



ANTI-PSORIATIC AND PHYTOCHEMICAL EVALUATION OF *THESPESIA POPULNEA* BARK EXTRACTS

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ABSTRACT

The plant *Thespesia populnea* (Malvaceae) traditionally claimed to be useful in the treatment of cutaneous affections such as scabies, psoriasis, ringworm, guineaworm, eczema and herpetic diseases. Oil prepared by boiling the ground bark in coconut oil is applied externally in psoriasis and scabies. However, there are no established scientific reports for its anti-psoriatic activity. Hence, the plant *Thespesia populnea* has been chosen to establish scientific data for its traditional claim as anti-psoriatic. This is first ever study on *Thespesia populnea* bark extract. Phytochemical investigation revealed the presence of carbohydrates, glycosides, tannins, flavonoids, triterpenoids, phytosterols, proteins and lipids/fixed oils in the bark of *Thespesia populnea*. Further, Thin layer chromatography studies supported their presence. Chemical tests performed, TLC studies carried out and UV & IR spectral data indicates that the isolated compounds TpF-1, TpF-2 might be flavonoids and TpS-2 might be a sterols. Three compounds TpF-1, TpF-2 & TpS-2 were isolated from the bark powder and an attempt was made to characterize them by physical, chemical and spectral data. Screening for anti-psoriatic activity was carried out by topical application of different extracts & isolated compounds (TpF-1, TpF-2 & TpS-2) of *Thespesia populnea* bark in the form of a cream using the Perry's scientific mouse tail model. Successive pet-ether extract showed maximum anti-psoriatic activity (increased orthokeratotic region by 25%) amongst the extracts tested where as the compound TpF-2 exhibited 38% increase in the same. From the above data, it can be said that, the plant *Thespesia populnea* is promising for further investigations to prove its anti-psoriatic activity.

Keywords : *Thespesia populnea*, Anti-psoriatic, Phytoconstituents, TpF-1, TpF-2 & TpS-2.

INTRODUCTION

Psoriasis is a common chronic inflammatory dermatosis. Person of all ages may develop the disease. Psoriasis is sometime associated with arthritis, myopathy, enteropathy, spondylitic heart disease or the AIDS. Psoriatic arthritis may be mild or may produce severe deformities resembling the joint changes seen in rheumatoid arthritis. Clinically, psoriasis most frequently affects the skin of the elbow, knees, scalp, lumbosacral areas, intergluteal cleft and glans penis¹. The most typical lesion is a well demarcated, pink to salmon colored plaque covered by loosely adherent scales that are characteristically silver white in color. Psoriasis can be one cause of total body

erythema and scaling known as erythroderma. Nail changes occur in 30% of cases of psoriasis and consist of yellow brown discoloration (often linked to an oil slick), with pitting, dimpling, separation of the nail plate from the underlying bed (onycholysis), thickening and crumbling. Psoriasis is either benign or localized (hands and feet) or generalized or life threatening, with associated fever, leukocytosis, arthralgias, diffuse cutaneous and mucosal pustules, secondary infection and electrolyte disturbances². Psoriasis may begin at any age, but in most cases, it begins between the ages of 10 and 20. Psoriasis affecting the body folds is common in individuals. Both sexes are equally affected. Psoriasis is an

autosomal, dominantly inherited dermatosis. The disease may flare up as a result infection of the upper respiratory tract (Streptococcal sore throat), bladder, teeth or kidneys. It may also be triggered by physical trauma (Kobner phenomenon). Currently available allopathic drugs have been associated with a number of side effects. Some drugs such as lithium, β -blockers and chloroquine are also provocative factors³. The management of Psoriasis (kitibha) is broadly divided into two parts viz. Purificatory (Sodhana) and Pacificatory (Samana) therapies⁴. Traditional Chinese medicine (TCM) is an alternative method of therapy that can be administered in oral, topical, or injectable forms. It emphasizes the importance of using many herbs that are combined in different formulations for each individual patient. Herbal medicine uses any plant part such as the root, bark, stem, seed, flowers, or leaves as a means for treatment. There are three basic functions that herbal medicines purportedly perform: elimination and detoxification, health management and maintenance, and health building⁵. Herbal medical practitioners can create many different formulas for different types of applications. Traditional Chinese Medicine (TCM) is of particular significance because it is a common choice of patients. Thus, it has become relevant for practicing dermatologists to be reasonably knowledgeable about this field⁶. Whether administering TCM or not, it is intrinsically imperative to have an accurate perspective. In this manner, one can be properly aware of possible adverse effects from use of TCM by the patient⁷. Although TCM has been used to treat a variety of skin diseases, of particular interest to us is psoriasis. Both topical and systemic use of

herbs has been administered to treat psoriasis, as well as a combination of herbal medications with UV-A⁸.

The plant *Thespesia populnea* (Malvaceae) traditionally claimed to be useful in the treatment of cutaneous affections such as scabies, psoriasis, ringworm, guineaworm, eczema and herpetic diseases. Oil prepared by boiling the ground bark in coconut oil is applied externally in psoriasis and scabies. However, there are no established scientific reports for its anti-psoriatic activity. Hence, the plant *Thespesia populnea* has been chosen to establish scientific data for its traditional claim as anti-psoriatic.

MATERIAL AND METHODS

The plant material was taxonomically identified and authenticated by **Prof. B.D. Huddar**, Professor and Head, Department of Botany, H.S.K. Kotambari Science College Hubli. The voucher specimen (04PG357, Siddharth) has been deposited in the herbarium section of the Pharmacognosy Division, K.L.E.S' College of Pharmacy, Hubli for future and further reference.

Preliminary phytochemical screening^{9,10}

Preparation of the extract

About 20 g of air dried powdered root was extracted with ethanol in a soxhlet extractor for 72 h. the aqueous extract was prepared by maceration with distilled water for 24 h to obtain the aqueous extract. Concentrated ethanol and aqueous extract in rotary vacuum evaporator. The extracts were screened for the presence of various phytoconstituents.

Test for alkaloids

Stirred a small portion of the solvent free petroleum ether, chloroform, ethyl acetate, alcohol and water extracts separately with a few drops of dilute hydrochloric acid and

filter. The filtrates were tested with various alkaloidal reagents such as Mayer's reagent (cream precipitate), Dragendorff's reagent (orange brown precipitate), and Wagner reagent (reddish brown precipitate).

Mayer's reagent: Few drops of Mayer's reagent were added in each extract and observed formation of the white or cream colored precipitates.

Dragendorff's reagent: Few drops of Dragendorff's reagent were added in each extract and observed formation of the orange yellow or brown colored precipitates.

Wagner reagent: Few drops of Wagner reagent were added in each extract and observed formation of the reddish brown precipitates.

Test for carbohydrates

Dissolve small quantities of alcoholic and aqueous extracts, separately in 4 ml of distilled water and filter. The filtrate may be subjected to various tests to detect the presence of carbohydrates.

Molisch's Test: To about 2 ml of extract few drops of α -naphthol (20% in ethyl alcohol) were added. Then about 1 ml of concentrated sulphuric acid was added along the side of the tube. Reddish violet ring appeared at the junction of two layers. Indicates the presence of carbohydrates.

Fehlings Test: 1ml of fehling's reagent (copper sulphate in alkaline conditions) was added to the filtrate of the root extract in distilled water and heated in a steam bath. Brick red precipitates appeared which confirm the presence of carbohydrates.

Test for glycosides

Hydrolysed another small portion of the extract with dilute hydrochloric acid for few hours in water bath and subjected the

hydrolysate with Liebermann-Burchard's, Keller-Killani and Borntrager's tests to detect the presence of different glycosides.

Keller-Killani Test: 1ml of glacial acetic acid containing traces of FeCl_3 and 1 ml of concentrated H_2SO_4 was added to the extract carefully. Colour appeared which confirm the presence of glycosides in the root extracts.

Borntrager's test: 1ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract. A black brown colour was obtained which show the presence of glycosides in the root extracts.

Test for phenolic compound and tannins

Take small quantities of alcohol and aqueous extracts separately in water and test for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%) and lead acetate test.

Ferric chloride test: On addition of ferric chloride solution (5%), colour was observed in all the three portions due to the presence of phenolic compounds. colour appeared which show the presence of phenolic compound.

Lead acetate test: Few drops of lead acetate solution (5%) were added to the alcoholic extract of the root. White precipitate was appeared which confirm the presence of phenolic compounds.

Test for flavonoids

Ammonia test: Filter paper strips were dipped in the alcoholic and aqueous solutions of the extract and ammoniated. The filter paper changed its colour to yellow which indicates the presence of flavonoids.

Pew test for flavonoids: To 1ml of the each extracts, a piece of metallic magnesium/zinc was added followed by addition of 2 drops of concentrated hydrochloric acid. A brownish

colour confirmed the presence of flavonoids in all the extract.

Test for proteins and free amino acids

Added a few ml of alcoholic and aqueous extracts in a few ml of distilled water and subjected to Million's, Biuret and Ninhydrin tests.

Millon's test: To 2 ml of filtrate, 5-6 drops of Million's reagent (solution of mercury nitrate and nitrous acid) was added. A red colour precipitate appeared which confirms the presence of proteins and free amino acids.

Biuret test: To the ammoniated alkaline filtrate 2-3 drops of 0.02% copper sulphate solution was added. A red colour was obtained which confirms the presence of proteins and free amino acids.

Ninhydrin test: To each of the filtrate, lead acetate solution was added to precipitate tannins and filtered. The Filtrate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and dried at 110o C for 5 minutes. Violet spots were seen which confirm the presence of proteins and free amino acids.

Test for saponin

Foam test: Dilute 1 ml of alcoholic and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponin.

Sodium bicarbonate test: To the few milligrams of extract few drops of sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

Test for phytosterol and triterpenes

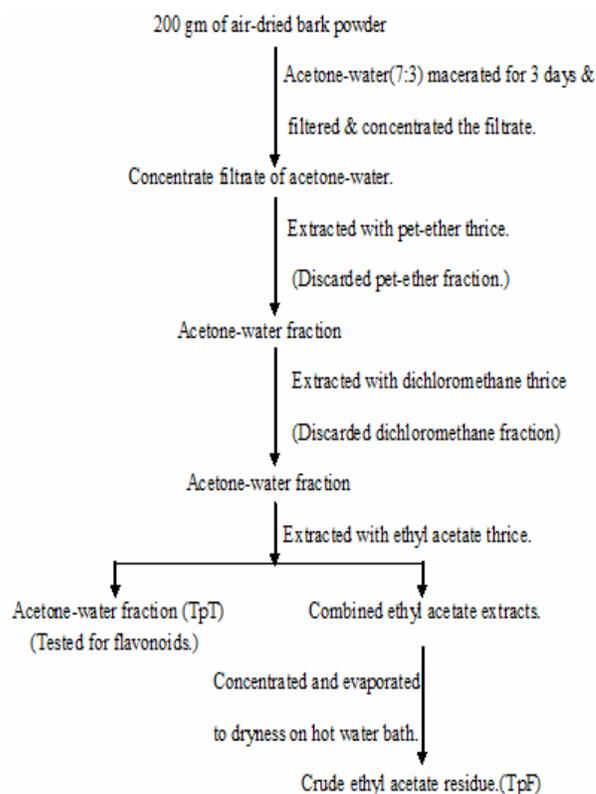
Liebermann-Burchard's test: The hydro-alcoholic extract was shaken with chloroform and few drops of acetic anhydride were added

chloroform extract along with a few drops of concentrated sulphuric acid from the side of the tube. The appearance of blue to brick red colour indicates the presence of sterol and triterpenes.

Hesse's reaction: The residue was dissolved in chloroform (4 ml) and an equal quantity of concentrated sulphuric acid was then along the side of the tube. The formation of the pink colored ring, which is on shaking diffused in both the layers, indicating the presence of sterols in the extract.

Isolation of Phytoconstituents

Scheme 1: Isolation of flavonoids and tannins¹¹



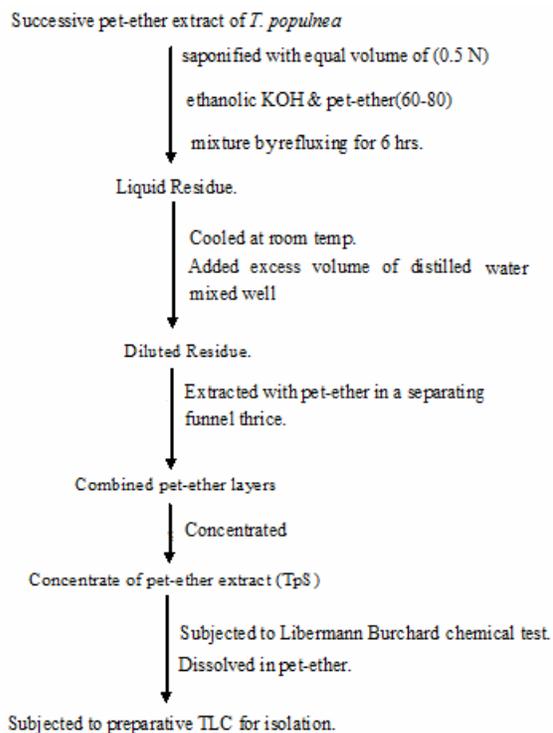
Identification of isolated compound(TpF)

Qualitative chemical identity tests

Shinoda test: Pink color Flvonoid may be present.

Zinc-HCl test: Pink color Flavonoid may be present.

Scheme 2: Isolation of Sterols¹²:



The non sprayed area(s) corresponding to the area(s) sprayed with Libermann Burchard reagent were scraped out, dissolved in pet-ether, filtered & the filtrate(s) were concentrated & evaporated on hot water bath to get respective residues.(TpS-1, TpS-2

Separation and purification

Column chromatography

Column chromatography is one of the most useful method for the separation and purification of both solids and liquids. Column chromatography is another solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase)¹³. The theory of column chromatography is analogous to that of thin-layer chromatography. The most common adsorbents - silica gel and alumina - are the same ones used in TLC. The sample is dissolved in a small quantity of solvent (the eluent) and applied to the top of the column¹⁴.

The eluent, instead of rising by capillary action up a thin layer, flows down through the column filled with the adsorbent. Just as in TLC, there is an equilibrium established between the solute adsorbed on the silica gel or alumina and the eluting solvent flowing down through the column. Column chromatography is generally used as a purification technique: it isolates desired compounds from a mixture¹⁵.

Details of column chromatography of crude ethyl acetate residue TpF:

Adsorbent: Silica Gel for column chromatography (30-60 mesh) activated at 110°C for 1hr.Length of the column: 45cm, Diameter of the column: Outer- 2.2cm, Inner- 1.8 cm.

Solvent system: Ethyl acetate: methanol: Water (100: 16.5: 13.5), Weight of crude flavonoid fraction taken: 390 mg. 60 gms of silica gel for column chromatography [laboratory grade] was activated in hot air oven at 110°C for 1hr. Above mentioned solvent system was used to pack silica gel in the glass column. The glass wool was fixed at bottom. The activated silica was charged in the column, in small, portion with gentle tapping after each addition, in order to ensure uniform packing. The small quantity of solvent was allowed to remain at the top of the column (about 4 cms). The air bubbles present in the column were removed by gentle tapping. Crude ethyl acetate residue (TpF) was dissolved in the solvent system and adsorbed on silica gel and allowed to dry. This mixture in a powder form was loaded to top of the column and eluted with above mentioned solvent system. 10 ml fractions were collected and each fraction was evaluated by TLC technique. These fractions

were grouped according to their homogeneity, judged from the TLC analysis. Two compounds (TpF-1) & (TpF-2) were separated.

Purification by re-crystallization:

Separated compounds were dissolved in methanol (pure) separately and evaporated it on the hot water bath. After 90% evaporation, powdery consistency appeared, filtered to separate it.

Evaluation for anti-psoriatic activity

Perry scientific mouse tail model¹⁶

This is accepted as a screening method for measuring anti psoriatic activity of drugs. The basis of this method is that topical treatment of a mouse-tail with anti- psoriatic drugs enhances orthokeratotic cell differentiation in the epidermal scales. This characteristic is utilized for direct measurement of drug efficacy in an animal model. Drugs are applied topically, once daily, 5 times in a week, for 2 weeks. Two hours after the last treatment the animal are sacrificed, longitudinal sections of the tail skin are made and prepared for histological examination (hematoxylin- eosin staining)As an indicator of orthokeratosis, the number of scale regions with a continous granular layer is counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.

Extracts tested

The ethanolic, successive pet-ether, butanolic, ethyl acetate, successive alcoholic and isolated compounds of *Thespesia populnea* bark were screened for anti psoriatic activity. Each extract (100 mg) & isolated compounds (50 mg) were formulated in the form of a cream, using liquid paraffin (10 ml) and bees wax (3 gm) and applied topically.

Standard used

Retino-A 0.05% (Tretinoin cream U.S.P.) - Janssen-Cilag Pharmaceuticals (Trademark of Johnson & Johnson, U.S.A.) Batch no: K 020, Mfg. Date: Oct. 2005, Exp. Date: Sep. 2007 in cream form was used as a standard.

Procedure¹⁷

Male Wistar rats weighing around 300 g are used. Proximal end of tail , an area on one side of the flank is irradiated for 20 min (1.5 J/cm²) at a vertical distance of 20 cm with UV lamps. A biphasic erythema is observed. Immediately after irradiation, initial faint erythema appears, disappearing within 30 min. The second phase of erythema starts 6 h after the irradiation and gradually increases, peaking between 24 and 48 h. The color is brownish-red, and the reaction is confined to the exposed area with a sharp boundary. By 48-72 h after irradiation, dark brown scale is formed on the erythematous lesion. Pieces of the scale are relatively thick.

Method of screening

Screening of various extracts viz. alcoholic, successive pet-ether, butanolic, ethyl acetate and successive alcoholic (in the form of cream) was carried out with reference to the standard. Extracts (in the form of cream) were applied topically, once daily, 5 times a week, for 2 weeks. Drugs are applied topically, once daily, 5 times in a week, for 2 weeks. Two hours after the last treatment animals were sacrificed, longitudinal sections of the tail skin were made and prepared for histological examination (hematoxylin- eosin staining) as an indicator of orthokeratosis the number of scale regions with a continous granular layer is counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.

RESULTS

Phytochemical Investigations: The amount of respective dried extract, physical characteristics

and qualitative chemical analysis of various extracts of *T. populnea* bark are as shown in Table 1 and Table 2, respectively.

Table 1: Amount of respective dried extract and physical characteristics of the various extracts of *T. populnea* bark

Extracts	Dry wt. gms	Color	Odour	Consistency
Alcoholic	16.88	Brownish red	Characteristic	Granular solid
SUCCESSIVE EXTRACTION				
Petroleum				
Ether (60-80°C)	6.33	Dull-Yellow	Characteristic	Granular solid
Butanolic	3.12	Blackish red	Characteristic	Lustrous solid
Ethyl acetate	0.150	Light brown	Characteristic	Powdery
95% Alcohol	8.68	Orange - red	Characteristic	Granular solid

Table 2: Qualitative chemical analysis of various extracts of *T. populnea* bark

Phytoconstituents	Alcoholic extract	Successive Extraction			
		PE	B-nol	EA	SAE
Alkaloids	-	-	-	-	-
Carbohydrates	+	-	-	+	+
Glycosides	+	-	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	-	+	+	+
Proteins & Amino acids	+	-	-	-	+
Sterols	+	+	+	-	-
Triterpenoids	+	+	+	-	-
Fixed oils	+	+	-	-	-

Key: PE = Petroleum ether extract (60-80°C), B-nol = Butanolic extract, EA = Ethyl acetate extract, SAE = Successive Alcoholic extract, "+" = Present, "-" = Absent.

Evaluation for anti-psoriatic activity¹⁶

Alcoholic extract, successive pet-ether, butanolic, ethyl acetate, successive alcoholic extract and isolated compounds (TpF-1, TpF-2 & TpS-2, at 50 mg dose) from *T. populnea* bark were screened for their possible anti-psoriatic activity using Perry's scientific mouse tail model. Extracts and isolated

compounds were applied topically in the form of a cream. Drug activity is defined by the increase in percentage of orthokeratotic regions (These are the regions in a cell having no nucleus and involved in protection from invaders like micro-organisms, UV rays, weak acids & bases). Successive extracts viz. pet-ether extract has increased the

orthokeratotic regions by 25% (Figure 4) where as, butanolic extract did by 15% (Figure 3) at the dose of 50mg. The standard drug Retin-A showed the increase by 72% (Figure 8). Isolated compounds

TpF-1, TpF-2 & TpS-2 increased orthokeratotic regions by 33% (Figure 6), 38% (Figure 7) & 30% (Figure 5) respectively in comparison to normal (Figure 2) and UV treated (Figure 1) skin.

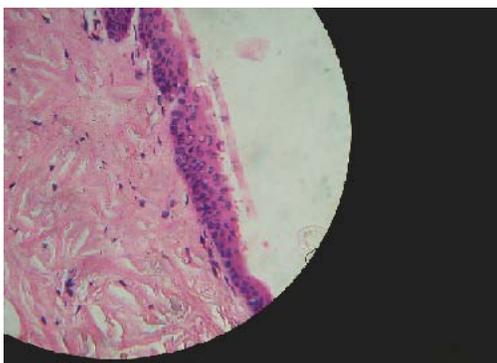


Figure: 1
3% Orthokeratotic region in UV treated skin.

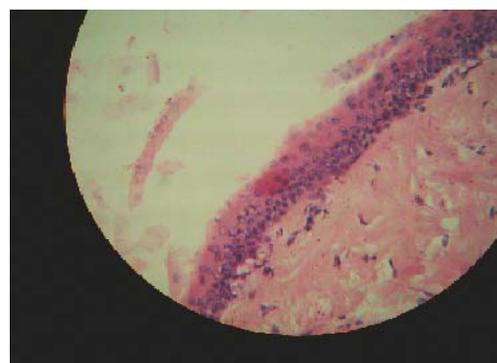


Figure: 2
5% Orthokeratotic region in normal rat tail skin.

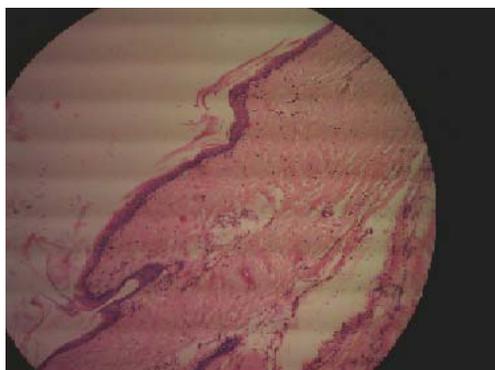


Figure: 3
15% Orthokeratotic region in butanolic extract treated rat tail skin.

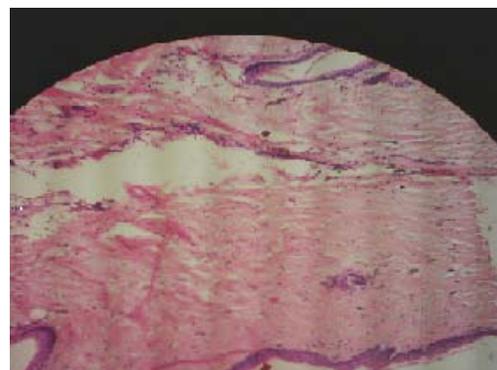


Figure: 4
25% Orthokeratotic region in pet-ether extract treated rat tail skin.

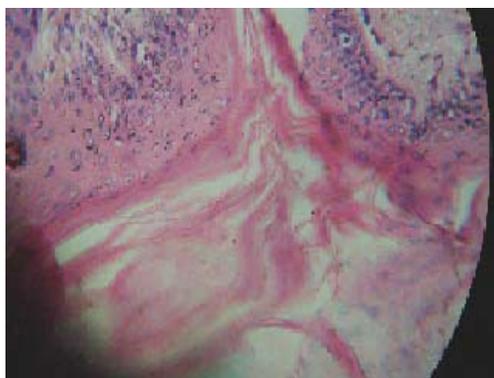


Figure: 5
30% Orthokeratotic region in isolated steroid (TpS-2) treated rat tail skin.

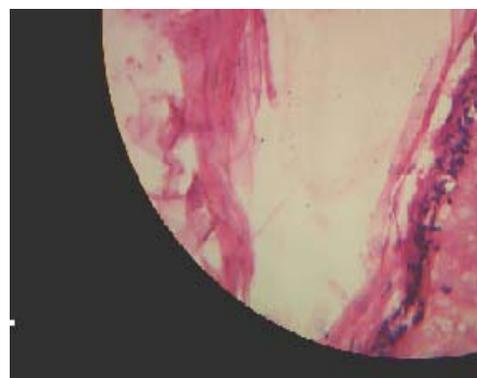


Figure: 6
33% Orthokeratotic region in isolated flavonoid (TpF-1) treated rat tail skin.



Figure: 7

38% Orthokeratotic region in isolated flavonoid (TpF-2) treated rat tail skin.

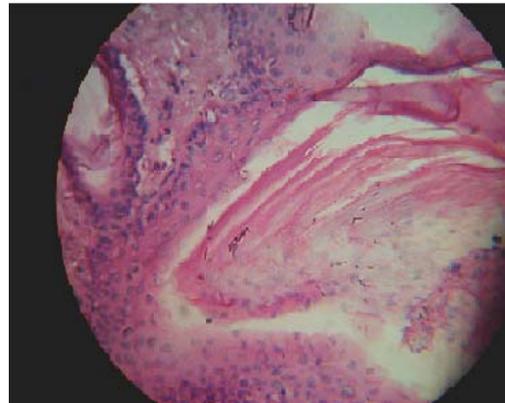


Figure: 8

72% Orthokeratotic region in reference drug Retino-A treated rat tail skin.

DISCUSSION

Phytochemical investigation *Thespesia populnea* bark extracts revealed the presence of carbohydrates, glycosides, tannins, flavonoids, triterpenoids, phytosterols, proteins and lipids/fixed oils. Three compounds TpF-1, TpF-2 and TpS-2 could be isolated from the bark powder and evaluated by chemical tests. Compounds TpF-1 & TpF-2 responded positively for Shinoda and Zn-HCl test for flavonoids Compound TpS-2 gave positive result for Libermann Burchard test. Screening for anti-psoriatic activity was carried out by topical application of different extracts & isolated compounds (TpF-1, TpF-2 & TpS-2) of *Thespesia populnea* bark in the form of a cream using Perry's scientific mouse tail model. Successive pet-ether extract has increased the orthokeratotic regions by 25% where as, successive butanolic extract did by 15%. The standard drug Retin-A showed the increase by 72%. Isolated compounds TpF-1, TpF-2 & TpS-2 increased orthokeratotic regions by 33%, 38% & 30% respectively in comparison to normal skin. From the above data, the plant *Thespesia populnea* bark is

promising for further investigations to prove its anti-psoriatic activity.

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