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Antipsoriatic activity and Cytotoxicity of ethanolic extract of *Psoralia corylifolia* seeds

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Abstract

Plan: *Traditionally in India and China, Psoralia corylifolia seed decoction used for treating psoriasis. However, the antipsoriatic activity of Psoralia corylifolia seeds has not been scientifically evaluated using mouse tail model.*
Methodology: *Ethanolic extract of Psoralia corylifolia seeds were analyzed by high performance thin layer chromatography (HPTLC) .The In vivo antipsoriatic activity of 95% of ethanolic extract of Psoralia corylifolia seeds was done by using mouse tail model for psoriasis and In vitro antipsoriatic activity was carried out by Sulphorhodamine B (SRB) assay using HaCaT human keratinocyte cell lines.*
Outcome: *HPTLC fingerprinting revealed the presence of gallic acid. The ethanolic extract shown IC50 255 µg/ml, with good antiproliferant activity when compared with Asiaticoside as positive control with potent IC50 value of 20.13 µg/ml. Psoralia corylifolia seed extract showed an overall antipsoriatic activity of 75.87%, when compared with standard tazarot gel activity 87.94% by using mouse tail model, confirmed its traditional use in psoriasis treatment.*
Keywords: *Psoralia corylifolia, Antipsoriatic activity, Cytotoxicity, SRB Assay, Mouse tail model.*

1. Introduction

Psoriasis is a common skin disease affecting 2 % of world population characterized by epidermal keratinocyte hyper proliferation, abnormal keratinocyte differentiation and immune cell infiltration [1-3]. Psoriasis is a recurrent and debilitating disease. The existing topical treatments such as emollients, coal tar and dithranol had lower efficacy and cosmetically unacceptable, while systemic therapies such as methotrexate, cyclosporine and acitretin had significant side effects [4]. Herbal remedies are promising in the management of dermatological conditions including psoriasis [5].

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Psoralia corylifolia Linn (Fabaceae) is an erect annual herb with broadly elliptic leaves, yellowish or bluish purple flowers and compressed, mucronate, dark chocolate to almost black coloured seeds [6].Traditionally in India and China, *Psoralia corylifolia* has been used for the treatment of stomachic,deobstruent,anthelmintic, diuretic, vitiligo and also certain skin diseases, such as leucoderma, psoriasis and leprosy [7].

Psoralia corylifolia contains psoralens which are capable of absorbing radiant energy. In ultraviolet range Photo-activation by Psoralens with (200–320nm) is known to ameliorate various skin disorders such as psoriasis, vitiligo and mycosis fungicides in humans[8]. *Psoralia corylifolia* has been traditionally used as an antipsoriatic agent. However the antipsoriatic activity of *Psoralia corylifolia* seeds has not been scientifically evaluated using mouse tail model. Our present article provides scientific evidence for traditional use of *Psoralia corylifolia* seeds.

2. Materials and Methods

2.1. Plant material collection and extraction

Fresh seeds of *Psoralia corylifolia* L. were collected from an herbal shop, Ooty, Nilgiri District, Tamilnadu, India on June 2009 and authenticated by Dr.S.Rajan, Field Botanist, Central Council for Research in Homoeopathy, Ooty, Nilgiris (District), Tamilnadu, India. Voucher specimens have been deposited at the Department of Phytopharmacy and Phytomedicine (TIFAC CORE HD), JSS College of Pharmacy, Rocklands, Ooty, India. To remove the dust particles *Psoralia corylifolia* seeds were washed with tap water, Shade dried and subjected to soxhletion by boiling 500 g of the leaf powder in 3000 mL of 95 % Ethanol for 30 min at 70°C in a soxhlet apparatus. The extract obtained was concentrated on a water bath to semisolid condition, which was further dried in an oven at 30°C on a shallow dish to constant weight to remove the solvent completely (yield, 25.65 % w/w).

2.2. Drugs and Chemicals

Tazarotene gel (0.1%) was obtained from local medical shop in Ooty. HaCaT human keratinocyte cell lines were obtained from National Center for Cell Science (Pune, India). All the reagents and chemicals used for the experiment were analytical grade. Precoated Thin Layer Chromatography plates were purchased from Merck (Germany). Dulbecco's modified Eagle's medium, fetal bovine serum and tri chloro acetic acid were purchased from Himedia chemicals (Mumbai, India).

2.3. Phytochemical screening

Preliminary phytochemical analysis of the extract was performed by simple chemical tests [9].

2.4. Selection of Marker

The solvent system selected for separation of gallic acid was chloroform: ethyl acetate: formic acid (7.5:6:0.5) by Thin Layer Chromatography. The detection wavelength was found to be 292 nm. The R_f value for gallic acid was found to be 0.44.

2.5. HPTLC fingerprinting

High Performance Thin Layer Chromatography was performed on silica gel 60 F_{254} plates using the mobile phase chloroform: ethyl acetate: formic acid (7.5: 6: 0.5). The standard solution was prepared by dissolving gallic acid (10mg) in methanol (10ml). The sample solution was prepared by dissolving ethanolic extract (100mg) of *Psoralia corylifolia* seeds in methanol (10ml)Standard and sample were applied in the form of band of 6 mm on the plate using Camag Linomat IV applicator at a distance of 14 mm from the edge of the plate at a quantity of 10 µL. The development was done to a distance of 80 mm in a pre-equilibrated Camag Twin-trough development chamber. Densitometric evaluation at 292 nm in absorbance mode using deuterium lamp was performed with the help of TLC scanner III. Gallic acid in the extract was identified by comparing the R_F value of standard with that of the sample. The gallic acid present in extract was calculated.

2.6. Sulphorhodamine B (SRB) Assay

In vitro antipsoriatic study was performed using SRB assay [10]. HaCaT human keratinocyte cell lines were used. Culturing of cell lines was done in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The monolayer cell culture was trypsinized and the cell count adjusted to $1.0x10^5$ cells/ml using growth medium in a 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells/well) was added. After 24 hours, the monolayer was washed once, when a partial monolayer was formed, the supernatant was taken, and 100 µl of drug dilution prepared with above media was added per well in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were recorded for every 24 hours. After 72 hours, 25 µl of 50% trichloro-acetic acid was added to the wells such that it forms a thin layer over the drug dilutions to form a overall concentration of 10%.The plates were then incubated at 4°C for one hour. The plates were flicked; culture was washed five times with tap water to remove traces of medium, drug and serum, and was then air dried. The air-dried plates were stained with SRB for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried.100µl of 10 mM tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using a microplate reader at a wavelength of 540 nm. Data obtained at different concentrations were used for IC50 calculations.

2.7. Pharmacological Screening
2.7.1. Experimental Animals

Institutional animal ethical committee permission was obtained (Registration No.: JSSCP/IAEC/M.PHARM/PHYTOPHARM/04/2009-2011) for carrying out the study in animals as per CPCSEA guidelines. Healthy male adult albino mice (25-30 g) were procured from the animal house of JSS College of Pharmacy, Ooty, Tamilnadu, India. Animals were allowed to acclimatize for 7 days prior to experiments being carried out. They were housed in polypropylene cages and fed on standard pellet diet and water ad libitum, and the animal house was maintained under controlled conditions (12 h light-dark cycle at 22±2°C).

2.7.2. Mouse tail model

In vivo antipsoriatic activity was determined using mouse tail model for psoriasis [11].

2.7.3. Animal Treatment

Animals were divided in to three groups of six each. The first group was negative control which was left untreated and the second group was positive control treated with tazarotene gel (0.1%). The third group was treated with the ethanolic extract of *Psoralia corylifolia* seeds. For the topical application the extract was diluted with water in the ratio of 1:4 and used. Animals were treated once daily for 14 days. About 0.5 ml of the extract or tazarotene was applied topically to the proximal part of the tail about 2.5 cms and allowed to remain in contact for 2 hrs. Then the tails were washed with water. Treatment was given once daily for 14 days. The animals were sacrificed using deep ether anaesthesia by cervical dislocation, two hrs after the last treatment and the proximal parts of their tails were cut and each group tails were stored in separate containers containing 10% formalin in saline.

2.7.4. Histopathological Evaluation

From the tail skin longitudinal histological sections were prepared and stained with hematoxylin eosin and specimens were analyzed for: (1) The individual scale horizontal length in between adjacent hair follicles including sebaceous glands (n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 60 measurements per treatment), (2) The fully developed granular layer horizontal length within an individual scale (n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 60 measurements per treatment), and (3) The vertical epidermal thickness between the dermo-epidermal junction and the lowest part of the stratum corneum (n = 5 measurements per scale, n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 300 measurements per treatment).

Taken together, from these calculations, the following three overall parameters were eventually used for the evaluation of the drug effects: (a) the degree of orthokeratosis, (b) the 'drug activity' and (c) the relative epidermal thickness.

$$\text{Drug Activity} = \frac{OK_{(s)} - OK_{(c)}}{100 - OK_{(c)}} \times 100$$

with OK (i.e. orthokeratosis) as the mean of the parameter explained under for a test substance (s) and the untreated control condition (c), respectively.

2.7.5. Statistical Analysis

Data obtained in the present study are presented as weighed mean ± standard error. In the mouse tail test for statistical comparisons, explorative probabilities were obtained by the Mann Whitney U test.
Statistical calculations were performed using Graph Pad Prism software. Values with $p < 0.05$ are considered significant.

3. Results

The yield of ethanolic extract of *Psoralia corylifolia* seeds was found to be 25.65 % w/w. The Phytochemical screening of the ethanolic extract of *Psoralia corylifolia* seeds revealed the presence of carbohydrates, triterpenes, fats & oils, resins, phenols, flavonoids, steroids and monoterpenes. HPTLC fingerprinting technique revealed the presence of gallic acid in *Psoralia corylifolia* seed extract. The amount gallic acid present in ethanolic extract of *Psoralia corylifolia* seeds was found to be 1.82 % w/w. The ethanolic extract of *Psoralia corylifolia* seeds shown IC50 255 µg/ml, with good antiproliferant activity when compared with Asiaticoside as positive control with IC50 value of 20.13 µg/ml. The ethanolic extract of *Psoralia corylifolia* seeds produced significant differentiation in epidermis as seen from its degree of orthokeratosis 85.36±3.56 when compared to control 17.30± 4.09%. It is equivalent to the standard tazarotene (0.1%) gel which showed 90.03 ± 2.00% degrees of orthokeratosis. Overall, the ethanolic extract of *Psoralia corylifolia* seeds showed 75.87% activity in the mouse tail model for psoriasis. In relative epidermal thickness, the 95% ethanolic extract of *Psoralia corylifolia* seeds showed significant increase when compared to control group.

4. Discussion

Gallic acid had anti fungal and anti viral activity. Ointments used to treat psoriasis and external haemorrhoids contain gallic acid [12]. Hence we used HPTLC fingerprinting technique for estimation of gallic acid in *Psoralia corylifolia* seed extract. The presence of gallic acid in extract can be correlated with the antipsoriatic activity of the *Psoralia corylifolia* seed extract. Psoriasis resulted from the hyper proliferation and abnormal differentiation of keratinocytes [13].

An antipsoriatic drug that targets the epidermis is a compound that ideally restores skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation, or both [14]. Substances like dithranol and vitamin D analogues that form keratinocyte differentiation are effective in bringing homeostasis of the epidermis in psoriasis conditions [14, 15]. The granular layer is greatly reduced or almost absent in epidermis of psoriatic leisions [16].

This parakeratosis condition is one of the most important hall marks of psoriasis. Granular layer formation around the epidermis is known as orthokeratosis condition [17]. The main principle behind the mouse tail test is conversion of parakeratosis to orthokeratosis [13]. Many herbs used in the treatment of psoriasis have been evaluated by this method, and were found to have significant effects [18-20]. More over the results of SRB assay correlates the antipsoriatic activity. Our findings gives scientific support to antipsoriatic activity of the *Psoralia corylifolia* seed extract.

5. Conclusion

Our study provides strong evidence that *Psoralia corylifolia* seed extract had potential antipsoriatic activity. Since psoriasis is a recurrent skin disease of multiple etiologies, we selected the drug of herbal origin to minimize the related side effects associated with synthetic drugs. Further we are going to isolate the lead molecules from *Psoralia corylifolia* seeds to give bring out best possible medicine with less side effects.

Conflict of interest: None

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Table 1 Inhibitory Concentration using Sulphorhodamine B Assay

SNO	*Sample*	*IC_{50} value (µg/ml)*
1.	Asiaticoside	20.13
2.	Ethanolic extract of *Psoralia corylifolia* seeds	255

All values are mean ± SEM (n = 3)

Table 2 Effects of 95% ethanolic extract of *Psoralia corylifolia* seeds on the degree of orthokeratosis and relative epidermal thickness as well as the 'drug activity' in the mouse tail model.

Treatment groups	*Degree of orthokeratosis (%)*	*Drug activity (%)*	*Relative epidermal thickness (%)*
Control	17.30±4.09	0	100.00±10.7
Standard Tazarotene (0.1%)	90.03±2.00*	87.94	103.56±4.7
Psoralia corylifolia seeds	85.36±3.56*	75.87	98.98±4.6

Values are expressed as mean ± S.E.M., * $P < 0.05$ with respect to control

Fig.1. Histopathological sections of mouse tail skin treated topically for 14 days, (original magnification 40×). (a) Control; (b) Tazarotene 0.1%, (c) 95% ethanolic extract of *Psoralia corylifolia* seeds. Note that granular layers less developed in most parts of the control specimen (a), Tazarotene induced orthokeratosis are clearly seen over the whole horizontal length of the scale as black layer, marked with an arrow (b). well developed granular layer is also seen in (c), which is treated with *Psoralia corylifolia* ethanolic extract.

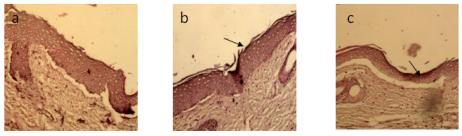
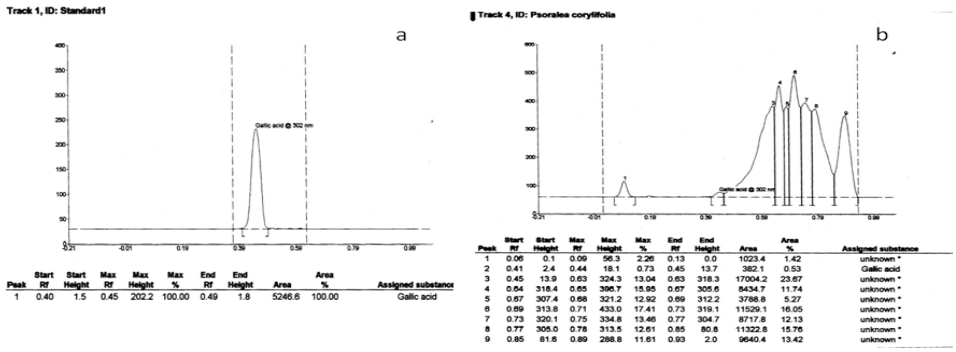


Fig.1**.** Histopathological sections of mouse tail skin treated topically for 14 days, (original magnification 40×). (A) Control; (B) Tazarotene 0.1%; (C) 95% ethanolic extract of *Psoralia corylifolia* seeds. Note that granular layer is less developed in most parts of the control specimen (A), Tazarotene induced orthokeratosis are clearly seen over the whole horizontal length of the scale as black layer, marked with an arrow. (B), well developed granular layer is also seen in (C), which is treated with *Psoralia corylifolia* ethanolic extract

Fig.2. HPTLC chromatogram in chloroform: ethyl acetate: formic acid (7.5: 6: 0.5). at 292 nm: (A) standard Gallic acid; (B) ethanol extract of *Psoralia corylifolia* seeds.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.40	1.5	0.45	202.2	100.00	0.49	1.8	5246.6	100.00	Gallic acid

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.1	0.09	56.3	2.26	0.13	0.0	1023.4	1.42	unknown *
2	0.41	2.4	0.44	18.1	0.73	0.45	13.7	382.1	0.53	Gallic acid
3	0.45	13.9	0.63	324.3	13.04	0.63	318.3	17004.2	23.67	unknown *
4	0.64	318.4	0.65	396.7	15.95	0.67	305.6	8434.7	11.74	unknown *
5	0.67	307.4	0.68	321.2	12.92	0.69	312.2	3788.8	5.27	unknown *
6	0.69	313.8	0.71	433.0	17.41	0.73	319.1	11529.1	16.05	unknown *
7	0.73	320.1	0.75	334.8	13.46	0.77	304.7	8717.8	12.13	unknown *
8	0.77	305.0	0.78	313.5	12.61	0.85	80.8	11322.8	15.76	unknown *
9	0.85	81.6	0.89	288.8	11.61	0.93	2.0	9640.4	13.42	unknown *

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