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## Qualitative phytochemical analysis of *Tinospora cordifolia* and *Withania somnifera*

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### Abstract

The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The present study involves the qualitative phytochemical analysis of two different medicinal plants: *Tinospora cordifolia* and *Withania somnifera* locally available in Pantnagar, region of Uttarakhand. The aqueous and ethanolic extract samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. Guduchi and ashwagandha are very renowned medicinal plant for its versatile pharmaceutical properties. For finding several compounds qualitative phytochemical analysis is very important. Two different solvents viz; water and ethanol were used to obtain extracts. These extracts were used for qualitative preliminary phytochemical analysis using standard chemical tests. Data indicates the presence of flavonoids, alkaloids, proteins, phenolic compounds, cardiac glycosides and tannins.

**Keywords:** Ethanolic, phytochemicals, *Tinospora cordifolia*, *Withania somnifera*, flavonoids

### Introduction

India has a rich resource of traditional herbal medicines to treat human and animal diseases. These have no or little side effects during treatment. Commonly used herbal extracts are from *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi) and *Emblia officinalis* (Amlaki) for the treatment of immunosuppressive conditions for humans and animal (Devasagaya, 2002) [1]. Remediation through plant materials would be cheaper, cost effective and eco-friendly with no deleterious effects. Various herbal products from *Hygrophila spinosa*, *Withania somnifera*, *Zingiber officinale*, *Solanum trilobatum*, *Psoralea corylifolia*, *Eclipta erecta*, *Ocimum sanctum*, *Picrorhiza kurroa*, *Phyllanthus niruri* and *Tinospora cordifolia* have the characteristics of growth promotion, anti-stress, immunostimulation, and anti-bacterial. The blend of herbal extract from natural sources enhances the level of duration of specific immune response, both cell mediated and humoral. It also helps in activation of the immune modulatory molecules in the body, act as agglutinating protein or as enzyme which selectively destroys bacterial cell walls without damaging host cells. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Doss, A (2009) [2].

*Tinospora cordifolia* is a large deciduous, creeping shrub belonging to the family Menispermaceae. It's indigenous to the tropical areas of India, Myanmar and Sri Lanka. More than 30-40 species of Giloy are present all over the world. It is commonly known as Guduchi, Giloy, Amrita and Gurucha. According to Drugs and Cosmetic Act of India (1940), giloy is considered as an ayurvedic drug. All parts of this plant like fruits, leaves, stem and seeds are useful. Most commonly stem is used and have been recognized to be of great use. Guduchi has many medicinal properties like antibiotic, immunosuppressant, anticancer, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritis, anti-allergic, anti-diabetic, Anti-toxic, Anti-HIV, antineoplastic, anti-oxidant, hypolipidemic, immunologic, anti-periodic, anti-stress, immunomodulatory etc.

A variety of active components like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides, have been isolated from the different parts of the plant body, including root, stem and whole plant. The crude values of Guduchi include protein (4.5-11.2%), high fibre (15.9%), carbohydrate (61.66%) and low fat (3.1%).

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Its nutritive value is 292.54 calories per 100 g. It has higher level of potassium (0.845%) (Helps in nerve impulse regulation), iron (0.28%), chromium (0.006%) and calcium (0.131%) (Regulate cardio and musculoskeletal system, blood coagulation) (Mutalik and Mutalik, 2011) [6].

*Withania somnifera* (winter cherry) is bitter – sweet, astringent, warming herb with a horse like smell, it acts mainly on the reproductive and nervous systems. It has sedative, rejuvenative and aphrodisiac effects. In addition to its dual energizing/calming effect, ashwagandha offers a number of benefits, i.e., supports a healthy immune system, calms mental processes, fosters healthy sleep patterns, benefits a healthy reproductive system in both males and females, supports sustained energy levels, strength, and vitality, including with physical activity, supports a healthy back and joints, promotes thyroid health, promotes healthy functioning of the adrenals. It possesses anti-inflammatory, anti-tumor, anti-stress, anti-oxidant, immunomodulatory, hemopoietic and rejuvenating properties. *W. somnifera* root is used as a Rasayana in the Indian ayurvedic tradition as a dietary supplement promoting rejuvenation, mental and physical health, as well as providing a defence against aging and challenging environmental factors. Sharma *et al.* (2010) [8] observed 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 (2011) [3] also reported that some herbal extracts are very effective against gills and skin flukes like *Benedenia seriola*. The main objective of the research work was to check the presence or absence of the phytochemical constituents in the two selected medicinal plants.

## Materials and methods

### Collection of guduchi, *Tinospora cordifolia*

During the experiment, the medicinal plant *Tinospora cordifolia* was collected from local areas and gardens of Pantnagar. The identification of plant Guduchi was done at Medicinal Plant Research and Development Centre (MRDC), Pantnagar. The selection of this plant was due to its various recognized medicinal properties. For the preparation of powder, leaves were collected and washed properly with fresh and clean tap water several times and then rinsed well in sterilized distilled water. They were then spread out on the polythene sheets and were air dried. After drying the leaves were ground in the ball miller to obtain fine powder. The powder was stored in the air tight containers for further use in the experiment.

### Collection of ashwagandha, *Withania somnifera*

Root powder of *Withania somnifera* was purchased from local market of Rudrapur. The powder was stored in the air tight containers for further use in the experiment.

## Phytochemical analysis

The qualitative phytochemical analysis was carried out to detect the presence of different phytochemicals in guduchi and ashwagandha powder. The procedures for the tests are as follows:

### Test for tannins

0.5 g of dried guduchi and ashwagandha powder respectively were taken in different test tubes. 20ml of distilled water was added and boiled in water bath at about 100°C. The solution

was filtered through Whatman No. 1 filter paper. After that add few drop of 0.1% ferric chloride ( $\text{FeCl}_3$ ). Development of brownish green or blue black coloration was indication of positive result.

### Test for Phenol [Ferric Chloride test]

Equal volume of guduchi and ashwagandha extract were taken in different test tube and then 5% of ferric chloride was added in each test tube. The appearance of dark green or bluish green color indicated the presence of phenol.

### Test for Saponin

2 g of dried guduchi and ashwagandha powder were taken in different test tubes, then add 20ml of distilled water and boil it for 2 min in water bath at 100 °C. The solution was filtered through Whatman No. 1 filter paper and 10ml of filtrate was taken in another test tube. Add 5 ml of distilled water and shake vigorously. The presence of persistent froth was taken as positive result.

### Test for Flavonoids

0.2 g of dried guduchi and ashwagandha powder separately in different test tubes were dissolved in 1% sodium hydroxide (NaOH). 10% HCl was added and change in the color of solution to yellow indicated the presence of flavonoids.

### Test for Cardiac Glycosides [Kellar-Kiliani test]

2 g of dried powder of guduchi and ashwagandha were taken in different test tubes. 5 ml of distilled water was added in each test tube and then it was boiled for 2 min in water bath at 100°C. The solution was filtered through Whatman No.1 filter paper. The 1ml of extract and 0.5 ml of glacial acetic acid was taken in another test tube. Few drops of 5% Ferric Chloride and few drops of conc.  $\text{H}_2\text{SO}_4$  were added. The appearance of greenish blue color was indicated as the presence of cardiac glycosides.

### Test for Steroids

2 g of dried powder of guduchi and ashwagandha were taken in different test tubes and then boiled with 2 ml of distilled water in water bath at 100 °C for 2 min. The solution was filtered through Whatman No.1 filter paper. The 200  $\mu\text{l}$  of extract and 10 volumes of chloroform and conc.  $\text{H}_2\text{SO}_4$  were added carefully along the sides of test tubes. The change in color of lower layer to yellowish with green fluorescence and reddish upper layer indicated the presence of steroids.

## Test for Alkaloids

### 1. Wagner's test

200  $\mu\text{l}$  of crude extract was taken in test tube. The few drops of Wagner's reagent were added to the inner side of test tube. A reddish brown precipitate was formed which confirmed the presence of alkaloids

### 2. Mayer's and Wagner's test

Equal amount of extract and 1% HCl were added and heated gently. Mayer's and Wagner's reagent were added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

### 3. Dragendorff test

0.2 g of dried guduchi and ashwagandha powder were taken in different test tubes. Add 10 ml of methanol individually and after few minutes, it was filtered with Whatman filter paper no 1. The 2 ml of filtrate in 1 ml of 1% HCl was taken and steam heated the solution for 2

minutes. Again the solution was filtered and 1 ml of filtrate was taken. Six drops of Mayer's reagent/ Wagner's reagent/ Dragendorff reagent were added. The change in color of precipitate to orange red/ brownish red/ creamish showed the presence of alkaloids respectively.

#### 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.

#### Preparation of Mayer's reagent

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

#### Preparation of Dragendorff reagent

##### Solution A

- 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 and made up its volume to 500 ml with distilled water.

#### Test for Reducing Sugar

1 ml of Fehling's solution A and B was added to aqueous extract of guduchi and ashwagandha powder, respectively. The solution was boiled in water bath for 5 to 10 minutes. The presence of non-reducing sugar was indicated by formation of brick red precipitation.

#### Results and discussion

##### Phytochemical analysis of Giloy (*Tinospora Cordifolia*)

The details of result for qualitative analysis of phytochemicals in water and ethanolic crude extract of giloy powder are presented in Table 1. Phytochemical screening of leaf extracts of *T. cordifolia* indicates the presence of alkaloids, cardiac glycosides, tannins, phenols, carbohydrates and flavonoids. The similar results are reported by Rani (2017) [7]. Madhavi *et al.* (2017) [5] carried out the phytochemical analysis of *Tinospora cordifolia* leaf and reported the presence of phytosterols, flavonoids, cardiac glycosides, alkaloids, phenolic compounds and tannins in methanolic and aqueous extracts. Similarly Yadav *et al.* (2011) [10] carried out phytochemical analysis of *Tinospora cordifolia*, *Bryophyllum Pinnatum*, *Terminali abellerica*, *Oldenlandia corymbosa*, *Xanthium strumarium*, *Ipomea aquatica* and *Ricinus communis* and observed the presence of phenols, flavonoids, proteins, tannin, carbohydrates and saponin.

**Table 1:** Phytochemical screening of Giloy (*Tinospora cordifolia*) leaves

S. No.	Secondary Metabolites	Water	Ethanol
1.	Saponin	+	—
2.	Alkaloids	+	+
3.	Phenolics	+	+
4.	Tannin	+	+
5.	Steroids	+	—
6.	Flavonoids	—	+
7.	Cardiac glycosides	+	+
8.	Carbohydrates	+	+
9.	Terpenoids	—	+
10.	Amino acid	—	+

##### Phytochemical analysis of Ashwagandha (*Withania somnifera*)

The results of preliminary phytochemical evaluation of ashwagandha root powder is presented in Table 2. Phytochemical screening of root powder of ashwagandha shows the presence of carbohydrates, starch, tannin, saponin, phenol, glycoside and alkaloid. Findings are similar to the results of the study made by Kushwah *et al.* (2015) [4] 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. Velu and Baskaran (2012) [9] investigated the antimicrobial activity and phytochemical screening of *Withania somnifera* in ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane and hot water. The results of preliminary phytochemical evaluation of ashwagandha root powder is presented in Table 2.

**Table 2:** Phytochemical screening of Ashwagandha (*Withania somnifera*)

S. No.	Secondary Metabolites	Water	Ethanol
1.	Saponin	+	+
2.	Alkaloids	—	+
3.	Phenolics	—	+
4.	Tannin	—	—
5.	Glycosides	—	+
6.	Carbohydrates	—	—
7.	Starch	+	+
8.	Amino acid	+	—
9.	Terpenoids	—	+
10.	Flavinoids	+	+

#### Conclusion

It can be concluded from the present study that phytochemical analysis of *Tinospora cordifolia* and *Withania somnifera* indicates the presence of flavonoids, alkaloids, proteins, phenolic compounds, cardiac glycosides and tannins. Our study revealed the presence of medicinally important constituents in these plant species. These herbal extract can be used for curing diseases without any side effects. These can also act as a source of useful drugs because of the presence of various phytochemical components.

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