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Formulation and Evaluation of Herbal Hair Growth Formulation of Ashwagandha in the Treatment for Alopecia

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ABSTRACT: Hair loss can be seen as a patchy, confluent, or diffuse pattern in alopecia. With a lifetime risk of 1.7%, the prevalence in the general population was estimated to be between 0.1% and 0.2%. Both men and women can have alopecia equally, but some studies have found that men are more frequently affected. At any age, it can happen. As a Rasayana, *Withania somnifera* (Ashawagandha) is a highly valued herb in the Indian Ayurvedic medical system (tonic). It is particularly utilised as a nervine tonic and for treating a variety of illness conditions. The relationship between ashwagandha and hair loss is still being researched in addition to all other applications.

It can be strongly inferred from the current research that Aswhagandha may have ingredients that help promote hair development. It was concluded that the highest withanolide content was found in methanolic extract, which, when combined with a herbal gel base, can promote hair growth without irritating the skin. According to the overall findings of this exploratory study, using this herbal hair growth formulation for a short period of time might dramatically reduce hair loss and may even stimulate new hair growth in some people.

Keywords: Alopecia, Ashawagandha, withanolide, *Withania somnifera*, minoxidil.

INTRODUCTION

Alopecia affects the scalp or body and is characterised by hair loss without any obvious evidence of inflammation. Alopecia areata is a common autoimmune disease that results in the loss of hair on the scalp and elsewhere (Hardy *et al.*, 1992). Patient with Alopecia areata in advanced phase and some of them converts into Alopecia totalis/Alopecia universalis (Seetharam, 2013). It accounts for 25% of all occurrences of alopecia worldwide and is one of the most prevalent types of hair loss that dermatologists report (Syed *et al.*, 2013). Scientist Cornelius Celsus was the first to describe it, and Sauvages invented the word "AA" in 1760 (Hunt and McHale 2005). The cycle of hair development is laborious, with the anagen phase being followed by the catagen and the telogen phases. The hair is actively growing during the anagen phase, while during the catagen phase, the lower portion of the hair follicle degenerates and is reabsorbed (Stenn and Paus 2001). A hair development cycle has three primary phases: anagen, catagen, and telogen. Telogen is the resting period, during which the hair is dormant and inactive. Following this phase, scalp hair follicle growth resumes.

The various types of allopathic drugs to treat hair loss but they have many side effects. Herbs are starting material for any medicine research. Approximately about 80% residents recommended herbal drugs for their beneficial effects along with fewer side effects as compared synthetic drugs (Thorat, 2010).

Withania somnifera (WS) pretreatment demonstrated significant protection against stress-induced stomach ulcers. Common names for ashwagandha include "Indian Winter cherry" and "Indian Ginseng". The Ashwagandha plant's root is ground into a powder that has been said to increase energy, lower inflammation, calm anxiety, and strengthen the body's immune system. Stress is frequently a factor in hair loss and shedding of hair (Pandey *et al.*, 2019). Adaptogens like ashwagandha can (in theory) prevent or stop hair loss by attempting to alleviate stress in the body. Strong antioxidant capacity may help to prevent hair loss (Likhitkar *et al.*, 2016). Inflammation, which may be a factor in conditions affecting your skin, joints, or other body parts, may be reduced by ashwagandha (Shalini *et al.*, 2017). Even though ashwagandha has a long history of use, the relationship between herb and hair loss is currently being researched (Alam *et al*., 2012).

Ashwagandha is botanically termed as *Withania somnifera* Synonym: Indian ginseng / winter cherry. *Withania somnifera* (L.)

Family: *Solanaceae.*


Fig. 1. *Withania somnifera* (Ashwagandha).

Chemical Constituents

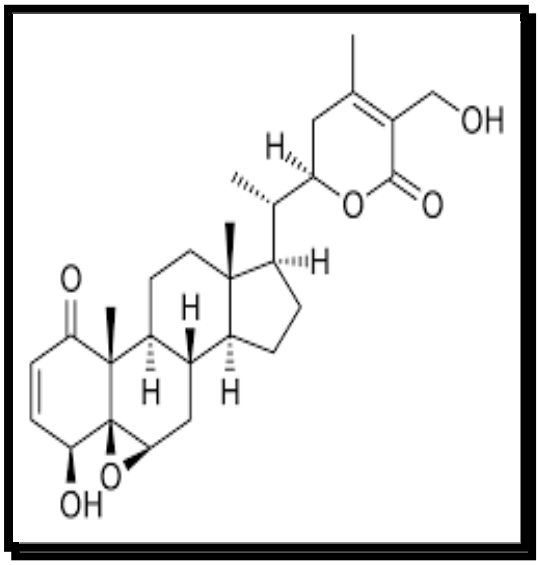

Fig. 2. Chemical structure of Withanolide A.

Alkaloids like isopellertierine and anferine, steroidal lactones like withanolides and withaferins, saponins with an extra acyl group like sitoindoside VII and VIII, and withanoloides with a glucose at carbon 27 ex: sitonidoside XI and X are the chemical components that are biologically active. Iron content is very high in *Withania somnifera* (Singh, 2008). Two primary withanolides, withaferin A and withanolide D, are thought to be responsible for a significant portion of Ashwagandha's pharmacological effect (Chaurasia *et al.*, 2013). Production of growth inhibitory factors by alkaloids may provide hair growth regulated mechanism cycle (Pena *et al.*, 1999).

Withanolide C- **Molecular Formula:** $C_{28}H_{39}ClO_7$

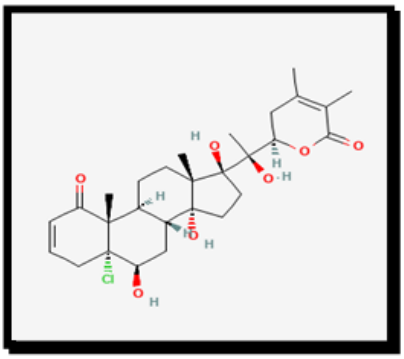

Fig. 3. Chemical structure of Withanolide C.

MATERIALS AND METHOD

Formulation

Method of preparation of gel:

Preparation of Aloe Vera Gel

Method of preparation: Formulations of WSR root extracts were made using aloe vera gel as a basis. Aloe vera's new leaves were used to extract the gel. The collected gel was then heated to 40°C, and to improve its stability, a mixture of stabilisers (ascorbic acid 0.5% w/w and sodium benzoate 0.5% w/w) were added to it. Once it had cooled, it was kept in a cool, dark place until it was needed for formulation development. A beaker containing 20 g of produced aloe vera gel had 0.2 g of dry extract added to it. Both were then combined to create a consistent gel (Nichola, 2008). 0.2 g of dried seaweed and 20 g of prepared aloe vera gel were combined. An uniform mixture was obtained by stirring. The Extract with highest withanolinide content on the basis of HPTLC evaluation was selected for further evaluation. Three different batches were prepared with tree different extracts, namely F1, F2 & F3. All batches of formulation prepared such were evaluated for organoleptic characterization and then optimized.

Table 1: Ingredients of Ashwagandha Gel.

Sr. No.	Drug	Botanical name/Scientific name	Part used	Quantity used
1.	Ashwagandha Extract	*Withania somnifera*	Roots	0.2 g
2.	Aloe Vera gel	*Aloebarbadensis miller*	Leaf	20 g
3.	Vitamin C	Ascorbic acid	Powder	0.5g
4.	Sodium benzoate	Benzoic Acid	powder	0.5g

HPTLC Analysis of root extracts of WS.

Chromatographic separation method. For the analysis, a CAMAG HPTLC system was outfitted with a TLC plate visualizer, TLC scanner 3, integrated software Win-CATS version 1.4.3, and a semiautomatic sample applicator. On silica gel 60 F254–precoated aluminium TLC plates (20 10 cm, Merck, Germany), the analysis was carried out. In a hot air oven at 120°C for 20 minutes, the prewashed plates with the same mobile phase were dried and activated. Using a semiautomatic application equipment (CAMAG Linomat 3) and a 10-L syringe, the sample aliquots were loaded as 8 mm bands on TLC plates at a rate of 10 nL s1 (Hamilton, Bonaduz, Switzerland).

HRMS Analysis. The indirect TLC–HRMS analysis of standard and sample band was compared. Both the samples and standard mass spectra showed the desired adducts (M + H) and (M + Na) of 20E. The HRMS analysis of standard withanolide A & withanolide C and sample was done in the positive mode of electrospray ionization. The standard withanolide A & withanoloide C and sample were having both adducts [M + Na] and [M + H]. The Withanolide sample was identified with two signals, $C_{28}H_{38}O_6$ + Na, [M + Na] (err = −0.9 ppm, score = 100) and $C_{28}H_{38}O_6$, [M + H] (err = −0.1 ppm, score = 100) m/z), while the plant sample extracted from the TLC plate showed the same two signals $C_{28}H_{38}O_6$, [M + Na] (err = −0.1 ppm, score = 100) and $C_{28}H_{38}O_6$, [M + H] (err = −0.0 ppm, score = 100) (50-1200 m/z). The adduct analysis of withaonile in sample and standard was having a score of 100 with an insignificant error. The comparative HRMS results of the sample with standards withanolide A&C and the reported literature support the identification of withanolide in Sampl (Alam *et al.*, 2012).

Hair growth activity *In-vivo*

Selection of Animals. The college's Institutional Animal Ethics Committee (IAEC) gave its approval to the study procedure, and the studies were carried out in accordance with CPCSEA standards.

The follicle cycle in rat is similar to human. Follicle develops and progress through actively growing phase, transitional phase and then resting phase (Rehana *et al.*, 2016).

Skin irritation test. Skin irritation test was performed on rats by following CPSCEA guidelines.

In-vivo hair growth study

Hair growth Study on rats: Selected animals were segregated in 3 groups (3 rats/group) control, test and standard. Hairs of rats on dorsal part in the area of 4x4cm were removed with the help of hair removing cream. control group was not applied with any medication, while test is applied with prepared Ashwagandha Gel and standard with 5 % Minoxidil solution daily for the period of 30 days. Rats were continuously observed for their hair growth during this period. Observations were noted on daily basis.

RESULTS AND DISCUSSION

The current study aims to assess gel's in-vivo hair growth capabilities. Physical characteristics, pH, spreadability, viscosity, an *in vitro* research to stimulate hair growth, and an in-vitro study to irritate skin were all used to characterise the created formulations.

Preformulation study:

Table 2: Organoleptic properties extract.

Sr. No.	Root powder	Characteristics
1.	Colour	whitish brown
2.	Odor	characteristic
3.	Taste	Bitter, pungent and sweet

Table 3: Extractive values of Ashwagandha (WS).

Sr. No.	Method of Extraction	Solvent	Sample Wt.	Extraction Values (%w/w)
1.	Sohxlet	Methanol	100	20.005
2.	Sohxlet	Ethyl Acetate	100	17.5
3.	Cold Expression	Sulphuric Acid	100	18.003

Table 4: Determination of solubility for Root extract.

Solvent	Solubility
Water	Soluble
Ethanol	Soluble
Chloroform	Soluble
Propyl Glycol	Soluble

HPTLC Analysis of root extracts and Optimization

Chromatographic separation method. This method is based on densitometry measurement at 366 nm in fluorescent mode, which has increased the sensitivity of measurements and avoided the use of derivatizing reagent. The spectral pattern study has suggested the RF as an important parameter. The fingerprinting of withanolide in the biomass of medicinally important herb *Withania somnifera* suggested multiuse of the method. The present analysis signifies methanolic extract as a rich source of withanolide compared to other extracts. The most of the antistress and hair growth supporting herbal drug formulation contains Ashwagandha as natural drug. The present validated HPTLC method may have importance in forensic and clinical pathology. The method has also significance in pharmaceutical and phytopharmaceutical industry as it is cost-effective rational approach for in-process quality control of plant raw materials.

The determination of an withanolide can be carried out with a small quantity of extract sample as this method can detect and quantify as low as about 4.67 and 14.16 ng band−1 . The withanolide content was found to be higher in the methanolic extract as compared to all other extracts. The indirect TLC–HRMS results of sample and standards support the identification of withanolide in gel formulation. This HPTLC indirect TLC–HRMS method can be applied in the screening and quantification of withanolides in crude plant powders, plant extracts, formulations, or in-process quality control in the phytopharmaceutical industry as well as for chemotaxonomy and phyllogenetic position of plant species.

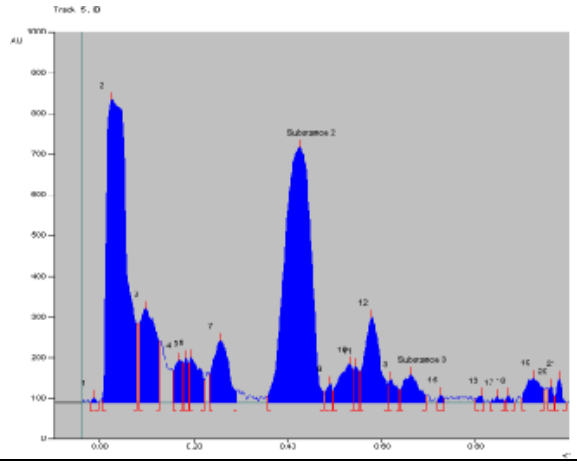

Fig. 4. track 5:Extract 3.

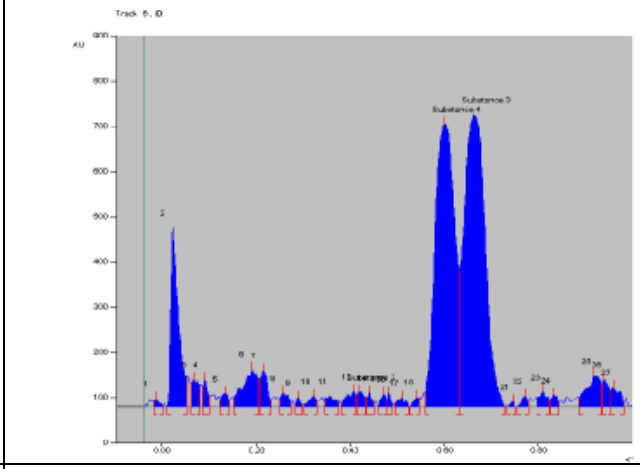

Fig. 5. track 6: Gel 1.

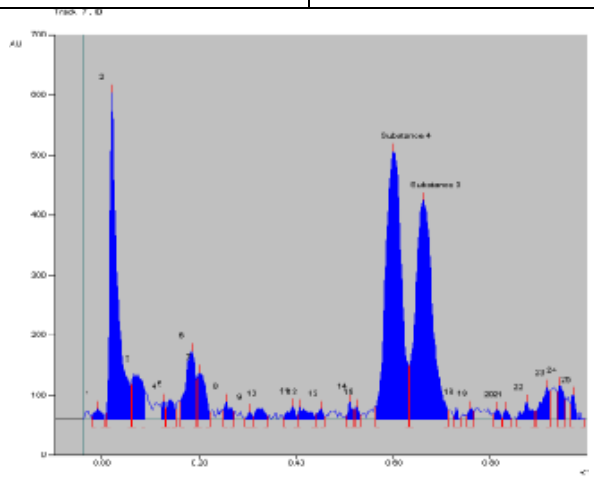

Fig. 6. track 7:Gel 2.

TLC-HRMS Analysis. **The sample and standard data from indirect TLC-HRMS support the identification of withanolide in gel formulation.**

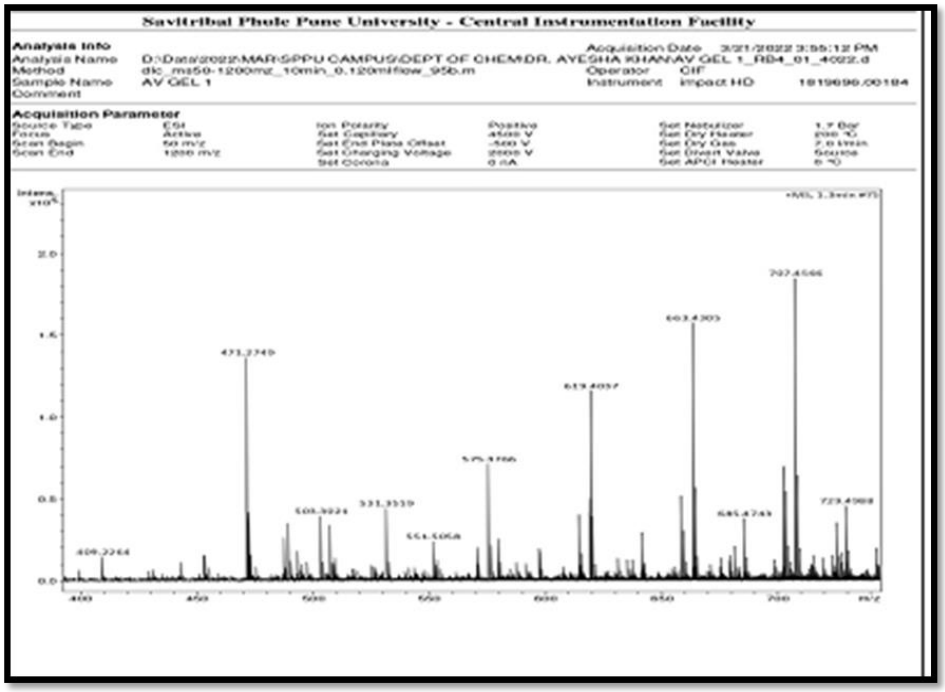

Fig. 7. HRMS data of prepared Gel 1.

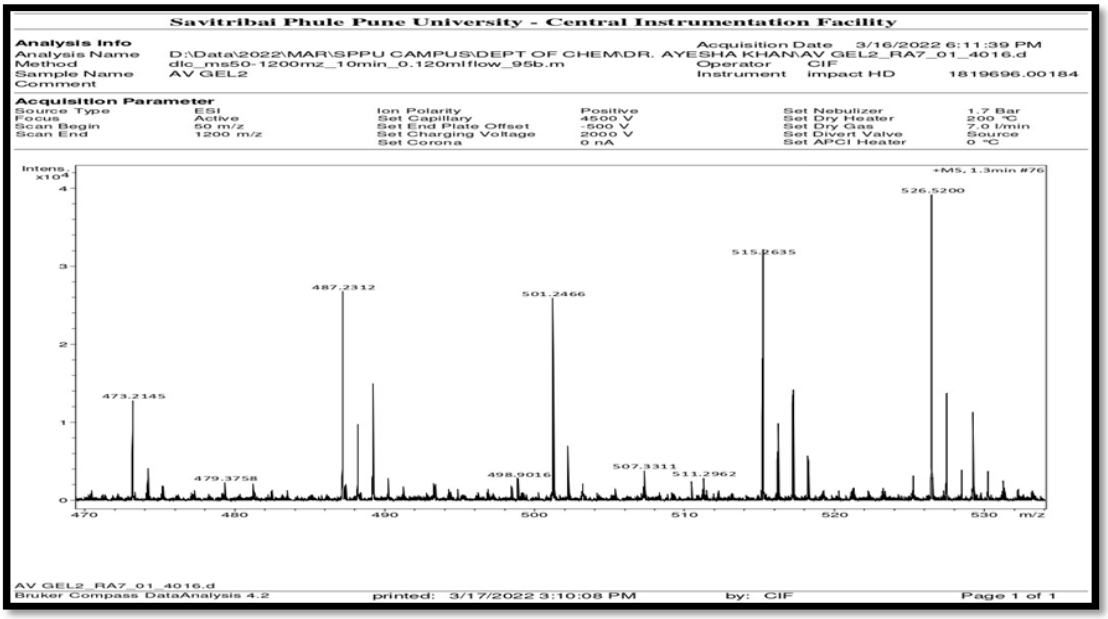

Fig. 8. HRMS data of prepared Gel 2.

Table 5: Determination of Withanolides using HPTLC method.

Withanolides	Concentration	HPTLC Fingerprinting of Withanolides
Extract 1	0.18 ± 0.013	Detected
Extract 2	0.67 ± 0.011	Detected
Extract 3	0.67 ± 0.016	Detected
Gel 1	0.83 ± 0.021	Detected
Gel 2	0.23 ± 0.008	Detected

Optimization of Batch: Methanolic extract from root extract produced positive results following analysis of all Extracts for their HPTLC evaluation. As a result of the extract's high withanolide contentability and optimization, it was used to create a gel for further testing on in vitro skin irritation and in vivo hair growth analysis.

Table 6: pH for root extracts formulation.

Sr. No.	Formulation	pH
1.	F1	6.4
2.	F2	6.8
3.	F3	6.7

Average pH = 6.7

Spreadability: From the combined graph of all formulations, it was determined that all of the created formulation demonstrated acceptable spreadability. The spreadability of the root extract gel formulation is presented in Table 11. After comparison with commercial gel formulations, all formulations exhibit good spreadability.

Table 7: Spreadability for gel formulations.

Sr. No.	Batch No	Spredability (%)
1.	F1	160
2.	F2	175
3.	F3	190
4.	M (marketed preparation)	175

Viscosity: Using a Brookfield viscometer, the prepared gel's viscosity was measured (Brookfield Engineering Laboratories). The formulation batch F3 gel formulation has the highest viscosity of all these formulations.

Table 8: Viscosity of gel formulation.

Batch	Viscosity
F1	1.96
F2	1.89
F3	1.75

Drug-Excipients compatibility study: Drug extract samples were kept at room temperature for 30 days while excipients were added in a variety of ratios. Physical changes were checked on the samples, but none were found in the mixture of extract and polymer.

Table 9: Physical Observations for Compatibility Study.

Batch	Caking		Liquification		Change in Colour	
	Starting	One month	Initial	One month	Starting	One month
F1	No	No Change	No	No Change	No	No Change
F2	No	No Change	No	No Change	No	No Change
F3	No	No Change	No	No Change	No	No Change

Skin Irritation Test: When the manufactured herbal gel was tested for its ability to irritate the skin, none of the formulations showed any signs of erythema or edoema even after 10 days of investigation (Table IV), indicating that the formulation was deemed to be safe.

CONCLUSIONS

Forensic and clinical pathology may benefit from the current HPTLC approach. The strategy is important for in-process quality monitoring of plant raw materials since it is a sensible, cost-effective solution in the pharmaceutical and phytopharmaceutical industries. The methanolic extract was found to have more withanolide than any of the other extracts. The identification of withanolide in the gel formulation is supported by the indirect TLC-HRMS results of the sample and standards. This HPTLC indirect TLC-HRMS approach can be used for chemotaxonomy and phyllogenetic position of plant species as well as the screening and quantification of withanolides in crude plant powders, plant extracts, formulations, or in-process quality control in the phytopharmaceutical industry.

The Aswhagandha may include ingredients that can stimulate hair development, according to the findings of an experiment on the activity of hair growth in rats. It is concluded that methanolic extract has the highest withanolide content and, when combined with a herbal gel base, can promote hair growth without irritating the skin. It demonstrated the shortest possible time for the growth of hair to begin and finish on denuded surfaces. In comparison to others, it was also discovered to increase hair length. Overall, the early study's findings indicate that using this herbal hair growth formulation for a brief period of time can dramatically reduce hair loss and, in some participants, even stimulate new hair growth.

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Conflict of Interest. None.

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