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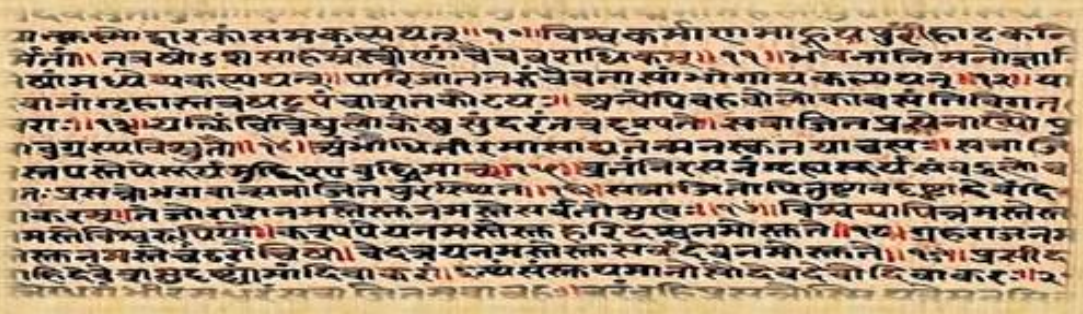

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CHROMATOGRAPHICAL STUDY OF WILD AND CULTIVATED SAMPLES OF ASHWAGANDHA (WITHANIA SOMNIFERA DUNAL)

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ABSTRACT:

Ashwagandha (Withania somnifera dunal) is especially important herb in TSM because of its multidimensional phytochemical profile. It contains more than 12 alkaloids and 35 withanolides. In the present study Ashwagandha cultivate by using Organic manure specifically Farmyard Manure and collected from wild source of same age. The chromatographic profile of both the samples were observed and compared for its values. The finding suggests that Chromatographically (HPLC- both the wild and cultivated Ashwagandha sample appears to be the equally potent so cultivated source is as an optimal source of ashwagandha.These bioactive compounds are known for their adaptogenic, anti-inflammatory, antioxidant, and neuroprotective properties, making Ashwagandha a versatile herb used to promote overall health, reduce stress, enhance cognitive function, and support immune function.

KEY-WORDS: chromatography, cultivation, wild and cultivated Ashwagandha

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INTRODUCTION:

Ashwagandha (Withania somnifera Dunal) is widely used, prioritised Ayurvedic herb having antistress, antioxidant, general tonic uses. More than 200 ayurvedic formulations use Ashwgandha as main ingredient. Its annual demand is 7000 tonnes which is increasing at 1500 tonnes per year. *Ashwagandha*, also known as Indian Ginseng, is a highly popular herb and widely used in lot of *Ayurvedic* formulations (more than 200), nutraceutical products and other herbal products[1]. Its annual demand is 7000 tonnes but its actual production is 1500 tonnes per year. The demand of this herb was estimated to be 9127.5 tons per annum in the year 2005. Based on the trend, the current demand of *Ashwagandha* per annum would be around 12500 tons.[2]

Opportunities for *Ashwagandha* cultivation: The global interest in this plant and the high demand for its roots provide ample scope to cultivate this plant on commercial scale. Other opportunities for cultivation include Present price for roots is attractive, crop gives economically remunerative returns in comparison to traditional crops, ease of cultivation under rainfed condition. But due to increasing demand & low production rates causes pressure on its wild germplasm to overcome this cultivate *Ashwagandha* in commercial manner. Majority of this demand is met from cultivated fields spread across various parts of India.Keeping yield as the important objective, many chemical fertilizers and pesticides are used during the cultivation. These have a probable effect on the ultimate phytochemical and pharmacological profile of the plant. Further, all growers of *Ashwagandha* do not use one standard package of cultivation practices. Therefore, *Ashwagandha* sourced from wild, **different type of cultivated fields are available in the market.**

Need of the Study: As stated above, *Ashwagandha* sourced from various different types of sources having different geo-climatic and ecological conditions are available for the use of end users. Plant growth and development, and often the nature and quantity of the secondary metabolites, are affected by temperature, rainfall, daylight, biotic interactions, etc. (Trease& Evans). Medicinal plants in high demand and which are unavailable in the wild to meet the demand requires being cultivated. Often cultivation results in improved quality raw material because of selective control and improvement of certain factors of plant growth.

But, the fundamental principle in determining the cultivation practices is the observation of conditions in wild where in the plant is flourishing and replicating them in field. Any change in these conditions can have an effect on the phytochemical and pharmacological behaviour of the plant. Since *Ashwagandha* is sourced from various kinds of sources, there is a logical possibility of encountering variations amongst them. Secondly, the purported therapeutic benefits of *Ashwagandha* as described in classics pertain to the *Ashwagandha* collected from wild. Therefore, it is essential to study the variations between *Ashwagandha* collected from wild, cultivated as per the NMPB suggested cultivation practices and cultivated in an organic manner and assess which source of

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Ashwagandha is **phytochemically most potent one.**

Aims & Objective:

1. To cultivate *Ashwagandha* in an organic manner, in the Herbal Garden (DhanwantariUpavan) of NIA, Jaipur.
2. To explore and find out a wild source of *Ashwangandha having same age.*
3. To collect the roots from the above two sources.
4. To undertake **Chromatographical** analysis of these two samples including qualitative and quantitative analysis of Withanaloides by using available protocols.
5. To explore phytochemical variations among these three samples.

Materials and methods

Materials: The root powder of wild and cultivated *Ashwagandha.*

Methods:

- A wild source for *Ashwagandha* was searched for sourcing the wild sample.
- Certified *Ashwagandha* seeds were procured from K.N.K. College of Horticulture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Mandsaur (M.P.)
- Cultivated *Ashwagandha* grown at research plot of the Herbal Garden (Dhanwantari Upavan), Jagga Ki Bavari, N.I.A., Jaipur
- *Ashwagandha* cultivated by following NMPB suggested package of practices (Inorganic Manner) and Organic manner
- A crop calendar was drawn as per the Pop's and the entire cultivation processes were

  Documented against it.
- The roots of the cultivated variety and wild were harvested as per the guidelines available.
- The roots were subjected to Post Harvest Management and stored as prescribed in GACP.

A) Collection of wild samples

Wild samples collected from west land and forest adjoining Dhanvantari upwan , NIA, jagga Ki Bawari, Jamdoli Road Jaipur, Rajasthan.

Collection of wild samples

- The Wild sample of *Ashwagandha* were Collected by the scholar herself after botanically identifying the plant source as *Withania somenifera Dunal.*The Wild sample was collected in the month of Jan-Feb from the forests adjoining Dhanvantari upwan , NIA, jagga Ki Bawari, Jamdoli Road Jaipur, Rajasthan.
- The maturity of crop is judged by drying out of leaves and yellow red berries as in similar way that of cultivated sample.
- The entire plant is uprooted for roots which are separated from aerial parts by cutting the stem 1-2 cm above the crown.
- The root was collected, cleaned and then cut into pieces. The samples were then dried under shade following proper post-harvest management and storage methods. The dried samples were polypacked and labeled for pharmacognostical and phytochemical evaluation.

B) Cultivation of *Ashwagandha*

1. Experimental details: The experimental details of the present investigation are as given below. Cultivated samples obtained through growing of *Ashwagandha* in Research Plot, Dhanwantari Upavan, Jagga Ki Bavari, N.I.A., Jaipur. Details are as given below.

(i) Planting Material: The certified seeds of *Ashwagandha* used as a planting material for the cultivated source in present investigation. The seeds of *Ashwagandha* were obtained from K.N.K. College of Horticulture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Mandsaur (M.P.)

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(ii) Crop: *Ashwagandha (Withania somenifera Dunal.)*
(iii) Variety: Jawahar Aswagandha-20
(iv) Commencement of Experiment: August, 2011
(v) Experimental Design: Block Design
(vii)Spacing: 60 cm x 60cm
(viii) Plot size: 4.2m x3.00 m
(ix) **Gross area**: 342 sq.m
(x) Treatment detail : Organic Manner Application of FYM@2 kg/m^2
(Xii) Cultivation Details

1. Land Preparation: The field selected for *Ashwagandha* cultivation was ploughed once with tractor drawn plough. Finally, the land was harrowed twice to bring the soil to fine tilth after receiving pre-monsoon rains. Stubbles and weeds were removed from the experimental site and land was leveled by the application of Pata.
2. Application of organic manure: Well decomposed FYM (@ 2 kg/m^2) and vermi-compost (@ 1.3kg/m^2 were uniformly incorporated into the soil at the time of preparation of land.
recommended dose of NMPB. Full dose of nitrogen and phosphorus was given at the time of sowing.
3. Seed Treatment: The certified seeds of *Ashwagandha* (Cultivar JA- 20) purchased from *K.N.K. Horticulture College, Mandasaur.* At the time of sowing organically growing *Ashwagandha* seeds treated with Tricoderma before sowing to protect seedlings from seed borne diseases.
4. Seed sowing: The seeds were sown in 19 August 2011 by line method. The seeds are sown about 1-3 cm deep. Light showers were given after sowing of seeds to good germination.
5. Irrigation: A light shower immediately after sowing of seeds in the field was given for proper establishment of seeds. There was no need of irrigation in monsoon period because of rainfall is good at this monsoon. Lifesaving irrigations was applied once in a month after monsoon.
6. Thinning: The seeds sown by the line method were thinned out by hand at 30-35 days after sowing to maintaining plant population at 60cm space between plant to plant and 60cm space between line to line.
7. Weeding: The weeding was regularly done to keep the experimental area clean and free from weeds. First weeding done after 30 days of sowing & second weeding after 30 days of first weeding
8. Plant protection measures: No serious pests and disease found during crop duration.
9. Harvesting: The roots of *Ashwagandha* harvested on 29 March 2012.
The maturity of crop was judged by drying out of leaves and yellow red berries.
The entire plant was uprooted for roots which were separated from aerial parts by cutting the stem 1-2 cm above the crown. The roots were cleaned and dried as a whole in the sun and store.
10. Post harvesting: The dried roots were beaten with a club to remove adhering soil and to break off thin, brittle, lateral rootlets. Lateral branches, root crown and stem remain are carefully trimmed with a knife. The roots were then cut transversely into small pieces (7 to 10 cm) or dried as it is in the sun. Berries were hand plucked separately threshed, crushed to take out the seeds and the seeds are dried & stored. The collected wild and cultivated samples of *Ashwagandha* powdered and used for chromatographical study.

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CHROMATOGRAPHIC STUDY:

High performance liquid Chromatography (HPLC) –HPLC is basically highly improved column chromatography where the solvent is made to move under high pressures instead of under gravity as is in the case of column chromatography. In HPLC the solute travels through the column to the detector at a particular time known as retention time. The output of HPLC is recorded as a series of peaks- each one representing a compound in the mixture passing through the detector and observing UV light. Therefore, under constant HPLC conditions multiple samples of the same herb should present similar peak when no chemical is identified these peaks can be used as a qualitative identifying tool known as fingerprint.[3]

The area under the peak is proportional to the amount of that particular compound which has passed through the detector therefore higher area means higher concentration. If a reference material is available then the peaks can be compared and identified as the particular chemical. The HPLC conditions were adopted from U.S. Pharmacoepia. [4]

PROCEDURE

1. Preparation of Sample solution: About 5.0 gm of *Ashwagandha* root, finely powdered was kept in a 250ml flask fitted with a reflux condenser. 50 ml of methanol was added to it and refluxed on a water bath for 10-15 min. It was cooled to room temperature and the 87 supernatant was obtained. This was repeated till the last extract becomes colourless. The extracts, filter, concentrate was combined under vaccum to about 40 ml and volume was adjusted with methanol up to 50 ml.

2. Preparation of mobile phase

Solution A: 0.14 gm of potassium dihydrogen phosphate was dissolved in 900 ml of water, 0.5 ml of phosphoric acid was added and then diluted with water to 1000ml and mixed.

Solution B: Filtered and degassed acetonitrile.

Table no.1 Mobile Phase: See the gradient table below.

Time (min.)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

3. Chromatographic system

Mode : **LC**
Detector : **UV** 227nm
Column : **Hypersil** (250x4.6) mm,5um, end –capped, packing L1
Temperature : 27± 1o
Flow rate : 1.5ml/min
Injection volume : 20µL

The relative retention times of the withanolide aglycones and glycosides are provided in the

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following table.

HPLC observations table

HPLC observation Table 2 [Chromatograms Values….]

Name of samples	Peaks #	Name	Retention time	Area	Area %
wild	1	Unknown1	8.992	607129	23.157
	2	Unknown2	10.700	382804	14.601
	3	Unknown3	11.460	348847	13.306
	4	Unknown4	17.302	527216	20.109
	5	Unknown5	17.822	755766	28.827
Total				**2621762**	**100.000**
	1	Unknown1	8.996	596500	22.986%
	2	Unknown2	10.700	378219	14.574%
	3	Unknown3	11.457	342247	13.188%
	4	Unknown4	17.306	521884	20.110%
	5	Unknown5	17.824	756263	29.142%
total				**2595113**	**100.000%**
Organically grown Ashwagandha	1	Unknown1	9.091	336839	14.874
	2	Unknown2	10.800	306537	13.536
	3	Unknown3	11.633	388436	17.153
	4	Unknown4	17.292	580446	25.632
	5	Unknown5	17.852	652317	28.805
total				**2264574**	**100.000**
	1	Unknown1	9.083	327703	14.907
	2	Unknown2	10.787	303349	13.799
	3	Unknown3	11.629	360016	16.377
	4	Unknown4	17.275	559809	25.465
	5	Unknown5	17.840	647475	29.453
				2198351	**100.000**

The data given in above table represent the chromatographic value of several withanolides.

DISCUSSION:

The above findings suggest that there is not so significant quantitative variation of withanolides among the wild and the organically grown *Ashwagandha* and to the virtual possible lack of control in time of harvesting from the wild source the authors suggest *Ashwagandha* cultivated with FYM is the optimal source of *Ashwagandha* for clinical use.

CONCLUSION:

Cultivated source is a reality and will be the sustainable source for Ashwagandha. Chromatographically both the wild and cultivated *Ashwagandha* grown with organic manures appears to be *equally potent*. Confirming the above suggestion of using the cultivated *Ashwagandha* as an optimal source of ashwagandha.

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