RESEARCH ARTICLE

Exploring Antipsoriatic Potential of *Aloe vera* Gel

Swapnil Shinde[1*], Shrinivas Mohite[2]

[1]*Shivaji University, Kolhapur, Maharashtra, India*
[2]*Rajarambapu College of Pharmacy, Kasegaon, Sangli, Maharashtra, India.*

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ABSTRACT

Research centers on conducting a phytochemical analysis of *Aloe vera* Linn leaf extracts, with an investigation into the existence of lead acetate solution, proteins, alkaloids, carbohydrates, flavonoids, cardiac glycosides, steroids, sulfur tests, and tannins. The gel formulation was tested for color, appearance, and homogeneity, and all batches produced gels that were easy to spread. Scores of 90% for the gel's extrudability, 80% for its quality, and 70% for its fairness were given. Inhibitory effects against HaCaT cell lines were observed with the synthetic herbal component *Aloe vera* Linn. These effects were minor when compared to the positive control 5-FU. Several aqueous plant extracts were tested for antipsoriatic activity using the Perry scientific mouse tail method. When compared to retino A cream, the aqueous extract of *Aloe vera* Linn gel (500 mg/kg) improved ortho keratotic regions by 59.26%. Results suggest that *Aloe vera* Linn leaf extracts may have a variety of functions, including antipsoriatic and therapeutic. To completely grasp the versatility of these plant extracts, additional research is necessary.

Keywords: *Aloe vera*, Herbal gel, Phytochemical analysis, Stability study, Antipsoriatic activity, HaCaT Cell line.

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INTRODUCTION

There are many different plant species that have been investigated for their potential to combat skin diseases.[1] Medical practitioners who specialize in herbal medicine are able to develop a wide variety of formulations for use in a variety of applications. It is becoming increasingly common to incorporate medicinal plants into the manufacturing process of pharmaceuticals due to the therapeutic efficacy and safety of these plants.[2] *Aloe vera* is a plant that is characterized by its stemless, perennial, drought-resistant, succulent nature. It has been utilized for therapeutic purposes since ancient times, according to recent reports.[3] The plant is a member of the family *Liliaceae* and has lance-shaped leaves that range from a stiff gray to a bright green color and contain a clear gel in the center of a mucilaginous pulp around the edges.[4] Recent studies have demonstrated that the pharmacologically active component of the *Aloe vera* leaf is concentrated in the gel and rind of a leaf. It has been demonstrated that the active compounds possess significant analgesic, antipruritic, wound healing, and anti-inflammatory activities.[5]

Many people use *Aloe vera* gel on their skin because of its calming and hydrating effects. Its polysaccharides form a protective layer on the skin, preventing dehydration and making it an excellent emollient for dry, irritated, or sunburned skin. It also has anti-inflammatory properties, such as acemannan, which reduces redness, swelling, and irritation associated with skin conditions like acne, eczema, and psoriasis.[6] It also contains antioxidants like vitamins C and E, beta-carotene, and flavonoids, which combat free radicals and prevent premature aging and oxidative damage. When combined with herbal extracts, *Aloe vera* gel can enhance the skin's nourishing effects, providing additional vitamins, minerals, and antioxidants, promoting overall health and vitality.[7] The present study deals with the formulation and characterization of herbal gel containing aloe.

MATERIALS AND METHODS

Collection of Plants

Aloe vera Linn, was collected from the local region of Sangli, Maharashtra state, India. Associate Professor Dr. Sanjay S. Sathe, Padmabhushan Dr. Vasantraodada Patil Mahavidyalaya, Tasgaon, Sangli, Maharashtra8 authenticated it.[8]

Animals

The mice used were male mature albinos weighing 25 to 30 g. In a controlled setting that maintained a 12-hour light-dark schedule while maintaining a temperature of 22 ± 2°C, kept in polypropylene cages and given pellet food and drink

**Author for Correspondence:* shindeswapnil2004@gmail.com

as needed. Before the studies were conducted, the animals were given a week to acclimate. In order to conduct the study on animals, the necessary Institutional Ethics Committee approval was sought in accordance with CPCSEA requirements (Registration No: IAEC/ ABCP /15/2015-2016).[9]

Aloe vera Linn Leaves Extraction

Collecting and washing *Aloe vera* Linn plants from the garden was the first step in making the aqueous extract. After the leaves were crushed, 500 mL of water was added, and the mixture was heated until it reached boiling point, maintaining a steady temperature of 100°C on average. The procedure was maintained until a volume of about 100 mL of the herbal extract was achieved. The extracts were refined by centrifugation.[10]

Qualitative Chemical Investigation of Extracts

In order to identify the different phytoconstituents, qualitative tests were performed on the extracts.[11]

Gel Formulation

As shown below, five distinct batches were created. In order to make 100 g of gel (or a blank gel), carbopol-934, glycerin, methylparaben, triethanolamine, and water were mixed in the appropriate amounts. The water needed for the gel formulation was split in half, with each half proportioned to 25 and 75%. A 25% active extract solution was prepared by dissolving glycerin and methylparaben in one portion of the water, while carbopol-934 was dissolved in the remaining 75% water. After thoroughly mixing the two solutions in the beaker, the pH was adjusted to 7.0 ± 0.5°C using tri-ethanolamine (Table 1).[12]

Physicochemical Evaluations

Color, appearance and homogeneity

Color, appearance, and homogeneity were the three main visual criteria used to evaluate the finished gel formulations.[13]

pH measurement

Using a digital pH meter, we verified that the gel compositions we had generated had the correct pH. Dissolved in 10 mL of distilled water, approximately one gram of gel was set aside to cool to room temperature. A calibrated digital pH meter was used to measure pH of every composition.

Spreadability

Sensory evaluation was used, based on volunteer response, to find out how spreadability of gel.

Viscosity study

A Brookfield viscometer was used to measure the viscosity of the gel compositions that were developed. A 100 g glass beaker was used to collect the sample, which was then tapped to remove any air bubbles or spaces. The samples were tested at a temperature of (25 ± 2°C) while the spindle was being spun at a rate of 20 r/min.

Extrudability

Standard capped collapsible aluminum tubes were used to determine extrudability. Aluminum tubes were filled with gel compositions and then sealed at the end. Weighing the tubes was done using a balance. Clamps were used to secure the tubes in place between two glass slides. Next, the cover was taken off, and a 500 g weight was set over the slide. Table 8 shows the results of the calculation for the amount of extruded gel.

- Amount of sample filled in tube = filled tube – Empty tube
- Amount of extruded sample = filled tube weight – the weight of tube after the experiment.

Microbiological activity

The test was carried out as per USP procedures.

Optimization of batch

Physical evaluations of the formulation batches' pH, viscosity, spreadability, and extrudability were examined and used to optimize the batches. The optimal batch for gel formulation was determined by analyzing the evaluation parameters of all batches.[14]

Stability study

Stability experiments evaluated the formulation's stability. In accordance with ICH standards, *Aloe vera* Linn formulations were tested under varying conditions of temperature and humidity. Samples were taken out at regular intervals and kept in an accelerated stability environment (40°C ± 2°C/75 ± 5% RH). They were then tested for color, appearance, homogeneity, pH, viscosity, spreadability, and microbiological activity.[15]

Stability Study Protocol

This protocol contains procedures to be followed for the stability study of gel formulation.

Purpose and stability commitment

- *Batch selection*

Out of 5 manufactured batches, 1 batch shall be subjected for stability study.

Container closure system

Gel formulation is packed in wide-mouth containers.

Storage conditions, time points and sample aliquot

The storage conditions are expressed in Table 2 after performing stability studies.

- *Note*
- In-house specifications for the finished product's shelf life should be followed by the test results.
- X = Initial analysis sample quantity is not considered in above stability loading details.
- A= Appearance, homogeneity, color, ph, viscosity, spreadability, extrudability and microbiological analysis.
- B = Appearance, homogeneity, colour, ph, viscosity, spreadability and microbiological analysis.

Pre-clinical studies

- *Skin irritation study*

In this experiment, Wistar rats (male or female, 150–200 g) were utilized. The unbroken skin was utilized. The rat had its hairs clipped three days before to the experiment. Experimental animals were administered the extract-containing gels. An

Table 1: *Aloe vera* linn gel formulation

Composition	*B1*	*B2*	*B3*	*B4*	*B5*
Aloe vera Linn (%)	2.5	2.5	2.5	2.5	2.5
Carbapol 934 (gm)	0.5	1.0	3.0	4.0	5.0
Methyl paraben (mg)	0.2	0.2	0.2	0.2	0.2
Glycerin	2.0	2.0	2.0	2.0	2.0
Triethanolamine (mL)	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water (mL)	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2: Storage conditions

	Long-term	*Intermediate*	*Accelerated*
Relative humidity (%)	60 ± 5	75 ± 5	75 ± 5
Temperature (°C)	25.0 ± 2	30.0 ± 2	40.0 ± 2
Test interval (months)			
0	A	A	A
1	B	B	B
3	B	B	B
6	B	B	B

animal serving as a control had a gel base put to its back. After seven days of treatment, the animals' skin was visually checked for signs of redness and swelling.

- *In-vitro study: Psoriatic skin cell line (HaCaT cell line)*

Using the SRB assay, an *in-vitro* antipsoriatic research was conducted. HaCaT human keratinocyte cell lines were utilized, which were obtained from NCCS, Pune. Cell lines were cultured in a medium that included 10% fetal bovine serum and was prepared according to Dulbecco's modified Eagle's protocol. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^4 cells/mL using growth media. Then, a 96-well plate was seeded with 0.1 mL of the diluted cell suspension, which corresponds to approximately 10,000 cells/well. The monolayer was rinsed one again after the first day. Each well plate was then filled with 100 µL of a drug dilution made using the medium mentioned earlier, after which the liquid above the partially formed monolayer was collected. The plates were examined under a microscope and the results were recorded every 24 hours. After 72 hours, a total concentration of 10% was reached by layering 25 µL of 50% TCA over the drug dilutions in each well. The next step was to incubate the plates at 4°C for one hour. In order to eliminate any remaining medium, medication, or serum, the culture was delicately mixed five times with tap water before being allowed to air dry. After allowing the plates to air dry for 30 minutes, SRB was used to stain them. In the next steps, we used 1% acetic vinegar to quickly wipe out the unbound dye four times. Allowing the plates to air dry was the next step. After adding 200 µL of 10 mM tris buffer, the plate should be read at 550 nm using the Elisa plate reader.

%Cytotoxicity = Reading of control - Reading of treated cells / Reading of control X 100

- *Perry scientific mouse tail method for psoriasis (In-vivo method)*

There were three sets of six mice each, with a weight range of 25 to 27 g. Close by components A local application of 1 g of the medication was made to the tails. Two groups were used in the study; one served as a control and was not treated, and the other was called a standard and was given 0.05% retinol A cream. Having made the gel, it was administered to the third group. When applying gel topically, start at the 2.5 cm mark and let it sit in a plastic cylinder secured with adhesive tape for two hours. The next step was a water wash of the tail. During the course of 14 days, treatment was administered once daily. Two hours subsequent to the final treatment, the animals were put to death by cervical dislocation under profound ether anesthesia. Individually group's tails were placed in individual containers covering 10% formalin solution after the proximal sections were clipped.

- *Orthokeratosis*

Ortho-+G Keratos- Keratin Keras (Horn) Osis-Condition

Microscopically- The surface of lesion displays hyperkeratosis & O-Keratosis: The condition of Keratin growing on the skin.

%orthokeratosis= length of granular layer/Length of scale

- *Drug activity*

An increase in the proportion of ortho keratotic regions - areas of cells that do not have a nucleus but play a protective role against external factors such microbes, ultraviolet light, and weak acids and bases - defines this condition. Staining with hematoxylin-eosin was performed on longitudinal histological slices prepared from tail skin. One thing that was measured in this specimen is the individual scale length, which is the distance between adjacent hair follicles and any sebaceous glands. The horizontal dimension along which the granular layer has completely grown. Measurement of epidermis thickness, from dermo-epidermal junction to stratum corneum base, was done using a total of ten scales per animal, six in each treatment group. The stratum corneum of the skin's surface. It is severely diminished or nonexistent on the surface of psoriatic lesions. Parakeratosis is the name given to this condition. One of the most prominent symptoms of psoriasis is this. Orthokeratosis is the process of the epidermis forming a granular layer. The fundamental idea behind mouse tail test is to change the condition of parakeratosis (Pk) to orthokeratosis.[16]

- Degree of Orthokeratosis(%)=Length of granular layer/ length of scale
- Drug activity= OK(s)-OK(c)/100-OK(c)×100 [where OK(s)- Degree of orthokeratosis of test substance, OK(c)- degree of orthokeratosis of control]

RESULTS AND DISCUSSION

Color, Solvent, Nature & %Yield

The details of obtained *Aloe vera* L (Leaf) are given in Table 3.

Phytochemical Screening of Leaf of *Aloe vera Linn*

By qualitative test it is shown that alkaloids, flavonoids, saponins and tannins are present.

Evaluation of Gel

The color, appearance and homogeneity of gel formulation was studied and the results obtained are mentioned in Table 4.

Measurement of pH, Viscosity and Spreadability

The results of pH, viscosity and spreadability are given in Table 5.

Extrudability Study

Extrudability studies were carried out and results were explained in Table 6.

Stability Study

We compared the results from each batch of formulations with respect to various characteristics. Overall data summary is mentioned in Table 7, which was studied at the initial (T0) time point. The Parameters included in study were color, homogeneity, appearance, pH, viscosity, spreadability, extrudability, and microbiological analysis.

Table 3: Solvents, extraction methods and respective yield

Extracts	*Solvent*	*Color*	*Nature*	*%yield w/w*
Aloe vera L (leaf)	Aqueous	Green	Semi-solid	9

Table 4: Color, appearance & homogeneity of gel formulation

Gel	*Color*	*Appearance*	*Homogeneity*
Aloe vera Linn gel (leaf)	Green	No extraneous particles	Good

In-vitro activity of *Aloe vera* Linn gel against HaCaT Cell line was studied and results are mentioned in Table 8.

Perry Scientific Mouse Tail Method

The original magnification is 40x, and these histopathological sections show skin from a mouse tail that was treated topically for 14 days. Became aware that the granular layer is underdeveloped in the majority of areas.

Using Perry's scientific mouse tail model, aqueous extracts of various plants were tested for potential antipsoriatic action.

Table 5: Viscosity of different batches of gel containing *Aloe vera Linn* (leaf)

Batches	*pH*	*Viscosity (cps)*	*Spreadability*
B1	6.6	4456	Poor
B2	6.9	5260	Good
B3	7.0	4360	Good
B4	6.7	4360	Good
B5	7.0	4600	Poor

Table 6: Extrudability of formulated gel containing *Aloe vera Linn*

Batches	*Wt. of gel in tube (g)*	*Wt. of gel extruded (g)*	*Extrudability amount (%)*	*Observation*
B1	10.7	9.08	84.90	Good
B2	10.5	8.97	85.50	Good
B3	10.8	9.26	85.75	Good
B4	9.7	9.07	93.50	Excellent
B5	10.8	8.91	82.50	Good

Table 7: Data summary of *Aloe vera* linn gel, at initial (T0) time point

Evaluation	*Acceptance criteria*	*Initial*	*1 month*	*3 months*	*6 months*
Colour	No difference compared to initial color	Greenish	√	√	√
Homogeneity	Free from extraneous particles	Good	√	√	√
Appearance	Report the results/For information purpose only	√	Good	Good	Good
pH	5.5–7.5	7.0	6.8	6.8	6.9
Viscosity (cps)	3500–5500	4360	4290	4160	4040
Spreadability	Report the results/For information purpose only	Good	Good	Good	Good
Extrudability (%)		85.75	NA#	NA#	NA#
Microbiological analysis					
Total aerobic microbial count (TAMC)	*NMT 10000 CFU/g	√	√	√	√
Total combined yeasts & molds count (TYMC)	*NMT 100 CFU/g	√	√	√	√
Escherichia coli		√	√	√	√
Salmonella		√	√	√	√
Staphylococcus aureus	Should be absent/g	√	√	√	√
Pseudomonas aeruginosa		√	√	√	√
Candida albicans		√	√	√	√

: Not applicable * : Not more than √: Complies

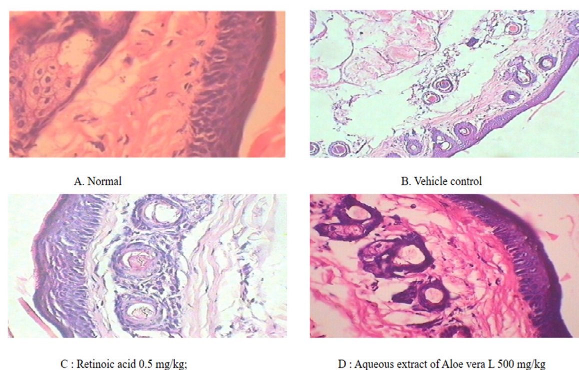

Figure 1: Longitudinal histological sections A. Normal; B: Vehicle control; C: Retinoic acid 0.5 mg/kg; D : Aqueous extract of *Aloe vera* L 500 mg/kg

Table 8: *In-vitro* activity of *Aloe vera* linn gel against HaCaT cell line

Compounds	*Reading*	*%inhibition*
Positive control (5 FU)	0.095	75.64
Negative control	0.390	---
100 µg/mL	0.098	74.8
50 µg/mL	0.105	73.07
10 µg/mL	0.121	68.977

Table 9: Effects of aqueous extract of different plants on the degree of orthokeratosis and 'drug activity' in mouse tail model

Groups	*Concentration*	*Degree of orthokeratosis (%)*	*Drug activity (%)*
Control	---	19.20 ± 3.06	0
Retino A cream	0.05%	70.01 ± 1.89	62.88
Gel containing *Aloe vera L* leaf extracts	500 mg/kg	48.14 ± 1.50	35.81

The numbers represent the average (± SEM) of 6 paired measurements. ANOVA and Dunnet's T-test (n = 6) determined statistical significance in the comparisons. Compared to the control group, $^{**}p < 0.01$, $^{*}p < 0.05$.

The extracts were applied topically in form of gel and cream. A drug's efficacy is measured by the proportion of orthokeratotic zones, which are areas of cells that do not contain a nucleus and play a protective role against harmful substances such as microorganisms, ultraviolet light, weak acids, and bases. The orthokeratotic areas were 5.92% larger than normal after being treated with a gel that contained 500 mg/kg of water-based *Aloe vera* Linn extract. Retinol A, the gold standard, demonstrated a 70.01% rise (Table 9, Figure 1).

CONCLUSION

The study is mostly about the phytochemical review of leaf extracts from *Aloe vera* Linn. The chemicals that make up the extracts were tested. Proteins, alkaloids, carbohydrates, flavonoids, heart glycosides, steroids, sulfur tests, tannins, acetic acid, potassium permanganate, and lead acetate solution were all discovered in the leaf extracts. The homogeneity, color, and appearance of the gel formulation were assessed. It was discovered that the gel was green, particle-free, and easily spreadable. The gel compositions, including *Aloe vera* Linn were tested for spreadability, viscosity, and pH, and all of the batches were determined to have easily spreadable gels. The gels were also evaluated for their extrudability, which was good (>90%), excellent (>80%), fair (>70%), and poor (>70%). The gel's extrudability was rated as good at 90%, good at 80%, and fair at 70%, according to the data summary at the initial time point.

The synthesized herbal compound *Aloe vera* Linn showed inhibitory effects against HaCaT cell lines, according to microbiological examination. The modest inhibitory actions of the herbal extract *Aloe vera* Linn were particularly noticeable when contrasted with the positive control 5-FU. Gel antipsoriatic activity against HaCaT cell lines was tested using the Perry scientific mouse tail method. To test the potential antipsoriatic action of various plant aqueous extracts, researchers used Perry's scientific mouse tail model & practical topical treatment to histological sections of mouse tail skin for 14 days. Drug activity was determined by rise in the proportion of orthokeratotic areas after topical application of gel-containing extracts or cream-containing extracts. While the conventional medicine retinol A cream raised ortho keratotic areas by 70.01% compared to a normal, gel containing aqueous extract of *Aloe vera* Linn (500 mg/kg) increased them by 59.26%. Additionally, the 'drug activity' and the degree of orthokeratosis were tested in mouse tail model in relation to aqueous extract of various plants.

The study concludes that extracts from *Aloe vera* Linn leaves may have antipsoriatic and medicinal uses, among other possible benefits. To fully understand the potential of these plant extracts for different uses, additional research is required.

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