RESEARCH ARTICLE

Ethnobotanical Exploration and Formulation Development of Cream Containing *Ammi majus* Linn and *Portulaca oleracea* Linn

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ABSTRACT

Stearic acid served as the emulsifier in the preparation of an O/W emulsion-based cream that also included oil-soluble components and water-based extracts of *Ammi majus* Linn and *Portulaca oleracea* Linn. The cream was evaluated for color, appearance, presence of foreign particles/grittiness, pH, viscosity, phase separation, and extrudability. A skin irritation study was conducted on Wistar rats, with intact skin and hair removed three days earlier experiment. Cream-containing extracts were applied to test animals, and the backs of animals were scrutinized for erythema and edema. To study psoriatic skin cells *in-vitro*, researchers used the HaCaT cell line.

Researchers also used the SRB assay to look for antipsoriatic effects *in-vitro*. Cultures of human keratinocyte cell lines HaCaT were carried out in a medium that included 10% fetal bovine serum and was otherwise identical to Dulbecco's modified Eagle's medium. In order to reach a concentration of 10%, 25 µL of 50% TCA was added to each well after 72 hours, creating a thin layer of ended drug dilutions. Phytochemical evaluation revealed that the extracts of *A. majus* Linn and *P. oleracea* Linn were soluble in water and had a yellowish solid color. The cream formulation showed good emulsionability, no foreign particles or grittiness, and no phase separation. The skin irritation test on Swiss albino rats showed that the standard drug (Retino A 0.05% cream) was free from irritation. The cream's acceptability for topical use was also observed.

Keywords: *Ammi majus* Linn; *Portulaca oleracea* Linn; Cream; HaCaT cell line.

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INTRODUCTION

Traditional medical systems around the world have long recognized the healing qualities of medicinal plants, and this recognition is only growing. There are a lot of plant species that have chemicals that have pharmacological actions that can cure a lot of different kinds of skin problems and other illnesses. The therapeutic safety, efficacy, diversity, accessibility, and sustainability of medicinal plants are just a few of the benefits they bring to the drug development process. Medicinal herbs are often thought of as safer for long-term usage and have fewer adverse effects than manufactured medications. They specifically target the biological pathways that contribute to the development of skin diseases due to the presence of bioactive chemicals within. Numerous formulations have been developed to address various skin problems and unique patient demands, thanks to the great diversity of medicinal plants. In areas where traditional medicine is prevalent, there is typically a greater availability and affordability of therapeutic plants compared to synthetic medications.[1]

A plant that can be consumed is called *Portulaca oleracea* L. In some Chinese provinces, it is also consumed as a vegetable source of nutrition.[2] The use of *P. oleracea* L. in folklore is extensive. Antiseptic, antiscorbutic, and antispasmodic are all uses for this substance. Chinese medicine makes use of it for a variety of purposes, including the prevention and treatment of diabetes and viral hepatitis. The results of a great number of research have demonstrated that this plant possesses a diverse array of pharmacological properties. Since ancient times, *Ammi majus* L., which is also known as ajwain or ova in India, has been utilized extensively in the treatment of a variety of skin conditions, including vitiligo.[3] Furocoumarins, such as xanthotoxin and bergaptons, are produced by this process. These furocoumarins are utilized in the formulation of creams and lotions that are used to treat skin problems. Present work

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based on the formulation of a semisolid O/W emulsion-based herbal cream.

MATERIALS AND METHODS

Collection of Plants

The *A. majus* Linn and *P. oleracea* Linn were placed from the local region of Sangli, Maharashtra state, India and authenticated by Associate Professor Dr. Sanjay. S. Sathe, Padma. Dr. Vasantraodada Patil Mahavidyalaya, Tasgaon, Sangli, Maharashtra.

Drying, Powdering and Aqueous Extraction of Plant Materials

A. majus Linn leaves and *P. oleracea* Linn leaves were collected, shade dried and powdered. Twice, in 1500 mL of water, 300 g of powder was heated to 70°C for 30 minutes to make the aqueous extracts. After passing over Whatman filter paper, the extract was allowed to evaporate and was subsequently concentrated to a semisolid state on the water bath under atmospheric pressure. Using a hot air oven, a small layer of this was added to a petri dish, and the resulting extract had a yield of 9, 10, and 9%, respectively.

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 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).

Qualitative Chemical Investigation of Extracts

In order to identify the different phytoconstituents, qualitative tests were performed on the extracts.[4,5]

Cream Preparation

Emulsion systems that are semisolid and described as opaque are what creams are. They are applied to the skin, hair, or mucous membranes. What kind of materials are in the internal phase and whether the cream is w/o or o/w determine rheologic parameters and the cream's homogeneity. We prepared a cream based on an O/W emulsion. Part A, the oil phase, was heated to 75°C and then mixed with stearic acid and other oil-soluble components and water extracts of *A. majus* Linn and *P. oleracea* Linn. Part B, the aqueous phase, was heated to 75°C after dissolving water-soluble preservatives and other components and water. The emulsifier was cooled afterward heating, and the oil phase was added to the water phase in parts while stirring constantly.[6] In Table 1, the cream's formula is given.

Table 1: Formula of cream

Oily phase(Part A)		*Aqueous phase (Part B)*	
Ingredients	*%w/w*	*Ingredients*	*%w/w*
Stearyl alcohol	5.0	Triethanolamine	2.0
Stearic acid	2.5	Propylparaben	0.04
White bees wax	1.5	Methylparaben	0.01
Mineral oil	5	Propylene glycol	5.0
Cetyl alcohol	6.5	Water	Up to 100
Aq. extract of *A. majus* Linn and *P. oleracea* Linn	2.5 + 2.5% each		

Evaluation

Cream was assessed for subsequent physical constraints[7]

Color and appearance

The cream was inspected visually for color and appearance.

Presence of foreign particles/grittiness

Using diffused light, approximately 500 mg of cream was put on a grease-free glass slide and examined for the occurrence of foreign particles.

pH of cream

A digital pH meter was used to find the formulation's pH. We tested 1 g of cream at 25 ± 2°C after weighing it and dissolving it in 10 cc of distilled water. A calibrated digital pH meter was used to measure the pH of every composition.

Viscosity

Spindle number S-64 of the Brookfield Viscometer II + model was used to determine the formulation's viscosity. 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 measured at 25 ± 2°C while the spindle was being rotated at 20 r/min.

Phase separation

The cream was stored, tightly sealed, in a dark, cool place (25–30°C) away from light. For 30 days, the phase separation was monitored meticulously every 24 hours. We made sure to check for any changes in phase separation.

Extrudability

The amount of force needed to extrude the material from a tube can be measured empirically using this test. Standard caped collapsible tubes were filled with the mixtures and then sealed. It was noted that the tube was weighed. Clamps were used to secure the tube between two glass slides. The glass slide was covered with a 500 g weight and the cap was then opened. The cream that was extruded was measured and recorded. It was determined what percentage of cream was extruded. (> 90%: excellent, >80%: good, > 70%: fair).

Amount of sample filled in tube = filled tube – Empty tube

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

Skin Irritation Study

In this experiment, Wistar rats (male or female, 150–200 g) were utilized. The unbroken flesh was utilized. The rat had its hairs clipped three days before the experiment. Experimental animals were administered the extract-containing gels. An 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.[8]

***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.[9] The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^4$ 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 once 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.[10,11] 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.[12] 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.[13] 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.[14,15]

RESULTS AND DISCUSSION

Phytochemical Evaluation

The solvents, extraction methods and obtained percentage yield are mentioned in Table 2.

Phytochemical Screening

A. majus L (Seed) and *P. oleracea* L (leaves) were found to contain alkaloids, flavonoids, and tannins.

Data for Cream Formulation

The developed cream was evaluated for various parameters and the obtained results are given in Table 3.

Skin Irritation Study

The skin irritation test was conducted on Swiss albino rats in an *in-vivo* experiment and results are given in Table 4.

The standard drug (Retino A 0.05% cream) was found to be free from irritation.

An observation indicates acceptability of this cream for topical use.

Table 2: Solvents, extraction methods and respective yield

Extracts	*Solvent*	*Color*	*Nature*	*% yield w/w*
A. majus L (Seed)	Aqueous	Yellowish	Solid	9
P. oleracea L (leaves)	Aqueous	Greenish	Solid	10

Table 3: Evaluation parameters of cream formulation

Cream	*A. majus Linn and P. oleracea Linn cream*
Color	Yellow-green
Appearance	No extraneous particles were observed
pH	5.5
Viscosity (CPS)	5500
Extrudability	Good
Presence of foreign particles/grittiness	No foreign particles were observed
Phase separation	No Phase separation

Table 4: Skin irritation study

Batch	*Number of animals*	*Erythema*	*Edema*
Control	06	Nil	Nil
Polyherbal cream containing *A. majus* Linn and *P. oleracea* Linn	06	Nil	Nil

CONCLUSION

The use of an oil-in-water emulsion system successfully created a semisolid cream. Stearic acid was used as an emulsifier, and several oil-soluble components were utilized. These components included white beeswax, cetyl alcohol, stearyl alcohol, mineral oil, and aqueous extracts of *A. majus* Linn and *P. oleracea* Linn. A full examination was performed on the cream, which included analyzing its color, appearance, pH, viscosity, phase separation, extrudability, and potential for causing skin irritation. Over the course of 30 days, the cream displayed a number of desirable qualities, such as a high degree of emulsionability, the absence of foreign particles or grittiness, and the absence of phase separation. Through both visual examination and extrudability testing, it was determined that the cream may be used for topical application without any adverse effects. In the course of skin irritation tests carried out on Wistar rats, it was discovered that the cream, which contained herbal extracts, did not cause erythema or edema. This led researchers to conclude that the cream might be considered safe for dermal application. Additionally, *in vitro* antipsoriatic experiments conducted with the HaCaT cell line exhibited encouraging results, indicating that the cream may have the potential to be useful in the management of psoriatic skin disorders.Taking everything into consideration, the findings indicate that the cream that was developed has the potential as a topical preparation for skin conditions, with the potential to provide therapeutic advantages while posing a minimum risk of discomfort. It is possible that additional research, including clinical trials, will assist in validating its efficacy and safety for practical implementation in dermatological practice.

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