



Qualitative screening of phytochemicals present in *Withania somnifera* root extract with pharmacological importance

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ABSTRACT

Withania somnifera, also known as "Ashwagandha", is a well-known perennial herb from India that belongs to the Solanaceae family. It is among the most valuable medicinal plants and has been widely utilised as therapeutic agents, such as anti-inflammatory, immunomodulatory, anti-stress, antibiotic, anticancer, anti-oxidant, sedative, alterative, and aphrodisiac. As the dried roots of *Withania somnifera* are widely used in the treatment of many disorders. Natural bioactive component flavonoids, alkaloids and phenolic compound are said to be present in the roots. The aim of the present study is to identify the presence of active phytochemical components like phenolic compounds, alkaloids, flavonoids, tannins, and amino acids in *Withania somnifera* root extracts. In order to determine the phytochemical constituents in the plants, the samples of aqueous and ethanolic extract were used for the phytochemical assessment by using standard chemical tests. Pharmacological studies and preliminary phytochemical study were conducted. The findings indicate the presence of tannins, amino acids, flavonoids, alkaloidal, and saponin. The current study will be useful for determining the quality and purity of a crude drug. According to our findings, Ashwagandha root powder is a great source of phytochemicals and nutraceuticals.

Keywords: *Withania somnifera*, Phytochemical Analysis, Qualitative, Ashwagandha, Root.

INTRODUCTION

Withania somnifera is a perennial herb native to India, Africa, and the Middle East. It belongs to the family (Solanaceae) and has been used for centuries for more than thousands of years Ayurvedic medicine. *Withania somnifera* is locally known as Indian Ginseng and Ashwagandha. For sustaining the health of humans and animals, plants have been a vital source of natural compounds. The biological effects of the plant include effects on the CNS and cardiopulmonary system, as well as anti-tumor, anti-inflammatory, anti-cancer, sleep-inducing, anti-stress, anti-oxidant helpful in memory-related conditions and insomnia, immunomodulatory, hemopoietic, and



Fig.1: Plant of *Withania somnifera*



Fig.2: Photographs of dried root of *Withania somnifera*

rejuvenating effects. (Singh *et al.*, 2010; Uddin *et al.*, 2012). Ashwagandha have been shown to have potent anti-inflammatory which reduces inflammation and pain and also an immune boosting plant which helps in to strengthens the immune system by boosting the white blood cell production serotonin, dopamine and GABA and neurotransmitters which can be regulated by ashwagandha to reduce the anxiety and improving the mood. Antioxidants in ashwagandha protect cells from damage caused by free radicals also it has the property to improve insulin sensitivity by making it potential treatment of diabetes. According to the World Health Organisation (WHO, 1998), medicinal plants are among the best sources for a variety of pharmaceuticals. Nearly 80% of people in developed countries use traditional medicine, which includes components derived from therapeutic plants. *Tinospora cordifolia* (Guduchi), *Emblica officinalis* (Amla), *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), are herbal extracts that are frequently used to treat immunosuppressive disorders in both human and animal patients (Devasagayam and Sainis, 2002). Plant-based remediation would be less expensive, more cost-effective, and environmentally beneficial with no negative impacts. It is important to take considerable measures for plant secondary metabolite screening to promote the use of herbal medications. There have been numerous reports of phytochemical screening in different species. (Chitravadivu *et al.*, 2009; Kumar *et al.*, 2010; Siddiqui *et al.*, 2009). *Withania somnifera* characteristics have been the subject of numerous pharmacological investigations to confirm their use as a multi-purpose

medicinal agent. (Nazir and Chauhan, 2018). Present study aims at the extraction and screening of various bioactive secondary metabolites present in the root of *Withania somnifera* which could account for their varied medicinal properties.

MATERIALS AND METHODS

The whole plant of *Withania somnifera* were collected from the Patna University campus, Patna, Bihar, India. In accordance with the pertinent Indian Pharmacopoeia monographs (2012), they were further prepared after identification and botanical authentication. The root of the plant was washed under running water, blotted with filter paper, and allowed to air dry in the shadow at room temperature. To create a fine powder, they were then ground in a mortar and pestle. The powder was kept in airtight containers to be used in the experiment later.

Chemical

All the chemicals used in the study were purchased from Merck (Germany)

Preparation of Ethanolic extract

The samples of dried root powder were then soaked in pure ethanol to prepare an ethanolic extract. *Withania somnifera* dried root powder (10g) was macerated in a close flask for 24 hours with 100 ml of pure ethanol, occasionally stirring during the first six hours. Next day the solution kept inside the ultrasonicator for one hour and squeezed into volumetric flask and stored

overnight. Next day the stored extract kept into rotary evaporator for one hour and the prepared sample were stored in refrigerator at -4°C for further analysis.

Preparation of water-soluble extract

A measured amount of *Withania somnifera* dried powder (10g) was soaked in 100 ml of purified water in a closed flask and left to rest for 18 hours with regular shaking. After that, solution kept at ultrasonicator for one hour and sample squeezed left overnight and the next day sample kept in rotary evaporator for one hour and the prepared sample were stored in refrigerator at -4°C for further analysis.

Phytochemical Evaluation

The Numerous standard protocols have been used to qualitatively examined the newly made aqueous extracts and ethanolic of *Withania somnifera* for the existence of phytochemical elements.

Detection of Alkaloids: Wagner's Test was used to check the presence of alkaloids. In this test reddish brown precipitate forms when added a few drops of Wagner's reagent to the test tube's inside after adding 200 µl of crude extract.

Preparation of Wagner's reagent

- Iodine -1.27grams
- Potassium iodide – 2gram
- Distilled water – 5 ml

For working solution, 100 ml of the distilled water was used to dilute the solution.

Detection of Tannins (Braymer's Test)

After dilution to 1 ml, each extract was treated with 1 ml of a 10% alcoholic ferric chloride solution, and the development of a blue or greenish colour was checked.

Detection of Flavonoids

1% sodium hydroxide (NaOH) was used to dissolve 200 mg of Ashwagandha root extract in individual test tubes. The presence of flavonoids is indicated by a change in colour from a deep yellow to colourless after adding a few drops of diluted hydrochloric acid.

Detection of Saponin

Froth Formation test (Kokate *et al.*, 1994): In the test container, 1 ml of the extract was mixed with 20 ml of distilled water. A graduated container was filled with 10 ml of the filtrate. Shake ferociously after adding 5ml of distilled water. Saponins are present when continuous froth forms.

Detection of Phenol

Ferric Chloride test (Kar, 2004): 5 ml of 1% ferric chloride is added to 5 ml of Ashwagandha filtrate in a test container. Phenol could be detected by the emergence of dark green or bluish green colour.

Detection of Reducing Sugar

Fehling's Test (Rosenthaler, 1930): 1ml of each extract of Ashwagandha mixed with 2ml of Fehling's solutions A and B. For five to ten minutes, the solution was brought to a boil in a water bath. Brick red precipitation formed in the presence of non-reducing sugar.

Detection of Glycosides

Kellar-Kiliani Test (Sim, 1968): Glacial acetic vinegar, which has traces of ferric chloride, is added to 1 ml of Ashwagandha extract in a test vessel. Along the test tubes' edges, gently 1 ml of strong sulfuric acid is added. The existence of glycosides is indicated by the appearance of a greenish blue colour indicates the presence of glycosides.

Detection of Proteins and Amino acid (1% Ninhydrin solution in Acetone)

In order to detect the presence of amino acids, 1 ml of each Ashwagandha extract was extracted, Ninhydrin solution was added 2 to 5 drops, and the mixture was then heated for 1 to 2 minutes in a water bath. The appearance of Violet colour indicates that the presence of protein.

RESULT AND DISCUSSION

In the present study, the qualitative analysis of phytochemicals presents in Ashwagandha root powder aqueous and ethanolic crude extracts were analysed and the results are presented in Table 1. The root powder of *Withania somnifera* found to contain carbohydrates, tannin, glycoside, phenol, and alkaloids. While ethanolic extracts show the presence of alkaloids, phenolic, glycosides, Saponin and phenolic compound, aqueous extracts contain amino acids, flavonoids, and Saponins and alkaloids. The findings are comparable to those of the research by Kushwah *et al.* (2015), which looked at both the qualitative analysis and quantitative of Ashwagandha root powder. They identified the presence of inorganic materials and heavy metals in the powdered root.

Table 1: Preliminary Screening of Crude Extract of *Withania somnifera* root extract in different medium

S.No.	Secondary metabolites	Colour Change	Aqueous	Ethanol
1	Alkaloids	Reddish brown	-	+
2	Flavonoids	Colourless	+	+
3	Saponin	Formation of persistent froth	+	+
4	Phenol	Dark green or bluish Green	-	+
5	Carbohydrate	Brick red	-	-
6	Glycosides	Greenish Blue	-	+
7	Amino acid	Purple	+	-
8	Tannins	Blue or greenish colour	-	-

PHARMACOLOGICAL STUDIES

Plants containing some natural occurring compounds which is called as phytochemicals that have been shown to have several health benefits. These chemicals are not considering as essential nutrients but it can have many significant impacts on the health and well-being. Some of the key reasons why phytochemicals are important because of its anti-inflammatory, hormonal regulation, immune system support and antioxidant activity. Some of the important phytochemicals present in the Ashwagandha is Alkaloids, Flavonoids, Tannins, Saponin. The pharmacological activity of the dried roots of *Withania somnifera*, which is recognized as a sedative in the Indian Pharmacopoeia, is attributed to the presence of several alkaloids.

Alkaloid: Therapeutically it is a well-known anti-inflammatory agent, anaesthetics, cardioprotective. Some of the well-known alkaloids are quinine, ephedrine, quinine, strychnine, nicotine. Tumours, nocturnal leg cramps caused by vascular spasms, and diarrhoea have all been known to be treated with alkaloids. These substances have sedative effects as well as antimicrobial action. In addition, alkaloids can be used to address palpitations and psychiatric conditions (Onike, 2012).

Flavonoids: It is a group of natural compounds that belong to the class of polyphenols, which are found in a wide range of fruits, vegetables, and other plant-based foods. A natural disease preventing, health promoting, dietary supplements.

Tannins: have various biological properties that make them potentially beneficial for human health. They have anti-inflammatory and antioxidant effects, which

may help against chronic diseases such as cancer, heart disease and diabetes. They also have antiviral, antibacterial, and antifungal properties that may help protect against infections.

Saponins: Saponins are a type of natural compound that is found in various plant-based foods, such as legumes, whole grains, and some fruits and vegetables. They are characterized by their foaming properties when mixed with water or other liquids, Saponins have various biological properties that make them potentially beneficial for human health. They have anti-inflammatory and immune-stimulating effects. According to Khare (2007), the free amino acids found in the root include glutamic acid, glycine, aspartic acid, cystine, proline, tyrosine, alanine and tryptophan.

CONCLUSION

The present study concluded *Withania somnifera* is rich source of various secondary metabolites that helps to cure diseases without any side effects. Numerous bioactive substances, including a wide range of phytoconstituents such as alkaloids, flavonoids, proteins, phenolic compounds, cardiac glycosides and tannins are present in various Ashwagandha root powder preparations. The findings of this phytochemical screening are further helpful for isolating different compounds from the herb for the cure of diseases and provide information in the identification and authentication of *Withania somnifera*.

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