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A PRELIMINARY PHYSICO-CHEMICAL ASSAY OF ASHWAGANDHA GRANULES A PILOT STUDY

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ABSTRACT
Withania somnifera, also known as Ashwagandha, Indian ginseng and winter cherry has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defence against disease and creating a sense of mental wellbeing. Various researches support the use of *Withania somnifera* for anxiety, cognitive and neurological disorders.
Ashwagandha contains steroidal compounds known as withanolides including withaferin A and withanolide A. These have been reported to be responsible for significant biological activities and are recommended as active markers for standardization. In the present study an attempt was made to evaluate the physico-chemical profile of Ashwagandha granules. Pharmacognostically authenticated *Withania Somnifera* root powder was used for the preparation of Ashwagandha granules and it was analyzed through qualitative and quantitative analysis for physico-chemical parameters. Fingerprints of Thin Layer Chromatography (TLC) and High- Performance Thin Layer Chromatography study (HPTLC) was also carried out.
KEYWORDS: *Withania Somnifera,* Ashwagandha granules, Physico-Chemical profile, Chromatography

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INTRODUCTION

Withania somnifera, also known as Ashwagandha, Indian ginseng and winter cherry has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defence against disease, arresting the ageing process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and creating a sense of mental well being.[1] It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects.[2]
Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support the use of *Withania somnifera* for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease. *Withania somnifera* chemo preventive properties make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. Recently *Withania somnifera* is also used to inhibit the development of tolerance and dependence on chronic use of various psychotropic drugs.[3]
Ashwagandha contains steroidal compounds known as withanolides (steroidal lactones with ergostane skeleton) glycol with anolides and alkaloids. These include withaferin A, withanolides G&D sitoindosides IX&X & with asomnine. Total alkaloids are about 0.2 %.[4-8] these have been reported to be responsible for significant biological activities and are recommended as active markers for standardization.[9-10]
There has been an exponential growth in the field of herbal medicine in the last few decades. It is getting

popularized in developing as well as in developed countries owing to its natural origin and lesser side effect. In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. But the scenario has changed now, herbal medicines are being manufactured on the large scale in Pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters.[11] Hence in the present study evaluation of pharmacognostical features and standardization of Ashwagandha granules was attempted following the scientific parameters including organoleptic characters, physicochemical analysis and chromatographic pattern.

AIMS AND OBJECTIVES

1. Pharmacognostical study of powdered drug – Ashwagandha
2. Physico-chemical analysis of Ashwagandha granules

Method of preparation of the Ashwagandha Granules

Fine powder was obtained after drying the roots of Ashwagandha and subjecting it to pulverization. Equal quantity of sugar was taken and syrup was prepared by adding sufficient quantity of water in mild flame with constant stirring till syrup reaches the thread like stage (Tantumatvam). Then Ashwagandha powder was added to the sugar syrup and mixed thoroughly to prepare a homogeneous blend. The blended mass was sieved through a 40# sieve to obtain granule form and kept it for drying in room temperature.

MATERIALS AND METHODS

Plant Material

The dried roots of *Withania somnifera* were collected from the Pharmacy, Gujarat Ayurved University, Jamnagar. The roots were pulverized and sieved through No. 80 and fine powder was collected. The fine powder was subjected to powder microscopy.

Pharmacognostical Study

Morphological, Organoleptic and Microscopic study of the powdered drug was done as per the guidelines of Ayurvedic pharmacopoeia of India[12] at Department of Pharmacognosy, I.P.G.T & R.A, Jamnagar. The powder was converted in to granules at the Pharmacy, Gujarat Ayurved University, Jamnagar.

Physico - chemical study

Ashwagandha granules were analyzed by using qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory of I. P. G.T & R. A., Gujarat Ayurved University, Jamnagar.

RESULTS & DISCUSSION

Pharmacognostical Study

Organoleptic Characters

Powder of the Ashwagandha root was whitish brown with characteristic odour, bitter and acrid taste.

Powder Microscopy

The dried powder of Ashwagandha (*Withania somnifera*) was mounted in the distilled water to detect microscopic characters. Starch grains, parenchyma cells, prismatic crystals, tracheids, fibers with pitted vessels, spiral vessels, cork in surface view and tangentially elongated cork was seen. When stained with phloroglucinol and conc. HCl pitted vessels, spiral vessels, pitted fibres and lignified fibres with pitted tracheids were seen. When stained in iodine solution cork, cortical parenchymatous cells embedded with simple & compound starch grains were seen (Plate 1). All the microscopic characteristics identified was equivalent to the standard profile.[12]

Physico-Chemical Study

Organoleptic characters

The characters of the sample are tabulated in table 1.

Physico-chemical parameters

The granules were evaluated for physico chemical parameters like total ash value, loss on drying, pH value, Sugar estimation (Total sugar, Reducing Sugar, Non - Reducing Sugar), acid soluble and water-soluble extractive values. The results are placed at table 2.

The Common parameters mentioned for Ashwagandha in Ayurvedic Pharmacopoeia of India are total ash, pH Value, water and alcohol soluble extractives.[13] On its basis the parameters like total ash content, water and methanol soluble extractives etc., were selected. Presence of more moisture content may create problems in preservation of the sample. Hence loss on drying was also selected as one of parameters. Sugar estimation was considered, as another parameter as the sample was in form of granules hence there is a possibility of significant sugar content.

Total sugar was found to be 50.86 % w/w suggesting presence of considerable amount of sugar in the sample. The water-soluble extractive and methanol soluble extractive values were found to be 69.6 % and 21 % respectively, indicating considerable amount of polar compounds in the sample.

Qualitative Test of Ashwagandha Granules

The methanol extract of the sample was analyzed qualitatively for different functional groups. Details are placed at table 3.

Thin Layer Chromatography

Methanol extract

Granules weighing 5 gm are taken with 100 ml of methanol kept for twenty-four hours. Filtrate was

prepared and evaporated till it gets dried in a flat-bottomed shallow dish and concentrated on water bath to volume of requirement.

TLC is mentioned as a primary tool for identification as part of monographs on all medicinal plants.[13] Alkaloid fraction was used for the spotting of the TLC plate (Silica gel G Precoated plates). Then the spotted TLC was run with the solvent systems Chloroform (9ml), and Methanol (1ml). And the resulting TLC pattern was viewed under long wave ultra violet light at 366 nm or Short wave ultra violet light at 254 nm (Table 4). Then after spraying with the Dragandroff's reagent and drying in a hot air oven 2 spots were viewed under daylight (Table 5)

TLC of alcoholic extract of drug on silica get "G" plate using Chloroform (9ml): Methanol (1ml) shows two spots Under 366 nm U.V at hRf 28 and 60. Where as in 254 nm no spots were seen. On running mobile phase over stationary phase, well distributed, distinct, clear spots were observed without clumping. Thin Layer Chromatography of alcoholic extract of Ashwagandha Granules after spraying Dragandroff's reagent followed by heating and then visualized in day light shows 2 prominent pink colour spots at hRf 44 and 84.

High Performance Thin Layer Chromatography

Methanol extract of Ashwagandha Granules were spotted on precoated silica gel GF 60_{254} Aluminium plate as 5mm bands, 5mm apart and 1cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Chloroform (9 ml) and Methanol (1ml) (v/v) (20ml) was used as a mobile phase. The development distance was 5.8cm (development time 30 min.).After development, Densitometric scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 Camag) (Fig 1). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s-1 (Table 6).Then the plate was sprayed with Dragandroff's reagent followed by heating and then visualized in day light shows 2 prominent spots (Table 7).

CONCLUSION

Ashwagandha is one of the most highly valuable herbs in the Ayurvedic medical system. The use of Ashwagandha in Ayurvedic medicine dates back over 3000 to 4000 years where it was widely extolled as a Rasayana. Ashwagandha is also considered a premiere anti-aging herb due to its rejuvenation and longevity enhancers. It is also one of the most widely used herbs by Ayurvedic practioners in the present era. Most of the formulations have proven to be clinically effective but many of the patient's have difficulty in taking the drug in powder form. Hence here the drug was prepared in granule form taking in consideration patient's compliance, as they are often a good choice for patients because of their ease of use, gentle taste and increased palatability.

The plant *Withania somnifera* was identified and authenticated pharmacognostically and was used as a unique ingredient. The formulation namely, Ashwagandha Granules was subjected to phytochemical, physico-chemical, TLC and HPTLC studies. It was inferred that the formulation meets the minimum qualitative standards as reported in the API at a preliminary level.

On the basis of our observations and experimental results, we created a method of preparation of Ashwagandha granules for the first time, which is economical in terms of time and labour. This study may be used as reference standard in the further quality control researches. Further studies may be carried out based on identification and separation of active ingredients with the help of Biomarkers.

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Table 1. Organoleptic characters of Ashwagandha granules

Sr.No	Parameters	Sample - Ashwagandha Granules
1	Texture	Rough
2	Colour	Brownish
3	Taste	Sweet, Bitter
4	Odour	Non Irritant

Table 2 – Physico-chemical parameters of Ashwagandha granules

Sr. No.	Parameters	Sample -Ashwagandha Granules
1.	Loss on drying	0.105 % w/w
2.	Water soluble Extract	69.6 % w/w
3.	Alcohol soluble Extract	21 % w/w
4.	Total Ash	6.19 % w/w
5.	pH Value	5.16(Acidic)
6.	Sugar Estimation	Total Sugar – 50.86%w/w Reducing Sugar – 10.76%w/w Non - Reducing Sugar – 38.2% w/w

Table 3 – Qualitative tests for Ashwagandha granules

Sr.No	Test	Name of Reagents	Results
1	Carbohydrate	Molisch's test	Positive
2	Steroid	Liebermann – Burchard test	Negative
3	Saponin Glycosides	Foam Test	Negative
4	Cardiac glycosides	Keller killiani test	Positive
5	Flavonoids	Shinoda test	Positive
6	Alkaloids	Dragandroff's test	Positive
7	Starch	Iodine test	Positive

Table 4: TLC of Methanol Extract of Ashwagandha Granules

Extract	Solvent system	Wavelength	Number of spots	hR_f value	Observation
Methanol extract	$CHCl_3$: MeOH (9:1)	366 nm	2	28 60	Blue spot Blue spot
		254 nm	No spots	-	-

Table 5: After spraying with Dragandroff's reagent

Extract	Solvent system	Spray	Number of spots	hR_f value	Observation
Methanol extract	$CHCl_3$: MeOH (9:1)	Dragandroff's reagent	2	44 84	Pink spot Pink spot

Table 6: HPTLC of Methanol Extract of Ashwagandha Granules

Extract	Solvent system	Wavelength	Number of spots	hR_f value
Methanol extract	$CHCl_3$: MeOH (9:1)	366 nm	2	6 92
		254 nm	4	6 10 24 92

Table 7: HPTLC - After spraying with Dragandroff's reagent

Extract	Solvent system	Spray	No. of spots	hR_f value	Observation
Methanol extract	$CHCl_3$: MeOH (9:1)	Dragandroff's reagent	2	6 90	Pink spot Pink spot

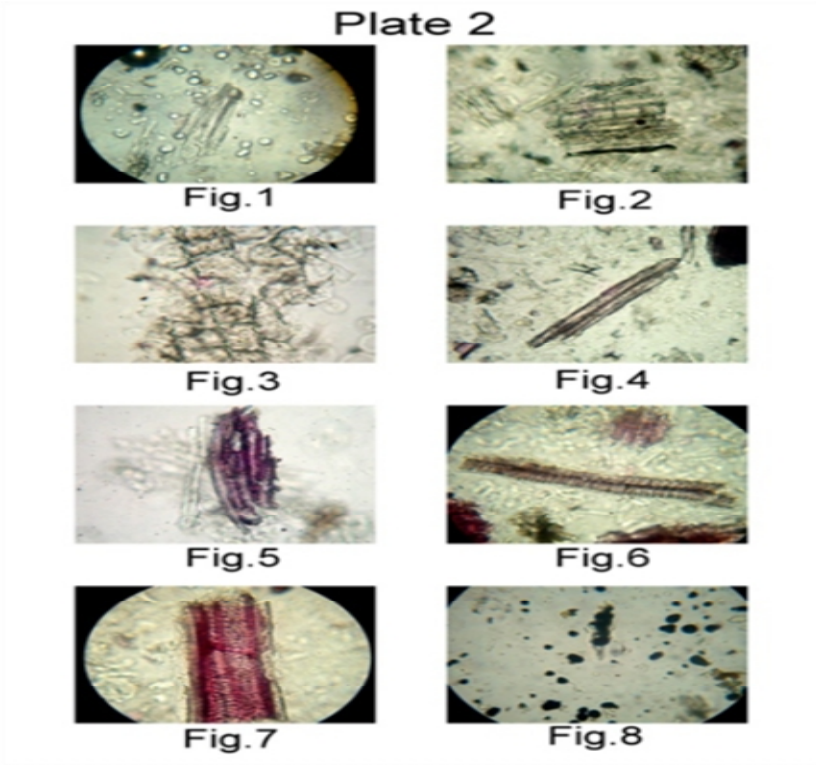
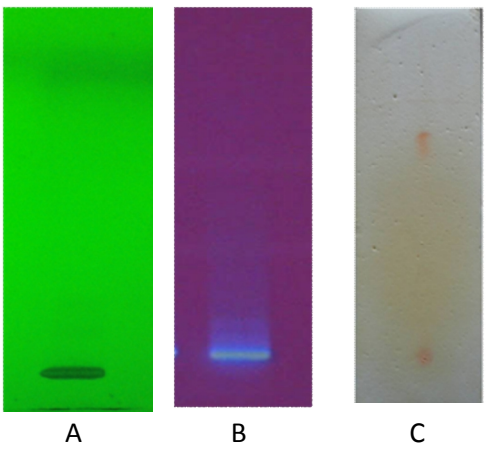


Plate No.1: Powder Microscopy of Withania somnifera root

Fig.1) Parenchyma cells with simple& compoundStarch grains (20X) Fig.2) Tangentially elongated parenchyma cells (20X) Fig.3) Parenchyma cells in surface view (20X) Fig.4) Pitted fibers (Stained 1) (20X) Fig.5) Lignified fibres with pitted tracheids (Stained 1) (20X) Fig.6) Spiral vessels (Stained) (20X) Fig.7) Pitted vessels (Stained) (20X) Fig.8) Simple & compound starch grains (Stained 2) (20X)
Stain 1- Phloroglucinol+Conc.HCl, Stain 2 – Iodine solution



A.254 nm UV light

B.366 nm UV light

C.After spraying with Dragandroff's reagent

Plate No. 2: TLC of Ashwagandha granules

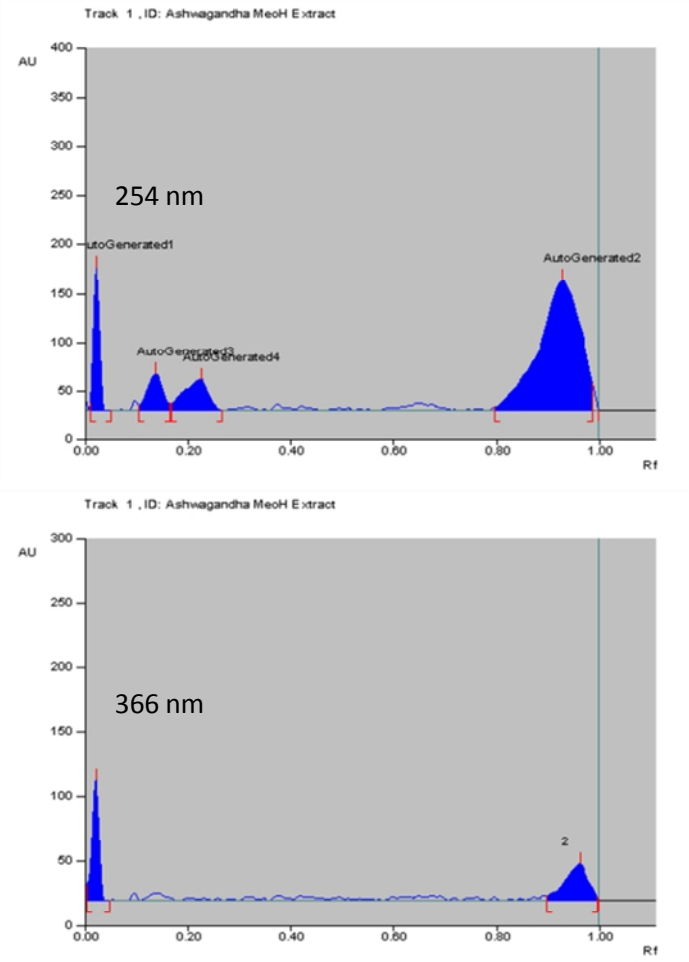


Figure 1: Densitogram curve of Ashwagandha Granules Extract in 254nm & 366nm

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