phytochemical analysis was performed on the petroleum ether, methanol and aqueous extracts of *Withania somnifera*. Petroleum ether extract was found to contain proteins, aminoacids, alkaloids, phenolic compounds, fats and oils. Methanolic extract contains carbohydrates, glycosides, alkaloids, flavonoids, saponins and phytosterols. Aqueous extract contains proteins, aminoacids, flavonoids and saponons. (Table 2).

Table 1: Physico-chemical investigation of *Withania somnifera*

S. No.	Quality parameters		Results
1	Moisture content		5.2
2	Ash value		
	A	Total ash value	6.2
	B	Acid insoluble ash value	1.4
	C	Water soluble ash value	2.9
3	Extractive values		
	A	Petroleum ether soluble extract	5.4
	B	Methanol soluble extract	5.7
	C	Aqueous soluble extract	7.8

Table 2: Phyto-chemical investigation of *Withania somnifera*

S. No.	Tests	Petroleum ether extract	Methanolic extract	Aqueous extract
1	Carbohydrates	-	+	-
2	Proteins and aminoacids	+	-	+
3	Glycosides	-	+	-
4	Alkaloids	+	+	-
5	Flavonoids	-	+	+
6	Phenolic compounds	+	-	-
7	Tannins	-	-	-
8	Saponins	-	+	+
9	Phytosterols	-	+	-
10	Fats and oils	+	-	-

4. Conclusion

Herbal based remedies serve as the important means of therapeutic medical treatment. The people are turning to usage of medicinal plants and phyto-chemicals in health care. India has one of the oldest cultural traditional uses of its herbal plants since from vedic period. Ayurveda, Unani, Siddha and other traditional systems of medicine are the ancient systems of medicine and utilize numerous numbers of medicinal plants. Phytochemical screening, biological screening of randomly collected plants and their phytochemical examination have proved to be helpful in discovering the new drugs.

Withania somnifera, generally known as Winter cherry, is very important medicinal plant belonging to family solanaceae. The present study concluded that the plant *Withania somnifera* contains variety of phytoconstituents. The physicochemical evaluation of *Withania somnifera* revealed that the standard quality and purity of drug. Phytochemical studies on the extracts of *Withania somnifera* showed presence of proteins, aminoacids, phytosterols, carbohydrates, flavonoids, saponons, fats and oils. This information may be further useful for isolation of various compounds from *Withania somnifera* for treatment of diseases in human beings.

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