

## Anti-Psoriatic Activity of *Smilax china* Linn. Rhizome

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### ABSTRACT

Submitted: 28/02/2012

Revised: 13/08/2012

Accepted: 17/11/2012

**Objectives:** The rhizome of the plant *Smilax china* Linn. has been used in Siddha and Ayurveda in the treatment of Psoriasis and various skin diseases. In the present study, the methanolic extract and ethyl acetate fraction of *Smilax china* Linn. rhizome was evaluated for antipsoriatic activity.

**Methods:** Mouse tail test was used for the evaluation of antipsoriatic activity. The methanolic extract and ethyl acetate fraction at a dose of 100 and 200 mg/kg b.w. were evaluated for antipsoriatic activity in Swiss albino mice using mouse tail test. The parameter studied in the mouse tail test was percentage orthokeratotic values. In vitro antiproliferant assay on HaCaT cell lines and determination of serum nitric oxide levels were also carried out.

**Result and conclusion:** The ethyl acetate fraction of *Smilax china* rhizome (200 mg/kg) showed maximum antipsoriatic activity (increased orthokeratotic region by 29%) when compared with methanol extract.

**Keywords:** *Smilax china*, Anti-psoriatic, ethyl acetate fraction, Quercetin, HaCaT cells.

### INTRODUCTION

Psoriasis is a common genetically determined chronic inflammatory skin disorder characterized by red, scaly and raised patches. It affects mainly knees, elbows and scalp. It is a lifelong disorder with unpredictable remissions and relapses. It affects the patients physically, mentally and socially<sup>1,2</sup>. In psoriasis, epidermal hyperproliferation, abnormal keratinocyte differentiation, angiogenesis with blood vessel dilatation and excess Th-1 and Th-17 inflammation can be observed. However, although genetic, immunological and environmental factors seem implied, the exact cause is not yet known and even today, psoriasis is not well understood<sup>3</sup>.

Affordability, availability, and side effects of prolonged use of allopathic drugs still remain a challenge and concern. Discovery of safer and more effective anti-psoriatic drugs remains an area of active research at the present time. One such plant was the rhizome of the plant *Smilax china* Linn. used in various diseases such as rheumatism, gout, epilepsy, skin diseases, chronic nervous diseases, syphilis, flatulence, dyspepsia, colic, neuralgia, constipation, helminthiasis, psoriasis and seminal weakness<sup>4,5</sup>.

The rhizome of *Smilax china* has not been evaluated for anti-psoriatic activity and there was no published report for its use in psoriasis treatment. Our study was solely based on a traditional Siddha healer in Tamil Nadu, India who is using rhizome of this plant in the treatment of psoriasis. Hence, in

the present study we evaluated the anti-psoriatic activity of the rhizome of *Smilax china* Linn.

### MATERIALS AND METHODS

#### Plant material

The Plant specimen for the proposed study was purchased from commercial source in Chennai, Tamil Nadu. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai. A voucher specimen (accession No. 168) was deposited in the Herbarium, Department of Pharmacognosy, School of pharmaceutical sciences, Vels University, Tamil Nadu, India, for future reference.

#### Extraction and Fractionation

About 500 gms of the powdered rhizome of *Smilax china* was extracted by soxhlet extractor using methanol as solvent at 70°C for 18 h five times. The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). The concentrated extract was again exhaustively defatted by refluxing with n-hexane and ethyl ether (15 h twice). The two fractions were negative for polyphenol and positive for sterol. Then the defatted bulk residue was successively extracted by refluxing with ethyl acetate (15 h twice) and this fraction was evaporated in a rotary evaporator under reduced pressure, freeze-dried and used for the study.

#### Phytochemical screening

Concentrated methanol extract and its fraction, n-hexane, ethyl ether and ethyl acetate fraction were screened for the presence of various phytoconstituents using standard procedure<sup>6</sup>.

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## TLC

To support preliminary chemical analysis, methanolic extract and its ethyl acetate fraction were subjected to TLC studies. A number of developing solvent systems were tried for fractions showing presence of flavonoids, but the satisfactory resolution was obtained in the mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1) and silica gel F as stationary phase. After developing, the plates were air dried and exposed to ammonia vapour, a detector specific for flavonoids<sup>7</sup>. The results were tabulated in Table 1 and shown in Figure 1.

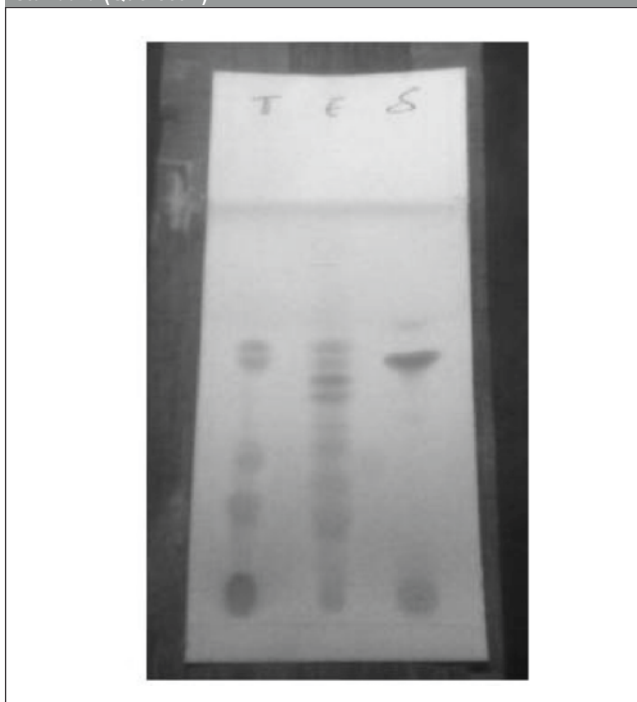
## HPLC analysis

Quantitative analysis of the flavonoid in the sample was performed using HPLC system. Analytical HPLC was performed on (D) a computer-controlled high-pressure-gradient LaChrom-HPLC-system (Merck-Hitachi), containing an Interface L-7000; two pumps L-7100 (one for each eluent); diode array detector L-7450; autosampler L-7200 with 100 $\mu$ L sample loop; solvent degasser L-7612; high-pressure gradient mixer; Rheodyne injection valve 7725i, 20 $\mu$ L sample loop; administration of the device, data recording and analysis was performed with the LaChrome Software version 4.0. The column was Thermo ODS Hypersil C18 (250 x 4.6 mm, 5  $\mu$ m) in isocratic mode. The separation was achieved using a mobile phase of acetonitrile - 0.1M phosphate buffer - glacial acetic acid (15: 85: 1, v/v/v) with pH adjusted to 4.0 using phosphoric acid at a flow-rate of 1.0 ml/min. The effluent was monitored using UV detection at a wavelength of 300 nm. The mobile phase was filtered through 0.45  $\mu$ m nylon filter prior to use<sup>8</sup>. The percentage content of flavonoids in the rhizome of *Smilax china* was calculated and the resultant graph is shown in Figure. 2 & 3.

## HPTLC analysis with known marker Quercetin

The methanolic extract and its ethyl acetate fraction of *Smilax china* rhizome was further subjected to HPTLC for the conformation of the active constituents. HPTLC was performed on 10 cm x 10 cm aluminum backed plates coated with silica gel 60F254 (Merck, Mumbai, India). Standard solution of quercetin and sample solution were applied to the plates as bands 8.0 mm wide, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by use of a Camag (Muttentz, Switzerland) Linomat V sample applicator equipped with a 100- $\mu$ L Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28  $\pm$  2 $^{\circ}$ C), with Toluene: Ethyl acetate: Formic acid (5 : 4: 1) (v/v/v), as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were dried with a hair dryer and then scanned at 254 nm with a Camag TLC Scanner with WINCAT software, using the deuterium lamp<sup>9</sup>. A stock solution of standard quercetin

Fig. 1: TLC of total methanolic extract, ethyl acetate fraction and standard (Quercetin)



T-Total Mathanolic extract, E-Ethyl acetate fraction, S-Standard(Quercetin)

Fig. 2: HPLC *Smilax china* rhizome

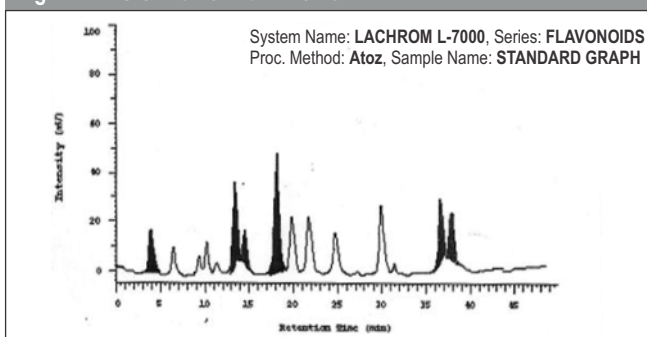
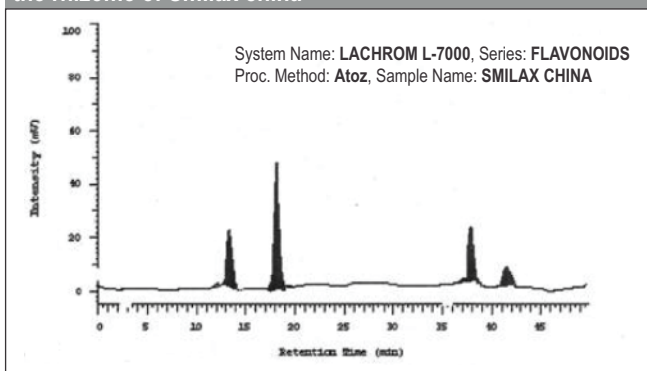


Fig. 3: HPLC of authentic samples of flavonoids present in the rhizome of *Smilax china*



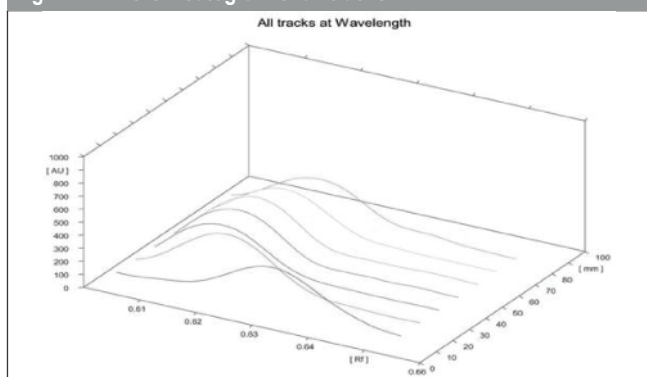
| No. Component Name | Ret. Time | Area   |
|--------------------|-----------|--------|
| 2 MYRICETIN        | 11.67     | 75454  |
| 3 QUERCETIN        | 13.45     | 644324 |
| 4 FORMONONETIN     | 39.34     | 54523  |
| 5 KAMFEROL         | 41.44     | 1121   |

(100µg/mL) was prepared in methanol. Different volume of stock solution 1, 2 and 3µL, were spotted on to TLC plate to obtain concentration 100 ng, 200 ng and 300 ng/spot of quercetin. Calibration curve range (100 - 300 ng/spot) for quercetin was found to be linear. The data of peak areas plotted against the corresponding concentration shown in Table 2 and in Figure 4, 5 & 6.

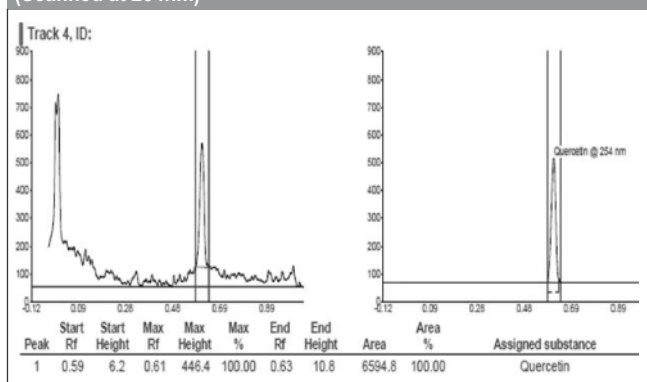
**Table 1: Thin layer chromatography of total methanolic extract and its ethyl acetate fraction of rhizome of *Smilax china* Linn**

| Test extract             | Solvent system      | Number of spots | R <sub>f</sub> values | Detecting agent |
|--------------------------|---------------------|-----------------|-----------------------|-----------------|
| Total methanolic extract | Toluene :           | 6               | 0.64                  | Ammonia vapour  |
|                          | Ethyl acetate:      |                 | 0.61                  |                 |
|                          | Formic acid (5:4:1) |                 | 0.55                  |                 |
|                          |                     |                 | 0.41                  |                 |
|                          |                     |                 | 0.32                  |                 |
| Ethyl acetate Fraction   | Toluene :           | 9               | 0.98                  | Ammonia vapour  |
|                          | Ethyl acetate :     |                 | 0.70                  |                 |
|                          | Formic acid (5:4:1) |                 | 0.67                  |                 |
|                          |                     |                 | 0.61                  |                 |
|                          |                     |                 | 0.55                  |                 |
|                          |                     |                 | 0.51                  |                 |
|                          |                     |                 | 0.42                  |                 |
|                          | 0.33                |                 |                       |                 |
|                          | 0.205               |                 |                       |                 |

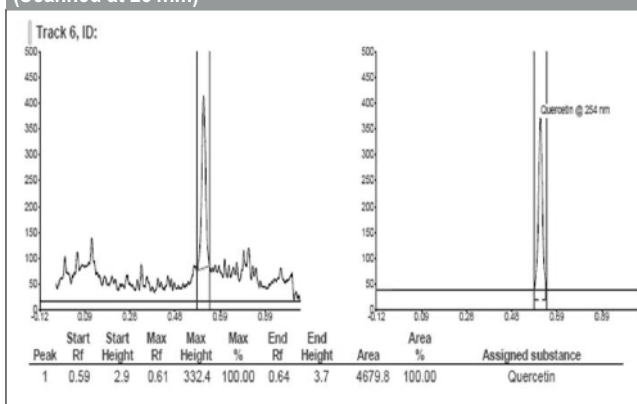
**Fig. 4: HPTLC Chromatogram of all tracks**



**Fig. 5: Peak display of methanol extract and reference (Scanned at 254nm)**



**Fig. 6: Peak display of Ethyl acetate fraction and reference (Scanned at 254nm)**



**Table 2: Peak table**

| Track ID | Sample ID              | Rf   | Height (mm) | Area (mm <sup>2</sup> ) | Assigned substance |
|----------|------------------------|------|-------------|-------------------------|--------------------|
| 1        | Reference              | 0.61 | 223.71      | 2408.81                 | Quercetin          |
| 2        | Reference              | 0.61 | 380.66      | 5284.61                 | Quercetin          |
| 3        | Reference              | 0.61 | 420.11      | 6535.12                 | Quercetin          |
| 4        | Methanol extract       | 0.61 | 446.36      | 6594.75                 | Quercetin          |
| 5        | Methanol extract       | 0.61 | 420.96      | 5828.01                 | Quercetin          |
| 6        | Ethyl acetate fraction | 0.61 | 332.25      | 4679.78                 | Quercetin          |
| 7        | Ethyl acetate fraction | 0.61 | 286.58      | 3828.66                 | Quercetin          |

## Animals

Healthy adult albino mice (25–30 g) obtained from the animal house of Vels University, Tamilnadu, India was used for the study. 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±2 °C) 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 (Registration No. XII/VELS/PCOG/01/2000/CPCSEA/IAEC/22.2.11). Animals were used for acute toxicity study and mouse tail test for Anti psoriasis study.

## Acute toxicity

Acute toxicity study–up and down procedure was carried out as per the guidelines by Organization for Economic Cooperation and Development (OECD) 423. Mice (6/group) were divided into five groups. The first 2 groups received oral doses of 1000, and 2000 mg/kg of methanol extract and 3<sup>rd</sup> and 4<sup>th</sup> groups received oral doses of 1000 and 2000 mg/kg of ethyl acetate fraction. The fifth group received saline (10 ml/kg) orally. Mortality was assessed 24 hours after administration. The animals were also observed for toxic symptoms and mortality was determined 24 hours after treatment<sup>10</sup>.

### **In-Vivo anti-psoriatic activity**

The mouse-tail model is based on the induction of orthokeratosis in those parts of the adult mouse-tail, which have normally a parakeratotic differentiation.

#### **Perry scientific mouse tail model**

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 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 continuous 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<sup>11</sup>.

#### **Extracts tested**

The methanolic extract and ethyl acetate fraction of *Smilax china* rhizome were screened for anti psoriatic activity. The methanol extract (100 mg and 200 mg) and ethyl acetate fraction (100 mg and 200 mg) were formulated in the form of a cream, using liquid paraffin (10 ml) and bees wax (3 gm) and applied topically. Retino-A 0.05% cream (Tretinoin cream U.S.P.) -Janssen-Cilag Pharmaceuticals (Trademark of Johnson & Johnson, U.S.A.) was used as a standard.

#### **Procedure**

Healthy adult albino mice weighing about 25-30 g was used. Screening of methanolic extract and ethyl acetate fraction were carried out with reference to the standard, Retino-A 0.05%. Extract and fraction were formulated in the form of cream and applied topically, once daily, 5 times 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 continuous 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. 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:

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

where OK = orthokeratosis:

The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit<sup>12</sup>.

#### **Measurement of epidermal thickness**

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 and the mean of different animals was calculated. The result of pretreatment with extract and fraction against psoriasis is shown in Figure 7.

#### **Nitric oxide scavenging assay**

Nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases.

Five mL of blood was collected from psoriatic animals induced by ultraviolet ray B, allowed to clot and serum was separated by centrifugation. As NO is an unstable molecule, it is rapidly converted to nitrates and nitrites in the body, Hence their concentration is parallel to NO levels. Nitrate and nitrite concentrations were then estimated by the Griess method. In this method nitrate is first reduced to nitrite which is treated with sulfanilamide and N-1-naphthyl-ethylene diamine. A red colored compound is formed after which its characteristic absorption spectrum is determined on spectrophotometry at 546 nm<sup>13</sup>. The result was shown in Table 3.

#### **In-vitro anti-psoriatic activity**

*In-vitro* antipsoriatic activity was carried out in HaCaT human keratinocyte cell line.<sup>[14]</sup> Human HaCaT keratinocytes were obtained from NCCS, Pune, India. The cells were seeded at a concentration of  $1.0 \times 10^5$  cells/ml in a 96 well microtitre plate and grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (BioWest). After 24 h, the supernatant was decanted and the monolayer was washed once. Then 100µl of test substance in various concentrations (25-400 µg/ml) was added to the cells in microtitre plates. Test compounds were prepared in dimethyl sulphoxide (DMSO) and then diluted with DMEM; the final concentration of DMSO was 0.2% in the culture medium. Each sample concentration was tested in triplicates. Controls were performed with DMSO or medium alone. Asiaticoside (Sigma) was used as positive control. The plates were then incubated at 37°C for 3 days in 5% CO<sub>2</sub>



atmosphere. Antiproliferant activity was assessed by performing the Sulphorhodamine B (SRB) assay. SRB assay was carried out according to the method of Skehan et al<sup>15</sup>. Cells were fixed by adding 25µl of ice-cold 50% trichloroacetic acid on top of the growth medium and the plates were incubated at 4°C for 1 h, after which plates were washed to remove traces of medium, drug and serum. SRB stain (50µl; 0.4% in 1% acetic acid) (Sigma) was added to each well and left in contact with the cells for 30 min after which they were washed with 1% acetic acid, rinsing 4 times until only dye adhering to the cells was left. The plates were then dried and 100µl of 10mM Tris buffer (Sigma) added to each well to solubilise the dye. The plates were shaken gently for 5min and absorbance read at 550nm using a micro plate reader (Biorad, USA).

#### Statistical analysis

Data was expressed as the arithmetic mean  $\pm$  SEM and was analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's "t" test. *P* value less than 0.05 (*P* < 0.05) was the critical criterion for level of significance.

### RESULT AND DISCUSSION

About 500 gms of the powdered rhizome of *Smilax china* was extracted using a soxhlet apparatus with methanol and fractionated with n-hexane, diethyl ether and ethyl acetate. The yield of methanol extract was about 4.4%w/w and its fraction n-hexane was about 0.05% w/w, ethyl ether was about 0.95% w/w and ethyl acetate was about 3.1%w/w.

#### Phytochemical screening

Preliminary phytochemical screening of methanol extract showed the presence of flavonoids, saponins, sterols, tannins, proteins and carbohydrates. The hexane fraction and ethyl ether showed positive results for steroids, the ethyl ether fraction also showed positive results for proteins and the ethyl acetate fraction showed positive results for flavonoids, tannins, terpenoids and carbohydrates. The total methanol extract and ethyl acetate fraction showed maximum phytoconstituents and hence were selected for the present phytochemical and pharmacological study.

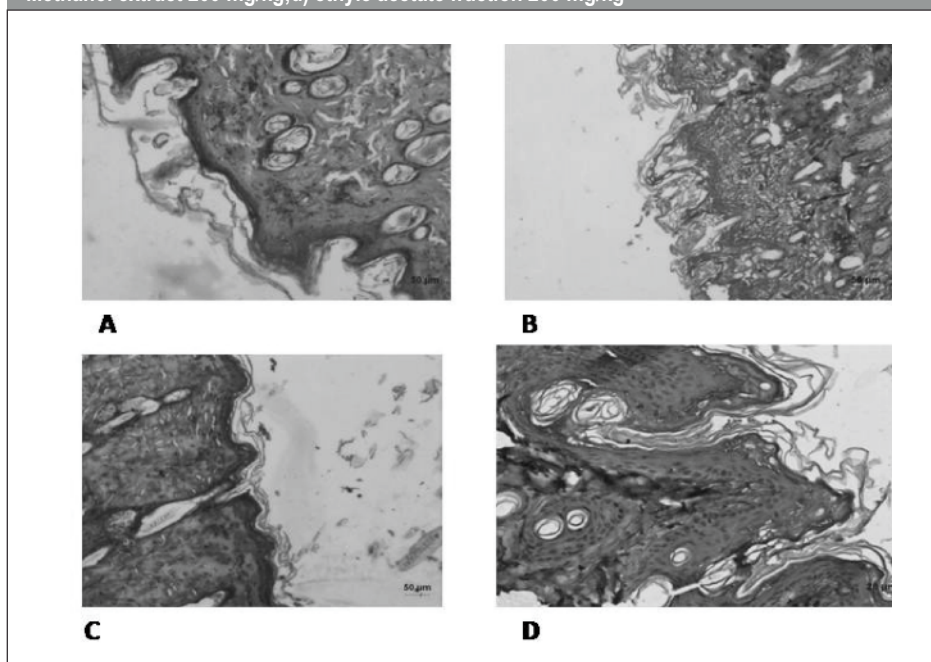
#### Thin Layer chromatography (TLC)

To support phytochemical screening, methanolic extract and its fractions were subjected to thin layer chromatography. The total methanolic extract showed six well separated spot, ethyl acetate fraction showed nine spots (Table 1) in the mobile phase Toluene: Ethyl acetate: Formic acid (4:5:1). TLC findings were in agreement with the data of qualitative chemical tests and the spots characteristic for flavonoids were observed (Figure 1).

#### High performance liquid chromatography

Comparing the HPLC chromatograms from the rhizome of *Smilax china* Linn showed 17 components (Figure 2). The main difference was in peak eluted at 13.45 min, 41.44 min, 11.67 min, and 39.34 min respectively. In the present investigation, 4 flavonoids were quantified at 254 nm using peak area by comparison to a calibration curve derived from

Fig. 7: Longitudinal histological section through the skin of Ultra Violet-B treated totally for 2 weeks, HE staining (original magnification 4X). a) Vehicle control; b) retinoic acid 0.5 mg/kg; c) Methanol extract 200 mg/kg; d) ethyle acetate fraction 200 mg/kg



**Table 3: Effect of methanol extract and ethyl acetate fraction on the degree of orthokeratosis and the 'drug activity' in the mouse tail test**

| Treatment  | Orthokeratosis (%) | Drug Activity (%) |
|--|--------------------|-------------------|
| Saline   | 4.07±3.2           | -                 |
| Retinoic acid (0.05 mg)                          | 65.06±1.09**       | 59**              |
| Cream containing methanol extract (100 mg)       | 19.09±2.90         | 6                 |
| Cream containing methanol extract (200 mg)       | 23.86±3.1          | 11*               |
| Cream containing ethyl acetate fraction (100 mg) | 20.68±2.10**       | 8                 |
| Cream containing ethyl acetate fraction (200 mg) | 29.51±3.50**       | 18*               |

Values are mean ± SEM of 6 parallel measurement. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6). \*\*P< 0.01, \*P< 0.05 when compared against control.

the standard, quercetin (0.968% w/w), kaempferol (0.082% w/w), myricetin (0.468% w/w), and formononetin (0.268% w/w). (Figure 3). The results above showed, therefore, that the rhizome of *Smilax china* is a rich source of the important biologically active flavonoids, quercetin, kaempferol, myricetin, and formononetin. The described HPLC procedure could be useful for the qualitative and quantitative analysis of flavonoids in plant materials

#### High performance thin layer chromatography

In the current study, the flavonoid quercetin was detected and quantified using HPTLC silica gel 60 F254 pre-coated plates with the mobile phase made of Toluene: Ethyl Acetate: Formic acid (5:4:1 v/v/v). The detection of quercetin was observed to be linear over a concentration range of 100 to 300 ng/mL-1 with correlation coefficient of 0.9751. The concentration of quercetin was found to be 0.298 mg/gm. The identity of quercetin in sample chromatograms was confirmed by comparison of the chromatogram obtained from the reference standard solution at various concentration 100 - 300 ng/spot at wave length 254 nm were shown in Figure 4. The Rf values were correspondingly calculated and shown in the Table 2. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation and quantification of botanical materials. It was conformed to the flavonoid quercetin as one of the active constituent for the plant of *Smilax china* Linn. rhizome. Polyphenols like quercetin attribute to the antioxidant property and works as anti-inflammatory, antioxidant, anticancer agents.

#### Acute toxicity

The methanolic extract and ethyl acetate fraction of *Smilax china* leaves were found to be safe in the doses used and no abnormality in the gross behavioral studies and no mortality were noted in the doses of 1g/kg and 2g/kg orally.

**Table 4: Nitric oxide radical scavenging property of Total methanolic extract and Ethyl acetate fraction**

| S. No | Treatment                          | % Inhibition   |
|-------|------------------------------------|----------------|
| 1     | Control-                           |                |
| 2     | Methanol extract (100 mg/kg)       | 43.63 ± 1.58** |
| 3     | Methanol extract (200 mg/kg)       | 51.35 ± 1.46** |
| 4     | Ethyl acetate fraction (100 mg/kg) | 58.80 ± 2.43** |
| 5     | Ethyl acetate fraction (200 mg/kg) | 62.67 ± 0.35** |

Values are mean ± SEM of 6 parallel measurement. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6). \*\*P< 0.01, when compared against control.

#### Evaluation for anti-psoriatic activity

The methanolic extract and ethyl acetate fraction of *Smilax china* rhizome were screened for their possible antipsoriatic activity using Perry's scientific mouse tail model. Extract and fraction 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). Methanolic extract (100 and 200 mg/kg) and ethyl acetate fraction (100 and 200 mg/kg) has increased the orthokeratotic regions by 19.09 and 23.86% respectively, where as 20.68% and 29.51% by ethyl acetate fraction (100 and 200 mg/kg) in comparison to normal. The standard drug Retino-A showed the increase by 65.06% (Table 3).

Granular layer of the epidermis is greatly reduced or absent in psoriatic lesions<sup>2</sup>. Parakeratotic condition is seen in the adult mouse tail which is one of the hallmarks of psoriasis<sup>16</sup>. Induction of orthokeratosis in the adult mouse tail is the basis behind the mouse tail test.<sup>(11)</sup> Many drugs presently used in the treatment of psoriasis have been evaluated by the mouse tail test and were found to have shown good efficacies<sup>17</sup>. Hence in the present study, we used the mouse tail test for evaluating the efficacy of methanolic extract and ethyl acetate fraction. In the mouse tail test, ethyl acetate fraction (100 and 200 mg/kg) produced increased orthokeratosis when compared to control and methanol extract (100 and 200 mg/kg) (Table 3). The phytochemical data showed the presence of increased amount of flavonoid content and it is suggested that the presence of flavonoids might be responsible for the anti-psoriatic activity, presumably through the anti-radical, anti-inflammatory and antiproliferative properties. Representative examples of the histological specimens underlying the histometrical investigation were shown in Figure 7.

#### Nitric oxide scavenging assay

Nitric oxide levels were significantly decreased (\*\*P< 0.01) in both methanol extract and ethyl acetate fraction treated

animals when compared with control (Table 4), suggesting the possible role of this mediator in the etiopathogenesis of psoriasis and the potential future use of NO inhibitors in the treatment of psoriasis.

Nitric oxide (NO) is increasingly recognized as an important intra and intercellular messenger. This heat-labile and unstable compound is synthesized in endothelial cells as well as neurons by constitutive NO synthase (cNOS), while inducible NO synthase (iNOS) is found in leucocytes, macrophages and mesangial cells. It is expressed by various inflammatory factors like IL1, TNF alpha etc. Once expressed it generates a sustained amount of NO. A small amount of NO produced by constitutive NOS in the endothelium is responsible for the relaxation of adjacent smooth muscles and prevents adhesion of platelets and leucocytes to the endothelium. This is the anti-inflammatory effect of NO.<sup>[18]</sup> However, when produced in large amount, NO can destroy tissues and impair immune response. Such high levels are demonstrated in immunological disorders like systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). Hence, inhibition of iNOS is an effective modality of treatment in these conditions Nitric oxide (NO), released from keratinocytes at high concentrations, is considered a key inhibitor of cellular proliferation and inducer of differentiation in vitro. Although high-output NO synthesis is suggested by the expression of inducible NO synthase (iNOS) mRNA and protein in psoriasis lesions, the pronounced hyperproliferation of psoriatic keratinocytes may indicate that iNOS activity is too low to effectively deliver anti-proliferative NO concentrations<sup>19</sup>. Kolb-Bachofen et al<sup>20</sup> demonstrated increased expression of iNOS in psoriatic plaque concluded that iNOS expression is involved in the pathogenesis of cutaneous inflammation of psoriasis. Cals-Gierson and Ormerod<sup>21</sup> have stated that NO is also known to stimulate epithelial cells to produce and release chemokines and other growth mediators such as vascular endothelial growth factor which appear to be important for keratinocyte proliferation and angiogenesis. All the above studies support the theory that in psoriasis, NO may be the mediator of inflammation and the driving force behind the pathogenesis.

#### ***In-vitro* antipsoriatic activity**

The cytotoxic effect of methanol extract and ethyl acetate fraction was evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation in psoriasis<sup>22-25</sup>. The methanol extract and ethyl acetate fraction both showed appreciable antiproliferant activity in HaCaT cell line. The ethyl acetate fraction (IC<sub>50</sub> value of 68.75±14.80µg/ml) was found to have more potent antiproliferant activity than methanol extract (IC<sub>50</sub> value of 102.5±10µg/ml). The results were validated

using asiaticoside as positive control. Asiaticoside showed a potent activity with IC<sub>50</sub> value of 31.40µg/ml.

Several lines of studies indicated that flavonoids such as quercetin possess antioxidant and free radical scavenging potential, anti-inflammatory activity and inhibit the growth of various cancer cell lines in vitro<sup>26,27</sup>. The phytochemical data showed the presence of increased amount of flavonoid content and it is suggested that the presence of flavonoids might be responsible for the anti-psoriatic activity, presumably through the anti-radical, anti-inflammatory and antiproliferative properties. However, the prospective studies to elucidate the exact mechanisms underlying the protective role of *Smilax china* against psoriasis are highly warranted.

#### **CONCLUSION**

We found from our field survey that the rhizome of *Smilax china* was used for the treatment of psoriasis by traditional Siddha healers in Tamil Nadu, India. Drugs that act by multiple mechanisms in combating psoriasis are more important than drugs acting by any single mechanism. This is because; psoriasis is a recurrent chronic inflammatory skin disorder with multiple etiologies. Ethyl acetate fraction of *Smilax china* rhizome showed good activity in the mouse tail test, antiproliferant activity and nitric oxide inhibition assay. From the above data, the plant *Smilax china* rhizome possesses anti-psoriatic activity which is in agreement with its traditional use.

#### **ACKNOWLEDGEMENTS**

Authors are extremely grateful to our Chancellor Dr. Ishari K. Ganesh, Vels University, for the facilities provided to complete this work successfully.

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