



PHARMACOGNOSTIC STUDY & PHYSICO-CHEMICAL ANALYSIS OF ASHWAGANDHA (*WITHANIA SOMNIFERA* LINN.) -ROOT

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Abstract

Withania somnifera family Solanaceae is commonly known as “Indian winter cherry” or “Indian Ginseng” or Ashwagandha in Hindi. It is the most important medicinal plant of Indian traditional System of Medicine. Plant root smells like horse (*Ashwa*), so it is called Ashwagandha (Means it gives the power of a horse). It is commonly used as a tonic and also to treat arthritis, leucoderma, constipation, insomnia, nervous breakdown, goiter etc. Crusted root paste is applied to reduce the inflammation at the joints. The present communication deals with a details account of the pharmacognostic study and physicochemical analysis carried out on root of the *Withania somnifera*. The study includes microscopy, powder microscopic studies, physicochemical tests, preliminary phytochemical screening, and development of HPTLC (High Performance Thin Layer Chromatography) fingerprints profile and heavy metal tests. Physicochemical tests were performed and found average values of root such as loss on drying at 105°C 5.12% w/w, total ash value 7.15% w/w, acid insoluble ash value 1.5% w/w, alcohol soluble extractive value 15.50% w/w and water soluble extractive value 25.14%w/w. Preliminary phytochemical tests were performed and found varous types of the phytoconstituents viz. Alkaloids, tannins, flavonoids, saponins and proteins. HPTLC (High Performance Thin Layer Chromatography) fingerprints profile of methanolic extract was done by using mobile phase toluene: ethyl acetate (7:3). Methanolic extract sample was applied, developed the plate and major spots R_f values with colour were recorded before derivatization at 254nm, 366nm and after derivatization at 366nm. Heavy metals (Pb, Cd, As, & Hg) were tested and found under WHO limits Established parameters can be used as standards for quality control and identification of the plant in herbal compound formulations and also preparation of a monograph of the plant.

Keywords: *Withania somnifera*, Physicochemical, HPTLC fingerprinting, Phyto-chemical screening, Pharmacognosy

INTRODUCTION

For millennia, people have used herbal remedies because they are safe, effective, and acceptable in their culture. Throughout history, people have used plants and items derived from them to treat and prevent disease, with differing degrees of effectiveness. Written records about medicinal plants date back at least 5000 years to the Sumerians and ancient records are

suggested earlier uses of medicinal plants. The reason for the herbal medicine popularity and acceptability is belief that all natural products are safe, non-toxic, less side effects, easily availability, and affordable prices and due to side effects of synthetic products. Therefore now a days, there is a revival of interest with herbal based medicine due to the increasing realization of the healthcare (Kamboj VP.,2000; and Kalia AN, 2005).

Withania somnifera family Solanaceae is commonly known as Ashwagandha or winter cherry. It is an evergreen herb and found in India, Middle East, and Africa etc. The plant is short shrub, branched, 0.5-1.5 meter tall. Leaves are elliptic, dully green, up to 12-15 cm long, 5-10 cm broad, flowers are bell shaped, green and small, fruit color greenish when ripe convert in to orange-red (Bhattacharya et. al., 1987; Sharma PV., 1999). Whole plant of the Ashwagandha is used to treat various ailments of human as well as animals root is considered a stimulant, tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, and thermogenic (Bhattacharya, 1995). Root smell like a horse so it is called Ashwagandha. It is frequently used for goiter, vitiated vata conditions, rheumatism, leucoderma, constipation, sleeplessness, neurological breakdown, emaciation in children (when given with milk, it is the finest tonic for children), debility from old age, and rheumatism (Mishra et. al., 2000). To lessen joint inflammation, use the paste that is created when water is crushed into roots (Bhandari, 1970). Additionally, it is used locally to painful swellings, ulcers and carbuncles (Misra B, 2004). The root is prescribed for both snake venom and scorpion stings when used in combination with other medications. There are several phyto-constituents *are contains in Ashwagandha* such as steroidal lactones (withanolides and withaferins) alkaloids (anahygrine, isopelletierine, cuseohygrine, anaferine, etc.), and saponins. Sitoindosides and acylsterylglucosides are found in Ashwagandha plant root and working as anti-stress agents. Active chemical constituents sitoindosides VII-X and Withaferin-A, have been shown to have significant anti-stress activity. Despite the numerous medicinal uses of the plant parts, there are no systematic pharmacognostical studies of this plant have, so far been carried out. Therefore the present work undertaken including morphological study, physicochemical analysis, phytochemical tests and High-Performance Thin Layer Chromatography.

MATERIALS AND METHODS

Collection of samples

Fresh plant of Ashwagandha was collected from the Arogyadham campus, Deendayal Research Institute in Chitrakoot, Satna (M.P.) India. Dr. Manoj Tripathi, Senior Scientist & Head (R&D Department), Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.), India, identified and verified the plant materials. Prepared the herbarium (voucher specimen, DRI/Arog./245), and placed in herbarium department, for further reference (Evans W.C., Trease, 2002). Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of High Performance Thin Layer Chromatography fingerprint profile.

Macroscopic and microscopic study

Macroscopic or organoleptic characters of Ashwagandha root like appearance, colour, odour and taste were evaluated. For transverse sections of Ashwagandha fresh root was taken

between the thumb and two fingers of right hand and sections were cut by using sharp razor held in the left hand, (for leaf used the potato pieces and put leaf in incision made on potato piece). Numerous sections cut and examined under microscope. The best sections were selected and transferred in to water containing watch glass, added the chloral hydrate solution. Sections were stained with phloroglucinol and hydrochloric acid (1:1) and the same was mounted in glycerin. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 211 with Digi-eye camera using Caliper plus version 4.2 software (Anonymous, 1996 and Khandelwal KR , 1998).

Powder microscopic study

The shade dried root of Ashwagandha was powdered and completely passed through 355 μm IS Sieve (old sieve number 44). about 2 g of powder washed thoroughly with potable water, poured out the water without loss of material. Mounted a small portion in glycerin were used to all characters of the Ashwagandha root. Small quantity of sample cleared by heating with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, another small quantity of sample stained with sudan red solution and mounted with glycerin, all mounted slides were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope and captured the images (Kokate C. K., 2006).

Physico-chemical tests

Physico-chemical tests were performed and set up the certain standards for Ashwagandha root in order to avoid the batch-to batch variation and also to check their adulteration and quality. The study was also giving an idea regarding the nature of phyto-constituents present, quality, safety and efficacy of drugs. Physico-chemical tests of drugs powder were carried out such as moisture content (loss on drying at 105⁰C), water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble extractive value (Anonymous, 1991).

Preliminary phyto-chemical analysis

Phyto-chemical tests for screening and identification of bioactive chemical constituents present in the *Ashwagandha* root, sample was performed the water and ethanolic extracts (Anonymous 2007 and Gupta A.K., 2003).

High Performance Thin Layer Chromatography (HPTLC) fingerprint profile

For High performance thin layer chromatography, about 5gm of root powder was extracted with 100 ml of methanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F₂₅₄ (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 μl Hamilton syringe. The sample, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates was developed using mobile phase consisting of toluene: *ethyl acetate* (7: 3v/v) near ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, Thin Layer Chromatography plate was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag

Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at UV light with Win cat software and R_f values noted (Mitra & Mehrotra, 1980 and Tripathi & Sikarwar, 2014).

RESULTS AND DISCUSSION

Macroscopic characters

Ashwagandha root external colour is brown, internal color white, taste, little sweet, and odor characteristics. Root cylindrical 15-25 cm long, 1 -3 cm in diameter, (**Figure 1-2**).



Figure- 1 Plant



Figure -2 Root

Microscopic characters

Detailed Transverse Section *Ashwagandha* root shows 3-8 rows of cork cells. Followed by 12 - 20 rows of cortical parenchymatous cells filled with simple and compound starch grains, calcium oxalate crystals. Uni to multiseriate medullary rays, xylem is wide consisting of thin walled fibres.

Powder microscopic characters

Under microscope *Ashwagandha* root powder showed thick walled cork cell, cortical parenchymatous cells containing simple and compound starch grains and spheroidal crystals of calcium oxalate, medullary rays cells containing simple starch grains that are up to 30μ , mular shaped, mostly single, occasionally groups of two or three with slit like or stellate hilum (**Fig3a-3c**).

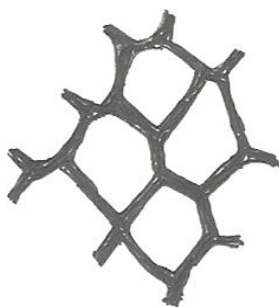


Fig.3a-Cork cell

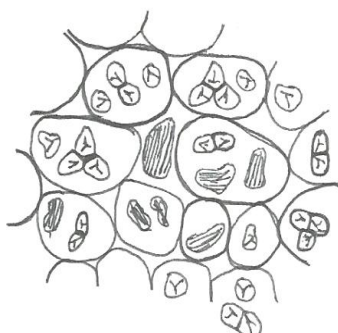


Fig.3b-Cortical parenchyma

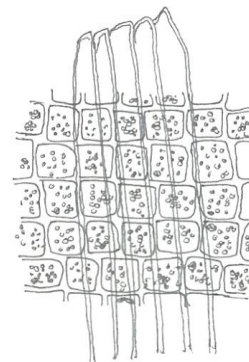


Fig.3c-Medullary rays

Physico-chemical tests

The physico-chemical tests such as Loss on drying on 105⁰C, water soluble extractive value and alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean (n=3) $\pm$ standard deviation in w/w. loss on drying at 105⁰C 5.12% w/w, total ash value 7.15% w/w, acid insoluble ash value 1.5% w/w, alcohol soluble extractive value 15.50% w/w and water soluble extractive value 25.14%w/w were found.

Preliminary phyto-chemical investigation

Preliminary phyto-chemical analysis was performed in water and ethanolic extracts of Ashwagandha root powder was carried out and outcomes are shown in **table 1**.

Table1 Preliminary phyto-chemical investigation of Ashwagandha root

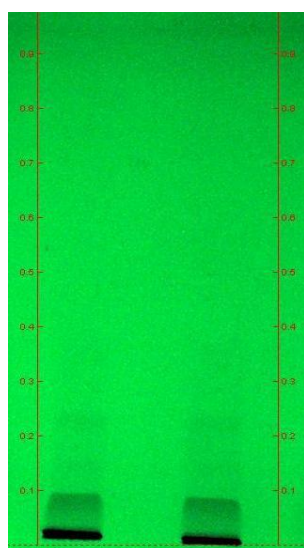
S. No.	Name of Experiments	Observation	Result
1.	Alkaloids		
	a) Mayer's test	Yellow colour appear	Present
	b) Wagner's test	Brown colour appear	Present
	c) Dragendorff's test	Orange colour appear	Present
2.	Carbohydrate		
	a) Anthrone's test	Dark colour appear	Present
	b) Fehling's test	Green colour appear	Present
	c) Molisch's test	No red – violet ring disappear	Present
3.	Proteins		
	a) Bieuret's test	Green colour appear	Present
	b) Millon's test	White ppt are not appear	Present
5.	Resins	Turbidity are seen	Present
6.	Saponins	Honey comb – like structure are form	Present
7.	Starch	Red colour is formed	Absent
8.	Flavonoid		Absent
	a) Ferric chloride test	Reddis pink colour is appear	Absent
	b) Alkaline reagent test	On addition of dilute acid yellow colour disappear	Absent
9.	Steroid		
	a) Salkowski's reaction	A red colour is disappear in the chloroform layer	Absent
10.	Glycoside		
	a) Borntrager's Test	Colour is change	Present
11.	Tannin	Greenish colour appear	Absent
	a) Lead acetate Test	Reddish brown bulky ppt. are formed	Absent

HPTLC finger print profile

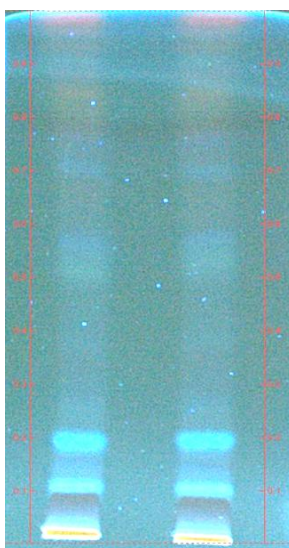
High performance thin layer chromatography (HPTLC) study of the methanolic extracts of Ashwagandha root spots applied in precoated TLC plate. by spotting test solution 6 μ l on pre-

coated silica-gel aluminum plate 60 F₂₅₄ (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 4 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. The plate was developed using a mobile phase consisting of *toluene: ethyl acetate* (7:5v/v). Linear ascending development was carried out in a 20x20cm twin through glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for the mobile phase (20 ml) was 30 min at room temperature. The length of the chromatogram run was 85 cm. Subsequent to the development, a thin layer of chromatography plate was dried at room temperature. The peak area for sample were recorded with the camera photo documentation system Camag Reprostar 3. Major spots R_f values with colour were recorded before derivatization at 254nm, 366nm and after derivatization at 366nm (Fig.4 & table 2).

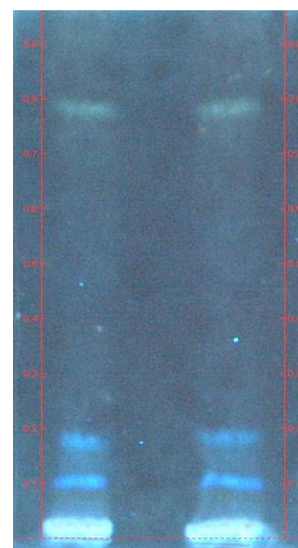
Figure: 4 HPTLC Fingerprint profile of test solution of Ashwagandha root



1 2
Fig. A: 254 nm
before derivatization



1 2
Fig. B: 366nm
before derivatization



1 2
Fig. C: 366nm
after derivatization

Where Track 1: test solution Ashwagandha root, Track 2: test solution Ashwagandha root

Table-2: R_f values of HPTLC fingerprints profile of Ashwagandha root			
R_f values	245nm before derivatization	366nm before derivatization	366nm after derivatization
R _{f1}	0.09 (black)	0.04(sky blue)	0.04(sky white)
R _{f2}	0.24 (light black)	0.08 (slaty brown)	0.10 (sky blue)
R _{f3}	-	0.12(sky blue)	0.18(sky blue)
R _{f4}	-	0.20(sky blue)	0.78(yellowish brown)
R _{f5}	-	0.95 (red)	-

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Ashwagandha root. The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. It was observed that the phytochemical higher present in aqueous extract than the ethanolic extracts. The results indicated that the alkaloids, flavonoid, saponins tannins and phenolic compounds were present in the plant root extracts. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug.

CONCLUSION

The importance and uses of herbal medicines are increasing day by day all over the world. The reason for the herbal medicine popularity and acceptability is belief that all natural products are safe, non-toxic, less side effects, easily availability, and affordable prices and due to side effects of synthetic products. Hence the standardization and quality control of medicinal plants present day need. Whole plant of the Ashwagandha is used to treat various ailments of human as well as animals. Root is considered a stimulant, tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, and thermogenic.

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