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Phyto Physicochemical Profile of *Withania somnifera* Dunal (Solanaceae)

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ABSTRACT

Introduction- The present article deals with study of phytochemical analysis of Withania somnifera Dunal roots. Withania somnifera also known as Ashwagandha or winter cherry. Various preparations of Ashwagandha (WS) are available in the market used in the treatment of many clinical conditions in India. **Objective-** Evolution of Physico-chemical values and phytochemical analysis of Ashwagandha Churna. **Materials and Methods-** The current investigation deals with extraction and detection or screening of active phytochemical compounds from different extracts of Withania somnifera root. Pharmacognostic studies, Physicochemical studies, Preliminary phytochemical studies was carried out. **Result and conclusion -** The result drown were 2% foreign mater was determined. Loss on drying 1.6%, total ash obtained was 9 %, acid insoluble ash was 1% and water soluble extractive was 12 % and Alcohol soluble extractive was 13 %. The phytochemical investigation revealed the presence of various phytochemical constituents such as alkaloids, flavonoids, carbohydrate, Steroids and Saponin Glycoside.

Keywords: Ashwagandha, Withania Somnifera, Phytochemical.

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INTRODUCTION

Withania somnifera Dunal. (Solanaceae) commonly known as Ashwagandha or winter cherry, is an important medicinal plant in Indian system of medicine. Various preparations of Ashwagandha (WS) are available in the market used in the treatment of many clinical conditions in India like, arresting the ageing process, procure the body in debilitated conditions, by creating a sense of mental wellbeing and increase physical fitness.[1]

The use of WS is increasing due to a number of chemical constituents present in it are found useful for health.[2]

Some important bioactive constituents of Ashwagandha roots are alkaloids, tannins and flavonoid and phenolic compounds.[3]

Objective-

Evolution of Physico-chemical values and phytochemical analysis of Ashwagandha Churna.

MATERIALS AND METHODS

Plant material authentication-

Plant materials of Withania Somnifera were collected from Teaching Pharmacy Sri Dharmasthala Manjunatheshwara college of Ayurveda & Hospital, Hassan as per the standard method.[4]

The identity of the plant was confirmed by Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara college of Ayurveda & Hospital, Hassan. No- SDMCAH-DG/E/2018/02, Date: 14.12.2018 and Biocyte Institute of Research & Development, Sangli, Maharashtra (Reg. No. - 1831300312031566). The plant was compared with voucher specimen in the institute. For further confirmation, the microscopic characteristics of this plant were studied and compared with available literature.

Foreign matter

The term "Foreign Matter" is used to designate any matter, which does not form a part of the drug as defined in the monograph.100g of the powdered drug is taken and spread out in a thin layer. Plant material collected should be free

from foreign matters like soil, insect parts or animal excreta. They are separated and weighed and the percentage is calculated.

Pharmacognostic studies

Macroscopic study

Macroscopic observation of roots of Withania somnifera was done. It comprised of shape, size, surface characteristics, texture, color, consistency, odour, taste, etc.[5]

Microscopic study

Transverse sections of Withania somnifera roots were taken by using a microtome. Permanent mount of stem was prepared using saffranin fast green stain by double staining technique.[6]

Preliminary phytochemical studies-

The roots of Withania somnifera Dunal. were coarsely powdered and extracted with methanol and water (6:4) using cold maceration technique. The extract is filtered and concentrated and dried in a Rota evaporator initially and then in vacuum desiccator. Preliminary phytochemical screening of extract was done for the presence of various phyto constituents by using standard procedure.[7]

Preparation of the extract-

The Ashwagandha powder was successively extracted in soxhlet apparatus with methanol, Acetone, chloroform, Ethyl acetate and hot water. The liquid extracts were collected in tarred conical flask. The solvent was removed by distillation. These extracts were used to study to various qualitative chemical tests and determine the presence of different phyto-chemicals.

Preliminary Phytochemical Screening

Phytochemical screening of the Withania Somnifera was done by the standard procedures prescribed by Kokate and Harborne.[8,9]

Estimation of physicochemical parameters

Various physicochemical parameters were studied in all the root samples using standard procedures.

Physicochemical studies

Physicochemical parameters were determined as per guidelines of WHO. Total ash value, loss on drying, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value were determined. [10, 11, 12.]

Determination of Total Ash Value: About 2 grams of air dried, powdered roots were accurately weighed and taken in a tarred silica crucible and incinerated in a muffle furnace by gradually increasing the temperature to 450^0 C to make it dull red hot and free from carbon. Cooled in a desiccator, weighed and the percentage of total ash was calculated.[13]

Loss on drying at 105 ^{0} C- 10 g of sample was placed in tarred evaporating dish. It was dried at 105 ^{0}C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.[14]

Acid insoluble Ash- To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ash less filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.[15]

Alcohol soluble extractive- Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.[16]

Water soluble extractive: Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105 ^{0}C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.[17]

Preliminary Phytochemical evaluation was done as per the standard methods

Test for Carbohydrates- Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used for the detection of carbohydrates.

1. **Fehling's test-** In 1 ml of the extract, one ml of Fehling's A and one ml of Fehling's B solutions were added in a test tube and heated in a water bath for 10 minutes. Formation of red precipitate indicates the presence of a reducing sugar. The filtrate was treated with one ml of Fehling's A and B, and heated in a boiling water bath for 5-10 min. Appearance of reddish orange precipitate shows the presence of carbohydrates.
2. **Benedict's test-** Few drops of Benedict's reagent was added to the test solution and boiled on water bath. Formation of reddish brown precipitate indicates the presence of sugars. Depending on the concentration of the reducing sugar, the amount and colour of the precipitate produced varied. A positive Benedict's test appears green, yellow, orange, or red.
3. **Molisch Test-** To 2.0 ml of the extract, 2 drops of Molisch reagent was added and mixed. 2.0 ml of concentrated sulphuric acid was added to this solution. Formation of the red violet ring at the junction of the solution and its disappearance on addition of excess alkali solution indicates the presence of carbohydrates.

Test for Protein-

1. Xanthoprotein test: Mix 3 ml test solution with 1 ml conc. Sulphuric acid. White Precipitate. is formed. Boil it. Precipitate turns yellow. Add Ammonium hydroxide Precipitate. turns orange. Protein is present.
2. Precipitation test: The test solution gives white colloidal Precipitate mixed with 5% lead acetate. White colloidal Precipitate.

Test for Tannins-

1. **Ferric chloride test:** To 2 ml of the test solution, add few drops of 5% Ferric chloride solution, deep blue-black colour is formed.

2. **Lead acetate test:** To 2 ml of the test solution, add few drops Lead acetate solution, white ppt. is formed.

Test for Alkaloids- Extracts were dissolved individually in dilute HCl and filtered.

1. **Mayer's test-** To 1.0 ml of the filtrate, 2 ml of the reagent was added. Formation of white or pale precipitate shows the presence of alkaloids.
2. **Wagner's test-** To about 1-2 ml of the filtrate, 2 ml of Wagner's reagent was added. Reddish brown colored precipitate indicates the presence of alkaloids.
3. **Hager's reagent:** The acetic test solution treated with Hager's reagent (Saturated picric acid solution) gives yellow precipitate.
4. **Ehrlich's test:** The acidic test solution treated with Ehrlich's Reagent shows two separate yellow and brown colored layers.
5. **Dragendroff's test-** The filtrate was treated with Dragendorff's reagent and the formation of orange precipitate indicates the presence of alkaloids.

Test for Steroids

1. **Acetic anhydride test-** 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H2S04. The change in colouration from violet to blue or green in samples indicates the presence of steroids.
2. **Liebermann-Burchard Test-** The extracts were dissolved in 2 ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red colour from blue to green indicates the presence of steroids.

Test for Saponin Glycoside-

1. Foam test: Shake the test solution vigorously with water. Persistent foam is observed.

Test for flavonoids

1. **Shinoda test (Magnesium hydrochloride reduction test)** - To the test solution (0.5 0- 1 ml), few reagent of magnesium ribbon were added and concentration hydrochloric acid was added drop-wise. Pink scarlet, crimson and red of occasionally green to blue colour appears after few minutes, if flavonoid is present in the sample.
2. **Alkaline reagent test-** To the test solution (0.5 – 1 ml), few drops of sodium hydroxide solution (10 percent) were added. Formation of an intense yellow colour, which turns colorless on addition of few drops of dilute acid, indicates presence of flavonoids.
3. **Zinc–hydrochloride test-** To the extract, a pinch of zinc dust was added followed by addition of concentrated hydrochloric acid along the sides of the test tubes. Appearance of magenta color indicates the presence of flavonoids.
4. **Lead acetate test-** The extract was treated with a few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids. Orange to crimson colour shows the presence of flavonones.
5. **Ammonia test-** 5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H2SO4. Formation of a yellow colouration in the extract indicates the presence of flavonoids. The yellow colouration disappears after some time.
6. **Ferric chloride test-** To the extract, a few drops of neutral ferric chloride solution was added, a blackish red colour forms, indicating the presence of flavonoids.

Test for Steroids-

1. **Acetic anhydride test-** 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H2S04. The change in colouration from violet to blue or green in samples indicates the presence of steroids.
2. **Liebermann-Burchard Test-** The extracts were dissolved in 2 ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red colour from blue to green indicates the presence of steroids.

RESULT AND DISCUSSION

Physicochemical studies

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.

The results of physicochemical parameter analysis of crude powder of *Withania somnifera* root are shown in Table 1. The average values are expressed as percentage of air-dried material.

Table 1: Physicochemical studies result

Sl. No	Test Done	Obtained Value	Standard value % w/w
1.	Foreign Matter	2%	Not more than 2 %
2.	Loss on Drying	1.6%	NMT 8%
3.	Total Ash	9 %	NMT 7 %
4.	Acid Insoluble Ash	1%	NMT 1%
5.	Alcohol Soluble Extractive	13%	NLT 15 %
6.	Water Soluble Extractive	12%	NLT 15%

Pharmacognostic studies

The pharmacognostic study is the major and reliable criteria for identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The detailed and systematic pharmacognostic evaluation would give valuable information for future studies.

Macroscopic studies

Roots straight, unbranched, thickness varying with age. roots bear fibre like secondary roots, outer surface buff to grey-yellow with longitudinal wrinkles, crown consists of 2-6 remains of stem base, stem bases variously thickened, nodes prominent only on the side from where petiole arises, cylindrical, green with longitudinal wrinkles, fracture, short and uneven, odor, characteristic, taste, bitter and acrid.

Figure 1: Root of Ashwagandha (*Withania somnifera*)

Microscopic studies

Cork exfoliated or crushed, when present isodiamatric and nonlignified, Cork cambium of 2-4 diffused rows of cells, Secondary cortex about twenty layers of compact parenchymatous cells, Phloem consists of sieve tubes, companion cells, phloem parenchyma, Cambium 4-5 rows of tangentially elongated cells, Secondary xylem hard forming a closed vascular ring separated by multiseriate medullary rays, a few xylem parenchyma, Vessels with bordered pits and horizontal perforations. Fibres aseptate with pointed ends. Starch grains abundant, simple, mostly spherical, reniform – oval with central hilum. Microcrystals in parenchyma cells.

Preliminary phytochemical studies

Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, carbohydrate, Steroids and Saponin Glycoside.

Table 2: Test for Carbohydrates

Test for Carbohydrates	Test result	Present or Absent
Fehling's Test	First yellow, then brink red ppt. is observed.	Present
Benedict's Test	Solution appears green, yellow or red depending on the amount of reducing sugar present in test solution.	Present
Molisch Test	Formation of the red violet ring at the junction of the solution and its disappearance on addition of excess alkali solution indicates the presence of carbohydrates.	Present

The dry weight of plant, typically composed of 50- 80% of the polymeric Carbohydrate Cellulose along with related structural material. Carbohydrates are the backbones of Nucleic Acids. These are synthesized in plants by photosynthesis from CO_2 and H_2O. The fundamental principles of life are catalyzed by the green plant pigment, Chlorophyll and enzyme system and liberate oxygen.

Table 3: Test for Proteins

Test for protein	Test result	Present or absent
Xanthoprotein Test	Precipitate turns yellow. Add Ammonium hydroxide Precipitate. turns orange.	Absent
Precipitation Test	White colloidal Precipitate	Absent

Proteins are highly complex molecules, which contain the elements of Carbon, Hydrogen, Nitrogen and occasionally Sulphur. They are synthesized by living cells and are essential part of the structure of the cell and its nucleus. The plant proteins are more easily isolated in crystalline form. Proteins are stored in plants in the form of aleuronic grains and are required for animals as the source of nitrogenous food. Proteins are hydrolysed to form simpler substance i.e. Amino acid.

Table 4: Test for Tannins

Test for Tannis	Test Result	Present or absent
Ferric chloride test	Deep blue- black colour is formed	Absent
Lead acetate test	white ppt.	Absent

Tannins are derivative of Benzoic acid, which are widely distributed in the Vegetable Kingdom. Tannin precipitates and combines with Proteins and the Protein-Tannin complex is resistant to proteolytic enzymes.

Table 5: Test for Alkaloids-

Test for Alkaloids	Test Result	Present or absent
Mayer's test	Cream colored precipitate	Present
Wagner's test	Reddish brown precipitate	Present
Hager's reagent	Yellow precipitate	Present
Ehrlich's test	Two separate yellow and brown colored layers	Present

Alkaloid may be defined as Organic Nitrogenous substances of plant origin exhibiting well defined physiological action. Alkaloids exhibit a variety of physical and chemical properties. The free alkaloids are insoluble in water but their salts are freely soluble. These are mainly three types, True alkaloids- Those having a Heterocyclic ring with Nitrogen atom. Proto alkaloids- They don't have Heterocyclic ring with Nitrogen atom. Pseudo alkaloids- They have Heterocyclic ring with Nitrogen atom but not derived from Amino acid. Both True and Proto alkaloids are derived from Amino acids.

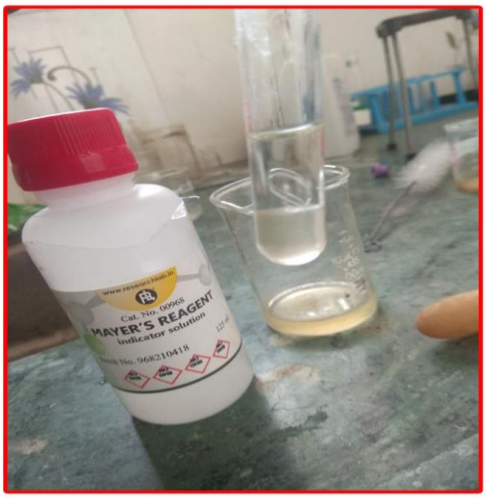

Fig. no-02-mayer's reagent

Fig. no-03- Wagner's Reagent

Figure 4: Hager's Reagent

Figure 5: Ehrlich's Reagent

Table 6: Test for Saponin Glycoside-

Test for Saponin glycoside	Test Result	Present or Absent
Foam Test	Persistent foam	Present

Glycosides are compounds, which upon hydrolysis give rise to one or more Sugars (Glycones) and a compound, which is not a Sugar (Aglycone or Genin). Glycosides are use was added as Cardiac stimulant; Laxative, Bitter Tonics, Hepatoprotective and some other have Expectorant properties.

Table 7: Test for Steroids

Test for Steroids	Test Result	Present or absent
Acetic anhydride test	The change Blue or green in samples indicates the presence of steroids	Present
Liebermann Burchard Test	The change of red colour from blue to green indicates the presence of steroids.	Present

Table 8: Test for flavonoids

Test for flavonoids	Test Result	Present or Absent
Shinoda test (Magnesium hydrochloride reduction test)	occasionally green to blue colour appears after few minutes, if flavonoid is present	Present
Alkaline reagent test	Formation of an intense yellow colour, which turns colorless on addition of few drops of dilute acid, indicates presence of flavonoids.	Present
Zinc–hydrochloride test-	Appearance of magenta color indicates the presence of flavonoids.	Present
Lead acetate test-	Orange to crimson colour shows the presence of flavonones.	Present
Ammonia Test	Formation of a yellow colouration in the extract indicates the presence of flavonoids.	Present
Ferric chloride test	blackish red colour forms, indicating the presence of flavonoids	Present

CONCLUSION

The present study was carried out with an aim of authenticity of the drug along with Physcio chemical and phytochemical analysis of Ashwagndha Churna. The result drown were 2% foreign mater was determined. Loss on drying 1.6%, total ash obtained was 9 %, acid insoluble ash was 1% and water soluble extractive was 12 % and Alcohol soluble extractive was 13 %.

The phytochemical investigation revealed the presence of various phytochemical constituents such as alkaloids, flavonoids, carbohydrate, Steroids and Saponin Glycoside.

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