Phytochemical evaluation of *Withania somnifera* extracts

Priyanka Arya and RS Chauhan

Abstract

*Withania somnifera* is popular perennial Indian medicinal plant belonging to family Solanaceae is commonly known as “Ashwagandha”, Asgand, Winter Cherry, Indian ginseng. It is one of the most valued medicinal plants which have been widely used as therapeutic agents such as anti-inflammatory, antibiotic, antitumour, immunomodulatory, anti-stress, anti-oxidant, sedative, alterative and aphrodisiac. The roots are reported to contain natural bioactive constituent alkaloid, flavonoids and phenolic compound. The present study comprises phytochemical evaluation to estimate the presence of carbohydrates, glycosides, flavonoids, tannins, phytosterols and phenolic compounds in *Withania somnifera* locally available in Pantnagar, region of Uttarakhand. The aqueous and ethanol extract samples were used for the phytochemical analysis by using standard chemical tests to find out the phytochemical constituents in the plants. Results revealed the presence of starch, amino acid, flavonoids, alkaloids, saponin. The present investigation will help in assessing the quality and purity of a crude drug and laying down pharmacopoeial standards for *Withania somnifera*. All studied standardization parameters like phytochemical screening provide the knowledge in the identification authentication of *Withania somnifera*.

Keywords: *Withania somnifera*, ashwagandha and antibiotic

Introduction

Plants have been a valuable source of natural products for maintaining human and animal health. Therapeutic through plant materials would be cheaper, cost effective and eco-friendly with no or less side effects on health. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs (WHO, 1998) [14]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Commonly used herbal extracts are from *Emblica officinalis* (Amla), *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi) and *Ocimum sanctum* (Tulsi) for the treatment of immunosuppressive conditions for humans and animal (Devasagayam and Sainis, 2002) [2]. Therapeutic efficacies of many indigenous plants, blend of herbal extract from plant and animal sources have been described by practitioners resulting enhances in the level of duration of specific immune response, both cell mediated and humoral immunity. Phytochemicals or bioactive constituents have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids, flavonoids and alkaloids. These compounds are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, agriculture, scientific research, veterinary and many other areas (Vasu et al., 2009) [12].

*Withania somnifera* (ashwagandha) is an erect, bitter – sweet, astringent, evergreen shrub. It mainly pose on the reproductive and nervous systems. It has sedative, revitalizes and aphrodisiac effects. Ashwagandha is prescribed in case of fatigue or exhaustion where it is reported to promote strength, vigor and acts as nature’s best adaptogen (an adaptogen strengthen the immune system, protects against mental and physical fatigue, fights stress, tension and regularizes all body functions). In addition to its the roots and leaves of the plant are used traditionally in the form of powder, decoction, oil etc. these have been used in conventional medicine against general enervation, hypertension, inflammations, asthma, cancer, tuberculosis, tumors, rheumatism, psoriasis, senility, smallpox, sores, syphilis, scabies, ringworm, typhoid, uterusis and wounds. It possesses anti-inflammatory, anti-tumor, anti-stress, anti-oxidant, immunomodulatory, hemopoietic and rejuvenating properties (Nazir and Chauhan, 2018) [8], Sharma et al. (2010) [10] found the stimulatory effect of dietary doses of *Withania somnifera* (Ashwagandha) root on immunity and disease resistance against *A. hydrophila* infection in Indian major carp, *L. rohita* fingerlings. Kolkovski and Kolkovski
(2012) [6] also observed that some herbal extracts are very effective against gills and skin flukes like *Benedenia seriolae*. It is mostly cultivated in many regions of India like Madhya Pradesh, Punjab, Gujarat and Rajasthan.

The main objective of the experiment was to check the presence or absence of the phytochemical constituents in the *Withania somnifera* (Ashwagandha) medicinal plants.

Materials and methods

Collection of ashwagandha, *Withania somnifera*

*Withania somnifera* dried root powder was purchased from local market of Pantnagar and Rudrapur, Uttarakhand. The powder was stored in the air tight plastic containers for further use in the experiment.

a) Preparation of alcohol soluble extract

Dried powder of *Withania somnifera* (5 g) was macerated with ethanol (100 ml) in a closed flask for 24 hours with occasional stirring during the first 6 hour. Then it was allowed to stand for 18 hour and then filtered swiftly to prevent any loss during evaporation. Evaporate approximately 25 ml of the filtrate in a porcelain dish and dried at 105°C and weighed.

b) Preparation of water soluble extract

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

Phytochemical Evaluation

The freshly prepared ethanolic and aqueous extracts of *Withania somnifera* were qualitatively analyzed for the presence of phytochemical constituents using the following standard protocol.

Test for Phenol

Ferric Chloride test (Kar, 2004) [4]

Take 2 ml of Ashwagandha filtrate in a test tube and then add 2 ml of ferric chloride (1%). The appearance of dark green or bluish green color indicated the presence of phenol.

Test for tannins

Add few drops of lead acetate solution in a test tube with 2 ml of filtrate. Yellowish coloration was indication of positive result.

Test for Saponin

Froth Formation test (Kokate et al., 1994) [5]

1 ml of extract was taken in test tube add 20 ml of distilled water. Take 10 ml of filtrate was taken in a graduated cylinder. Add 5 ml of distilled water and shake vigorously. Formation of persistent froth indicates the presence of saponins.

Test for Flavonoids (Harbourne, 1973) [3]

Alkaline Reagent Test

To the 200 mg of extract in a test tube add few drops of Sodium hydroxide solution. Then add few drops of dilute hydrochloric acid, change in the color from deep yellow colour to colourless indicates the presence of flavonoids.

Test for Alkaloids

a. Wagner’s Test

To 200 μl of crude extract add few drops of Wagner’s reagent to the inner side of test tube. A reddish brown precipitate was formed which confirmed the presence of alkaloids.

Preparation of Wagner’s reagent

- Iodine : 1.27g
- Potassium iodide : 2g
- Distilled water : 5ml

The solution was further diluted in 100 ml of distilled water for working solution.

b. Mayer’s Test

Equal amount of extract and 1% hydrochloric acid were added and heated gently. Mayer’s and Wagner’s reagent were added to the mixture. Cream colored of the resulting precipitate was taken as proof for the presence of alkaloids.

Preparation of Mayer’s reagent

- Mercuric chloride : 13.6 parts
- Potassium iodide : 50 parts
- Distilled water : 940 parts

c. Dragendorff’s Test

In 2 ml of filtrate add 1 ml of 1% hydrochloric acid and steam heated the solution for 2 min. filtered the solution and take 1 ml of filtrate. Add six drops of Dragendorff’s reagent. The change in color of precipitate to orange red/ brownish red showed the presence of alkaloids.

Preparation of Dragendorff reagent

Solution A

- BSN (Bismuth subnitrate) : 17g
- Tartaric acid : 200g
- Distilled water : 800ml

Solution B

- Potassium iodide : 160g
- Distilled water : 400ml

After that solution A and B were mixed. A working standard was prepared by taking 50 ml of this solution and adding 100g of tartaric acid. Add distilled water to make up its volume 500 ml.
Test for Reducing Sugar  
Fehling’s Test (Rosenthaler, 1930) [9]  
2 ml each of Fehling’s solution A and B was added to 1 ml extract of ashwagandha powder. The solution was heated in water bath for 10 minutes. The formation of brick red precipitation indicated the presence of non-reducing sugar.

Results and Discussion  
Phytochemical analysis of Ashwagandha (Withania somnifera)  
The details of result for qualitative analysis of phytochemicals in water and ethanolic crude extract of ashwagandha root powder are presented in Table 1. Phytochemical screening of root powder of Withania somnifera indicates the presence of carbohydrates, starch, tannin, saponin, glycoside, phenol and alkaloid. Aqueous extract contains amino acids, flavonoids and saponins while ethanolic extracts indicate the presence of saponin, alkaloids, phenolics, glycosides, starch, terpenoids and flavonoids. Findings are similar to the results of the study made by Kushwah et al. (2015) [7] who examined both the quantitative and qualitative analysis of ashwagandha root powder. They also determined the presence of heavy metals as well as inorganic matter in the root powder.

Table 1: Phytochemical screening of Ashwagandha (Withania somnifera)  
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Secondary Metabolites</th>
<th>Aqueous</th>
<th>Ethanol</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Phenolics</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Amino acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Starch</td>
<td>+</td>
<td>+</td>
</tr>
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</table>

Conclusion  
The present study concluded that the plant Withania somnifera contains variety of phytoconstituents like flavonoids, alkaloids, proteins, phenolic compounds, cardiac glycosides and tannins. The present study revealed the presence of medicinally important constituents which can be confirmed the utilization of root for therapeutic medical treatment of diseases without any side effects. This phytochemical screening provides the knowledge in the identification authentication of Withania somnifera and results further useful for isolation of various compounds from herb for the treatment of diseases.

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References  