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COMPARATIVE STUDY OF *WITHANIA SOMNIFERA* L. HERBAL FORMULATIONS BY HPTLC METHOD

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ABSTRACT
Ashwagandha belong to Solanaceae family has long been used as an Ayurvedic herb against a variety of human diseases. Ashwagandha churna and vatti are an important Ayurvedic formulation containing Withania somnifera L., as one of the prime ingredient of formulation. The combination of drugs and the dosage differ from person to person depending on severity of disease and physiological condition of the body. This research presents a high performance thin layer chromatography based standardization and development of quality of Ashwagandha formulations by using biomarkers compounds. In the process of developing a standardised methodology for quality control of Ayurvedic formulations, the presence of bioactive markers is feasible, and its verification through the HPTLC fingerprint profile is the best technique to distinguish and assess the quality of the finished formulation.

KEYWORDS: Ashwagandha, Churna, HPTLC fingerint, Formulation.

INTRODUCTION

Plant material and herbal remedies derived from them represent substantial portion of the global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary.

WHO has emphasized the need to ensure the quality control of medicinal plant products by using modern techniques and by applying suitable standards. Internationally several pharmacopoeias have provided monograph stating quality parameters and standards of many herbs and herbal products.Standardization problem arises from the complex composition of drugs which are used in the form of whole plant, parts of the plants and plant extracts.

Herbal drugs are inevitably inconsistent because their composition and hence their standardization, may be influenced by several factors such as age and origin, harvesting period, method of drying and so on. To eliminate some of the causes of inconsistency, one should use cultivated rather than wild plants which are often heterogeneous in respect of the above factors and consequently in their content of active principles.

The major reasons for these lacunae are.

- In the olden days Ayurvedic formulations were prepared by vaidyas as and whenever necessary in fresh form for administration.
- Active constituents vary from species to species depending on the geographic and seasonal variations.
- The combination of drugs and the dosage differ from person to person depending on severity of disease and physiological condition of the body.
- Most of the Ayurvedic preparations have to be consumed with specific vehicles such as ghee, milk, honey, water etc.
- In many cases even though medicines are the same, the vehicles are different for different conditions.

Ayurveda treatment consists of not just medicines but also restricted and recommended diet, which varies in different ailments. These are some of the unique features with Ayurveda which is absent in allopathy wherein the dose is rationalized and comes as fixed dose in combination or as individual drug.

This decade witnessed a boom in Ayurvedic formulations that are marketed for mass consumption in the form of general tonics, memory enhancers, immune stimulants, blood purifiers, beautification agents, etc. No doubt it has revolutionized the Ayurvedic concepts that

were lying hidden, but at the same time the quality control aspects should not be neglected. Undesirable effects due to excess usage need proper monitoring.

MATERIAL AND METHOD

Chemicals

Herbal formulations (Ashgandhchurna, and Ashwagandha vatti), Withania roots extract, methanol, toluene, ethyl acetate, chloroform, iodine and formic acid.

Apparatus and instrument

CAMAG HPTLC, Sonicator, TLC chamber, Iodine flask, Rota evaporator, Lyophilizer Freeze Dryer and UV chamber.

Preparation of extract

Made the coarse powder of dried roots (sieve no. 2000/355). Took out the 100 gm of coarse powder of roots in 1000 ml of beaker and added 800 ml of methanol. Extraction was carried out by UAE method up to one hour at the constant temperature of 40˚C, evaporated by distillation and same procedure was followed for all formulations.

PHYSICOCHEMICAL PARAMETERS

Determination of total ash

About 2 g of powdered drug was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The crucible was incinerated gradually by increasing temperature by making it red hot until free from carbon. The procedure was repeated to get constant weight. The same procedure was followed for the formulation also.

Determination of water soluble ash

The total ash obtained was boiled for five minute with 25 ml of distilled water, the insoluble matter was collected in an ash less filter paper, incinerated at a temperature not exceeding 450˚C and the same procedure was followed for the formulation also.

Determination of acid insoluble ash

The total ash obtained was boiled for five minute with 25 ml of 2N hydrochloric acid. The insoluble matter was collected in an ash less filter paper, washed with hot water, ignited, cooled in desiccators and weighed. The same procedure was followed for the formulation also.

Determination of pH

One gram of root powder was placed in a 100ml volumetric flask and made up to 100 ml by adding distilled water. The solution was sonicated for 10 minutes and pH was measured by digital pH meter.

Determination of Moisture content

The root powder was taken and dried for 24 hours at 105°C in an oven. To obtain a uniform sample weight, the sample's moisture content was evaporated. The dried samples were cooled and weighted. Moisture content was calculated using formula.

$$\text{Moisture Content (\%w/w)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

HPTLC ANALYSIS

Test sample preparation

5 gm of formulation was taken in 250 ml beaker, 75 ml of methanol (25 ml x 3) was added and Sonication for 30 minutes. Combined all the solutions and concentrated up to 25ml, finally filtered the solution through Whatmann filter paper. This filtrate was used for HPTLC analysis. Various concentration of test solutions were used for quantification of ashwagandha constituents.

Standard preparation

Stock solutions of Withanolide-A (0.16 mg/ml) were separately prepared by dissolving 1.5 mg accurately weighed standards in small amounts of methanol and made up the volume to 10 ml in a standard volumetric flask. The stock solutions were further diluted as per requirement for the preparation of working solutions.

RESULTS AND DISCUSSION

Physico-chemical parameters of extract and formulations

Table 1: Physico-chemical parameters of Ashwagandha root extract.

S. No.	Parameters	Results
1	Total ash % (w/w)	5.9
2	Water soluble ash % (w/w)	2.9
3	Acid insoluble ash % (w/w)	1.01
4	pH (5% w/v aqueous solution)	5.4
5	Loss on drying at 105 ˚C % (w/w)	6

Table 2: Physico-chemical parameters of Ashwagandha churna formulation.

S. No.	Parameters	Results
1	Total ash % (w/w)	7
2	Water soluble ash % (w/w)	3
3	Acid insoluble ash % (w/w)	3
4	pH (5% w/v aqueous solution)	4.7
5	Loss on drying at 105 ˚C % (w/w)	7

Table 3: Physico-chemical parameters of Ashwagandha vatti formulation.

S. No.	Parameters	Results
1	Total ash % (w/w)	6.8
2	Water soluble ash % (w/w)	3.2
3	Acid insoluble ash % (w/w)	2.05
4	pH (5% w/v aqueous solution)	5.2
5	Loss on drying at 105 ˚C % (w/w)	4.8

HPTLC ANALYSIS OF EXTRACT AND FORMULATIONS

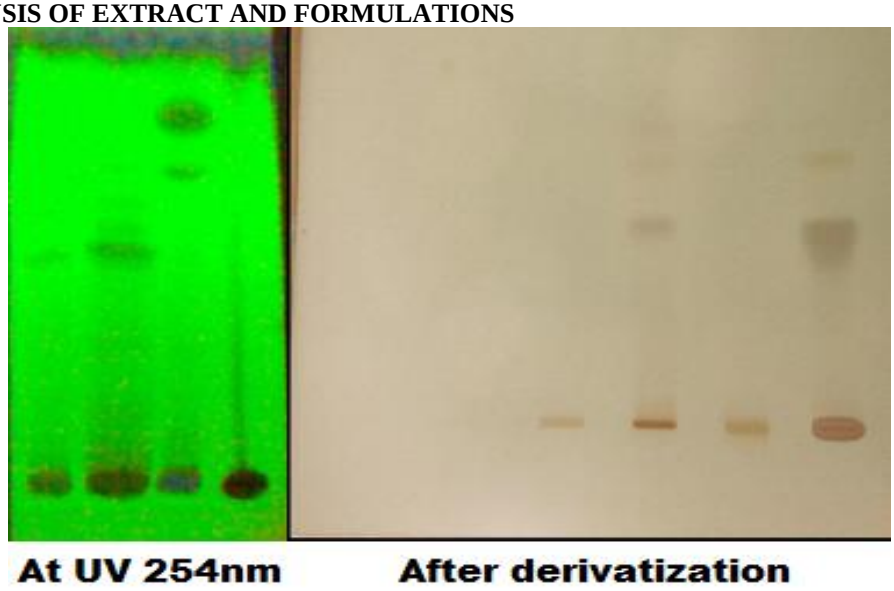

Fig. 1: HPTLC profile of Methanol extract of root, Ashwagandha churna, Ashwagandha vatti and Withanolide-A as a biomarker compounds. Track 1- Extract; Track 2-Ashwagandha churna, Track 3 – Ashwagandha vatti; Track 4 –Withanolide-A; (10 µl). Solvent system: Toluene: Ethyl acetate: Formic acid (5:4:1 v/v).

Table 4: Withanolide-A content in extract and formulations (%w/w).

S. No.	Particulars	Results
1	Root extract	0.43
2	Ashwagandha churna	0.03
3	Ashwagandha vatti	0.04

CONCLUSION

Modern techniques of analysis is extremely important for Standardization and development of reliable quality protocols for Ayurvedic polyherbal formulations. The generated physical characteristics, physiochemical parameters, HPTLC fingerprint profile with bioactive marker compounds will be used as important factors in the quality control of herbal formulations. Evaluation and quantification of bioactive marker as Withanolide-A can be used by pharmaceutical industry for standardization and quality control of Ashwagandha formulations. Standardization protocol of herbal formulation is very important tool for control the quality, purity, efficacy and safety of the Ayurvedic formulations. The Rf value of Withanolide-A is 0.45 in biomarker and formulations were found scanning under UV light at 254 nm.

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