

Preliminary Phytochemical Evaluation of Root of Natural Grown *Withania somnifera* of Northern Part of India

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ABSTRACT

The Ethno medicine involves the use of different plant extracts or the bioactive constituents of vital importance in the health application at an affordable cost and study of such ethno medicine keenly represents one of the best avenues in searching new economic plants for medicine. Keeping this view in mind the present investigation is carried out in *Withania somnifera* roots collected from the medicinal plant garden of NIAPR, Patiala, Punjab, India. The physicochemical characters and as well as Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids and Quinone.

Key words: *Withania Somnifera*

INTRODUCTION

The traditional Indian system of medicine has a long history¹ of use of plants in various forms. They lack adequate scientific documentation, particularly in light of modern scientific knowledge. These natural compounds are the basis of modern drugs that we are using today. There are many families of phytochemicals those help the human body in a variety of ways. Phytochemicals may protect human from various diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive² properties. Phytochemicals are basically divided into two groups³, primary and secondary metabolites according to their functions in plant metabolism. Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins and so on. Ashwagandha (*Withania somnifera*) is such a medicinal plants with immense uses. It is a small, woody shrub in the Solanaceae family that grows about two feet in height. It is also known as Ashwagandha, Indian ginseng and winter cherry⁴. It is an important herb used in the Ayurveda⁵ and other indigenous medical systems for over 3000 years. This plant is being used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent, and more recently to treat bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia⁶. Clinical trials and animal research support the use of Ashwagandha for anxiety, cognitive and neurological disorders⁷, inflammation, and Parkinson's disease. Ashwaganda's have properties which make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. Ashwaganda is also used therapeutically as an adaptogen for patients with nervous exhaustion, insomnia, and debility due to stress, and as an immune stimulant in patients with low white blood cell counts. It is a native medicinal plant grown all over north-western⁸ and central India. It is an important ancient

plant, the roots of which have been deployed in Indian traditional systems of medicine, ayurveda and unani. It grows well in dry and sub-tropical regions. Ashwagandha is a hardy and drought tolerant plant. Madhya Pradesh, Gujarat, Haryana, Maharashtra, Punjab, Rajasthan and Uttar Pradesh are the main producing states of this crop in the country⁹.

As a result of this wide growing range, there are considerable morphological and chemotypical variations in terms of local species. The present study is aimed to produce the data of different bioactive constituents presents in the natural grown species and some of the physico-chemical parameters¹⁰. As well as the present work unfolds some valuable information and important findings which can establish a scientific standard on identification, purity and quality etc. and may strengthen the Pharmacopoeial standards for this traditional drug.

MATERIALS AND METHOD

The roots of species *Withania somnifera*. were collected from the medicinal plant garden of NIAPR, Patiala, Punjab and authenticated in Department of Pharmacognosy, NIAPR, Patiala, Punjab, India. After authentication, the fresh, roots were collected in bulk in the month of September, during autumn. The roots were washed thoroughly, dried in shade and then milled into coarse (half dust) powder by mechanical grinder. The sample was subjected to phytochemical analysis.

Extraction of Plant Material: The dried roots were ground into a fine powder and the total mass was subjected for extraction by a hot percolation method with water, ethanol and Methanol in soxhlet apparatus for 72 hrs respectively. Each solvent extraction step was carried out for 24 hrs. and after extraction the extracts were concentrated by evaporation and stored at 4°C for further study.

Preliminary Phytochemical Screening^{11, 12:} The

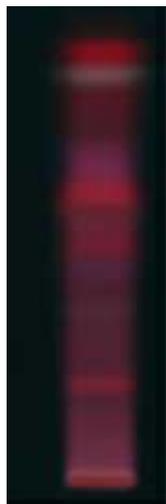
Table 1 : Results of Phytochemical screening of different extract of *Withania somnifera* Root

Class of Phytocompounds	<i>Withania somnifera</i>		
	W	E	M
Steroids	+	+	+
Terpenoids	+	-	-
Alkaloids	+	+	+
Flavanoids	+	-	+
Coumarins	-	-	-
Saponins	-	-	-
Tannins	+	+	+
Phenol	+	+	+
Anthraquinone	+	+	+
Quinone	-	-	-
Catechin	+	-	-

Table 2 : Physicochemical Parameters of *Withania somnifera* Root

Sl. No.	Parameters	Results
1.	Loss on Drying	2.34 %w/w
2.	Total Ash	5.31 %w/w
3.	Acid-insoluble Ash	0.74 %w/w
4.	Water-soluble Ash	1.13 %w/w
5.	Alcohol-soluble Extractive	26.43 %w/w
6.	Water-soluble Extractive	89.64 %w/w
7.	pH (10% aqueous suspension)	4.69

Fig. 1. Chromatogram of Aswagandha root extract visualized at UV 366nm.



phytochemical screening of the extracts was done using standard procedure as described below and the observations were presented in table no.1.

1. Steroids and Terpenoids: 10 mg of the extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of concentrated Sulphuric acid. Blue colour in chloroform layer which changes to green shows the presence of steroids, whereas the appearance of pink colour in chloroform layer shows the presence of terpenoids.

2. Alkaloids: 10 mg of the extract was dissolved in concentrated HCl and filtered. A few drops of solution were poured into the center of watch glass. Mayer reagent was added along the sides of the watch glass with the help of a glass rod. Formation of a gelatinous white precipitate at the junction of two liquid shows the

presence of alkaloids.

3. Flavonoids: 10 mg of the extract was dissolved in methanol. Magnesium turnings were added into this followed by concentrated HCl. A magenta colour shows the presence of Flavonoids.

4. Coumarins: 10 mg of the extract was dissolved in methanol and alcoholic KOH was added. The appearance of yellow colour which decolorizes while adding conc HCl shows the presence of Coumarin.

5) Saponins: Extract was dissolved in water and shaken well. Froth which last for a long time shows the presence of saponins.

6) Tannins: 10 mg of the extract was boiled with 1 ml water for 30 min. The extract was filtered clear and to this 0.5 ml 2% gelatin was added. A curdy white precipitate indicates the presence of tannin.

7) Phenolic compounds: Extract was dissolved in alcohol and 1 drop of neutral ferric chloride was added to this. The intense colour indicates the presence of phenolic compound.

8) Anthraquinone: To the extract Magnesium Acetate solution was added the pink colour developed indicates the presence of Anthraquinone.

9) Quinone: Few mg of the substrate in alcohol is treated with sulphuric acid. The colour developed indicates the presence of Quinone.

10) Catechin: Few mg of the substrate in alcohol is treated with a few drops of Ehrlich reagent and a few drops of concentrated HCl. The pink colour developed indicates the presence of catechin.

(B) Evaluation of quality control parameters^{13,14,15}

1. Organoleptic parameters: Organoleptic parameter like color, odor and taste were carried out.

2. Physicochemical parameters: Physicochemical standards were generally used for deciding the quality and purity of the drug source. The parameters viz. pH of aq. suspension, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, water-soluble extractive values were calculated and recorded (Table 2). These extractive values revealed that the plant material have higher molecular weight components. Higher water extractive value further indicated the presence of highly polar chemical constituents in the plant drug

3. Determination of pH: The pH value of a solution was determined by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according to the manufacturer's instructions. First the apparatus was calibrated using buffer of 4 and 7 pH. 10 g powdered extract was taken and dissolved in 100 ml demineralized water. The electrodes were immersed in the solution and the pH was measured (Anonymous, 1996).

Thin Layer Chromatography¹⁶: Preparation of sample solution: An accurately weighed 0.1gm of dried root powder was extracted in 10 ml of chloroform:ethanol(1:1), warmed at ~70°C temperature for 30 minutes and kept overnight in sealed mouth for total extraction. Then it was filtered using whatman no. 1 in a dry 50 ml volumetric flask and the volume was made up to the mark with methanol to get a sample of 2000 ppm.

Chromatographic conditions:

1. Chemicals used: All the chemicals used here in the experiment were of GR grade.

2. HPTLC Condition: TLC plate: Silica gel 60 F₂₅₄ of E Merck cat No. 1.05554.0007

Development Chamber: Twin trough glass chamber (Camag, Switzerland)

3. Method: On a 3cm x 10cm TLC plate an aliquot of the sample solution was applied as 8 mm band at 10 mm from the base of the plate. Then it was developed in Twin trough glass chamber up to 80 mm using the mobile phase hexane : ethyl acetate : methanol : formic acid : ethyl formate (5.5 : 4 : 0.5 : 0.5 : 0.5).. The developed plate was dried in air and subsequent photography at UV 366 nm were taken.

RESULT AND DISCUSSION

The present study carried out on the plant samples revealed the presence of medicinally important bioactive compounds. The phytochemical characters of the plants investigated were summarized in the Table: 2. The water extract of *Withania somnifera* was found to contain steroids, terpenoids, flavonoids, phenol, quinones and catechin, ethanol extracts show steroids, tannins, phenol, quinines, methanol extracts exhibit only tannins, phenol and quinones. Moreover the physico-chemical data give the characteristics properties of the plant material as well as it indicate the quality of the drug or the raw materials.

REFERENCES

- Lodhi R, Bagga A, Traditional Indian Systems of Medicines, Annals of the Academy of Medicine, 2000; 29(1): 37-41.
- Colleen Carkeet, Grann Kerry, Randolph R. Keith, Phytochemicals, Edn 1, vol.1, CRC Press, Boca Raton, 2012, 115-133.
- Kennedy O David, Wightman .L.Emma, Herbal Extract and Phytochemicals, Advances in Nutrition, 2011; 2: 32-50
- Sharma, MP, Ahmad, J, Hussain, A, Khan, S. 1992. Folklore medicinal plants of Metwat (Gurgaon district), Haryana, India. *Int J Pharmacog.* 30(2): 129-134.
- Chopra, Deepak and Simon, David. *The Chopra Center Herbal Handbook: Forty Natural Prescriptions for Perfect Health.* New York: Three Rivers Press, 2000. ISBN 0609803905, 9780609803905.
- Tirtha, Swami Sadashiva. *The Āyurveda Encyclopedia: Natural Secrets to Healing, Prevention & Longevity.* Bayville: Ayurveda Holistic Center Press, 2005. ISBN 0-9658042-2-4.
- Deocaris, Custer C., Kaul, Sunil C. and Wadhwa, Renu. Biogerontology 9: From proliferative to neurological role of an hsp70 stress chaperone, mortalin. *SpringerLink.*
- Acharya, D. Shrivastava, A. 2008. Indigenous Herbal Medicines (Tribal Formulations and Traditional Herbal Practices). Aavishkar Publishers Distributors, Jaipur. ISBN: 978-81-7910-252-7.
- Sharma, MP, Ahmad, J, Hussain, A, Khan, S. 1992. Folklore medicinal plants of Metwat (Gurgaon district), Haryana, India. *Int J Pharmacog.* 30(2): 129-134.
- Egon Stahl (2009) Thin Layer Chromatography, Second Edition, Springer International, 854
- Finar IL. (1975). Organic chemistry-Stereo chemistry & the chemistry of natural products, fifth edition, Vol. 2, Long man group Ltd., 276.
- Harborne JB. (1973). Phytochemical method-A guide to modern techniques of plant analysis. Springer, 43-45.
- Lohar DR. (2007). Protocol for testing of Ayurvedic, Siddha & Unani medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare: Pharmacopoeial laboratory for Indian medicines, Ghaziabad, 47-52.
- Anonymous. (2002). Quality control methods for medicinal plant materials. World Health Organization,

- Geneva. AITBS publisher and distributors, New Delhi, 16. Stahl E. (2009). Thin Layer Chromatography - A Laboratory Handbook, 2nd edition, Springer (India) Pvt. Limited, 77.
15. Anonymous. (1996). Indian Pharmacopoeia, Vol. II, Government of India, Ministry of Health and Family Welfare, New Delhi, A-81-83, 95, 736.