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

Iqra Nazir and Rikhi S Chauhan

**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 *Emblica 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 *Hygrophiha spinosa*, *Withania somnifera*, *Zingiber officinale*, *Solanum trilobatum*, *Pсорalea corylifolia*, *Eclipta erecta*, *Ocimum sanctum*, *Picorrhiza 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 Gurcha. 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%).
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 (FeCl₃). 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 tubes 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-Kiliiani 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. 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. H₂SO₄ 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 µl of extract and 10 volumes of chloroform and conc. H₂SO₄ 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 µl 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) [8] 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) [9] 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>
<thead>
<tr>
<th>S. No.</th>
<th>Secondary Metabolites</th>
<th>Water</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Amino acid</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

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>
<thead>
<tr>
<th>S. No.</th>
<th>Secondary Metabolites</th>
<th>Water</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
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</tr>
<tr>
<td>3</td>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannin</td>
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<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>Starch</td>
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<td>+</td>
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<tr>
<td>8</td>
<td>Amino acid</td>
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<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
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<tr>
<td>10</td>
<td>Flavonoids</td>
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<td>+</td>
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</tbody>
</table>

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.

Acknowledgement
The authors are grateful to the Head, Department of Aquaculture and Dean, College of Fisheries, G.B. Pant University of Agriculture & Technology, Pantnagar for providing laboratory and field facilities for conducting present study. The fellowship grant provided by University Grants Commission as Maulana Azad National Fellowship to the first author is great fully acknowledged.
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