PHYTOCHEMICAL SCREENING OF WITHANIA SOMNIFERA LEAF EXTRACT IN DIFFERENT SOLVENTS.

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ABSTRACT

Withania somnifera (Ashwagandha) is an important rasayan herb and widely known as Indian ginseng in Ayurveda. In India, various parts of the plant such as leaf, root have been used to treat various diseases including sexual and nervous disorders, cancer, diabetes, ulcer, stress, arthritis and immunological disorders. The present study comprises of phytochemical screening of leaf extract of withania somnifera in different solvents. The phytochemical investigation was carried out to evaluate presence of alkaloids, carbohydrates, glycosides, tannins, flavonoids, phenolic compounds & phytosterols in different extract of withania somnifera leaf. Results shows the presence of alkaloids, carbohydrate, saponin, flavonoids, fat, oil, phytosterols. The present phytochemical screening of plant extract will be helpful in assigning the quality of crude drug or its bioactive potentials to treat several remedies.

Keywords: Withania somnifera, ashwagandha, phytochemical screening.

INTRODUCTION

Since a long period of time, plants have been an important source of natural products for treatment of various diseases. According to world health organization [1] medicinal plants are the best source of variety of drugs. Plants have been used to treat humans, animals and plant diseases also herbal diseases are used to cure human diseases [2,3].

Withania somnifera belongs to the family Solanaceae grows widely in eastern region of India and used for the treatment of arthritis, tuberculosis, cancer, and sexual transmitted infections [4]. Therefore, the present study was planned to find the various phytochemicals present in different extracts of leafs of Withania somnifera.

MATERIAL AND METHODS

Preparation of extracts: Withania somnifera plant was collected from Government agriculture college indore. The samples were authenticated by Botany Department P.M.B Gujarati Science College, Indore. The plant material was washed under running tap water, shade dried and powdered using mechanical grinder.

Alcoholic Extraction

Alcoholic (alcohol and acetone) plant extract was prepared by Soxhlet extraction method [5]. About 100 gram of powder material was uniformly packed in to thimble and run in the soxhlet extractor. It was allowed to extracted with methanol for the period of about 48 hours. Prepared
extracts were filtered with the help of filter paper and concentrated by vacuum rotator evaporator. The residue was dried and kept in refrigerator at 4°C for further analysis.

**Aqueous Extraction**
For aqueous extraction, 20 grams of leaf powder was dissolved in 200ml of distilled water in a conical flask, boiled at 100°C in a water bath for 8 hours and the filtered through Whatmann No. 1 filter paper. The filtrate was then condensed and stored in room temperature for phytochemical analysis.

**Preliminary phytochemical analysis**
The preliminary phytochemical screening was followed by medicinal chemistry [6].

**Test for tannins and phenolic compounds**
**Ferric chloride test**
5% W/V solution of ferric chloride in 90% alcohol and used for detection of phenols.
**Lead acetate test**: 25% of basic lead acetate solution is used for detection of flavonoids.

**Test for Proteins & amino acids**
**Biuret test**
To 2 ml of test solution was taken with equal value of 10% NaOH and one drop of 10% CuSO₄ solution. A violet colour formation indicates that the presence of peptide linkage.

**Ninhydrin test**
To 1ml of ninhydrin solution, add 1 ml test solution and heat. Formation of violet colour indicates the presence of X-amino acids. Ninhydrin is a powerful oxidizing agent, which causes Oxidative decarboxylation of X-amino acids yielding CO₂, NH₃ and aldehyde. The reduced ninhydrin then reacted with the liberated ammonia forming blue complex proteins and hydroxyl proteins produce a yellow rather than a purple colour with ninhydrin.

**Test for alkaloids**
**Dragendorff’s Reagent**
It is used for the detection of alkaloid. Boil 14g of sodium iodide with 5.2g basic bismuth carbonate in 50 ml glacial acid for few minutes. Allow it to stand overnight and filter off the precipitate of sodium acetate crystals. To 40 ml of the red brown solution is amber coloured bottle. When needed added 20 ml acetic acid to 10 ml of this stock solution and make up to 100 ml with water.

**Hager’s Reagent**
A saturated aqueous solution of picric acid used for detection of alkaloids.

**Test for Carbohydrate**
**Fehling’s test**
2 ml of Fehling’s solution A and 2ml of Fehling’s solution B mixed together and added to 2 ml of sample solution. Boiled for 2 minutes and cooled yellow precipitate shows the presence of reducing sugars.

**Barfoed’s test**
2 ml of Fehling’s solution A and 2 ml of Fehling’s B mixed together and added to 2 ml of the sample solution. Boiled on water bath. Brick red precipitate at the bottom of the test tube shows the presence of monosaccharides.
Test for glycosides
Legal test
Sample was treated with small amount of pyridine in a test tube. A pinch of sodium nitroprusside solution was added. If blood red colour appears. Then alkaloid was present in the sample.

Sodium nitroprusside test
About 0.5-1 ml of sample was taken in a test tube. A pinch of sodium nitroprusside powder and 2-3 drops of sodium hydroxide solution (10 percent) were added. Test tube is shaken and allowed to stand for 2-3 minutes. Appearance of red colour indicates presence of glycosides in the sample.

Test for flavonoids

Shinoda test
To the test solution few reagent of magnesium ribbon were added and concentrated HCL was added drop-wise. Pink scarlet, crimson and red green to blue colour appears few minutes then it shows the presence of flavonoids.

Alkaline reagent test
To the test solution few drops of NaOH solution (10 percent) were added. Formation of an intense yellow colour, which turns colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.

RESULT AND DISCUSSION
Preliminary phytochemical analysis of aqueous, ethanolic and acetone extracts of leaves of *Withania somnifera* shows the presence of alkaloids, flavonoids, phenolic compounds, proteins, carbohydrate and glycosides. (Table no.1).

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Phytochemicals</th>
<th>Ethanol extract</th>
<th>Acetone extract</th>
<th>Aqueous extract</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Phenolic compounds</td>
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<td>Proteins</td>
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</tr>
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<td>5</td>
<td>Carbohydrates</td>
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<td>+</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>
CONCLUSION
The results obtained from this study shows the presence of following phytochemical like alkaloids, flavonoids, phenolic compounds, proteins, carbohydrate and glycosides in leaf extract of Withania somnifera in different solvents.

REFERENCES
2. Goun E, Cunningham G, Chu D, Nguyen C, Miles D. Antibacterial and antifungal activity of Indonesian ethnomedicinal plants. Fitoterapia 74(6); 2003; 592-596.