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# Pharmacognostic Evaluation of Withania Somnifera Medicinal Plant

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**Abstract:** *Withania somnifera Linn. (Solanaceae) is an erect, evergreen and perennial shrub. Traditionally it has been used in treatment of rheumatism, gout, hypertension, nervine and skin diseases. It has been widely used as sex stimulant and rejuvenator and is considered as vigor and strength promoting drug. The main constituents of ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The leaves contain steroidal lactones, which are commonly called as "withanolides". Pharmacognostical and Phytochemical studies of W. somnifera Linn. Leaves were carried out in present study. The present investigation aims to evaluate the pharmacognostical studies such as morphology, macroscopy, microscopy, Transverse section (TS) of leaf and Powder characteristics of Ashwagandha. The physic-chemical parameters such as loss on drying, total ash content, sulphated ash, acid insoluble ash and extractive values were determined. Phytochemical parameters of Ashwagandha plant were analysed in accordance with Ayurvedic Pharmacopeia of India. The results drown were Loss on drying 5.0%, total ash obtained was 4.0%, Sulphated ash 6.0%, acid insoluble ash 0.9%, alcohol soluble extractive value 13% and water-soluble extractive value 12%. The phytochemical investigation revealed the presence of various phytochemical constituents such as alkaloids, flavonoids, carbohydrates, proteins, tannins, Steroids and Saponin Glycoside.*

**Keywords:** Withanolides, Rejuvenator, Alkaloids, Withanine, Steroidal lactone

## I. INTRODUCTION

Indian traditional medicine and medicinal plants are also considered as vital sources for new drug development. In order to make traditional a mainstream system, several measures have been taken to incorporate traditional medicine in evidence based clinical practice. According to the Indian Medicinal Plants Factsheet (NMPB 2020), out of the ca. 17,000 flowering plant species in India, more than 7000 plants species are known to have been used traditionally, as medicinal plants. Ayurveda, more than 3000 years old system of medicine has widespread acceptance. More than 90% formulations used in the Ayurveda, Siddha and Unani systems of medicine are plant based and about 22% of the MAP-production is sourced through cultivation.

Ashwagandha is a famous medicine herb from Ayurveda. In the ayurvedic medicine system, it is also known as "Withania somnifera L. Dunal" and "Indian Ginseng". It is a versatile herb that is talked about as having a nourishing tonic effect like ginseng and various health-promoting effects on the roots and fruits. It belongs to the evergreen shrub of the Solanaceae family. It is native to India as well as the Middle East and Africa too.

Ashwagandha root has been used habitually for more than 4000 years and has been used as a medicine for health promotion, disease prevention, treatment, energy enhancement, and nourishing tonic herb. In Ayurveda, ashwagandha is also known as a "rejuvenating drug".

Ashwagandha means "smell like a horse" in the Sanskrit language. It is derived from the unique pungent odour from the fresh roots of Ashwagandha. The scientific name suffix "Somnifera" means "sleeping", which means that this herb has a sedative effect. There are species of ashwagandha available in the world due to variations in the soil, climate and

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many more. It has been cultivated on a large scale for commercial purposes in India since the latter half of the 19th century, becoming a major production area.

Withania somnifera L. Dunal (Ashwagandha) is incredibly revered herb of the Indian Ayurvedic system of medication as a Rasayana (tonic). It is used for numerous types of sickness processes and Especially as a nervine tonic. Since antiquity, it's used against myriad of clinical conditions and, in fact, its history of use as a medication dates back to AD 6000. The plant contains a spread of various categories of chemical constituents like alkaloids, internal secretion lactones, and flavonoids. These chemical moieties are answerable for numerous biological activities of the plant. Laboratory studies incontestable the plant to be antioxidant, anti-inflammatory, antitumor, neuroprotective, antimicrobial, antistress, medicament, and cardioprotective. It possesses a good array of health edges thanks to the presence of many bioactive metabolites in it, in the main happiness to a category known as Withanolides (a cluster of internal secretion lactones with a basic C-28 framework).

Ayurvedic medicines have been continuously using Ashwagandha as one of the active ingredients for centuries due to its pleotropic effects namely antiinflammatory, immuno-modulatory and antistress properties.

The present investigation aims to evaluate the pharmacognostical studies such as morphology, macroscopy, microscopy, Transverse section (TS) of leaf and Powder characteristics of Ashwagandha. The physicchemical parameters such as loss on drying, total ash content, sulphated ash, acid insoluble ash and extractive values were determined. Phytochemical parameters of Ashwagandha plant were analysed in accordance with Ayurvedic Pharmacopeia of India.

## II. PLANT PROFILE

Figure 1: whole plant of Withania somnifera l. dunal

Botanical name : Withania somnifera L. Dunal

Family : Solanaceae

Vernacular names : Ashwagandha, Asgand, Asgand Nagori, Ashvakandika, Balada, Balaja, Gandhapatri, Vajigandha, Vajikari, Vajiini, Winter cherry, Indian winter cherry, Indian ginseng.

Geographical distribution : Africa, South Africa, Cape Verde Islands, Canary region, Arabia and Middle East Region Like India, southern China and Sri Lanka.

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Morphological Characters : It is a dense, hairy, erect, grayish-tomentose herb or Under-shrub, grows up to a height of 1.5 meter. It's all parts are covered with whitish, stellate trichomes.
Stems : Branches ligneous, tomentose at the apex covered with Minute stellately hairs.
Leaves : Leaves are simple, alternate or sub opposite, ovate, entire, Basis cunate, 10 cm long.
Flowers : Flowers are inconspicuous, greenish or lubrid-yellow, in Axillary, umbellate cymes.
Berries : Berries are small, globose, and orange-red when mature, enclosed in the persistent calyx.
Seeds : Seeds are yellow, reniform.
Roots : The roots are stout, long tuberous, fleshy, and whitish-Brown.
Ethnomedical uses : The plant has anti-oxidant, anti-tumour, anti-inflammatory, anti-Bacterial, fungicidal, Anthelmintic, anti-convulsant, anti-Stress, immunomodulatory properties.

## III. MATERIALS AND METHODS

### 3.1. COLLECTION OF SAMPLES OF ASHWAGANDHA POWDER

The sample of Ashwagandha powder were purchased from local raw material traders at Kuttiyappa Stores, Krishnagiri and the brand name was 7006 Herbals Siddha Shastric Medicine which was manufactured by Arul Soapnut Powder Company, 20, Bye-Pass Road, Avaniyapuram, Madurai – 625012, Tamilnadu.

### 3.2. PHARMACOGNOSTICAL EVALUATION STUDIES

#### 3.2.1. Macroscopic Evaluation Study

Macroscopic observation of leaves of Withania somnifera L. Dunal was done. It comprised of shape, size, surface characteristics, texture, colour, consistency, odour, taste, etc.

#### 3.2.2. Microscopic Evaluation Study

(a) Transverse section of leaves of Withania somnifera L. Dunal were taken by using a microtome Permanent mount of leaf was Prepared using safranin fast green stain by double staining technique.
(b) Powder characteristics of Withania somnifera L. Dunal were studied.

### 3.3. PHYSIOCHEMICAL EVALUATION STUDIES

Physicochemical parameters were determined as per guidelines of WHO. Loss on drying, total ash value, Sulphated ash, Acid insoluble ash and extractive values were determined.

#### 3.3.1. Determination of Loss on Drying (LOD)

Weigh about 1.0 gm of powdered drug into a weighed china dish. Dry in the hot air oven at 105℃, until two consecutive weighing do not differ by more than 0. 5 mg. Cool in a desiccator and weigh. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as % w/w with reference to air dried sample.

#### 3.3.2. Determination of Total Ash Value

Weigh sample in previously dried and weighed china dish, Heat the china dish carefully over a small flame to char the material, ignite in a muffle furnace at 550 ± 25℃. Grey ash is obtained, cool in desiccators, if wetting show ash to be carbon free, remove dish from desiccator, weigh the dish and repeat the operations for two successive weighing. Record the lowest mass, calculate the result.

#### 3.3.3. Determination of Sulphated Ash

Take 1.0 gm of powdered drug in an accurately weighed china dish, ignite gently at first until the substance is thoroughly charred. Cool, moisten the residue with 1ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignite in muffle furnace at 800℃ ± 25℃ until black particles have disappeared. Allow the china dish to

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cool, add a few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighing does not differ by more than 0.5 mg.

### 3.3.4. Acid Insoluble Ash Value

Refer to the total ash procedure till ashing, add 25ml of dilute hydrochloric acid. Heat on a water bath for 10minutes, cool and filter the contents of the dish, wash the filter paper with water until the washings are free from the acid, after the filter paper is free from the acid, Return the filter paper in a china dish, keep it in an oven at 100± 2℃ for 3hours, ignite in a muffle furnace at 550 ± 25℃ for one hour, Cool the china dish in a desiccators and weigh. Repeat this process for two successive weighing. Record the lowest mass, Calculate the result.

### 3.3.5. Alcohol Soluble Extractive Value

Alcohol soluble extractive Weigh accurately 4g 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.

### 3.3.6. Water Soluble Extractive Value

Water soluble extractive Weigh accurately 4g 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°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice, and take the average value

## 3.4. PRELIMINARY PHYTOCHEMICAL STUDIES

### 3.4.1. Test for Protein

| S.No | Test for Protein | Procedure |
|---|---|---|
| 1 | Xanthoprotein Test | Mix 3.0 ml of test solution with 1.0 ml of concentrated sulphuric acid. A white precipitate is formed. Boil it. The precipitate turns yellow. Add ammonium hydroxide. The precipitate turns orange. This indicates the presence of protein. |
| 2 | Precipitation Test | The test solution gives a white colloidal precipitate when mixed with 5% lead acetate. Formation of white colloidal precipitate indicates the presence of protein. |

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### 3.4.2. Test for Carbohydrates

| S.No | Test for Carbohydrate | Procedure |
|---|---|---|
| 1 | Fehling's Test | To 1.0 ml of the test solution, 1.0 ml of Fehling's A and 1.0 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. |
| 2 | Benedict's Test | Few drops of Benedict's reagent were added to the test solution and boiled on a water bath. Formation of reddish-brown precipitate indicates the presence of sugars. A positive Benedict's test appears green, yellow, orange, or red. |
| 3 | Molisch Test | To 2.0 ml of the test solution, 2 drops of Molisch reagent were added and mixed. 2.0 ml of concentrated sulphuric acid was added. Formation of a red violet ring at the junction of the solution and its disappearance on addition of excess alkali solution indicates the presence of carbohydrates. |

### 3.4.3. Test for Tannins

| S.No | Test for Tannins | Procedure |
|---|---|---|
| 1 | Ferric Chloride Test | To 2.0 ml of the test solution, add a few drops of 5% ferric chloride solution. A deep blue-black colour is formed. |
| 2 | Lead Acetate Test | To 2.0 ml of the test solution, add a few drops of lead acetate solution. A white precipitate is formed. |

### 3.4.4. Test for Alkaloids

| S.No | Test for Alkaloids | Procedure |
|---|---|---|
| 1 | Wagner's Test | To about 1–2 ml of the filtrate, 2.0 ml of Wagner's reagent was added. Reddish brown coloured precipitate indicates the presence of alkaloids. |
| 2 | Hager's Test | The acetic test solution treated with Hager's reagent (saturated picric acid solution) gives yellow precipitate. |
| 3 | Dragendorff's Test | The filtrate was treated with Dragendorff's reagent and the formation of orange precipitate indicates the presence of alkaloids. |

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### 3.4.5. Test for Steroids

| Test for Steroids | Procedure |
|---|---|
| 1. Liebermann Burchard Test | The test solution was dissolved in 2.0 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. |

### 3.4.6. Test for Saponin Glycoside

| Saponin Glycoside | Procedure |
|---|---|
| 1. Foam Test | Shake the test solution vigorously with water. Persistent foam is observed. |
| 2. Keller–Killiani Test | To 2.0 ml of test solution, add glacial acetic acid, one drop of 5% $FeCl_3$ and conc. $H_2SO_4$. Reddish brown colour appears at the junction of the two liquid layers and the upper layer appears bluish green. |

### 3.4.7. Test for Flavonoids

| Test for Flavonoids | Procedure |
|---|---|
| 1. Alkaline Reagent Test | To the test solution (0.5–1.0 ml), few drops of sodium hydroxide solution (10 percent) were added. Formation of an intense yellow colour, which turns colourless on addition of few drops of dilute acid, indicates presence of flavonoids. |
| 2. Lead Acetate Test | The test solution 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 flavanones. |

## 3.5. PHARMACOLOGICAL STUDIES

### 3.5.1 Hypoglycaemic Activity

Many studies incontestable that Ashwagandha reduces glucose levels. A tubing study found that it's inflated hormone secretion and improved peripheral tissue sensitivity to hormone. Transina, one ayurvedic formulation that contain Ashwagandha as active ingredient are according to decrease streptozotocin (STZ)- induced symptom in rats. This hypoglycaemic result could also be attributed to duct Gland Island radical scavenging activity as a result of the hyperglycaemic activity of STZ could be a consequence of decrease in duct gland island cell enzyme SOD activity resulting in the build-up of chronic aerophilic free radicals in island beta cells.

### 3.5.2. Anti-Inflammatory Activity

Withaferin A exhibits fairly potent anti-arthritic and anti-inflammatory activities.

Medicine activity has been attributed to biologically active steroids, of that Withaferin A may be a major part. It's as effective as Cortef sodium succinate dose for dose. In contrast to hydrocortisone-treated animals that lost weight, the animals treated with Withaferin A showed gain in weight in rheumatoid syndrome. It's attention-grabbing that Withaferin A appears to be strenuous than Cortef in adjuvant-induced inflammatory disease in rats, an in-depth experimental approximation to human arthritis. In its oedema inhibiting activity, the compound gave an honest dose response within the dose vary of 12-25 mg/kg weight of anomaly rats intraperitoneally and one dose had an honest length of action, because it may effectively suppress the inflammation when four hours of its administration.

### 3.5.3. Antioxidant Activity

Ashwagandha was tested for its anti-aging properties in an exceedingly doubleblind test. A gaggle of one zero one healthy males, 50-59 years previous got the herb at an indefinite quantity of three grams daily for one year. The themes older important improvement in haemoprotein, red somatic cell count, hair animal pigment, and seated stature. 3.5.4.

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Anti-Convulsant Activity Administration of Ashwagandha was found to scale back jerks and convulsion in seventieth and 100% animals severally with dose of 100mg/kg and reduction within the severity of Pentylene Tetrazole (PTZ)-induced convulsions was evident from EEG wave pattern. Ashwagandha showed reduction in severity of motor seizures elicited by electrical stimulation in right basilateral amygdaloidal nuclear advanced through bipolar electrodes. The protecting impact of Ashwagandha in convulsions has been according to involve GABAergic mediation.

### 3.5.5. Immunomodulatory Activity

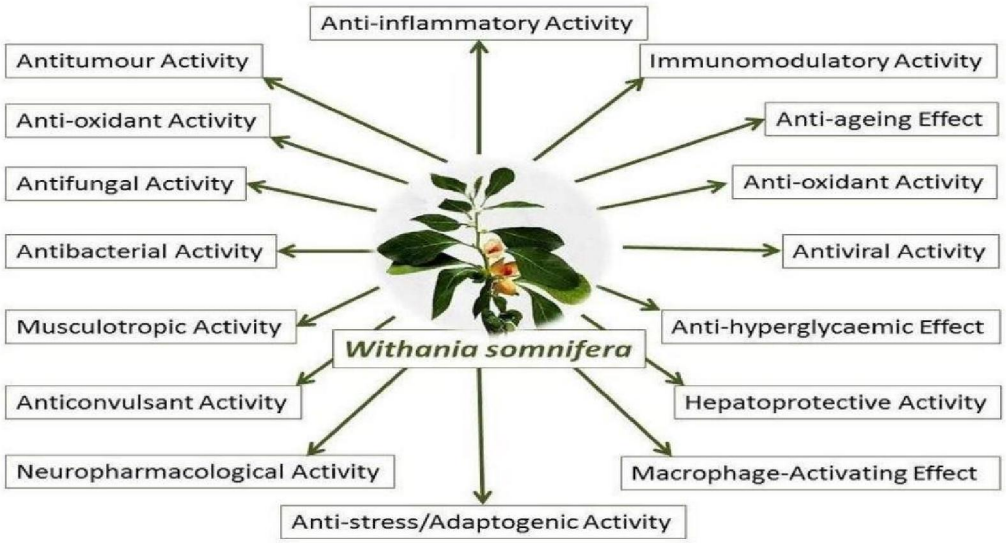
Ashwagandha showed a big modulation of immune reactivity in animal models. Administration of Ashwagandha was found to forestall myelo-suppression in mice treated with 3 immunological disorder medicine viz. cyclophosphamide, azathioprine, and Prelone. Treatment with Ashwagandha was found to considerably increase haemoglobin concentration, RBC count, thrombocyte count, and weight in mice.

### 3.5.6. ANTI-CARCINOGENIC ACTIVITY

Ashwagandha is according to own anti-carcinogenic effects. Analysis on animal cell cultures has shown that the herb decreases the amount of the nuclear issue kappa B, suppresses the animate thing growth death issue, and potentiates apoptotic signalling in cancerous cell lines. One of the foremost exciting of the doable uses of Ashwagandha is its capability to fight cancers by reducing growth size. To investigate its use in treating varied varieties of cancer, the anticancer effects of Withania somnifera L. Dunal are studied by researchers.

### 3.5.7. ANTI-EPILEPTIC ACTIVITY

A study was designed to investigate the neuroprotective role of Withania somnifera L. Dunal (Ashwagandha), also known as Indian ginseng, against glutamate induced toxicity in the retinoic acid differentiated rat glioma (C6) and human neuroblastoma (IMR-32) cells. The neuroprotective activity of the Ashwagandha leaves derived water extract (ASH-WEX) was evaluated. Cell viability and the expression of glial and neuronal cell differentiation markers was examined in glutamate challenged differentiated cells with and without the presence of ASH-WEX. The study demonstrated that RA-differentiated C6 and IMR-32 cells, when exposed to glutamate, undergo loss of neural network and cell death that was accompanied by increase in the stress protein HSP70. ASHWEX pre-treatment inhibited glutamate-induced cell death and was able to revert glutamate-induced changes in HSP70 to a large extent.



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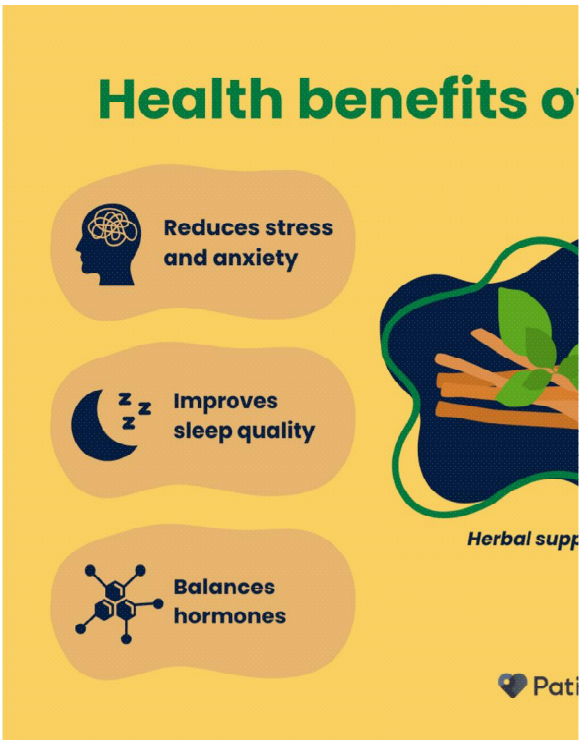


Figure 3: Benefits of Withania somnifera l. dunal

## IV. RESULTS AND DISCUSSION

### 4.1. PHARMACOGNOSTICAL EVALUATION STUDIES

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

#### 4.1.1. Macroscopic Evaluation

| Characteristic | Description |
|---|---|
| Colour | Green |
| Taste | Bitter and acrid |
| Odour | Pungent barn-like, earthy fragrance |
| Leaf Arrangement | Spiral |
| Leaf Venation | Pinnate |
| Leaf Persistence | Evergreen |
| Leaf Type | Simple |
| Leaf Blade | 5–10 cm |

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| | |
|---|---|
| Leaf Shape | Ovate |
| Leaf Margins | Entire |
| Leaf Textures | Hairy, Medium |
| Leaf Scent | Pleasant |

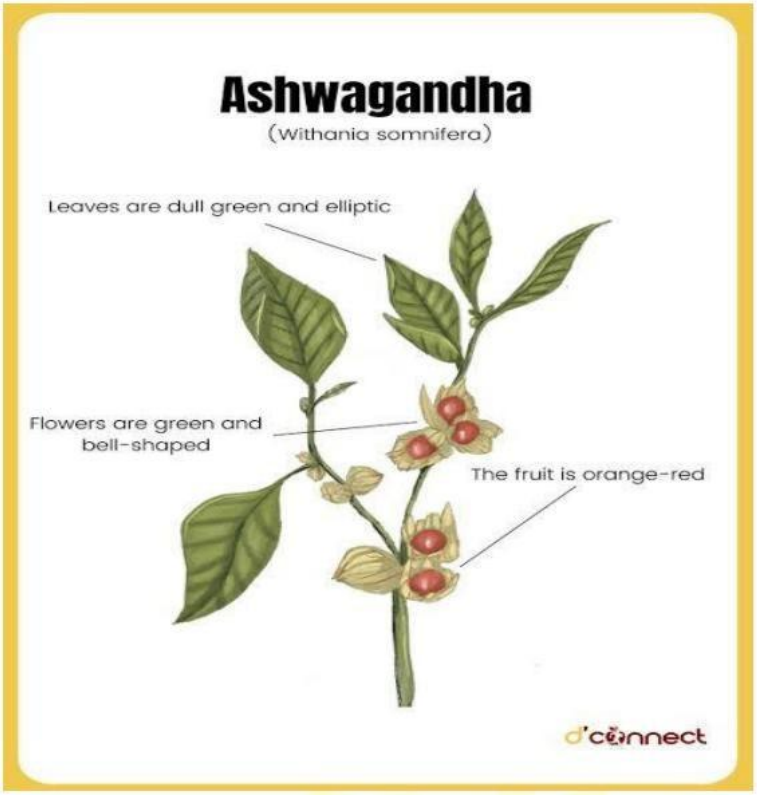


Figure: 4 Macroscopy of Withania somnifera L. Dunal

**4.1.2. Microscopic Evaluation**

The microscopical exam were provided on the cross sections or on the surface preparations of the Aswagandha leaf. The anatomical structure of the leaf is bifacial, dorsiventral. The epidermis is covered with multicellular, dendroid trichomes with pitted terminal cells and the pits are not prominent, but shorter and more abundant on the lower epidermis. The stomata surrounded by 4–6 epidermal cells are on the both epidermises, in the spongy mesophyll we distinguish a lot of calcium oxalate rosettes, the vascular bundles are collateral and, in the midrib, region enclosed in an endodermis with collenchyma above and below.

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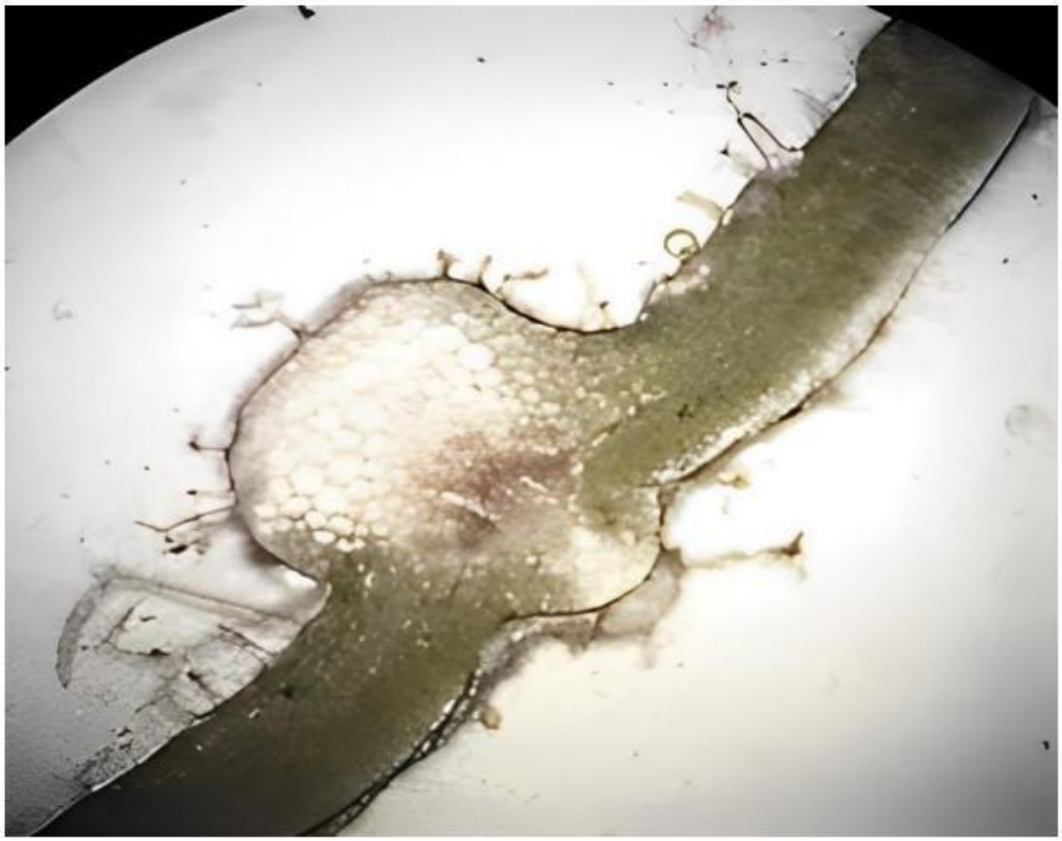

Figure: 5 T.S of Mature Leaf of Withania somnifera L. Dunal

**POWDER MICROSCOPY**

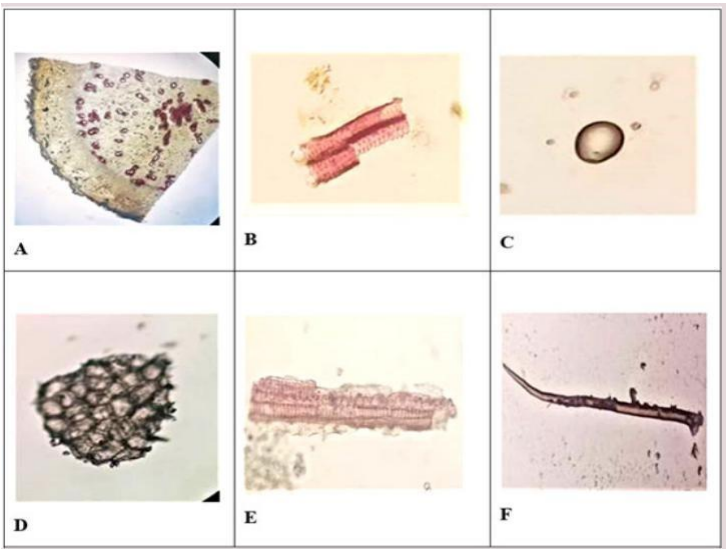


Figure 6: Powder Microscopy of Withania somnifera L. Duna

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A – Xylem Vessels
B – Tracheid
C – Cork Cells
D – Fibres
E – Starch Grains

### 4.2. PHYSIOCHEMICAL EVALUATION STUDIES

The quantitative determination of some pharmacognostical 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 L. Dunal are shown in Table 1. The average values are expressed as percentage of air - dried material.

Table 1. Physiochemical analysis of Withania somnifera L. Dunal

| S.No | Parameters | Results (% W/W) |
|---|---|---|
| 01 | Loss on Drying | 5.0% |
| 02 | Total Ash | 4.0% |
| 03 | Sulphated Ash | 6.0% |
| 04 | Acid Insoluble Ash | 0.9% |
| 05 | Alcohol Soluble Extractive Value | 13% |
| 06 | Water Soluble Extractive Value | 12% |

### 4.3. PRELIMINARY PHYTOCHEMICAL STUDIES OF Withania somnifera L. Dunal

Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, carbohydrates, proteins, tannins, Steroids and Saponin Glycoside.

Table 2. Preliminary Phytochemical Analysis of Withania somnifera L.Dunal

| S.No | Parameters | Results (% W/W) |
|---|---|---|
| 01 | Loss on Drying | 5.0% |
| 02 | Total Ash | 4.0% |
| 03 | Sulphated Ash | 6.0% |
| 04 | Acid Insoluble Ash | 0.9% |
| 05 | Alcohol Soluble Extractive Value | 13% |
| 06 | Water Soluble Extractive Value | 12% |

Result: +: Present

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Figure: 7 Phytochemical Screening of Withania somnifera L. Duna

## V. SUMMARY AND CONCLUSION

The Ashwagandha is a highly valued medicinal plant. This is useful in alternative systems of medicine as a natural drug in treating infectious diseases. Withania somnifera L. Dunal harbours very rich source of phytochemicals which are present in roots, stems, leaves and fruits. The phytochemicals such as steroidal lactones, alkaloids, tannins, flavonoids, phenolic compounds etc. can be extracted from different parts. The polar solvents may extract more compounds in comparison to non- polar solvents.

The extensive survey of literature revealed that Withania somnifera L. Dunal is an important source of many pharmacologically and medicinally important chemicals, such as withaferin, sitoindosides and various useful alkaloids. In Indian variety thirteen Dragendroff positive alkaloids have been reported. The withanolides are the most searched chemical constituents of WS and till date around 138 withanolides with both β and α side chain has been reported apart from various amino acid and other normal plant constituents. The plant has also been widely studied for their various pharmacological activities like antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, anti-inflammatory, antitumor properties. Various other effects like immunomodulation, hypolipidemic, antibacterial,

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cardiovascular protection, sexual behaviour, tolerance and dependence have also been studied. Although the results from this review are quite promising for the use of Withania somnifera L. Dunal as a multi-purpose medicinal agent, several limitations currently exist in the current literature.

The present study was carried out with an aim of authenticity of the powdered drug along with Physio-chemical and phytochemical analysis of Ashwagandha plant. The results drown were Loss on drying 5.0%, total ash obtained was 4.0%, Sulphated ash 6.0%, acid insoluble ash 0.9%, alcohol soluble extractive value 13% and water-soluble extractive value 12%. The phytochemical investigation revealed the presence of various phytochemical constituents such as alkaloids, flavonoids, carbohydrates, proteins, tannins, Steroids and Saponin Glycoside.

Further studies (or) Research is based on Presence of Alkaloids having Antioxidant activity from extract of plant leaf of Withania somnifera L. Dunal.

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