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Evaluation of herbal ointment containing ethanol extract of *Plecranthus amboinicus* root for the management of psoriasis

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Plant, *Plecranthus amboinicus* (Lour.) Spreng, belonging to the family Lamiaceae, commonly known as 'Karpuravalli' in Tamil language is widely used in folk medicine to treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases. The present study aimed to evaluate antipsoriatic effect of the ethanol extract of *Plecranthus amboinicus* root. Ointment containing ethanolic extract of *Plecranthus amboinicus* root was prepared and evaluated for antipsoriatic activity using complete Freund's adjuvant (CFA) and formaldehyde induced animal model. Psoriasis is induced by applying mixture of 0.1 mL of prepared CFA and formaldehyde mixture (1:10 ratio) topically for 7 days on the dorsum surface of the skin of Swiss albino mice. Antipsoriatic effect of 0.5% and 1.0% (w/w) ointments containing ethanolic extract of *Plecranthus amboinicus* root was evaluated in terms of Psoriasis severity index (PSI) by the phenotypic features (redness, scales and erythema) and histological features (epidermal thickness). The result showed that there was a significant increase in the orthokeratinocyte layer and a significant reduction in the epidermal layer of skin in the *in vivo* mice model with a progressive reduction ($p^{**}<0.01$) in the severity of psoriatic lesions (redness, erythema, and scales) from day 7 to 21st day and significant ($p^{*}<0.05$) decreased epidermal thickness and increased orthokeratotic regions in animals treated with 0.5% and 1.0% (w/w) ointments of *Plecranthus amboinicus* root. The present investigations revealed that *Plecranthus amboinicus* root possess potent antipsoriatic activity, confirming their traditional use in skin disorders.

Keywords: Complete Freund's adjuvant, Herbal ointment, *Plecranthus amboinicus,* Psoriasis

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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]. In the psoriatic state, the epithelialization occurs in about 36 h and so the division of keratinocytes which reduces to 10 days (240 h). This phenomenon collectively leads to hyper keratinized state. As psoriasis is an immune-disorder, also associated with over expression of pro-inflammatory cytokines and abnormal proliferation of keratinocytes, therapeutic agents that either modulate the immune system or normalize the differentiation program of psoriatic keratinocytes are suggested for treating psoriasis[2,3]. The course of treatment available for treating psoriasis includes the use of the combinations of conventional methods like using coal tar preparations, dithranol, calcipotriol, topical corticosteroids and controlled UV radiations. However, serious side effects are associated with them. Systemic treatment is considered if extensive psoriasis fails to respond to local measures. For many people with psoriasis, existing treatments are not effective, appropriate or may not be accessible due to cost. In general, herbal formulations are less expensive and are known to minimize the risk of side effects and therefore, provide a viable alternative for psoriasis treatment[4].

Plectranthus amboinicus (Lour.) Spreng. is a perennial herb belonging to the family Lamiaceae which occurs naturally throughout the tropics and warm regions of Africa, Asia and Australia[5]. This herb has therapeutic and nutritional properties attributed to its natural phytochemical compounds which are highly valued in the pharmaceutical

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industry[6,7]. It is widely used in folk medicine to treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases[8,9]. The literature survey revealed the occurrence 76 volatile and 30 non-volatile compounds belonging to different classes of phytochemicals[10]. However, till date there are no validated scientific reports for its antipsoriatic activity. Therefore, in light of ethnopharmacological facts of the plant, the aim of the present study was to investigate the antipsoriatic potential of ethanolic extract of *Plectranthus amboinicus* (Lour.) Spreng in CFA and formaldehyde-induced psoriatic model.

Materials and methods

Plant materials

The Plant specimen for the proposed study were collected by the month of November from the different places of Chennai, Tamil Nadu and authenticated by Dr P Jayaraman, Director, Plant Anatomy Research Center (PARC) Tambaram and Chennai-65. A voucher specimen No. PARC/2017/2156 has been deposited for further references.

Extraction

The roots of *Plecranthus amboinicus* were isolated and subjected to drying followed by grinding. Coarsely dried powder was defatted with petroleum ether (60-80°C) for 72 h to remove fatty materials and then extracted with ethanol (95%) using Soxhlet apparatus for 36 h, the extract was collected, filtered through Whatman filter paper, concentrated in vacuum under reduced pressure using a rotary vacuum flash evaporator and the dried extract was stored in airtight container at 4°C for further study. The percentage yield of the extract was calculated.

Phytochemical analysis

The ethanol extract was subjected to preliminary phytochemical analysis for the identification of various phytochemical constituents present using standard methods[11].

Thin layer Chromatography (TLC)

To support preliminary chemical analysis, ethanolic extract was subjected to TLC study. A number of developing solvent systems were tried for fractions showing presence of flavonoids 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

Simple ointment containing the ethanol extract of *Plecranthus amboinicus* (0.5% w/w 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°C. The aqueous phase was prepared by boiling the extract, glycerin and the water at 70°C. On reaching 70°C the aqueous phase was slowly added to the oleaginous phase with continuous stirring and was left to cool[12].

Evaluation of ointment

The physical parameters such as colour of the ointment, its pH, viscosity of the ointment, spread ability and wash ability were evaluated as per standard procedure[13].

Animals

Adult Swiss albino mice weighing about 25±2 kg of age 10 weeks were obtained from the Institutional Animal house. Animals were housed in polypropylene cages and were left 7 days for acclimatization to animal room maintained under controlled condition (a 12 h light–dark cycle at 22±2ºC) on standard pellet diet and water ad libitum. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with due approval from the Institutional Animal Ethics Committee (IAEC). IAEC approved the protocol (Registration no. XXI/VELS/PCOL/ 14/2000/CPCSEA/IAEC/01.12.2017).

Acute dermal toxicity

The acute dermal toxicity of prepared ointments was evaluated according to the Organization for Economic Cooperation and Development guidelines no. 402. Swiss albino mice were divided into two groups each group with consisting of 6 animals.

Table 1 — Formulation of Ointment

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

Twenty-four hour prior to the test, hair were removed from the 10% of the body surface area on dorsum portion. Starting dose 2000 mg/kg, body weight of prepared 0.05% and 0.1% (w/w) ointments was applied topically on the shaved area. The treated animals of both groups were monitored for 14 days for redness, erythema, and changes in fur, sleep pattern, behavior pattern and mortality[14].

Evaluation of anti-psoriatic activity
CFA and formaldehyde induced model
Induction of Psoriasis

A stable mixture of CFA and formaldehyde (1:10 ratio) was prepared. Hair on the dorsum portion (nearly 2 cm×2 cm) of each mouse were removed using depilatory cream (Reckitt Benckiser, Inc., UK). A volume of 0.1 mL of the prepared mixture was applied topically on the shaved area of all test animals (n =10), at day 1, 2, and 3.

Animals were observed for psoriatic lesions daily for 7 days. An objective scoring system was developed based on the clinical psoriasis area and severity index. 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. The cumulative score (sum of redness, erythema, and scaling) served as a measure of the psoriasis severity index (PSI) (scale 0–12). At the end of the study, animals were anesthetized using ketamine, and specimen of skin was collected and preserved in glass vials containing 10% formalin solution for histological examination. Longitudinal sections of mice skin specimen (about 5 μm thickness) were prepared by microtomy and stained with hematoxylin and eosin (H and E) dye for histological examination[15].

Antipsoriatic activity of the prepared herbal ointment

Psoriasis was induced in the animals by topically applying the mixture of CFA and formaldehyde as mentioned in "Induction of psoriasis." After the induction, all the animals were re-randomized before treatment to reduce the error in mean PSI between the groups. Disease-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) ointments respectively. Animals were treated after induction of psoriatic lesions once daily for 3 weeks. Reduction in the symptoms of psoriasis was evaluated by scoring the severity of psoriatic lesions every week.

Drug activity is defined by the increase in percentage of orthokeratotic regions. Ten sequential scales were examined for the presence of a granular layer induced in the previously parakeratotic skin areas. The induction of orthokeratosis in those parts of the adult mouse tail, which have normally a parakeratotic differentiation, was quantified 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:

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

where OK = orthokeratosis

The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit[16].

Histological examination

After treatment with the prepared ointment, the animals were subjected to ketamine overdose and the dosal 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 heamatoxylin. It was obtained by measuring the distance between the dermoepidermal 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 *Plecranthus amboinicus* was found to be 4.80%w/w. The qualitative phytochemical analysis of the extract showed the presence of flavonoids, glycosides, alkaloids, tannins, triterpenoids, polyphenols, carbohydrates, and proteins.

Thin Layer Chromatography (TLC)

The TLC studies of the ethanol extract of *Plecranthus amboinicus* showed the presence of well separated 5 cleared spot with the Rf values of range 0.12-0.81.

Formulation of herbal ointment

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

Evaluation of formulated herbal ointment

The various physicochemical parameters utilized to evaluate the prepared ointment formulations are shown in Table 2. The pH of the formulations lies in the normal pH range of the human skin (6.8±1). Both the formulations did not produce any skin irritation, i.e., erythema and edema for about a week when applied over the skin. Spread ability of all formulations was found to vary from 6.5 to 6.6, indicating an easy in spreading over the skin and therefore the active ingredient, which was *Plecranthus amboinicus* extract, would be released for a local effect. In general, all formulations met the acceptable conditions of consistency for application.

Antipsoriatic activity

Induction of Psoriasis

Topical application of 0.1 mL of CFA and formaldehyde for 7 days resulted in the development of induced psoriasis on the dorsum portion of the mice. Several phenotypic changes such as redness, erythema, and silvery scales on exposed area were marked visually and found an increase in severity progressively, whereas the cumulative score, PSI was significantly ($p<0.05$) increased on the 7th day of induction, shown in Table 3.

Increased epidermal thickness, hyperproliferation of keratinocytes, granulocyte infiltration, the presence of Munro's microabscess, capillary loop dilatation, elongation of rete ridges and absence of the granular cell layer (parakeratosis) were observed through histological examination in CFA- and formaldehyde-treated mouse skin as compared to normal mouse skin (Fig. 1). All these phenotypic and histological features have a close resemblance with the human plaque psoriasis.

Effect of test formulations on CFA and formaldehyde-induced Psoriasis

Application of 0.5% and 1.0% (w/w) ointments containing ethanol extract of *Plecranthus amboinicus* were applied once daily for 3 weeks in CFA-formaldehyde induced psoriasis, and the severity of psoriatic lesions was evaluated by visual and histological examinations. In visual examinations, the severity of psoriatic lesions (redness, erythema, and scales) was gradually increased in the untreated group (Group I) throughout the experimental period. Cumulative score was significantly ($p<0.05$) increased manifold in Group I on 21st day in comparison to other groups. In Group II, topical application of Retino-A cream (0.05%) progressively reduced ($p<0.01$) the severity of redness, erythema,

Table 2 — Physical evaluation of formulated ointment

Physiochemical parameters	Observation (0.5%w/w)	Observation (1.0%w/w)
Colour	Mild brown	Brown
Odour	characteristic	characteristic
pH	5.8	5.7
Consistency	Smooth	Smooth
Spreadability	6.3	6.2
Washability	Good	Good
Non irritancy	Non irritant	Non irritant

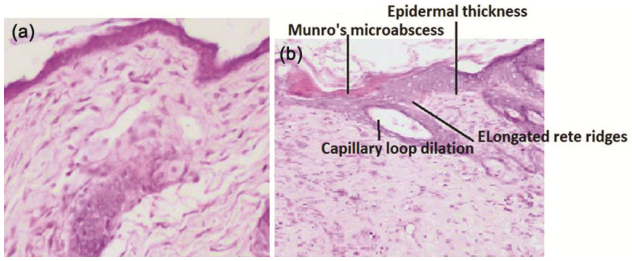

Fig. 1 — Longitudinal histological sections of mouse skin (H and E, ×40), (A) section of normal mouse skin and (B) section of complete Freund's adjuvant- and formaldehyde-treated mouse skin.

Table 3 — Examination of redness, erythema, and scales in CFA and formaldehyde-treated mice

Day	Redness	Erythema	Scales	Cumulative score (PSI)
1	0.86±0.06	-	-	0.86±0.06
2	1.04±0.04	-	-	1.04±0.04
3	1.46±0.08	0.58±0.05	-	2.04±0.13
4	1.94±0.04**	1.08±0.06	0.64±0.05	3.66±0.15
5	2.48±0.08	1.52±0.04	1.48±0.06	5.48±0.18
6	2.94±0.04	2.84±0.08	2.82±0.04	6.60±0.16
7	3.14±0.08	3.38±0.04*	3.16.±0.08**	7.68±0.20*

'alues are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparison test. The alues were considered significant at *p<0.05; **p<0.01. PSI: Psoriasis Severity Index

scales and cumulative score from day 7 to 21st day confirming the therapeutic effect of a standard drug on psoriatic lesions. Application of 0.05% (w/w) ointment in Group III, showed a gradual decrease in redness, erythema, and scales along with a significant reduction ($p<0.05$) in the cumulative score in comparison to the Group I. Application of 0.1% (w/w) ointment in Group IV animals was found to possess a significant reduction ($p<0.01$) on psoriatic lesions such as redness, erythema, scales and cumulative score showing the therapeutic efficacy of *Plecranthus amboinicus* roots on CFA-formaldehyde induced psoriatic animal model (Fig. 2).

Topical application of Retino-A cream (0.05%) in Group II has increased the orthokeratotic regions significantly (**$p<0.01$) by 60.97% and Group III and IV treated with ointment (0.5% & 1.0% w/w) containing ethanol extract of *Plecranthus amboinicus* roots has increased the orthokeratotic regions by 19.51% and 36.58% respectively in comparison to normal. 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 prepared ointment, 1.0% w/w treated group showed significant (*$p<0.05$) decrease in epidermal thickness.

Ethanol extract of *Plecranthus amboinicus* roots treated group showed remarkable therapeutic effect by reducing epidermal thickness significantly (*$p<0.05$) in terms of reduced parakeratosis and granular layer retention, indicating the reduced hyperproliferation of keratinocytes and initiation of keratinization process. The results were shown in Table 4 and Fig. 3.

Early and active psoriatic lesions are characterized by intraepidermal penetration of activated polymorphonuclear leukocytes, which causes the uncontrolled production of reactive oxygen species, leading to peroxidative damage to membranes of the skin and contributing to the exacerbation of lesions. Reactive oxygen species may also activate

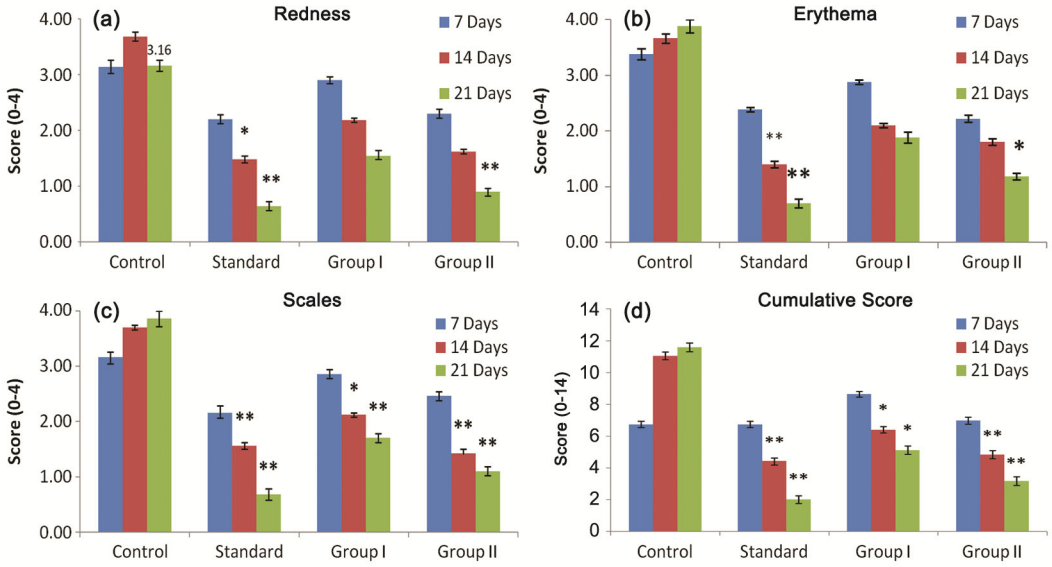

Fig. 2 — Effect of herbal ointment containing ethanol extract of *Plecranthus amboinicus* on the redness, erythema, scales and cumulative score in CFA-formaldehyde induced model. Values are mean ± SEM of 6 parallel measurements. Data were analyzed by one-way ANOVA followed by Tukey Kramer multiple comparison test. The values are *$p< 0.05$; **$p< 0.01$ when compared against control

Table 4 — Antipsoriatic activity of Herbal ointment containing ethanol extract of *Plecranthus amboinicus* 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%)	34.33±1.20	19.51%	84.92±2.31
Test II (1.0%)	48.67±1.45	36.58%	69.67±1.45*

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.

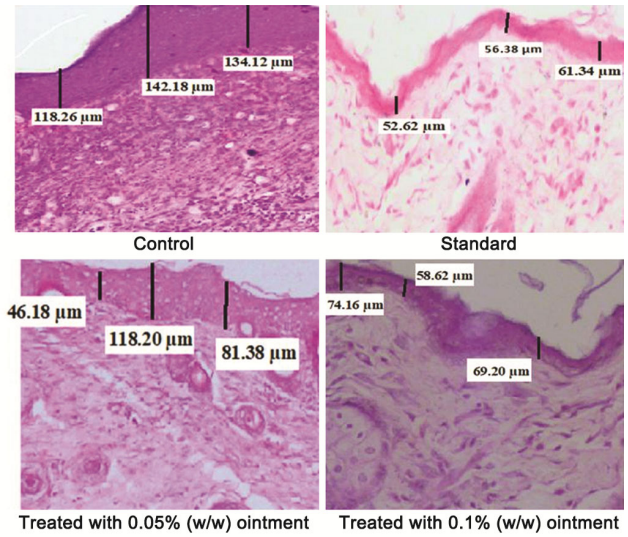

Fig. 3 — Longitudinal histological sections of mouse skin of different groups (H and E, ×40).

phospholipase A2 and thus increase the release of mediators of arachidonic acid. Prostaglandin E2 produced by the cyclooxygenase pathway also contributes to psoriasis by dilating capillaries in the dermis, increasing leukocyte infiltration, and stimulating keratinocyte cell growth[17]. During induction of psoriasis, topical application of CFA and formaldehyde provoked several pro-inflammatory reactions (redness, erythema, and scales) in the mice skin. Fully developed psoriatic lesions were characterized by inflammatory erythematous papules covered with dry silvery and red scales; this condition shows a close resemblance with parakeratotic condition associated with an evident sign of plaque psoriasis that indicated precise immune response produced by the CFA.

In case of positive control group, section showed regular elongation of rete ridges, capillary loop dilation with minimal grade lesion of diagnostic Munro's micro abscess and marked increase in epidermal thickness as compared to other groups. The group treated with standard drug has shown a significant reduction in the mean epidermal thickness and also significant retention of the stratum granulosum, the absence of movement of neutrophils. The formulation (1% w/w) significantly decreases total thickness of the epidermis indicating that the presence of ethanol extract of *Plecranthus amboinicus* in the formulation has an influence to retard the hyper proliferation of the keratinocytes, the presence of the granulosum layer indicates its ability to suppress the altered process of differentiation of the keratinocytes. Flavonoids, triterpenoids and polyphenolic compounds are well known as potent antioxidants and for their anti-inflammatory, antiproliferative, immunomodulatory and free radical scavenging activities[18,19]. These characteristics of polyphenolic phytoconstituents may be beneficial for the treatment of diseases with multiple etiologies such as psoriasis. Phytochemical screening of the *Plecranthus amboinicus* revealed the presence of the rich amount of flavonoids and polyphenols, which may be responsible for their protective role in the management of psoriasis. The results of the present study support the use of *Plecranthus amboinicus* in traditional Indian medicine can be used as an easily accessible source of natural antipsoriatic agent and can be useful in some skin problems.

Conclusion

The plant *'Plecranthus amboinicus'* belonging to the family Lamiaceae commonly known as 'Karpuravalli' in tamil possess many number of activities like anti inflammatory, anti microbial, anti neoplastic and also in skin disorders. The ethanol extract of the root of *Plecranthus amboinicus* was evaluated for antipsoriatic activity in CFA and formaldehyde induced animal model. After inducing, the animals were treated with the ointment (0.05% and 0.1% w/w) containing ethanol extract of *Plecranthus amboinicusv* 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 *Plecranthus amboinicus* possesses antipsoriatic activity which is in agreement with its traditional use.

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