

## Formulation and Evaluation of Herbal Ointment of *Berberis aristata* for Inhibition of Psoriasis-like Symptoms

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To prepare an herbal ointment using an ethanolic extract of *Berberis aristata* and assess the anti-psoriatic effectiveness of the finished product. We were able to make multiple ointments that were each categorized as F-1, F-2, and F-3 by adding varying concentrations of stearic acid and cetyl alcohol by learning about various formulation types, such as oil in water. The analysis of many factors, including pH, viscosity, spreadability, and stability, was used to evaluate all formulations. A formulation of ethanolic extract (F-3) showed antipsoriatic action. No evidence of a separate phase or ease of removal was found in any of the formations, but they all demonstrated good spreadability, consistency, appearance, and pH. Additionally, during irritancy trials, the formulation F-3 did not cause redness, oedema, erythema, or irritation. The use of this formulation on the skin is safe. As a result, the study implies that the extract's and the ointment's base's compositions are safer and more stable, however, they might also have synergistic effects.

**Keywords:** Antipsoriatic; *Berberis aristata*; Ethanolic extract; Formulation; Ointment.

In some groups, psoriasis is infrequent or uncommon, probably because of genetic causes. In a survey at a teaching hospital in Nigeria, very few psoriatic patients were identified, and the condition is reportedly uncommon among Eskimos. Indians from North or South America hardly ever exhibit it. The prevalence of psoriasis is equal in males and females, according to the current consensus, despite numerous research in the past showing a varying sex incidence. The age of onset and mean age of affected males and females varied, according to Steinberg and colleagues; the clinical disease manifested in females at a younger age, and the mean age of statistically studied groups of females with psoriasis was three to four years lower than that of comparable males. Ointments, which

are topical formulations, are more well-liked by patients since they have higher patient compliance<sup>1</sup>. Ointment are the semisolid dosage preparations intended for topically infected diseases which carries water, waxes, hydrocarbons and volatile oils as a semisolid<sup>2</sup>. Protection, antibacterial, emollient, antipruritic, keratolytic, and astringent characteristics are all uses for ointments<sup>3</sup>. Plants were being used as medicine much before recorded history. According to a World health organisation (WHO) report, almost 25% of human prescription drugs are made from plants, while 80% of people still use the conventional medical system<sup>4</sup>. The WHO has created guidelines to aid its members in creating national old-style medicine policies. WHO recognises the value of medicinal plants

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for the provision of public health services in developing countries<sup>5</sup>. Herbalism has traditionally been practised outside of the realm of mainstream medicine, but it is growing in popularity as new studies and research demonstrate how successful they are at diagnosing, treating, and preventing disease<sup>6</sup>. Because of the skin's accessibility, size and exposure area medication delivery system through the skin has been considered a promising approach. The primary objective of this study was to formulate and evaluate anti-psoriatic by combining ethanol extract of *Berberis aristata* with additional components to provide a variety of skin advantages, including antibacterial and antiseptic properties.

## MATERIALS AND METHODS

### Identification, collection and authentication of plant material

Plants of the *Berberis aristata* Linn. species were harvested in March 2021 from the Garhwal district of Uttarakhand. The root bark was cleaned and allowed to air dry. The scientist In-charge of the Botanical Survey of India, Allahabad (Central region), India, performed the authentication. The department of botany received an herbarium for the future reference (Accession No.: 104540).

### Preparation of Extract from Root-barks

The fresh root barks of *Berberis aristata* was collected in March 2021, the root barks was washed and dried at RT (Room temperature), then converted into a coarse powder by the help of grinder and passes through a sieve number 18 to get the uniform size. To get rid of fat and other pigments, powdered root bark was defatted using petroleum ether (40–60%). Using a Soxhlet device, the defatted dry root barks were further extracted with ethyl acetate and subsequently with ethanol. The extracts were dried completely in a vacuum oven until all traces of ethanol were eliminated. In a refrigerator set between 2-8 °C, the extracts were stored<sup>7</sup>.

### Formulation of Herbal Ointment

The topical ointment was prepared by using an ethanolic extract of the *Berberis aristata* (EEBA). A combination of appropriate preservatives (Methylparaben and Propylparaben) were used to create the EEBA in concentrations

of 1%, 2%, and 4%, and the ointment was kept in the refrigerator until further usage. Weighed and melted the required ingredient given in the table below. A sufficient amount (i.e. 1 gm for 1%, 2 gm for 2% and 4 gm for 4%) of ethanolic extract of the root bark was added to this and thoroughly mixed to create a homogenous material<sup>8</sup>. Table 1 represents the different polyherbal ointments' components.

### Physicochemical evaluation parameters

The following preliminary testing of formulations at various doses was done:<sup>9</sup>.

#### Colour and aroma

Visual inspection was used to assess colour and smell or aroma.

#### pH

Using a digital pH metre, the pH of several formulations was determined. 100 millilitres of distilled water were used to dissolve one gramme of ointment, which was allowed to stand for two hours. Each formulation's pH was measured triple times, and the average results are shown in the table.

#### Spreadability

Spreadability of the formulation was determined by an apparatus suggested by Multimer with some modifications. It consists of a wooden block having a pulley at one end with fixed glass slide on block. An excess of ointment (3 g) placed on ground plate. The ointment was sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. A 1 kg weight was placed on the top of the two plates for 5 min to expel air and to provide a uniform film of the ointment between the plates. Excess of ointment was scrapped off from the edges. The top plate was then subjected to pull of 240 g. With the help of spring attached to the hook and time required by the top plate to cover a distance of 10 cm was noted.

#### Extrudability

A straightforward methodology was used to carry out this study. Collapsible tube was used to evaluate the extrudability of the formulated ointment. The formulated ointment filled into the hopper and tubes were filled with ointment carefully. The extrudability of each ointment formulation was measured by the weight in grammes required to extrude an ointment ribbon of 0.5 cm in length in 10 seconds.

### Consistency

The consistency of the formulations and the presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. Immediate skin feel (including stiffness, grittiness, and greasiness) was also evaluated.

### Diffusion study

The agar nutritional medium was made to conduct the diffusion investigation. The ointment was put inside a board with a hole in the middle of it. It was noticed and evaluate, how long it took the ointment to diffuse.

### LOD

The LOD was calculated by putting the formulation in a petri dish and drying it at 105°C.

### Solubility

Solubility was determined by dissolving a required amounts of prepared ointments were tried to solubilizes in 10 ml of solvent such as water, boiling water, ether, alcohol etc.

### Washability

For evaluating the washability 0.5 g ointment were applied to the skin and then after some time ointments were wash with running water and then the result was analyzed by manual inspection.

### Non-irritancy Test

For evaluating the irritancy, 0.5g of formulated ointment was applied to the human skin and wait for some time, then the results were predicted.

### Stability study

The developed ointment formulations were subjected to stability study as per the International Conference on Harmonization (ICH) guidelines. The formulated ointment was filled in the collapsible tubes and stored at different temperatures and humidity conditions, namely, 25°C±2°C /60%±5% Relative Humidity (RH), 30°C±2°C /65%±5% RH, and 40°C±2°C /75%±5% RH for a period of 1 months.

### Evaluation of Antipsoriatic activity

#### Antipsoriatic Activity

Animal ethical work was approved by the Institutional Animal Ethical Committee (IAEC). CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) examined all experimental protocols and procedures utilized in this work. Wistar rats of either sex, weighing 180–220 g, were used for the experiment when they were 8–11 weeks old. Until the completion of the 23-day experiment, animals had unrestricted access to water and a conventional meal.

There were five groupings created from the animals. In each group, there was six rats. Using a delicate hair removal treatment, the backs of all the animals were carefully shaved. All animals, had their backs shaved after 24 hours and then specific amount of imiquimod cream applied using an applicator brush for 7 days in a row<sup>10</sup>.

**Table 1.** Composition of *Berberis aristata* extract-based ointment

Ingredients	F1 (g)	F2 (g)	F3 (g)
Ethanollic Extract	1.000	2.000	3.000
Stearic acid	1.200	1.200	1.200
Triethanolamine	0.165	0.165	0.165
Rose water	0.400	0.400	0.400
Paraffin oil	0.300	0.300	0.300
Moisturizer	1.200	1.200	1.200
Cetyl alcohol	0.100	0.100	0.100
Methylparaben	0.018	0.018	0.018
Propylparaben	0.002	0.002	0.002
EDTA	0.010	0.010	0.010
Water	q.s.	q.s.	q.s.



**Fig. 1.** Simple Ointment Base

- **Group-I:** Rats in the control group received normal feeding and a basic ointment base. Imiquimod 5% cream at a dose of 62.5 mg (equivalent to 3.125 mg API) was applied topically to the shaved backs of this group of animals for 7 days in a row to induce the condition. For seven days, rats were given acetate in their water to increase the skin inflammation caused by imiquimod<sup>11</sup>.
- **Group-II:** Rats in the standard control group received the same psoriasis treatment as Group I animals. Retino-A 0.025% was administered topically for the following 16 days starting on the eighth day<sup>12</sup>.
- **Group-III:** Similar to the animals in Group II, this group also had psoriasis induced. 1% w/w of a herbal preparation was applied topically for 16 days starting on the 8th day.
- **Group-IV:** Similar to the animals in Group II, this group also had psoriasis induced. In-house herbal preparation 2% w/w was administered topically for 16 days starting on the 8th day.
- **Group-V:** Similar to the animals in Group II, this group also had psoriasis induced. 4% w/w in-house herbal preparation was applied topically for 16 days starting on the 8th day.

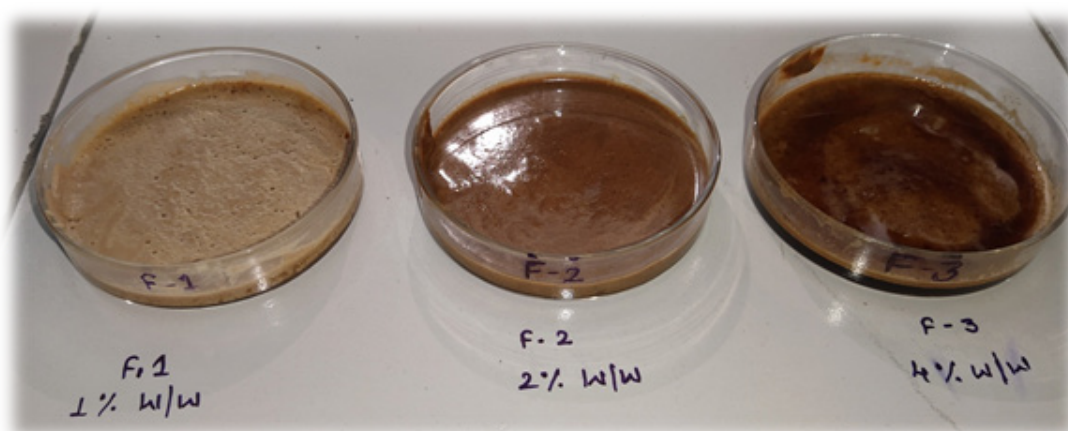
A digital micrometre was used to measure the thickness of the skin every other day (SLB Works). Skin irritation was measured by the increase in skin thickness<sup>13</sup>.

#### Scoring Severity of Inflammation

Based on the Psoriasis Area and Severity Index, scoring system was created to assess the degree of inflammation on the back skin. On a scale from 0 to 4, erythema, induration, and desquamation were rated separately: '0' mean:

**Table 2.** Physicochemical evaluation of formulated formulation

Physicochemical Parameters	F1 (1% w/w)	F2 (2% w/w)	F3 (4% w/w)
Colour	Off Brown	Light Brown	Dark Brown
Odour	None	None	None
Consistency	Slightly dry	Smooth	Smooth
pH	7.02	6.88	6.70
Spreadability (Seconds)	4.26	4.33	4.26
Extrudability	3.60 g	3.40 g	3.65 g
Diffusion study (after 60 min)	0.7cm	0.72cm	0.65cm
LOD	30%	30%	28%
Solubility	Boiling water	Boiling water	Boiling water
Washability	Easy to wash	Good	Good
Non-irritancy	None	None	None
Stability (2°C, 25 °C, 37 °C)	Stable	Stable	Stable



**Fig. 2.** Different Concentrations of Ointments

none; ‘1’ mean: faint; ‘2’ mean: moderate; ‘3’ mean: marked; ‘4’ mean: very marked<sup>14</sup>.

**Histopathological Examination of Skin**

10% formalin in saline was used to preserve the isolated skin in its containers. Animal skin was cut into longitudinal histological slices and stained with hematoxylin-eosin<sup>15</sup>.

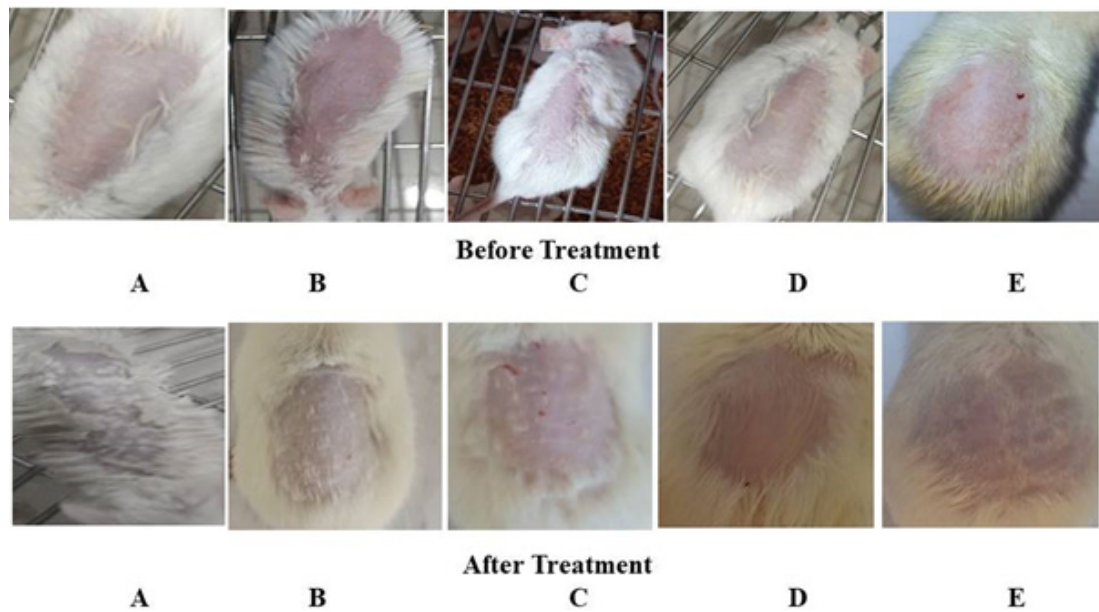
**Effect of Imiquimod (IMQ) induced psoriasis in rats**

Morphological discoveries Imiquimod treatment resulted in scales and redness on the skin of mice.

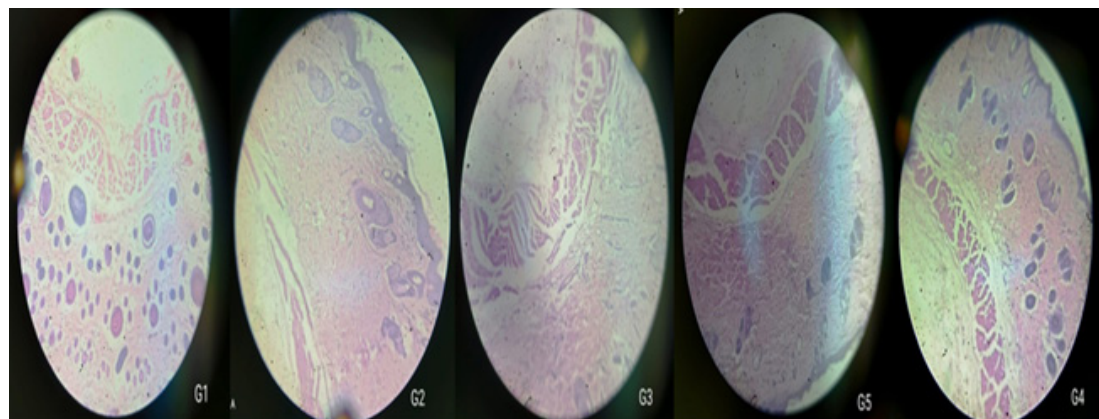
**RESULTS AND DISCUSSIONS**

**Physicochemical evaluation of formulated formulation**

The emulsifying ointment was used as the substrate to create herbal ointments in the current investigation. The formulations’ physical characteristics were then assessed. These physicochemical characteristics were acceptable. The formulations appeared to be stable based on the stability analyses that were conducted. In tabular form, the maximum dosages of compounded ointments are displayed (Table No. 2)



**Fig. 3.** Assessment of psoriasis (Before and after the treatment)



**Fig. 4.** Histopathological Examination

G-1: Control; G-2: Standard; G-3: F-1 Treated; G-4: F-2 Treated; G-5: F-3: Treated

### Psoriasis Area and Severity Index Assessment (PASI)

Throughout the trial, all rats' morphological characteristics and health states, including food and water consumption, behavioral indicators, b.w., cardiovascular indicators and respiratory patterns, were normal. The dorsal skin was seen to have erythema, scaling, and thickening two to three days after the IMQ treatment began (Figure 3). After that, the severity of the ill rats' psoriasis-like symptoms gradually worsened until 16<sup>th</sup> day or end of the experiment. The dorsal skin of rats in Group I treated daily with a ointment base, however, does not exhibit any symptoms of inflammation (Figure 3). Before starting treatment with a retinoid-A and extract-based ointment formulation, the independent PASI scores after IMQ application from days 1 to 7 showed a continuous rise in inflammation levels. The intensity of PASI scores peaked seven days after IMQ therapy, indicating that the mice treated with the drug successfully induced psoriasis-like dermatitis.

However, starting on day 9, which is equivalent to the second day of treatment, there was a statistically significant reduction in psoriasis-like symptoms. On the 2<sup>nd</sup> day following the start of the retino-A and F-1, F-2, and F-3 formulation treatment. Up until day 16, these symptoms continuously got better. Figure 3 shows the individual PASI scores and the total scores of all groups from days 1 to 16. The formulation treated groups shows a substantial inhibitory effect on IMQ-induced psoriasis like dermatitis as compared to the IMQ-treated group. Group IV (F-3 formulation) of rats showed the greatest improvement in the PASI inflammatory symptoms. The Retino-A treatment group's significant decrease in PASI scores was equivalent to the 4% w/w EEBA treated group.

### Histological Slides

The evaluation of several parameters, including inflammatory cells, neutrophils, etc., was made easier by the histology slides [Figure 4]<sup>16</sup>. A degenerative change in skin tissue was seen in the skin section treated or induced with imiquimod. This alteration includes the thickness of the epidermis, increase the no. of keratinization, decline the portion of infection and orthokeratosis, how much extension of ridges, vessels dilation with microabcess and vacuolization, they all

shows the severity of the skin tissue. The skin tissue cytoarchitecture of animals given retinol and 4% w/w EEBA ointment was normal, indicating degenerative changes.

### CONCLUSION

*Berberis* has been utilised for its several therapeutic characteristics, including antibacterial, antifungal, and anti-inflammatory ones, since ancient times. As a result, this ointment may be used as a simple dose form to make effective use of these medicinal characteristics. The goal of the current study was to formulate and assess herbal ointment. To achieve a good yield of extract for this, the herbal extracts were made using a straightforward extraction technique using Soxhlet, with no harm to the chemical contents or their activity. The ointment was made using the fusion method, which ensured that the herbal extract and ointment base were mixed uniformly and remained stable throughout storage. The physicochemical properties have been investigated, and the findings for spreadability, extrudability, washability, solubility, loss on drying, and other features are satisfactory. Additionally, the formulation was left for four weeks to undergo stability testing at various temperatures, including 2, 25, and 37 degrees Celsius. In terms of spreading ability, diffusion research, or irritating effect, there were no alterations found. Beginning on day 9, there was a statistically significant reduction in psoriasis-like symptoms in the Antipsoriatic trial. The graph shows the individual PASI scores as well as the total scores for every group from days 1 through 16. The IMQ-induced psoriasis-like dermatitis was significantly inhibited when compared to the IMQ-treated group and the EEBA-treated groups. The 4% EEBA (F-3) treated group of rats showed a decrease in the PASI inflammatory signs. The Retino-A treatment group's significant decrease in PASI scores was equivalent to the 4% w/w EEBA treated group. *Berberis* has become an effective antipsoriatic drug over the years <sup>(17)</sup>. *Berberis aristata* has a huge antioxidant effect and is suitable for the treatment of various diseases, such as diabetes, diarrhea, and hormone disorders <sup>(18)</sup>. However, the pro-apoptotic effect of this alkaloid is terrible. *Berberis aristata* contains alkaloids like berberine, berberine inhibits the proliferation

and metastasis of a variety of psoriasis, such as leukemia, colorectal, prostate, lung, glioma and ovarian psoriasis <sup>(19)</sup>, although there are certain obstacles that greatly affect the anti-infective properties of berberine. The main reason for this limited apoptotic effect is its poor absorption and limited biological activity. However, with the advancement of nanotechnology, these problems can be overcome <sup>(20)</sup>.

#### Conflict of Interest

There is no conflict of interest.

#### Funding Sources

There is no funding sources.

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