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Antipsoriatic activity of *Cassia auriculata* L. flowers in Freund's adjuvant-formaldehyde induced animal model

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The plant *Cassia auriculata* L. belonging to the family Caesalpiniaceae (Leguminosae) used in the Ayurvedic system of medicine for the treatment of diabetes, ulcers, leprosy, conjunctivitis, liver and skin diseases. The present study aimed to evaluate the antipsoriatic effect of the ointments 0.5 and 1.0% (w/w) containing ethanol extract of the flowers of *C. auriculata* using complete Freund's adjuvant (CFA) and induced animal model and also evaluated for its physical parameters. Antipsoriatic effect of 0.5 and 1.0% (w/w) ointments was evaluated by the phenotypic features (redness, erythema, and scales) in terms of psoriasis severity index (PSI) and histological features (epidermal thickness and degree of orthokeratosis). Evaluation of physical parameters for the prepared ointments showed satisfactory results with an acceptable condition of consistency for application. 0.5 and 1.0% (w/w) ointments treated animals showed a significant (**P* <0.05) increase in the orthokeratinocyte layer and a significant (***P* <0.01) reduction in the epidermal layer of skin treated with 1.0% (w/w) ointment with a progressive reduction (***P* <0.01) in the severity of psoriatic lesions (erythema, redness, and scales) from day 7 to 21st day. The present investigations revealed that the flowers of *Cassia auriculata* possess antipsoriatic activity, confirming their traditional use in skin disorders.

Keywords: *Cassia auriculata* L., Complete Freund's Adjuvant, Herbal ointment, Psoriasis.

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Introduction

Psoriasis is an inflammatory disease of the skin that affects as many as 125 million people worldwide. It is characterized by pink colour plaques and white flaky skin due to increased levels of pro-inflammatory cytokines and over-proliferation of keratinocytes at the basal layer of the epidermis[1]. As psoriasis is an immune-disorder, also associated with overexpression of pro-inflammatory cytokines and abnormal proliferation of keratinocytes, the therapeutic agents that may be able to inhibit proliferation of psoriatic keratinocytes or able to modulate the immune system are suggested for treating psoriasis[2]. For many people with psoriasis, existing treatments are not effective, appropriate or may not be accessible due to cost. As herbal formulations are considered to be less expensive with minimal side effects, a review by Deng *et. al.* highlighted possible alternative medicine for the treatment of psoriasis[3].

Generally, *Cassia* species are well known for their laxative and purgative constituents and are also used for the cure of skin diseases. *Cassia auriculata* L. (Caesalpinaceae), commonly known as Tanners Senna, is a common, highly branched shrub with large bright yellow flowers distributed wildly in dry regions of the central provinces and western peninsula of India[4]. The plant as a whole has been extensively used in *Ayurvedic* and *Siddha* practice as antidiabetic, antidysenteric, antimicrobial and for various skin diseases from ancient times[5-7]. Therefore, in light of ethnopharmacological facts of the plant, to validate its use in skin diseases scientifically, an attempt has been made to investigate the therapeutic potential of ethanolic extract of *C. auriculata* flowers in chronic inflammatory skin disease, psoriasis in Complete Freund's adjuvant (CFA) and formaldehyde-induced psoriatic model.

Materials and Methods

Plant materials

The plant specimen for the proposed study were collected in the month of November 2018 from Kancheepuram, Tamil Nadu and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research

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Center, (PARC) Tambaram and Chennai. A voucher specimen No. PARC/2017/2155 has been deposited for future references.

Extraction

The flowers of *C. auriculata* were isolated and subjected to drying followed by grinding. To remove fatty materials, the coarse powder was extracted with petroleum ether (60-80 °C) for 72 hours, followed by extraction using a Soxhlet apparatus with 95% ethanol for 36 hours. The collected ethanol extract was filtered and concentrated using a rotary vacuum flash evaporator in a vacuum under reduced pressure[8]. The dried extract was stored in an airtight container at 4 °C and the percentage yield of the ethanol extract was noted.

Phytochemical analysis

The ethanol extract was subjected to preliminary phytochemical analysis for the identification of various phytochemical constituents present using standard methods[9].

Thin-layer chromatography (TLC)

To support preliminary chemical analysis, the ethanolic extract was subjected to TLC study. A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent system Toluene:Ethyl acetate:Formic acid (5:4:1). After developing, the plates were air-dried and exposed to iodine vapour.

Formulation of ointment

Topical therapy is the standard of care for treatment of mild to moderate disease. A large proportion of patients would benefit from topical therapy, which can be initiated at the primary care level[10]. Hence, herbal ointment containing ethanol extract of *C. auriculata* (0.5 and 1.0% w/w) was prepared using wool fat, hard paraffin, yellow soft paraffin and cetosteryl alcohol as oleaginous phase and extract, glycerin and water as the aqueous phase as per the formula in Table 1. The oleaginous base was prepared by heating the wool fat, hard paraffin and the cetosteryl alcohol at 70 ^{o}C. The aqueous phase was prepared by boiling the extract, glycerin and the water at 70 ^{o}C. On reaching 70 ^{o}C the aqueous phase was slowly added to the oleaginous phase with continuous stirring and was left to cool[11].

Evaluation of ointment

The physical parameters such as the colour of the ointment, pH, viscosity of the ointment, spreadability and washability were evaluated as per standard procedure[12].

Table 1 — Formulation of ointment

S. No	Ingredients	Quantity for 0.5% w/w	Quantity for 1.0% w/w
1	Yellow soft paraffin	26.45g	26.45g
2	Hard Paraffin	26.45g	26.45g
3	Wool Fat	21.45g	21.45g
4	Cetosteryl alcohol	10.15g	10.1g
5	Water	5 mL	5 mL
6	Glycerine	10 mL	10 mL
7	Ethanolic Extract	0.5%	1.0%

Animals

Adult Swiss albino mice weighing about 25±2 g of age 10 weeks were obtained from the Institutional Animal house. Animals were housed in polypropylene cages and were acclimatized under controlled condition (a 12 h light-dark cycle at 22±2 ^{o}C) on standard pellet diet and water *ad libitum* for 7 days. As per the guidelines of CPCSEA, all animals were maintained with due approval from the Institutional Animal Ethics Committee (IAEC). The protocol was approved by the IAEC (No. XXI/VELS/PCOL/14/2000/CPCSEA/IAEC/01.12.2017).

Acute dermal toxicity

According to the Organization for Economic Cooperation and Development guidelines (402), the acute dermal toxicity of the formulated herbal ointments was evaluated. Swiss albino mice were divided into two groups, each group consisting of 6 animals. Hairs were shaved on the 10% dorsal surface of the animal body 24 hours prior to the test. The 0.05 and 0.1% (w/w) of formulated ointments of dose 200 mg/kg body weight was applied on the shaved area topically. The treated animals of both groups were monitored for 14 days for redness, erythema, and changes in fur, sleep pattern, behaviour pattern, and mortality[13].

Evaluation of anti-psoriatic activity

Complete Freund's adjuvant and formaldehyde induced model - Psoriasis induction

A mixture of CFA and formaldehyde in the proportion of 1:10 ratio was prepared. Using depilatory cream, hairs on the dorsal region (2 cm × 2 cm) were removed (Reckitt Benckiser, Inc., UK) from the animal. About 0.1 mL of the prepared mixture was applied on the shaved area topically of all test animals (n = 10), for 3 days.

Psoriasis induced animals were observed for the presence of psoriatic lesions for 7 days daily. Based on the psoriasis area clinically and severity index, an objective scoring system was developed. Redness, erythema, and scales were scored independently on a scale from 0 to 4: 0-none; 1-slight; 2-moderate; 3- marked; and 4-very marked. Psoriasis severity index (PSI) was measured in terms of the cumulative score (sum of redness, erythema, and scaling) (scale 0 – 12). Animals were anaesthetized using ketamine at the end of the study, skin specimen was collected and preserved in glass vials containing 10% formalin solution and subjected to histological examination. Longitudinal sections of mice skin specimen (about 5 μm thickness) were prepared by microtomy and stained with hematoxylin (H) and eosin (E) dye for histological examination[14].

Antipsoriatic activity of the prepared herbal ointment

A mixture of CFA and formaldehyde (1:10 ratio) was applied topically to induce Psoriasis. All the animals were re-randomized before treatment to reduce the error in mean PSI between the groups after the induction. Psoriasis induced animals were divided into four groups of 6 each (n = 6). Group I (Untreated), Group II (Positive control) treated with Retino-A cream (0.05%), Group III and IV were treated with 0.05 and 0.1% (w/w) of prepared ointments, respectively. Animals were treated with the ointment after induction of psoriasis for 3 weeks once daily. Antipsoriatic effect of the ointment was evaluated in terms of the severity of psoriatic lesions score every week indicating the reduction in the psoriatic symptoms.

Increase in percentage of orthokeratotic regions indicates drug activity. Presence of a granular layer induced was examined in 10 sequential scales which show previously parakeratotic skin areas. The induction of orthokeratosis, which have normally a parakeratotic differentiation in psoriatic condition, was quantified by measuring the length of the granular layer (A) and the length of the scale (B). The proportion (A/B)×100 represents the % orthokeratosis per scale, and the drug activity (DA) was calculated as follows:

$$DA = \frac{\text{mean OK of treated group} - \text{mean OK of control group} \times 100}{100 - \text{mean OK of control group}}$$

where OK = orthokeratosis.

The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit[15].

Histological examination

After treatment with the prepared ointment, the animals were subjected to ketamine overdose and the dorsal skin part was removed and stored in 4% formalin. Then the skin samples were given for histopathological study which was performed using eosin stain and haematoxylin. It was obtained by measuring the distance between the dermo-epidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales.

Statistical analysis

Values were represented as mean±SEM. Data were analyzed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparison test using Instat-V3 software. P values <0.05 were considered significant.

Results and Discussion

Preliminary phytochemical screening

The percentage yield of ethanol extract of *C. auriculata* was found to be 10.20% w/w. The qualitative phytochemical analysis of the extract showed the presence of flavonoids, alkaloids, tannins, glycosides, terpenoids, phenols, carbohydrates, and proteins.

Thin-layer chromatography

The TLC studies of the ethanol extract of *Cassia auriculata* showed the presence of well-separated 6 cleared spot with the Rf values of range 0.07-0.89.

Formulation of herbal ointment

Simple ointment 0.5 and 1.0% (w/w) containing the ethanol extract of *C. auriculata* was prepared as per the formula given in Table 1.

Evaluation of formulated herbal ointment

The prepared ointment containing ethanol extract of *C. auriculata* was evaluated for various physico-chemical parameters. The pH of the ointment lies within the normal pH range of the human skin (6.8±1). The prepared ointments, when applied over the animal skin for a week, did not produce any skin irritation in terms of erythema and oedema. Spreadability of the prepared ointments was found to be in the range 6.5 to 6.6, revealing an easy in spreading over the skin and therefore the active ingredient in the ointment would be released for a local effect. In general, all formulations met the

acceptable conditions of consistency for application. The results are shown in Table 2.

Antipsoriatic activity

Induction of psoriasis

Psoriasis was induced in mice by applying 0.1 mL of the mixture of CFA and formaldehyde for 7 days on the dorsal portion of the animal. On the 7th day of induction, silvery scales, erythema and redness were observed on exposed area and the severity increased progressively. On the 7th day of psoriatic induction, the cumulative score, PSI was significantly (*P <0.05) increased (Fig. 1).

Histological examination of psoriasis induced in mouse skin by the topical application of CFA and formaldehyde exhibited several pro-inflammatory reactions such as redness, erythema, scales, elongation of rete ridges, presence of Munro's microabscess and dilatation of capillary loop, increased epidermal thickness, keratinocytes proliferation and absence of the granular layer (parakeratosis) when compared with normal animal skin (Plate 1). All these phenotypic and histological features in mice resemble a sign of psoriatic lesions in human plaque psoriasis.

Table 2 — Physical evaluation of formulated ointment

Physico-chemical parameters	Observation (0.5% w/w)	Observation (1.0% w/w)
Colour	Yellow	Brown
Odour	Characteristic	Characteristic
pH	6.7	6.8
Consistency	Smooth	Smooth
Spreadability	6.5	6.6
Washability	Good	Good
Non irritancy	Non irritant	Non irritant

Effect of test formulations on complete freund's adjuvant and formaldehyde-induced psoriasis

Ointments 0.5 and 1.0% (w/w) containing ethanol extract of *C. auriculata* extract were applied once daily for 3 weeks in psoriasis induced animals and the severity of psoriatic lesions was calculated by visual and histological examinations. In untreated animal group (Group I), the severity of psoriatic lesions was gradually increased when examined visually throughout the experimental period and cumulative score (PSI) was increased manifold on 21st day when compared with other groups. In standard group of animals (Group II), topical application of standard drug Retino-A cream (0.05%) reduced the severity of psoriatic lesions and cumulative score progressively (**P <0.01) from day 7 to 21st day confirming the therapeutic effect of a standard drug, retinoid a derivative of vitamin A on

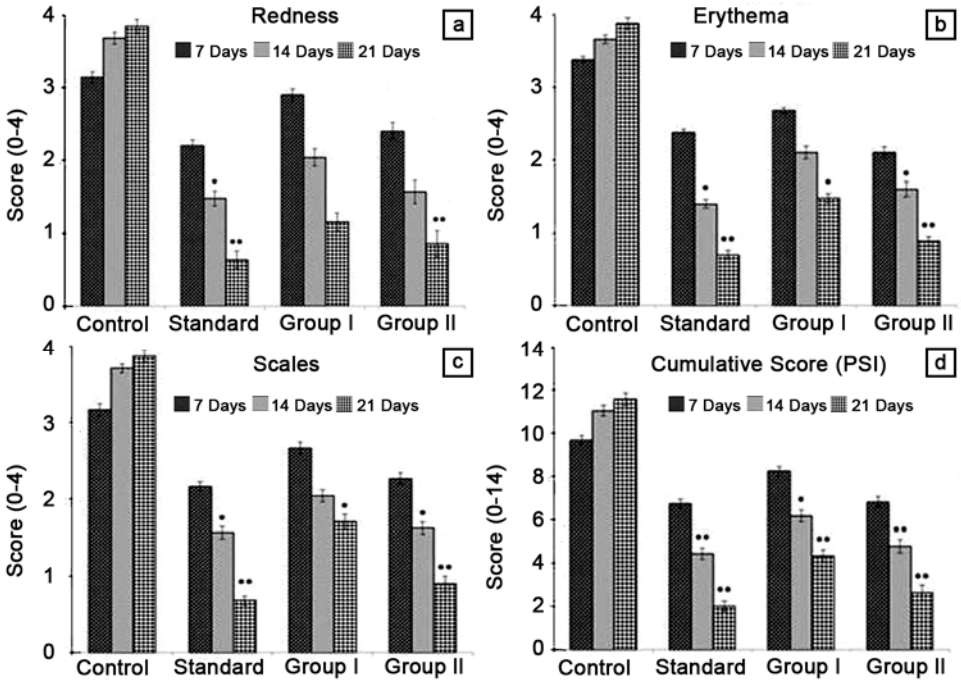

Fig. 1 — Effect of herbal ointment containing ethanol extract of *Cassia auriculata* on the redness, erythema, scales and cumulative score in CFA-formaldehyde induced model. Values are mean±SEM of 6 parallel measurements. Data were analyzed by one-way ANOVA followed by Tukey Kramer multiple comparison test. The values are *P <0.05; **P <0.01 when compared against control.

psoriatic lesions reduces inflammation by reducing the formation of cytokines and interleukins. Animals treated with 0.5% (w/w) ointment (Group III), showed a gradual decrease in redness, erythema, scales (psoriatic lesions) and a significant reduction (***P* <0.01) in the cumulative score when compared with control group. Animals treated with 1% (w/w) ointment (Group IV), showed a significant reduction (***P* <0.01) in redness, erythema, scales and cumulative score (Fig. 1).

Animals treated with standard drug (Group II) has increased the orthokeratotic regions significantly (**P* <0.05) by 60.97% and animals treated treated with 0.5 and 1.0% w/w ointment (Group III and IV) containing ethanol extract of *C. auriculata* flowers has increased the orthokeratotic regions by 28.04 and 43.90% respectively in comparison to control group. Increased epidermal thickness (approximately 2 fold increase) was observed in control group, whereas the group treated with standard showed significant (***P* <0.01) decrease in epidermal thickness. Among the tested ointment, 1.0% w/w treated group showed significant (***P* <0.01) decrease in epidermal thickness indicating reduced parakeratosis, retention of granular layer, reduced hyperproliferation of keratinocytes and initiation of keratinization process. The results were shown in Plate 2 and Table 3.

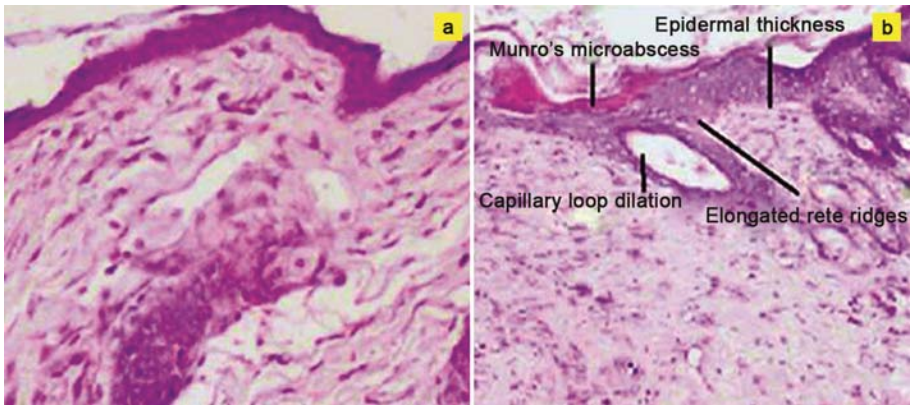

Plate 1 — Longitudinal histological sections of mouse skin (H and E, ×40), a) section of normal mouse skin and b) section of complete Freund's adjuvant- and formaldehyde-treated mouse skin.

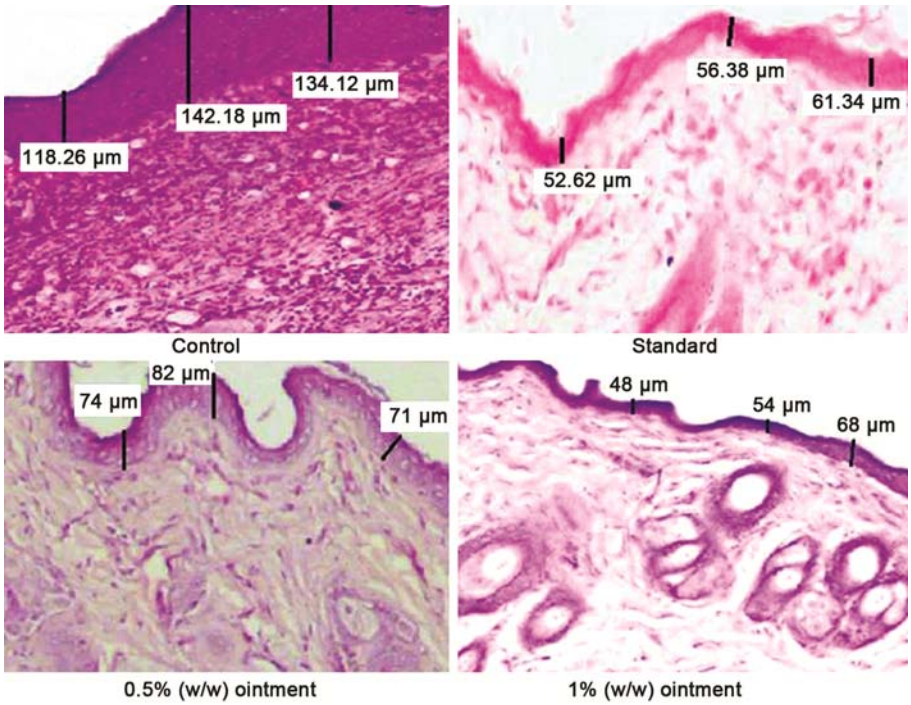

Plate 2 — Longitudinal histological sections of mouse skin of different groups (H and E, ×40).

Table 3 — Antipsoriatic activity of herbal ointment containing ethanol extract of *Cassia auriculata* in CFA and formaldehyde induced model

Group	Degree of orthokeratosis (%)	Drug activity (%)	Mean epidermal thickness (μm)
Control	18.33±0.88	-	128.34±1.20
Standard	68.33±0.88*	60.97%**	54.78±1.73**
Test I (0.5%)	45.42±0.94*	28.04%	79.84±1.40
Test II (1.0%)	1.08*	43.90%	60.56±1.84**

Values are mean±SEM of 6 parallel measurements. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test. The values are *P <0.05; **P <0.01 when compared against control.

The psoriatic lesions in early stage are characterized by intraepidermal penetration of activated polymorphonuclear leukocytes that leads to the uncontrolled production of reactive oxygen species causing peroxidative damage to the skin membranes which leads to the exacerbation of psoriatic lesions. Reactive oxygen species activate phospholipase A2 and increases arachidonic acid mediators release. Psoriasis is also contributed by the production of prostaglandin E2 by the cyclooxygenase pathway through dilating capillaries in the dermis, increasing infiltration of leukocyte and stimulating the growth of keratinocyte cell[16]. Flavonoids, triterpenoids, and polyphenolic compounds are well known for their potential antioxidant effect and for their immunomodulatory, anti-inflammatory, anti proliferative, and free radical scavenging activities[17]. Hence, polyphenolic phytoconstituents may be beneficial for the treatment of diseases with multiple etiologies such as psoriasis. Preliminary phytochemical analysis of the *C. auriculata* flowers showed the presence of the rich amount of polyphenols and flavonoids, which may be responsible for their protective role in the management of psoriasis and may be due to the synergistic effect of phytoconstituents. The results of the present study support the use of *Cassia auriculata* in traditional Indian medicine and can be used as an easily accessible natural source for treating psoriasis, a chronic inflammatory skin disease.

Conclusion

The ethanol extract of the flowers of *Cassia auriculata* was evaluated for antipsoriatic activity in complete Freund's adjuvant and formaldehyde induced animal model. After induction of psoriasis, the animals were treated with the ointment (0.5% and 1% w/w) containing ethanol extract of *Cassia auriculata* flowers and the prepared ointment alleviated the sign of psoriasis along with mean PSI, which may be due to the presence of the polyphenols (flavonoids and tannins). Hence, we conclude that the plant *Cassia auriculata* flowers possesses antipsoriatic activity which is in agreement with its traditional use.

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Conflict of interest

Authors state that they have no conflict of interest.

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