

Gas Chromatography-Mass Spectrometry Elucidation and Antipsoriatic Activity on Developed Herbal Formulations from *Carissa congesta*, *Catharanthus roseus*, *Annona squamosa*, and *Polyalthia longifolia* Plant Extracts

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ABSTRACT

Background: *Annonaceae* and *Apocynaceae* families are known for their plethora of pharmacotherapeutic potential. **Objectives:** To evaluate antipsoriatic potential (ultraviolet [UV]-induced photodermatitis and tail model) and gas chromatography-mass spectrometry (GC-MS) studies on developed formulations from the plant extracts.

Materials and Methods: Formulations of ethanolic extracts of *Carissa congesta* and *Catharanthus roseus* and petroleum ether extracts of *Annona squamosa* and *Polyalthia longifolia* were evaluated for antipsoriatic activity (UV-induced photodermatitis and tail model) at 250 and 500 mg/kg. GC-MS studies were done subsequently after 6 months. Formulation batches were tested by different tests, viz., pH, viscosity, spreadability, washability, homogeneity, grittiness, drug content, Sudan red, bleeding, and sensitivity tests. **Results:** Histopathology studies indicated the absence of Munro's microabscess, regular elongation of rete ridges, capillary loop dilatation, granular cell layer, and prominent parakeratosis at 500 mg/kg. Formulations of the plant extracts did not reveal toxic components by GC-MS interpretation. No greasiness was observed during washing. Uniform homogeneity was observed with neither clumps nor large aggregates. The dispersed globules appeared red against colorless water background. Bleeding time was found to be stable during the experimentation. **Conclusion:** Developed topical plant formulations showed antipsoriatic potential on selected models. The GC-MS studies confirmed the presence of no drastic changes in the quantity of active constituents assuring its stability.

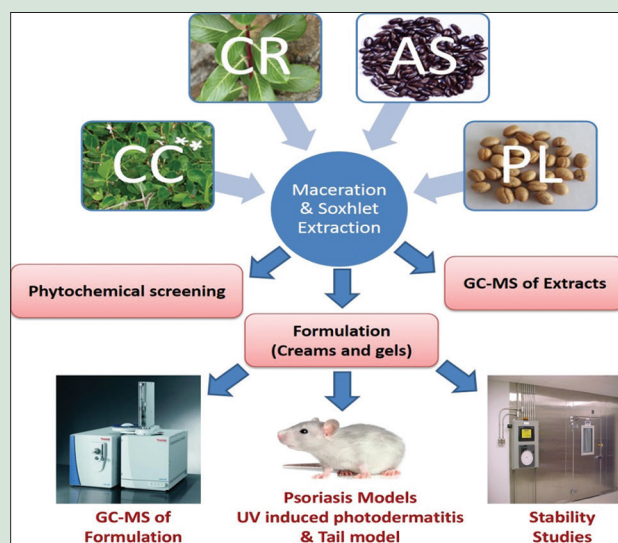
Key words: *Annona squamosa*, *Carissa congesta*, *Catharanthus roseus*, gas chromatography-mass spectrometry, *Polyalthia longifolia*, psoriasis

SUMMARY

- The current article focuses on developed formulations from plant extracts. Formulation batches were designed and tested by different tests as per the standard protocols. Optimized formulations with good results were screened for gas chromatography-mass spectrometry profiling to check the toxic components. Antipsoriatic activity was studied at different dose levels for the relevant formulations.

Abbreviations Used: FCK: *Carissa congesta* cream 250 mg (2.5%) and 500 mg (5%); FCV: *Catharanthus roseus* cream 250 mg (2.5%) and 500 mg (5%); FGK: *Carissa congesta* Gel 250 mg (2.5%) and 500 mg (5%);

FGV: *Catharanthus roseus* gel 250 mg (2.5%) and 500 mg (5%); FCA: *Annona squamosa* cream 250 mg (2.5%) and 500 mg (5%); FCP: *Polyalthia longifolia* cream 250 mg (2.5%) and 500 mg (5%); FGA: *Annona squamosa* gel 250 mg (2.5%) and 500 mg (5%); FGP: *Polyalthia longifolia* gel 250 mg (2.5%) and 500 mg (5%); GC-MS: Gas chromatography-mass spectrometry.



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INTRODUCTION

The plant kingdom has been regarded as a treasure house of potentially natural origin drugs, and there has been upsurge increase in awareness regarding their importance.^[1] The *Annonaceae* is a family of flowering plants consisting of trees, shrubs, or rarely lianas. *Carissa congesta* (CC) leaves have been reported for carissic acid, triterpene carandinol, betulinic acid, β -sitosterol-3-O- β -D-glucopyranoside, oleanolic acid, ursolic acid, and 4-hydroxybenzoic acid. Its leaf decoction is used against fever, diarrhea, and earache. Ayurvedic preparations are made from it such as *Marma Gutika*, *Hridaya Mahakashaya*, *Kalkantaka Rasa*,

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Kshudra Karvanda Yoga, and *Marichadi Vati*.^[2] *Catharanthus roseus* (CR) contain alkaloids, viz., vinblastine, vincristine, vindesine, vindeline, tabersonine ajmalicine, vinceine, vineamine, raubasin, reserpine, and catharanthine.^[3,4] Leaves have been used in the treatment of menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, menstrual disorders, anti-allergic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, anthelmintic, skin diseases, hypolipidemic, bleeding, diarrhea, and antiviral properties.^[5]

Apocynaceae is a family of flowering plants with trees, shrubs, herbs, stem succulents, and vines. *Annona squamosa* (AS) possesses potent bioactive principles in all its parts and used as antimicrobial, insecticidal, anti-tumor, antioxidant, analgesic, anti-inflammatory, antiulcer, etc.^[6,7] *Polyalthia longifolia* (PL) has been known in the Indian traditional system of medicine and has its role in inflammation, bacterial infection, skin diseases, antitumor, antioxidant, antimicrobial, etc.^[8,9]

There was observed that wide variation in the global prevalence of psoriasis has been depicted in review by the international population-based studies. It has been noticed that geographic location influences the likelihood of having psoriasis as well as disease prevalence tends to increase with increasing distance from the equator. Prevalence of psoriasis across India varies from 0.44% to 2.8%, with children having 4.4% of total patients.^[10] A diverse range of synthetic conventional treatments has been available for psoriasis. However, conventional psoriasis treatment may not be reliable as it only provides temporary relief. The objective of natural or holistic therapy was to get to the root of the problem. Natural remedies are more acceptable with the belief that they are safe, cost-effective, and having fewer side effects. Available marked formulations are combinations of multiple herbs used in the treatment.^[11]

Citing the context of situation, the present research article attempts to develop a topical formulation of cream and gel with the ethanolic air-dried leaves extract of CC and CR and petroleum ether dried seed extract. Monoherbal topical plant formulations were evaluated by antipsoriatic activity. At the end of 6 months, gas chromatography-mass spectrometry (GC-MS) technique revealed no toxic components from the formulations, thereby indicating its the stability Graphical Abstract.

MATERIALS AND METHODS

Plant collection, identification, and extraction

The fresh leaves of CC and CR (No. 3/187/2017/Adm-1755/17-172 and No. 3/187/2017/Adm-1755/17-173) were collected from Mumbai, while AS and PL seeds (No. 3/187/2013/Adm. 1692/080 and No. 3/187/2017/Adm-1752/17-170) were collected from Raigad district (MS), India. All the plants were authenticated by Agharkar Research Institute, Pune. The leaves of CC and CR were air-dried in the absence of sunlight, powdered with a mechanical grinder, and passed through sieve no. 40. Soxhlet extraction (25 g) with 80% ethanol was done at a temperature of 50°C for 21 h. AS and PL seeds were homogenized to coarse powder. 200 g of ground seeds was soaked and macerated in 1000 ml of petroleum ether for 7 days at room temperature. All the extracts were evaporated on a water bath at temperature after filtering it with a Whatman filter paper. Extracts were concentrated and stored in the refrigerator at 4°C for future use.

Phytochemical analysis

The GC-MS studies were carried out to confirm the presence of phytoconstituents in the ethanolic (CC and CR) and petroleum ether (AS and PL) extracts.^[12-14]

Formulations development

The base of cream and gel was optimized, and then the extracts were added.^[15,16] Creams were prepared using oil phase and adding the

appropriate quantities of cetyl alcohol (36%), isopropyl myristate (24%), and menthol (1.5%). The water phase was separately prepared by adding the extracts (2.5% and 5% each), tween 80 (1.7%), methylparaben (0.2%), propylparaben (0.02%), and distilled water. Both the phases were heated to a temperature of 60°C. The water phase was added to the oil phase and appropriately stirred on a magnetic stirrer until a semi-solid consistency was obtained. Finally, triethanolamine was added to adjust the pH of the formulations. Gels were prepared with carbopol 934 (1%) and added to small quantity of distilled water and soaked for 24 h. The extract (250 and 500 mg each) along with other excipients were added and stirred on a magnetic stirrer until consistency was obtained. Finally, triethanolamine was added to adjust the pH of the formulations.

Evaluation of the formulations

The formulations were evaluated based on the following parameters.

Solubility studies

The solubility of extracts in various solvents was observed by adding small amounts of extracts to a fixed amount of solvents.

Drug-excipient compatibility studies

Physical mixture of the drug and excipient in the ratio of 1:1 was evaluated for the solid-state compatibility under the following conditions: dry- and wet-closed vials were kept at 30°C/60% RH and 40°C/75% RH for 1 month. Physical mixtures were observed for changes in color and odor.

Physical evaluation

The physical parameters such as appearance and color were assessed.

pH

5 ± 0.01 g of the formulations was weighed accurately and 45 mL of water was added, dispersing the formulations in it. The pH of the suspension was determined at 27°C using a digital pH meter (DKB Instruments).

Viscosity

A viscometer (Brookfield Digital Viscometer LV DVE 230, USA) was used to measure the viscosity (in Cps) of the creams and gels. The spindle (T bar, S 96) was rotated at 3 rpm. Samples of the cream and gel had been kept to settle 30 min before taking measurements at the specific temperature.

Spreadability

1 g of the respective formulations of cream or gel was taken and sandwiched between two tiles. A weight of 200 g was placed above it for 30 s. After 30 s, the upper tile was removed. The formulations were found to spread on the tile. The total amount of formulations spread on the tile was measured with the help of a scale, by measuring the diameter. These measurements were taken thrice, and the average values obtained were put in the formula mentioned below:

$$S = M \times L/T$$

Where

S = spreadability

M = weight applied on tiles

L = length/diameter of the formulations spread

T = Time of contact (s).

Washability

The ease of removal of the formulations applied was examined by washing the applied part with tap water.

Homogeneity

All the developed formulations were tested for homogeneity by visual inspection, appearance, and presence of any aggregates.

Grittiness/particle uniformity

Grittiness and uniformity of particles in the formulations were observed microscopically.

Drug content

One gram of formulations was weighed and added to a 100 mL volumetric flask and the volume was made up with ethanol. From this stock solution, appropriate dilutions were prepared. The resulting solutions were then filtered using the Whatman filter paper and subjected to spectrophotometric estimation using ultraviolet (UV)/visible spectrophotometer by Shimadzu 1800-Double beam spectrophotometer. The drug content was estimated based on the calibration curve obtained for the respective drug/extracts.

Sudan red test (for cream)

Sudan red was mixed with the cream. A drop of the cream was placed on a microscopic slide with a cover slip and examined under the microscope. If the dispersed globules appear red against the colorless background, the cream was oil-in-water (O/W) type. The reverse condition occurred in water-in-oil type cream, i.e., the dispersed globules appear colorless in the red ground.

Bleeding test

The formulations had been kept for a specified period of time in the refrigerator and at room temperature, and the bleeding of liquid from the formulations was observed.

Animal and experimental design

The antipsoriatic activities of cream and gel formulations were evaluated based on two models.^[17-19] The protocols (VESCOP/05/2017 and VESCOP/06/2017) were approved by the IAEC Committee. Albino Wistar rats (250–300 g) were procured from Bombay Veterinary College, Parel, Mumbai. The unique cage number and individual numbers of the rat were marked on the tail with an inedible marker pen. The rats were marked (toward the tip of tail) with the temporary animal numbers at start of acclimatization. The rats were marked with permanent animal numbers (toward the base of tail) with different-color indelible marker pen before the start of external application. They were selected and grouped manually. Under laboratory conditions, the treated animals were monitored for 14 days for changes in fur, eyes, behavior, and toxic reactions. Rats without any visible signs of illness were used for the formulation experimental studies.

Acute toxicity studies were undertaken as per the Organization for Economic Corporation and Development (OECD) guidelines 402. Before the 24 h experimentation, fur was removed from the dorsal area of the trunk of the rats by clipping or shaving. Since the extract was herbal, 2000 mg/kg (topically 10% of the body surface area) of creams and gels was given to three animals per group. Cream and gel were held in contact with the skin with a porous gauze dressing and non-irritating tape for 24 h. The condition of mortality and clinical signs were observed during the first 30 min and at approximately 2–6 h after application of the formulations on day 0 and twice daily during the period of 14 days. The animals were treated with dose levels of 250 and 500 mg of O/W creams and gels. UV-induced photodermatitis rat skin and psoriasis rat tail models groups were positive control; standard cream and gel; bases of cream and gel; creams and gels of CC, CR, AS, and PL (250 and 500 mg).

Ultraviolet-induced photodermatitis rat skin

The hair on the dorsal skin of rats was carefully shaved. Except for an area of 1.5–2.5 cm on the depilated skin, the entire animal was covered with a UV-resistant film. The uncovered area of 1.5–2.5 cm was irradiated for 15 min at a vertical distance of 20 cm with a UV lamp. Application of the

formulations was started 12 h after irradiation and continued for 3 days. A schedule of two applications/day at intervals of 12 h was maintained. On the 3rd day, 2 h after the last treatment, the animals were sacrificed with carbon dioxide in a euthanasia chamber. Skin biopsies were taken immediately, fixed in 10% formalin. Tissue sections (4 µm thick) were stained with hematoxylin and eosin and studied by direct microscopy. Parameters evaluated were Munro's microabscess, regular elongation of rete ridges, capillary loop dilatation, absence of granular cell layer, and prominent parakeratosis.^[20]

Psoriasis rat tail

The animals were treated once daily, 5 times a week for 2 weeks. Tails were treated locally with the test and standard formulations, by applying to the proximal part of the tail. For the contact time of 2 h, a plastic cylinder was slipped over the tail and fixed with adhesive tape. At the end of contact time, the cylinders were removed. Animals were treated once daily, 5 times a week, for 2 weeks. Two hours after the last treatment, the animals were sacrificed with carbon dioxide in a euthanasia chamber and the tails were prepared histologically with 10% formalin and hematoxylin-eosin stain. Parameters evaluated were Munro's microabscess, regular elongation of rete ridges, capillary loop dilatation, absence of granular cell layer, and prominent parakeratosis.^[21]

Stability studies

The stability studies were performed as per the ICH guidelines at 30°C ± 2°C/65% relative humidity (RH) ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH. The parameters evaluated for any significant change were the appearance, color, pH, viscosity, spreadability, and drug content of the formulations.

Gas chromatography-mass spectrometry

Developed formulations of the plant extracts were subjected to the performance of stability at the end of 6 months to assess toxic components.

RESULTS AND DISCUSSION

Table 1 depicts the physical evaluation of plant extracts. From CC, components obtained were oleic acid, squalene, etc., CR showed the presence of n-hexadecanoic acid, ethyl ester, Vitamin E, 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy]methyl] ethylester, phytol, 3-epivindoline, and oleic acid.^[11] Five different compounds were analyzed from AS seeds, viz., oleic acid; hexadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester; 9-octadecenoic acid (Z), 2-hydroxy-1-(hydroxyl-methyl) ethyl ester; 9,12,15-octadecatrienoic acid-2-[(trimethylsilyl)-oxy]-1-[(trimethylsilyl)oxy]methyl] ethyl ester (Z, Z, Z); and n-hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester.^[12] PL seeds revealed compounds such as 16-hentriacontanone, squalene, n-hexadecanoic

Table 1: Physical evaluation of plant extracts

Parameter	Observations			
	CC	CR	AS	PL
Appearance	Coarse powder	Slightly sticky powder	Oily liquid	Oily liquid
Color	Dark brownish green	Dark green	Colorless	Pale yellow
Odor	Characteristic	Strong tea-like odor	Odorless	Characteristic

CC: *Carissa congesta*; CR: *Catharanthus roseus*; AS: *Annona squamosa*; PL: *Polyalthia longifolia*

acid, trans-13-octadecenoic acid, 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester.^[13] The extracts were found to be readily soluble in ethanol, methanol, and clove oil. They were compatible with the excipients used in the formulations. Table 2 shows the data on formulation evaluation.

All the formulations were found to be homogeneous [Figure 1]. No clumps or large aggregates were found to be present. The dispersed globules appeared red against the colorless ground. Hence, the creams were O/W type. No bleeding was observed in any of the formulations kept under test, indicating the formulations to be stable. The site of application did not show any inflammation or rashes, indicating that the formulations were non-irritant to skin. The acute dermal toxicity studies

of the formulations were determined according to the OECD guidelines no. 402. The formulations were safe up to the dose of 2000 mg/kg. All the treated animals were found to be absolutely normal with respect to behavioral changes as well as fur and eyes observations. No mortalities were observed. All surviving animals had gained body weight on day 14 as compared to day 0. All the animals appeared normal throughout the experimental period. Based on the results obtained, suitable doses of 250 and 500 mg were chosen for testing the activity in the formulations for the antipsoriatic animal models.

Histopathologically, numbers of features were observed in fully developed lesions in psoriasis such as Munro's microabscess, regular elongation of rete ridges, capillary loop dilatation, absence of granular cell layer, and prominent parakeratosis. Positive control group showed regular elongation of rete ridges with marked capillary loop dilatation and lesion of Munro's microabscess in the parakeratotic layer. The section also showed increase in relative epidermal thickness. In the case of the standard group, a slight lesion of Munro's microabscess, capillary loop dilatation, and elongation of rete ridges were observed. At 250 mg formulations, no significant response in comparison to positive control and standard groups was observed. At 500 mg formulations, there was absence of Munro's microabscess, capillary loop dilatation, as well as elongation of rete ridges in the sections. Hence, it was concluded that 500 mg shows good therapeutic potential [Table 3 and Figures 2, 3].

There was no significant change in any of the evaluated parameters for the specified period of time. The drug content was also found to be within the specified limits. Hence, it can be concluded that the formulations were stable at the specific conditions of temperature and humidity. CC cream and gel showed the presence of benzoic acid, 4-hydroxy-; cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1 α ,2 β ,5 α)]-; 2,8,9-trioxo-5-aza-1-Silabicyclo[3,3,3]undecane, 1-methyl-; I-propyltetradecanoate; n-hexadecane; 1-hexadecanol; isopropyl palmitate; oleic acid and oxazolidinone, 4,4-dimethyl-; cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1 α ,2 α ,5 β)-; thiomorpholine, N-(2-hydroxyethyl)-; bicine; hexadecanoic acid, 2-hydroxy-1-

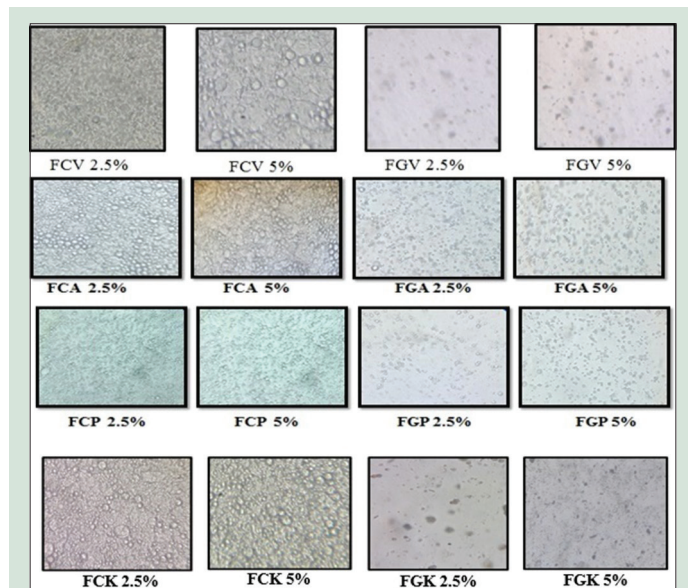


Figure 1: Microscopic images showing grittiness

Table 2: Formulations evaluation of the plant extracts

Formulation	Color	pH	Viscosity (Cps)	Spreadability (g/s)	Drug content (%)
CC cream					
2.5%	Light brown	6.82	222000	37	94.2
5%		6.78	182000	40	96.1
CC gel					
2.5%	Dark coffee-like	6.91	297500	40.2	95
5%	brown	6.75	201000	42.2	96.7
CR cream					
2.5%	Pale green	7.1	197800	38.2	96.1
5%		6.83	199500	41.6	97.4
CR gel					
2.5%	Coral green	6.67	191600	39.06	95.9
5%		6.77	193800	41.53	96.8
AS cream					
2.5%	White	6.96	162800	33.33	95.73
5%		6.95	127000	34.86	97.86
AS gel					
2.5%	White	6.73	186900	33.53	96.8
5%		6.8	222600	36.20	97.06
PL cream					
2.5%	White	6.88	147200	34.66	96.5
5%		6.87	136600	36.44	97.66
PL gel					
2.5%	White	6.93	78130	35.33	94.33
5%		6.84	73200	37.33	95.83

CC: *Carissa congesta*; CR: *Catharanthus roseus*; AS: *Annona squamosa*; PL: *Polyalthia longifolia*

(hydroxymethyl) ethyl ester; 2-piperidinone, N-[4-bromo-n-butyl], respectively. CR cream and gel showed the presence of tetradecane, isopropyl myristate, hexadecan-1-ol, trans-9- and oleic acid and decane, 6-ethyl-2-methyl-; tridecane and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester. AS cream and gel showed the presence of oleic acid; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; 1-hexadecanol and oleic acid; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester. PL cream and gel showed the

presence of n-hexadecanoic acid; 1-hexadecanol and 9-octadecenoic acid (Z); 2-hydroxy-1-(hydroxymethyl) ethyl ester.

Selected plants may be existing in the treatment of psoriasis as combination in the development of herbal formulations. However, we have attempted developing monoherbal formulations from these plant extracts. Psoriasis has been termed as “psora” meaning itch. It is a common inflammatory condition of the human skin characterized by focal to coalescing raised cutaneous plaques with consistent scaling as well as variable erythema.

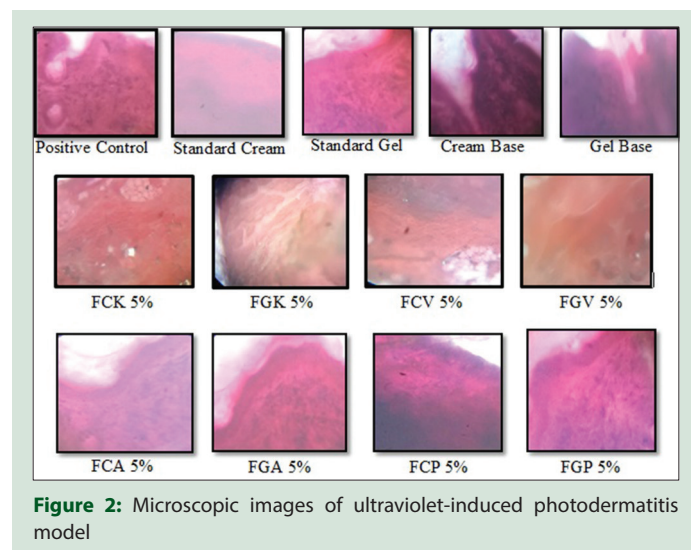


Figure 2: Microscopic images of ultraviolet-induced photodermatitis model

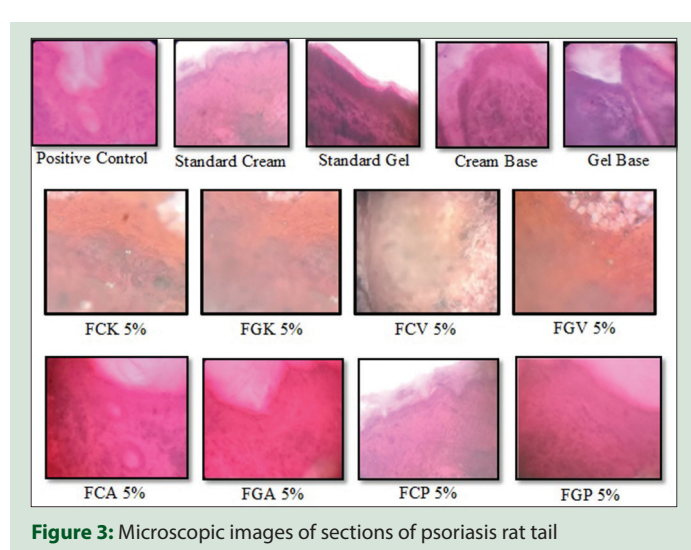


Figure 3: Microscopic images of sections of psoriasis rat tail

Table 3: Histopathological examination

Groups	Ultraviolet-induced photodermatitis model			Psoriasis rat tail		
	Munro micro abscess	Elongation of rete ridges	Capillary loop dilatation	Munro micro abscess	Elongation of rete ridges	Capillary loop dilatation
Positive control	++	+++	+++	++	+++	+++
Standard cream	+	+	+	++	+	++
Standard gel	+	+	+	+	+	+
Cream base	+	++	++	+	++	++
Gel base	+	+	++	+	++	++
CC cream						
2.5%	++	++	+	+	+	+
5%	-	-	-	-	-	-
CC gel						
2.5%	+	+	++	+	+	++
5%	-	-	-	-	-	-
CR cream						
2.5%	+	+	+	++	+	+
5%	-	-	-	-	-	-
CR gel						
2.5%	++	++	+	++	++	++
5%	-	-	-	-	-	-
AS cream						
2.5%	+	+	+	+	+	+
5%	-	+	-	-	+	-
AS gel						
2.5%	+	++	+	+	++	+
5%	+	-	-	-	-	-
PL cream						
2.5%	+	++	+	+	++	+
5%	-	-	-	-	+	-
PL gel						
2.5%	+	++	+	+	++	+
5%	+	-	-	-	-	-

+: Mild or slight grade lesion; ++: Moderate grade lesion; +++: Severe grade lesion; -: No lesion in section; CC: *Carissa congesta*; CR: *Catharanthus roseus*; AS: *Annona squamosa*; PL: *Polyalthia longifolia*

Psoriasis occurs when the immune system considers the skin cells as a pathogen and begins to send faulty signals via signaling molecules, such as cytokines, chemokines, and growth factors. The severity of psoriasis can be divided into benign, moderate, and severe psoriasis.^[20-22]

Histopathologically, psoriasis has been judged on the basis of epidermal hyperplasia along with the elongation of the rete ridges, a less discrete epidermal granular layer, and persistence of the keratinocytes nuclei in the stratum corneum of the epidermis. In psoriasis, epidermal hyperproliferation, abnormal keratinocyte differentiation, angiogenesis with blood vessel dilatation, excess Th-1 and Th-17 inflammation, and leukocytic infiltration of the dermis and epidermis have been observed.^[20]

Topical delivery is an attractive route for local and systemic treatment of psoriasis. It can penetrate deeper into the skin and give better absorption. They were deemed more effective and less toxic due to the biphasic composition and structure. A topical preparation avoids the gastrointestinal irritation, prevents the metabolism of the drug in the liver, and increases the bioavailability of the drug. Topical preparations give its action directly at the site of action. At 250 mg formulations, there were no satisfactory responses, but at 500 mg formulations, there was absence of Munro's microabscess, capillary loop dilatation, as well as elongation of rete ridges in the sections. Hence, it was concluded that 500 mg shows good therapeutic effects. The GC-MS studies done after 6 months stability period indicated that creams and gels were stable and no harmful degradation products were formed during the stability period.^[16,22-24]

CONCLUSION

The pH of the formulations was found to be 6.5–7 and polymers were compatible with all the extracts. The creams and gels were found to be homogeneous, nongritty, non-irritant, no-phase separation, and stable at the specified conditions of temperature and humidity. The topical formulations revealed antipsoriatic activity. Hence, it can be predicated that upcoming studies can be more focused on plant-related remedies for psoriasis. GC-MS studies provide confirmatory evidence about no toxic degradation components.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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