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Phytochemical evaluation and anti-psoriatic activity of the ethanolic extract of the leaves of *Thespesia populnea*

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Psoriasis is a chronic, mild and common inflammatory skin condition. Still an ideal treatment for psoriasis, effective, safe, convenient, and economical is not available. In this scenario, the search for suitable alternative treatments with minimal side effects is necessary. Plants can be effective and alternative in this regard. Therefore, this article discusses the leaves of the plants *Thespesia populnea* (Malvaceae) that are traditionally used in the treatment of psoriasis. The present study aimed to assess anti-psoriatic activity. The dried leaves of the plants were subjected to soxhlation with 95% ethanol and phytochemical studies were performed. The anti-psoriatic activity was evaluated by the Mouse-Tail model. It is a relatively sensitive and reproducible morphometric method that allows quantitative evaluation of the effects of anti-psoriatics through epidermal differentiation. Extracts were applied topically at a dose of 500mg/kg over 14 days and at the end, the animals were sacrificed, longitudinal histological sections were made of the tail skin and the degree of orthokeratosis was determined. It was significantly (P <0.05) increased by the ethanolic extract of *Thespesia populnea* (52.86±2.86) compared to the control (17.30±4.09). In relative epidermal thickness, the ethanolic extract of *Thespesia populnea* (92.68±8.8) showed a significant difference (P <0.05) compared to the control (100±10.7). The data obtained suggest that the selected plant has anti-psoriatic activity and confirms its traditional use in the treatment of psoriasis.

Keywords: Alcoholic soxhlation method, Ascorbic acid, Mouse tail model, Orthokeratosis, Psoriasis, *Thespesia populnea*

Psoriasis is a long - term genetic disease[1]. It is a chronic and inflammatory disease. It's an autoimmune disease. Psoriasis is a greek word meaning 'itching'. About 0.3-3 % of the population worldwide are affected by psoriasis[2]. It is usually a skin disease that usually affects the skin such as elbows, knees, and scalp *etc*. It has red patches covered with a silvery scaly surface. The lesion is usually affected in the outermost layer of the skin, *i.e*. the epidermis. It initiates the (lower) layer of the epidermis and initiates the production of keratinocytes. Keratinocytes are immature skin cells that produce keratin, rapid growth of keratinocyte immune cell proliferation (immature skin cells) and inflammation to the lower (basal) layer on the skin surface[2,3]. It is recurrent disease that affects environmental factors, such as weather or stress, and genetic factors that predispose them.

Importance of herbal medicine

In recent decades, herbal medicine has been used for various disease treatments. About 80% of the world's population still mainly uses herbal medicines that have a less toxic effect than synthetic drug[4]. Digoxin is isolated form *Digitalis lanta*. It imparts the property of cardiac glycosides. Aspirin is isolated form Willow bark and other plants containing salicylate, it confers the property of analgesic and antipyretic. Herbal extracts can be used for wound healing and inflammation due to active terpenoids and flavonoids and others[5].

Thespesia Populnea (L.) Corr

Thespesia populnea commonly called Portia tree [6]. It is a member of the Malvaceae family, found mainly on the coasts of India, China and Australia. It has been folk used to treat many diseases such as type 2 Diabetes mellitus (T2DM), hypertension, cancer, bacterial and viral infection, chronic skin diseases (psoriasis, scabies, ring worm, eczema and other skin infections)[7].

Habitat and cultivation

Thespesia populnea is also (known as Bhendi) a small (12 m high) fast growing evergreen tree found in tropical areas of India, China, and Australia. All parts of the plants are used in traditional medicine for

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conventional treatments. The leaves are alternately petiolate, dark green in color. Leaf size about 8-16 cm long on a petiole of equal length[8]. Bark size about 4.0 mm thick, grey to brown and fibrous [7]. Flowers are yellow. The Fruits are flattened, spherical brown capsules with a sticky yellow sap and about 10 hairy seeds [8]. Seed propagation and vegetative method have been used for the growth of *Thespesia populnea*.

Chemical constituents present in *Thespesia populnea*

Thespesia populnea contains active constituent which gives the property of anti-psoriatic activity are Gossypol, 7 Hydroxy-2,3,5,6-tetrahydro-3,6, 9-Trimethylnaphto [1,8-B,C] Pyran-4,8-Dione, Kaempferol, Quercetin, Kaempferol 3-glucoside, Quercetin 3-glucoside, rutin, Nonacosane, lupenone, myricyl alcohol, lupeol, β-sitosterol and β-sitosterol-β-D-glucoside, 5, 8-dihydroxy-7-methoxyflavone, 7-1ydroxyisoflavone and Thespone, Mansonones D E and F Populneol, Thespesin[9]. *Thespesia populnea* flowers contains[10], Flavonoids, Alkaloids, Tannins, Anthraquinones glucosides (Table 1).

Flavonoid (Populneol)[11]: It has the property of vitamin –C activity and powerful anti-oxidants [12,13]than the traditional vitamins such as quercetin[14]. Gossypol[11]: Anti-inflammatory property. Gossypetin[11]: It gives the properties anti-oxidant properties and eradication of swelling[15].

Kaempferol[11]: Properties of antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective[16].

Thespesia populnea traditionally claimed that useful in the treatment of cutaneous affections such as scabies, psoriasis, ringworm, Guineaworm, and eczema disorders[2]. The topical application of different extracts & isolated compounds (TpF-1, TpF-2 & TpS-2) of *Thespesia populnea* was used for anti-psoriatic activity[7].

Table 1 — The results of quantitative phytochemical tests for *Thespesia populnea*

Tests	*Thespesia populnea*
Alkaloids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Triterpenes	+
Fats & Oils	+
Resins	+
Phenols	+
Tannins	+
Flavanoids	+
Proteins	+
Mucilages	–
Steroids	–
Diterpenes	–
Monoterpenes	–

Bark extraction cream is used for the clinical study of Perry's scientific mouse tail model (increases in orthokeratosis region). The plant, *Thespesia populnea* has been anti-psoriatic activity. Fruit and leaves of the plant exhibited potency of wound healing activity. The aqueous extraction of *Thespesia populnea* fruit has the properties of wound healing activity in the excision wound and incision of wound *etc*[17].

Materials and Methods

Plant material: Fresh leaves of *Thespesia Populnea* (L.) Webb & Berth. (Malvaceae) were collected from Sims Park, Coonoor (Nilgiris District, Tamil Nadu, India) on June 2009 and authenticated by Dr S. Rajan, Field Botanist, Central Council for Research in Homoeopathy, Ooty (Nilgiris District, Tamil Nadu, India).

Preparation of extraction

Thespesia populnae (Malvaceae) leaves was extracted from manually, and extracted with 95% ethanol as a solvent in a soxhlet apparatus for 30 min at 70C. The phytochemical extract was stored for 7 days at room temperature and filtered[18,19]. The dark green coloursemi solid extract was obtained and percentage yield (8.94 % *w/w*).

Phytochemical screening test

Preliminary phytochemical analysis of the extract was screened for the presence of various phyto-constituents test[20,21].

Pharmacological screening

Select healthy adult male albino mice (25-30 g) obtained from animal house of JSS college of pharmacy (Ooty, Tamil Nadu, India), were used in study. Mice were housed in polypropylene cage and were fed on a standard pellet diet and water *ad libitum*. Room should be maintained under controlled condition (12 h light –dark cycle at 22 + 2C)[16,20,21]. Animals were allowed to acclimatization (to new condition) for 7 days prior to experiments being carried out. Institutional animals ethical committee permission was obtained as per CPCSEA guidelines (Registration No.: JSSCP/IAEC/M.PHARM/PHYTO-PHARM/04/2009- 2011) for carrying out the study in animals.

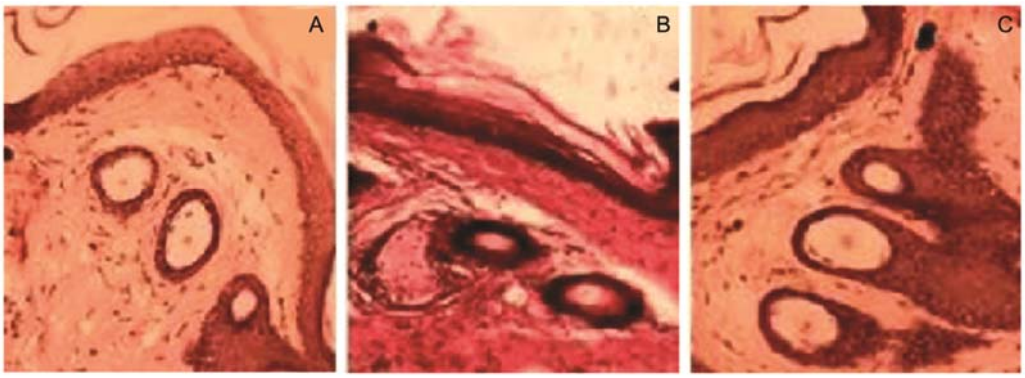

Fig 1. — Histopathological sections of mouse tail skin treated topically for 14 days, (set the original microscope magnification 40x). (A) Control, (B) tazarotene 0.1% and (C) 95% ethanolic extract of *Thespesia populnea*(semi solid form). Note that: (a) the granular layer is less developed in most parts of the control specimen; (b) tazarotene induces orthokeratosis is clearly seen over the whole horizontal length of the scale as a black layer; (c) a well-developed granular layer is also seen, in mouse tail skin treated with *Thespesia populnea ethanolic* extract

Mouse tail model

Mouse tail model is used for the assessment of *in vivo* antipsoriatic activity of psoriasis. 6 animals each were taken in three groups[22]. The group one is a negative control which is left untreated and the group two is a positive control treated with tazarotene gel (0.1%). The extract of *Thespesia populnea* is used to treat group three. Water is used to dilute the extract in the ratio of 1:4. Animals were treated for fourteen days. 0.5 mL of the tazarotene or the extract was applied to the proximal part of the tail, topically and it remains in contact for 2 h. Water is used to wash the tails. After 2 h deep ether anesthesia is used to sacrifice the animals and the proximal parts of their tails were cut. The tails were stored in 10% formalin in saline in separate containers[23,24].

Histopathological evaluation

From the mouse tail longitudinal histological section was prepared, hematoxylin – eosin is used to stain it. The cells of the mouse tails were prepared and analyzed (Fig. 1).

- The section of horizontal length of an individual scale laying in between adjacent hair follicles including sebaceous glands (n = 10 scales per animal, n = 6 animals per treatment group).
- The granular layer which is entirely developed horizontal length within an individual scale (n = 10 scales per animal, n = 6 animals per treatment group).
- The epidermal thickness which is between the dermo - epidermal junction and stratum corneum the lowest part (n = 5 measurements per scale, n = 10 scales per animal, n = 6 animals per treatment group).

For the evaluation of the drug effects, the calculation is done on the basis of above 3 following parameters:

(a) the degree of orthokeratosis,
(b) the 'drug activity' and
(c) the relative epidermal thickness.

Drug activity =OK(s)–OK(c)/100–OK(c) × 100

OK (*i.e*. orthokeratosis) as

(s) =the mean of the parameter explained under for a test substance and

(c) =the untreated control condition, respectively.

Statistical analysis

Weighed mean ± standard error is the presented probability of the above calculation. The method for statistical comparisons and explorative probabilities in mouse tail test were obtained by the method of Mann Whitney U test. Graph Pad Prism software is used for the statistical calculation. P <0.05 values are considered as the significant.

Results and Discussion

The phytochemical yield extract of *Thespesia populnea* was found to be 8.94% *w/w*. The main constituents reveled in the presence of flavonoids (populneol), alkaloids, tannins, anthraquinones glycosides, gossypol, gossypetin, kaempferol was found in *Thespesia populnea. Thespesia populnea extraction* produced significant differentiation in epidermis as showed from its degree of orthokeratosis (65.31 ±2.59***) 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 (Table 2). Overall, the *Thespesia populnea* extract was found to be 65.31 % activity in the mouse tail model method is used for the treatment for psoriasis. HPTLC analysis of Quercetin in *Thespesia populnea* by ethanolic extract with Rf value

Table 2 — Effects of 95% ethanolic extract of *Thespesia populnea* on the degree of orthokeratosis and relative epidermal thickness as well as on "drug activity" in the mouse tail model

Groups	% Orthokeratosis	Drug activity	% Relativity epidermal thickness
Control	17.30±4.09	0	100.00±10.7
Standard	90.03±2.00	77.54	103.56±4.7
Thespesia populnea	65.31±2.59	58.05	138.5±15.6

Values are mean ± standard deviation.*P <0.05 with respect to control

(0.95) is matching with standard Quercetin with Rf value (0.98) by peak area comparison. The presence of Quercetin in the extract was confirmed.

Conclusion

Our study can provide strong evidence of the anti-psoriatic property present in *Thespesia populnea* leaves. Psoriasis is the most common skin disorder or long term genetically or an inflammatory disease. Herbal medicines are safe, tolerable, effective and less side effects than synthetic drugs. The 95% ethanolic extract of *Thespesia populnea* leaves (semi-solid) was used to topical application and beneficial for the treatment of psoriasis[24,25].

Conflict of interest

All authors declare no conflict of interest.

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