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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *WITHANIA SOMNIFERA* (L.) Dunal ROOT

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ABSTRACT

Background: *Withania somnifera* (L.) Dunal is a plant of Solanaceae family which is commonly known as *Ashwagandha*, Indian ginseng, and winter cherry. It is a thick hairy herb which is found in the forests of Mandsaur and Bastar in Madhya Pradesh, the foothills of Punjab, Himachal Pradesh, Uttar Pradesh and western Himalayas in India. *Ashwagandha* attains the special name because its root smells like horse ("*Ashwa*") and believe to provide power like horse when consumed. *Ashwagandha* is very famous plant for *Rasayana*, *Balya* and *Vrishya* property in *Ayurveda*. It has several activities including immunomodulation, anti-cancer, anti-epileptic, anti-ageing, antioxidant, cardioprotective, neuroprotective, hypoglycemic, hypocholesterolemic activities, memory enhancer and in common an effective adaptogen. The aim of present article is to put forward the pharmacognostical evaluation of root of *Withania somnifera* (L.) Dunal. **Methods:** Macroscopic evaluation, microscopic evaluation, physicochemical evaluation, extractive values, phytochemical analysis, T.L.C. study were carried out using root of *Withania somnifera* (L.) Dunal and data was obtained. **Results:** Data pertaining to the above cited evaluations was recorded for root of *Withania somnifera*. **Conclusion:** All the values hence obtained were subjected to comparison with their corresponding standard values as mentioned in API. It was observed that all the values were under their normal range.

KEYWORDS: *Withania somnifera*, *Ashwagandha*, *Rasayana*, *Balya* and *Vrishya.*

INTRODUCTION

Ashwagandha (*Withania somnifera*, fam. Solanaceae) is commonly known as "Indian Winter cherry" or "Indian Ginseng". It is a thick hairy herb which is found in the forests of Mandsaur and Bastar in Madhya Pradesh, the foothills of Punjab, Himachal Pradesh, Uttar Pradesh and western Himalayas in India. It is cultivated in Madhya Pradesh, Rajasthan and other drier parts of the country.[1]

It is one of the most important herb of Ayurveda used for millennia as a *Rasayana* for its wide ranging health benefits. *Ashwagandha* is commonly available as a *churna*, a fine sieved powder that can be mixed with water, ghee (clarified butter) or honey. It enhances the function of the brain and nervous system and improves the memory. It improves the function of the reproductive system promoting a healthy sexual and reproductive balance.[2] Ashwagandha root are a constituent of over 200 formulations in Ayurveda, Sidhha and Unani medicine which are used in the treatment of various physiological disorders.[3]

The root of Ashwagandha is regarded as tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. The root smells like horse ("*ashwa*"), that is why it is called Ashwagandha (on consuming it gives the power of a horse).[4] Various clinical & experimental studies proved its utility as anti-anxiety, anti-stress, anti-inflammatory, antidepressant, antioxidant, antiageing, anticarcinogenic, antibacterial, adaptogenic, hemopoetic & immodulation activity along with cognition enhancing & memory improving activity with effect on Parkinson disease, neuritic regeneration and synaptic reconstruction.[5]

Withanolides and alkaloids are the major secondary groups characterized from W. somnifera and are of great medicinal interest. Large numbers of withanolides have been isolated from its roots and leaves which attribute the medicinal property of this plant. Withaferin A represented the first natural lactone of the withanolide series isolated from its shoots. Most of the pharmacological activities of this plant are due to two main withanolides, withaferine A and withanolide D.[6] Due to its wide therapeutic importance it is worthwhile to obtain various qualitative and quantitative standards of drug to prevent its adulteration. Hence the aim of present article is to put forward the pharmacognostical evaluation of root of *Withania somnifera* (L.) Dunal.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Ashwagandha roots were collected by scholar. The botanical authentication of this plant was carried out in B.S.I., Jodhpur (Rajasthan), Letter no. BSI/ AZRC/ 1.12014/ Tech. / 2016-17-(PI.Id.) / 497, Date-14.10.2016, by Mr. Vinod Maina (Scientist- D & Head of Office).

The roots of *Ashwagandha* were collected from Nagaur district (Rajasthan) on 15 september 2016 in rainy season and washed thoroughly with purified water, chopped, dried under shade at room temperature, powdered and stored in polythene bags till further analysis.

Fig. No. 1: Showing crude drug sample (Root of *Ashwagandha*) used in this study.

(1) Macroscopic study: The collected sample i.e rhizomes of *N. jatamansi* were powdered and studied organoleptically, with naked eye and magnifying lens, with the help of Pharmacognostical procedure i.e. Appearance, size, shape, colour, and odour and findings were recorded.

(2) Microscopic study: All fresh samples (root of *Ashwagandha*) were cut in very thin slices with the help of blade and were dipped in water for some time to make them soften. After that staining was done with safranin. After staining, mounting was done on microslides. In this process, sections were transferred on slides & glycerine was added on sections. Then coverslip was put on sections, excess water was wiped out & then the slides were observed in microscope & photos were taken.

(3) Determination of Moisture Content: Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105ºC for 5 hours, and weight of sample was calculated for every 30 minutes, until the weight of the sample came out to be constant, no

variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.[7]

(4) Determination of pH: The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per litre. It practically means the quantitative indication of the acidity or basic nature of a solution. pH value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.[8]

(5) Determination of Extractive values: Determination of Alcohol Soluble Extractive: Alcohol-soluble extractive value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.[9]

Determination of Water Soluble Extractive: Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.[10]

(6) Determination of Total Ash: The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface. Total ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.[11]

(7) Determination of Acid Insoluble Ash: Acid insoluble ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.[12]

(8) Determination of Water Soluble Ash: Water soluble ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India.[13]

(9) Phytochemical screening[14]: Freshly prepared extracts were subjected to preliminary phytochemical screening. Presence of carbohydrates (Molisch's Test, Benedict's test, Barfoed's test, Fehling solution test), alkaloids (Mayer's test, Dragondroff's test, Wagner's Test, Hager's Test), amino acids (Ninhydrin test), proteins (Biuret test, Xanthoprotic test, Millons test), saponins (Foam test), glycosides (Borntragor's test), Phenolic Compound, flavonoids (Shinod test), steroids (Salkowaski test) and tannins (Ferric chloride solution, Lead acetate test, Pot. Dichromate test) were tested.

(10) Chromatography: Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi- quantitative estimation.

Preparation of test sample- The coarsely powdered dried plant materials (1 g) were successively extracted on small scale with methanol (10 ml) at 90°C 1 hrs using Reflux condenser.

Stationary phase- T.L.C. plate coated with 0.25 mm layer of silica gel 60 F254 with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width).

Activation of pre-coated Silica gel 60 F_{254}: Plates were dried in hot oven at 1050 C for one and half hour.

Solvent system- *Cyclohexane: dichloromethane: Ethyl acetate: Methanol* (5:1:4:0.4)

Procedure- Apply 10µl Methanolic extract of *Withania somnifera* on a TLC plate as bends of 10mm. develop the plate to a distance of 8cm from the line of application. Dry the plate in air and spray with solution of *Vanillin sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible.[15]

Rf Value: Measured and recorded the distance of each spot from the point of its application and calculated Rf value by dividing the distance travelled by the spots with the distance travelled by the front of the mobile phase.

RESULT AND DISCUSSION

Table 1: Macroscopical examination.

S. no.		Root	Root powder
1.	External features	Roots are long tapering, uniform, brittle, short and starchy	Smooth fine powder
2.	Shape	Cylindrical, green with longitudinal wrinkles	-
3	Fracture	Short and uneven	-
4.	Color	Yellowish white	Yellowish brown
5.	Odour	Characteristic	Characteristic
6.	Taste	Bitter	Bitter

MICROSCOPICAL EVALUATION OF ROOT

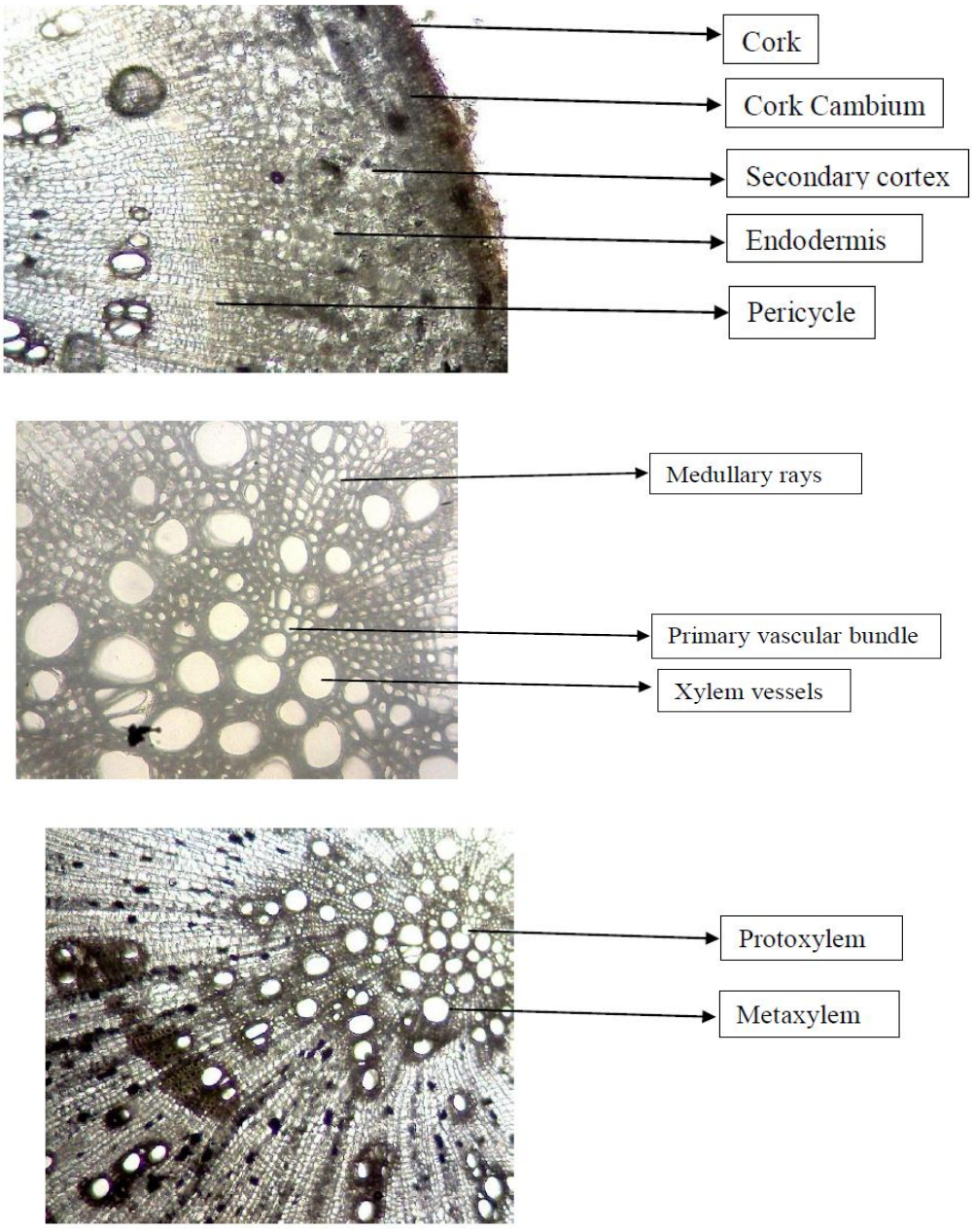

Fig 2: Microscopy of Root of *Withania somnifera.*

Transverse section of root shows cork exfoliated or crushed and non-lignified cells present. Cork cambium of 2-4 diffused rows of cells. Secondary cortex about 15-20 layers of compact parenchymatous cells. Phloem consists of sieve tubes, companion cells, phloem parenchyma. Secondary xylem hard forming a closed vascular ring separated by medullary rays, a few xylem parenchyma.

Table 1: Physico-Chemical Analysis.

Sr.No.	Physicochemical standards	Results % w/w	API standard value (API part-1, Vol.I)
1.	Moisture content	7.58%	Not mentioned
2	P^H Value	5.33	Not mentioned
3.	Water soluble extractive value	24.6%	NLT 15%
4.	Alcohol soluble extractive value	16.8%	NLT 15%
5.	Total ash	6.07%	NMT 7%
6.	Acid insoluble ash	0.92%	NMT 1%
7.	Water soluble ash	1.78%	Not mentioned

Moisture content is water holding capacity of a sample, high moisture content in a sample indicates that it may decrease the stability of the sample. Moisture content in roots was 7.58%.

pH is a method of quantity analysis of acidic and basic nature of drug. pH of roots was 5.33 which is acidic in nature.

Extractive value show soluble content present in sample. Water soluble content present in roots was 24.6%. Alcohol soluble content present in roots was 16.8%.

Total Ash is a quantity analysis technique to determine siliceous material and inorganic substance in a sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of inorganic substance in Ash. Roots have Total Ash 6.07%, Acid Insoluble Ash 0.92% and Water Soluble Ash 1.78%.

Table 2: Qualitative Phytochemical Tests Of Extracts Of Root Of *Withania Somnifera.*

Carbohydrate test			
Sr. no.	**Name of test**	**Water extract**	**Alcohol extract**
A.	Molisch test	+ve	-ve
B.	Benedict test	+ve	-ve
C.	Barfoed's test	-ve	-ve
D.	Fehling test	+ve	-ve
2. Alkaloids			
A.	Dragondrof test	-ve	-ve
B.	Wagner's test	-ve	-ve
C.	Hager's test	+ve	-ve
3. Amino acids			
A.	Ninhydrine test	+ve	-ve
4. Proteins			
A.	Biuret test	-ve	-ve
B.	Xanthoprotic test	+ve	+ve
C.	Millon's test	+ve	+ve
5. Saponin			
A.	Foam test	+ve	+ve
6. Glycosides			
A.	Borntragor's test	+ve	+ve
7. Phenolic compound			
A.	Phenolic test	-ve	-ve
8. Steroids			
A.	Salkowaski reaction	+ve	-ve
9. Tannin			
A.	FeCl3 test	-ve	-ve
B.	Lead acetate test	+ve	-ve
C.	Potassium dichromate test	-ve	-ve
10. Flavanoids			
A.	Shinod test	-ve	-ve

Table 3: Chromatography of Methanolic Extract of Root.

TLC	Extract	Mobile phase	Derivatization	Rf values
	Methanolic extract	*Cyclohexane: dichloromethane: Ethyl acetate: Methanol* (5:1:4:0.4)	*Vanillin sulphuric acid reagent*	0.38, 0.43, 0.49, 0.60, 0.78, 0.90, 0.96

CONCLUSION

The morphological examination which includes macroscopic and microscopic characterization is preliminary study to decide authenticity of drug. Physico-chemical analysis and phytochemical analysis give qualitative information about the purity and the standard of the crude drug. The TLC is a very important parameter for herbal drug standardization and for proper authentication of the medicinal plants.

Macroscopic, organoleptic and microscopic characteristics of the *Ashwagandha* root were in accordance with those are given in Ayurvedic pharmacopeia of India Part I, Volume I.

Moisture content, total Ash values, water soluble extractive value, alcohol soluble extractive values of root powder were recorded. All the values were found similar to the standard values mentioned in API.

On phyto-chemical analysis of *Ashwagandha* root, it was reported that carbohydrates, reducing sugars, ketone functional groups, alkaloids, proteins with aromatic amino acids, protein with secondary and primary amines, amphipathic glycosides, tannins, anthraquinone glycosides, phenolic compounds, steroids, and flavonoids were present in aqueous and alcoholic extract.

The above phyto-chemical analysis shows that *Ashwagandha* root contain both primary & secondary metabolites which are required for plant's growth & are present in a plant when plant is fully grown. It means sample was collected at the perfect time with its full growth & are authentic one.

CONFLICTS OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Harish kumar singhal et al., A review on Ashwagandha [Withania somnifera (L.) Dunal], Int J Ayu Pharm Chem, 2014; 1(1).
2. Singh, Narendra et al. "An Overview on Ashwagandha: A Rasayana (Rejuvenator) of Ayurveda." *African Journal of Traditional, Complementary, and Alternative Medicines* 8.5 Suppl (2011): 208–213. *PMC*. Web. 2 Sept. 2017.
3. Mamidi, Prasad, and Kshama Gupta. "Journal of Pharmaceutical and Scientific Innovation."

4. Sharma PV. Ashwagandha, Dravyaguna Vijana, Chaukhambha Viashwabharti. Varanasi, 1999; 763–765.
5. Harish kumar singhal et al., A review on Ashwagandha [Withania somnifera (L.) Dunal], Int J Ayu Pharm Chem, 2014; 1(1).
6. Bano, Anisha, et al. "A Systematic and Comprehensive Review on Withania somnifera (L.) Dunal-An Indian Ginseng." *Br J Pharm Res*, 2015; 7.2: 63-75.
7. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush, 2007; Appendices-2.2.9.
8. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush; 2007. Appendices-3.1.3
9. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush, 2007; Appendices-2.2.6.
10. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush, 2007; Appendices-2.2.7.
11. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush, 2007; Appendices-2.2.3.
12. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush, 2007; Appendices-2.2.4.
13. The Ayurveda Pharmacopeia of India: Part I, Vol-I. New Delhi: Govt. of India, Ministry of health and family welfare, Dept. of Ayush, 2007; Appendices-2.2.5.
14. Prof. G. S. Lavekar. Laboratory guide for the analysis of Ayurveda and siddha formulations. New Delhi: CCRAS, Dept. of Ayush, Ministry of health and family welfare, Govt. of India; 2010.
15. Luxmini. K.P.A.M.K et al. Comparative Pharmacognostic Study of Different Parts of Withania somnifera and its Substitute Ruellia tuberosa. World Journal of Agricultural Research, 2015; 3(1): 28-33. Doi: 10.12691/wjar-3-1-7.