



Revista Brasileira de Farmacognosia

BRAZILIAN JOURNAL OF PHARMACOGNOSY

www.journals.elsevier.com/revista-brasileira-de-farmacognosia



Original article

Anti-psoriatic activity of flavonoids from *Cassia tora* leaves using the rat ultraviolet B ray photodermatitis model

Vijayalakshmi. A^{a,*}, Madhira Geetha^b

^aDepartment of Pharmacognosy, SRM College of Pharmacy, SRM University, Tamil Nadu, India.

^bVirugambakkam, Chennai, Tamil Nadu, India.

ARTICLE INFO

Article history:

Received 18 November 2013

Accepted 13 April 2014

Keywords:

Cassia tora

Antipsoriatic

UV-B induced photodermatitis model

Flavonoids

A B S T R A C T

The plant *Cassia tora* L., Fabaceae, traditionally, is claimed to be useful in the treatment of psoriasis and other skin diseases. In order to evaluate this information, antipsoriatic activity of three flavonoids, namely luteolin-7-O- β -glucopyranoside (1), quercetin-3-O- β -D-glucuronide (2) and formononetin-7-O- β -D-glucoside (3), isolated from the ethanol extract of *C. tora* leaves were investigated using UV-B induced photodermatitis model. Further, the flavonoids present in the ethanol extract were identified using HPLC by comparing their retention time with known standard luteolin, quercetin and formononetin. In the UV induced photodermatitis model, histopathological analysis of the section revealed the absence of Munro's microabscess, elongation of rete ridges, and capillary loop dilation in ethanol extract (400 mg/kg), isolated compound 2, 3 and standard group. The ethanolic extract (400 mg/kg) and isolated compounds 1, 2 and 3 exhibited a significant ($p < 0.01$) percentage reduction of relative epidermal thickness when compared with a positive control. In the HPLC analysis, three flavonoids were identified by comparison of the retention times of standard marker, namely luteolin, quercetin and formononetin. We concluded, using animal model, that the flavonoids from *Cassia tora* leaves have significant antipsoriatic activity.

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Introduction

Psoriasis is a genetically determined chronic inflammatory skin disease characterized by red, scaly and raised patches, and it affects 2.3% of the worldwide population. Psoriasis is a disorder of the skin, which occurs when the immune system sends out faulty signals, resulting in the speeding-up of the skin cell's cycle (Azfar and Gelfand, 2002). Histologically, psoriasis is characterized by marked keratinocyte hyper-

proliferation, a dense inflammatory infiltrate of T cells and neutrophils, and vascular dilation and proliferation. The primary defect in psoriasis patients was believed to be an abnormal epidermal cells proliferation (Fry, 1988). In psoriasis, abnormal keratinocyte differentiation, angiogenesis with vasodilation and excess Th-1 and Th-17 inflammation can be observed (Pelle et al., 2005). Psoriatic skin is also characterized by an advanced state of lipid peroxidation (Yildirim et al., 2003), thus, it has been suggested that an antioxidant treatment

* Corresponding author.

E-mail: aviji_1975@rediffmail.com (A. Vijayalakshmi).

could be part of a more specific and effective therapy for the management of this skin disease (Young et al., 2008).

Recent literature supports the fact that polyphenolic compounds found in most plants potentially have a positive effect on many chronic diseases (Middleton et al., 2000; García-Perez et al., 2010). Natural polyphenols, recognized as potent antioxidants, are multifunctional molecules that can act as anti-inflammatory and antiproliferative agents through the modulation of multiple signaling pathways. This characteristic could be advantageous for the treatment of multi-causal diseases, such as psoriasis. Polyphenols are ubiquitous constituents of plants and possess a broad spectrum of biological activities such as immune system activities, oxygen radical scavenging, antimicrobial, anti-inflammatory, and antitumor activities (Grimm et al., 2006). We therefore tried to investigate the possible positive effects of the leaves of *Cassia tora* and isolated flavonoids on a UV-B induced photodermatitis model of psoriasis. *C. tora* is a wild crop that grows in most parts of India as a weed, and has been traditionally used for the treatment of psoriasis and other skin diseases (Anonymous, 1992; Vaidyaratnam, 1997). The present study aimed to investigate the anti-psoriatic potential of standardized ethanol extract (70% v/v ethanol) and the flavonoids isolated from the leaves of *C. tora* on a UV-B induced photodermatitis model, with the purpose of establishing scientific evidence for its ethnobotanical uses.

Materials and methods

General experimental procedures

UV spectra were recorded using methanol as solvent on a Shimadzu UV-1601 PC spectrophotometer. Infrared spectra were recorded in a KBr matrix using a Perkin-Elmer spectrometer-1. ¹H NMR spectra were recorded on a Bruker Avance III 500 spectrometer (500 MHz) and ¹³C NMR on a JEOL GSX 400 spectrometer (400 MHz). Melting points were determined using a Büchi B-540 analyzer. Column chromatography was performed over Silica gel 60 (60-120 mesh, Merck).

Plant material

The plant specimen for the proposed study was collected in Chennai, Tamil Nadu during the month of September. It was identified and authenticated by Dr. P. Jayaraman, Director of the Plant Anatomy Research Centre, Tambaram, Chennai. A voucher specimen was deposited for further reference under N° PARC/2011/2141.

Extraction and isolation

About 750 g of the powdered leaves of *C. tora* was extracted using a Soxhlet apparatus with ethanol (70% v/v) (18 h). The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator).

The crude ethanol extract (23 g) obtained was subjected to chromatography (Silica gel 120 mesh, 500 g) with gradient elution using solvents of increasing polarity: *n*-hexane, chloroform, ethyl acetate and methanol. A total of 72 fractions were eluted. A Shinoda test was carried out to confirm the presence of flavonoids, and thin-layer chromatography (TLC) was carried out using benzene:methanol:ammonia (9:1:0.1) solvent system. The spot was visualized by spraying with ammonia, a reagent specific for flavonoids. The eluates ethyl acetate, ethyl acetate:methanol (40:60) and methanol were positive for flavonoids producing pink color with Shinoda test. The fractions 1-40 were negative for flavonoids. The fractions 41-46, 62-69 and 84-94 exhibited single spot with R_f values of 0.61, 0.64 and 0.76, respectively. Fractions with similar spots and positive for flavonoids were pooled together and concentrated to obtain compound **1**, **2** and **3**. The yield of each compound was 710 mg, 640 mg, and 1 g for compounds **1**, **2** and **3**, respectively. The isolated compounds were purified by recrystallization with methanol.

Acid hydrolysis of compounds 1, 2 and 3

Each compound was refluxed in 2N HCl (5 ml) for 1 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards and UV spectral analysis using the usual shift reagents. Sugar moieties were detected on cellulose TLC plates with ethyl acetate-methanol-water-acetic acid (13:3:3:4), using aniline phthalate as spraying reagent.

Spectral analysis

The compounds (**1**, **2** and **3**) obtained from the ethanol extract of *C. tora* leaves were identified using spectral data.

Compound 1 (luteolin-7-O-β-glucopyranoside)

A yellow amorphous powder with chromatographic properties: R_f 0.54 (benzene:MeOH:NH₄OH); deep yellow fluorescence (AlCl₃) and green color with FeCl₃ spray reagents. UV λ_{max} MeOH nm: 269, 336; + NaOAc: 273, 369; + NaOAc + H₃BO₃: 270, 339; + AlCl₃: 259 sh, 278, 295 sh, 351, 387 sh; + AlCl₃ + HCl: 256 sh, 280, 293 sh, 345, 387 sh; + NaOH: 269, 302 sh, 379. IR (KBr) λ_{max} 3340 (OH), 1634 (C=C), 1507, 1374 (aromatic ring) cm⁻¹. Negative ESI-MS (C₂₁H₂₀O₁₁) m/z 447 [M-H]⁻; 285 [M-H-162]⁻ = [aglycone-H]⁻. ¹H NMR (500 MHz, DMSO-d₆): δ 12.97 (1H, s, H-bounded OH-5), 7.44 (1H, dd, J = 8.1, 1.8 Hz, H-6'), 7.42 (1H, d, J = 1.8 Hz, H-2'), 6.90 (1H, d, J = 8.1 Hz, H-5'), 6.78 (1H, d, J = 2.1 Hz, H-8), 6.74 (1H, s, H-3), 6.44 (1H, d, J = 2.1 Hz, H-6), 5.08 (1H, d, J = 7.2 Hz, H-1'), 3.72 (1H, br d, J = 9.9 Hz, H-6''a), 3.55 to 3.10 (5H, m, H-2'', 3'', H-4'', 5'', 6''b). ¹³C NMR (100 MHz, DMSO-d₆): δ 183.82 (C-4), 166.8 (C-2), 165.8 (C-7), 162.96 (C-5), 158.2 (C-9), 149.7 (C-4'), 148.2 (C-3'), 121.9 (C-1'), 119.8 (C-6'), 114.9 (C-5'), 112.6 (C-2'), 105.84 (C-10), 104.74 (C-3), 102.8 (C-1''), 99.4 (C-6), 94.6 (C-8), 78.7 (C-5''), 77.9 (C-3''), 74.82 (C-2''), 70.8 (C-4''), 61.9 (C-6'').

Compound 2 (quercetin-3-O-β-D-glucuronide)

A yellow amorphous powder with chromatographic properties: R_f 0.61 (benzene:MeOH:NH₄OH); it acquired a red color with Mg-HCl, olive green with alcoholic Fe³⁺, golden yellow color

with NH_3 and NaOH . UV λ_{max} MeOH nm: 256, 275sh, 303sh, 370; +NaOMe 248sh, 325, 414; + AlCl_3 271, 302sh, 335, 460; + AlCl_3 -HCl 267, 303sh, 330, 429; +NaOAc 272, 332, 387; and +NaOAc- H_3BO_3 , 263, 304sh, 340 nm. IR (KBr) λ_{max} 3058 (OH), 1682 (C=C), 1507, 1334 (aromatic ring) cm^{-1} . Negative ESI-MS ($\text{C}_{21}\text{H}_{27}\text{O}_{13}$) m/z 477 $[\text{M} - \text{H}]^-$. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 12.52 (1H, s, H-5), 8.06 (1H, br s, H-2'), 7.38 (1H, d, $J = 8.2$ Hz, H-6'), 6.80 (1H, d, $J = 8.2$ Hz, H-5'), 6.37 (1H, br s, H-8), 6.23 (1H, br s, H-6), 5.32 (1H, d, $J = 6.5$ Hz, H-1''). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 177.4 (C-4), 172.2 (C-6'), 164.3 (C-7), 161.1 (C-5), 157.1 (C-2), 156.7 (C-9), 148.4 (C-4), 144.6 (C-3'), 133.7 (C-3), 121.3 (C-1'), 122.6 (C-6'), 117.3 (C-2), 115.2 (C-5'), 104.3 (C-10), 101.6 (C-1''), 99.7 (C-6), 93.1 (C-8), 76.9 (C-3''), 74.7 (C-5''), 74.4 (C-2''), 71.6 (C-4'').

Compound 3 (formononetin-7-O-glucoside)

Pale yellow powder with MW 431, mp 204-206°C; UV (MeOH) λ_{max} 208, 259, IR (KBr) ν_{max} cm^{-1} : IR 3402 cm^{-1} (OH) 2924, 2517, 2170, 1625 (C = O in flavon), 1530. TLC R_f : 0.76 (benzene: MeOH: NH_4OH). UV λ_{max} MeOH nm: 254, 268, 346; + NaOMe: 263, 309 sh, 398; + NaOAc + H_3BO_3 : 268, 293 sh, 370; + AlCl_3 : 276, 298 sh, 386; + AlCl_3 /+ HCl: 270, 289 sh, 353, 387; + NaOH: 243 sh, 270, 304 sh, 392. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 8.44 (1H, s, H-2), 8.08 (1H, d, $J = 8.8$ Hz, H-5), 7.54 (1H, d, $J = 6.6, 2.3$ Hz, H-6'), 7.28 (1H, d, $J = 2.2$ Hz, H-8), 7.14 (1H, dd, H-6), 7.10 (1H, d, $J = 9$ Hz, H-5'), 5.14 (1H, d, $J = 7.2$ Hz, H-1''), 3.79 (3H, s, OCH_3), 3.70 to 3.31 (5H, m, H-2'', 3'', 4'', 5'', 6''). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ 173.7 (C-4), 160.2 (C-7), 158.8 (C-4'), 156.9 (C-9), 154.4 (C-2), 130.8 (C-2', C-6'), 125.6 (C-1'), 125.3 (C-3, C-5), 118.4 (C-10), 115.3 (C-6), 113.7 (C-3', C-5'), 104.4 (C-8), 102.1 (C-1''), 77.4 (C-5''), 74.6 (C-2''), 73.4 (C-3''), 61.4 (C-6''), 54.7 (OCH_3).

HPLC analysis

The qualitative analysis of the sample was performed according to the method of Boligon et al., 2012. The Jasco HPLC system consists of a pump (model Jasco PU2080, intelligent HPLC pump) with an automatic injection system programmed at 20 μl capacities per injection. The detector consists of a UV/VIS spectrophotometer (Jasco UV 2075) at a wavelength of 270 nm. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. The column was Thermo ODS Hypersil C18 (250 \times 4.6 mm, 5 μm) in isocratic mode. The separation was achieved using a mobile phase of methanol, water and phosphoric acid (100:100:1, v/v/v) at a flow-rate of 1.5 ml/min. The effluent was monitored using UV detection at a wavelength of 270 nm. The mobile phase was filtered through 0.45 μm nylon filter prior to use.

Sample preparation

Powdered sample of *C. tora* leaves was weighed and transferred to a 250 ml flask fitted with a reflux condenser. About 78 ml of extraction solvent ($\text{EtOH}:\text{H}_2\text{O}:\text{HCl}$, 50:20:8) was added, refluxed on a hot water bath for 135 min, cooled at room temperature and transferred to a 100 ml volumetric flask. About 20 ml of methanol was added to the 250 ml flask and sonicated for 30 min, afterwards the solution was filtered and the filtrate was transferred to the 100 ml volumetric flask, the residue was washed on the filter with methanol. The wash was collected

in the same 100 ml volumetric flask and diluted to volume. Identification is based on retention times and on-line spectral data in comparison with authentic standards.

Animals

Healthy male Wistar rats (120-170 g) and Swiss albino mice (25-30 g) obtained from the institutional animal housing facilities were used for the study. Animals were housed in polypropylene cages and were left seven days for acclimatization to animal room, which was kept under controlled conditions (a 12 h light-dark cycle at $22 \pm 2^\circ\text{C}$) and fed on standard pellet diet and water *ad libitum*. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with approval from the Institutional Animal Ethics Committee ([CPCSEA] IAEC/52/2012).

Acute toxicity

Acute toxicity studies were carried out using mice as per Economic Co-operation and Development OECD 425 guidelines (2002). Mice (six animals per group) were divided into five groups. Animals were treated orally with ethanol extract at a dose of 2000 mg/kg body weight and isolated compound 1, 2 and 3 at a dose of 500 mg/kg body weight. The animals were observed for clinical signs and mortality for a period of 15 days, and body weight changes were recorded every week. The tested sample was found to be safe and did not produce any mortality after the stated time.

Rat UV ray photo dermatitis model for psoriasis

In the "ultraviolet ray photodermatitis model for psoriasis", the exposure of the rat's skin to UV radiation using a UV-B bulb (wavelength 280-315 nm) induced proinflammatory reaction in the skin that resembles the one observed in psoriasis. This was evident by the altered skin parameters; the most important of which are the increase in epidermal thickness to almost double the normal size, absence of stratum granulosum and the movement of neutrophils towards the epidermis, all symptoms typical of psoriasis. Although the causative factor and the mechanism underlying the chronic manifestation of psoriasis is not clearly understood, there is scientific agreement that the end result is the initiation of a T-cell lymphocyte mediated immunological response that is proinflammatory in nature. The inflammatory reaction is restricted to the area of the skin where the antigen exists.

Irradiation of the depilated rat skin with ultraviolet radiation is known to produce a biphasic erythema. Immediately after irradiation, initial faint erythema appears, and disappears within 30 min. The second phase of erythema starts 6 h after irradiation and gradually increases, peaking between 24 to 48 h. This reaction is confined to the exposed area and has a sharp boundary. It develops a brownish-red color. By 48 to 72 h, silvery white scales appear on the erythematous lesion. These scales are relatively thick and begin to fall beyond the 72 h. Although the erythematic reaction is induced artificially, many of the pathological features resemble those seen in psoriasis vulgaris. The close resemblance of inflammatory

process produced by ultraviolet radiation to the one exhibited in psoriasis provides us with a good model to investigate drugs that have a potential to reduce the inflammatory reaction associated with psoriasis (Michael et al., 2005).

Procedure

The hairs of one side of the flank of the rat were clipped with scissors followed by careful shaving, taking precaution to avoid injury to the skin. The animals were then placed on a curved wooden block and their legs tied around it, to avoid contact with the floor. This arrangement prevented the movement of the animal during its subsequent exposure to UV radiation. Except for an area of 1.5 × 2.5 cm on the depilated skin, the entire animal was covered with a UV resistant film. The uncovered area of 1.5 × 2.5 cm was then irradiated for 20 min with a UV-B lamp kept at a vertical distance of 20 cm from the skin (Vogel et al., 2002). The animals were divided into seven groups (six animals per group). The control group animals received normal saline (10 ml/kg, *p.o.*) and standard group received retinoic acid (0.5 mg/kg, *p.o.*). The remaining groups were treated orally with the ethanol extract of *C. tora* leaves (200 and 400 mg/kg) and compounds **1**, **2** and **3** (50 mg/kg) once daily, five times a week, 12 h after irradiation, for two weeks. Two hours after the last treatment animals were sacrificed; longitudinal sections of the tail skin were made and prepared for histological examination with hematoxylin-eosin staining (Nakaguma et al., 1995; Singhal and Kansara 2012).

Histopathological examination

Sections were examined for the presence of Munro's microabscesses, elongation of rete ridges, and capillary loop dilation by direct microscopy. The vertical epidermal thickness between the dermoepidermal junction and the lowest part of the stratum corneum ($n = 3$ measurements per scale, $n = 3$ scales per animal, $n = 6$) were examined. The percentage relative epidermal thickness of all the groups was calculated in comparison to the positive control group (100%; $n = 54$ measurements per treatment). It was also examined for Mean thickness of stratum corneum and stratum granulosum.

All measurements were made at a magnification of 400× using a digital camera attached to an Olympus microscope, and used software to take measurements.

Statistical analysis

Values were represented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA), and group means were compared by the Tukey-Kramer Multiple Comparison test using InStat-V3 software. *p* values < 0.05 were considered significant.

Results and discussion

The therapeutic potential of flavonoids and the necessity for scientific validation in popular medicine have prompted increased interest in the field. The ethanol extract of *Cassia*

tora L., Fabaceae, leaves was defatted with petroleum ether and subjected to column chromatography over silica gel to yield 54 fractions. Three flavonoids were isolated viz., luteolin-7-O- β -glucopyranoside (**1**), quercetin-3-O- β -D-glucuronide (**2**) and formononetin-7-O- β -D-glucoside (**3**).

Compound **1** was isolated as a yellow amorphous powder of yellow color with alkalis, pink color with Mg-HCl, olive green with Fe³⁺ and answered Molisch's test. Acid hydrolysis afforded aglycone, luteolin, and sugar, glucose. The aglycone was yellow under UV and UV/NH₃, characteristic of a flavonol with free 5-OH and had λ_{\max} (MeOH) 259, 266, 365nm. The UV spectrum of the aglycone in methanol and changes observed after the addition of shift reagents indicated that there is a free hydroxyl group present at C-5 position and the 7-hydroxyl group was substituted.

The ¹H NMR showed a singlet at δ 12.97, as evidence for H- bonded OH-5. The H-3 resonance was assigned at 6.74 (s) as an evidence for ring C in flavone. In the aliphatic region, a β -anomeric proton doublet was located at 5.08 ($J = 7.2$), and one of the two diastereomeric CH₂-6'' was assigned at 3.72 (9.9 Hz, H-6''a), proving β -4C-stereostructure for a glucoside moiety.

The ¹³C NMR spectrum showed fifteen signals typical for luteolin-7-O-substituted aglycone including five key signals at δ 183.82 (C-4), 165.8 (C-7), 149.7 (C-4'), 148.2 (C-3') and 104.74 (C-3). The full assignment of all ¹H and ¹³C-resonances were confirmed by comparison with previously published data (Agrawal and Bansal, 1989).

The structure of the glycoside luteolin 7-O- substituted aglycone was further evidenced by mass spectrum. The mass spectrum of the glycoside shows prominent peaks at m/z 447 [M - H]⁻ and at 285 [M-H-162]⁻ aglycone-H. The molecular formula was determined as C₂₁H₂₀O₁₁, m/z 447 by Negative ESI-MS. Therefore, the compound **1** was established as luteolin-7-O- β -D-glucopyranoside.

Compound **2** was isolated as a yellow amorphous powder and gave a red color when in contact with Mg-HCl, olive green with alcoholic Fe³⁺, and a golden yellow color with NaOH. The aglycone was yellow under UV and UV/NH₃, characteristic of a flavonol with free 5-OH, it also had λ_{\max} (MeOH) 256, 275sh, 303sh, 370 nm. The UV spectrum of the aglycone in methanol and changes observed after the addition of shift reagents indicated that there is a free hydroxyl group present at C-5 and C-7 position and the 3-hydroxyl group was substituted.

In the ¹H NMR spectrum (500 MHz, DMSO-d₆) of the glycoside quercetin 3-O-glucuronide, the signal appearing at δ 12.5 ppm corresponds to the -OH at C-5. The signal at δ 9.8-10.5 ppm appears due to the hydroxyl proton at C-7. The doublet appearing in the region δ 8.06 ppm (d, $J = 8$ Hz) and δ 7.3 ppm corresponds to the proton at C-2' and C-6', while the proton of C-3' appears at δ 6.17 ppm. The signal appearing at δ 6.8 ppm (d, $J = 8$ Hz) corresponds to C-5' proton. C-8 proton due to meta coupling with C-6 proton appears as a doublet at δ 6.3 ppm (d, $J=2.2$ Hz). C-6 proton, due to meta coupling with C-8 proton serves as a supporting evidence for the structure of the flavonol glycoside quercetin 3-O-glucuronide, which is provided by the ¹³C-NMR (100MHz, DMSO-d₆) spectral data. The ¹³C NMR spectrum of II showed the presence of signals at δ 172.2 for C-6'' of the glucuronic acid that supported the FAB-mass data. The structure of the glycoside quercetin-3-O-

glucuronide was further evidenced by mass spectrum. These data are identical with those for quercetin-3-O- β -D-glucuronide reported elsewhere (Li et al., 2011).

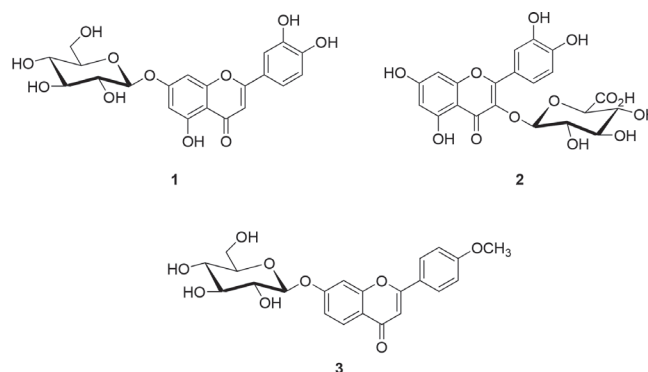
The mass spectrum of the glycoside shows prominent peaks at m/z 477 $[M - H]^-$ and at 301 $[M-H-178]^-$ aglycone-H. The molecular formula was determined as $C_{21}H_{18}O_{13}$, m/z 478 by Negative ESI-MS. Therefore, the compound 2 was established as quercetin-3-O-glucuronide.

Compound 3 was isolated as a pale yellow amorphous powder, displayed a yellow color with alkalis, a pink color with Mg-HCl, an olive green color with Fe^{3+} and answered Molisch's test. Acid hydrolysis of 3 yielded aglycone, formononetin, and sugar, glucose. This was confirmed by co-chromatography with authentic samples. The UV spectrum (λ_{max} in MeOH, 261 nm and 330 nm) showed the characteristic absorption of an isoflavone. No UV bathochromic shift was observed with $AlCl_3$ suggesting the absence of free 5-OH group, and the absence of bathochromic shift on addition of sodium acetate suggests that there is a free hydroxyl group presented at C-7 position, as the 7-hydroxyl group was substituted.

The 1H NMR spectrum of compound 3 also suggests an isoflavone skeleton with a signal at 8.44 (1H, s, H-2) and one methoxy group with signal at 3.79 (3H, s). The 1H NMR signals of two coupled doublets ($J = 8.8$ Hz) at 8.08 and 7.14 were characteristic of 2-ortho-related H-5 and H-6 protons of ring A. 1H NMR signal at 7.28 (1H, s) indicates non-coupled proton at C-8. The 1HNMR spectrum also supported the presence of one sugar moiety with the proton signals at δ H 5.14 (1H, d, $J = 7.8$ Hz, Glc-H-1'') related to glucose. 1H NMR signal at 3.79 (3H, s) indicated a methoxy group. Presence of signals at 7.10 (2H, dd, $J = 6.6, 2.3$ Hz, H-3' and H-5') and at 7.54 (2H, dd, $J = 6.6, 2.3$ Hz, H-2' and H-6') indicated the presence of the methoxy group at C4' in ring B.

The identity of the sugar was confirmed by ^{13}C NMR (Table 1). The ^{13}C NMR spectrum of compound 3 showed the presence of signals at δ 102.1 for C-1'' of the glucose, that supported the FAB-mass data (the fragmentation pattern showed a peak at m/z 268 $[M+H-162]^-$ due to the loss of glucose). The structure of the glycoside formononetin-7-O-substituted aglycone was further evidenced by mass spectrum. The mass spectrum of the glycoside shows prominent peaks at m/z 429 $[M-H]^-$ and at 268 $[M+H-162]^-$ aglycone-H. The molecular formula was determined as $C_{22}H_{22}O_9$, m/z 430 by Negative ESI-MS.

Therefore, the composition of compound 3 was established as Formononetin-7-O- β -D-glucoside.



HPLC analysis

Qualitative analysis of the ethanol extract of the *Cassia tora* leaves demonstrates the presence of seven components. In the present investigation, three flavonoids were identified at 254 nm using peak area by comparison to a retention times of the standard marker, luteolin, quercetin and formononetin. The main difference was in peak, which eluted at 12.07 min, 19.62 min and 31 min respectively. These results show therefore, that *Cassia tora* leaves are a rich source of the important biologically active flavonoids, luteolin, quercetin and formononetin. The described HPLC procedure could be useful for the qualitative analysis of flavonoids in plant materials.

Acute toxicity study

During the acute toxicity study, the ethanolic extract, as well as compounds 1, 2 and 3, was administered orally and animals were observed for mortality and behavioral responses. There was no mortality observed even at 2000 mg/kg for the extract and at 500 mg/kg for the compounds. All the animals were normal and there were no gross behavioral changes till the end of the observation period.

Table 1

Effect of ethanol extract and isolated compounds of *Cassia tora* leaves on Histopathological features on U.V.-B-induced psoriasis in rats.

Treatment	Munro's micro-abscess	Elongation of rete ridges	Capillary loop dilation
Positive control	+++	+++	+++
Standard	-	-	-
Ethanol extract 200 mg/kg	-	+	+
Ethanol extract 400 mg/kg	-	-	-
Compound 1 (50 mg/kg)	-	-	+
Compound 2 (50 mg/kg)	-	-	-
Compound 3 (50 mg/kg)	-	-	-

Note: + mild or slight grade lesion; +++ severe grade lesion; - no lesion.

Rat UV ray photo dermatitis model for psoriasis

Screening of anti-psoriatic activity of ethanol extract and isolated flavonoids of *C. tora* leaves was carried out using ultraviolet-B-induced psoriasis in rat. The irradiated rat skin treated with ethanol extract and isolated flavonoids of *Cassia tora* leaves showed a significant reduction in the total epidermal thickness, as well as a significant retention of the stratum granulosum, the absence of movement of neutrophils further substantiates that the ethanol extract and isolated flavonoids has been very useful in containing the changes that occur in the skin due to irradiation.

Histopathologically, number of features was observed in fully developed lesions in psoriasis (positive control group) such as Munro's microabscess, regular elongation of rete ridges, and capillary loop dilation which were shown in Fig. 1 and Table 1. Mean thickness of the epidermis, stratum corneum and stratum granulosum in the standard and the groups that were treated with the ethanol extract of *Cassia tora* leaves and compound 1, 2 and 3 are presented in Table 2 and Fig. 2. In comparison to positive control group, all other groups led to significantly decreased relative epidermal thickness.

Overall, the irradiation of rat skin with UV-B has shown good changes in the epidermis, resembling psoriasis.

Skin is the largest exposed organ of body and an easy target for allergic and immunologic reactions. Skin ailments for dermatitis, urticaria, angioedema, psoriasis, among others, are immune-mediated disorders, and are chronic, inflammatory, and proliferative in nature (Meeuwis et al., 2011). Psoriasis got the attention of scientists and became an important thrust area of scientific studies due to its severe effect on the quality of life, expensive treatment and toxicity and/or side effects of the available medication (Krueger et al., 20). Medicinal plants are known to be safe for human health and are widely employed by the traditional healers for the treatment of various diseases including psoriasis. Medicinal plants are known to be a rich citadel of variety of chemical compounds and have attracted researcher's attention to find new treatment for psoriasis (Kaur and Kumar, 2012).

The section of positive control group, showed regular elongation of rete ridges, capillary loop dilation with minimal grade lesion of diagnostic Munro's microabscess and marked increase in relative epidermal thickness as compared to other groups. In the ethanol extract (200 mg/

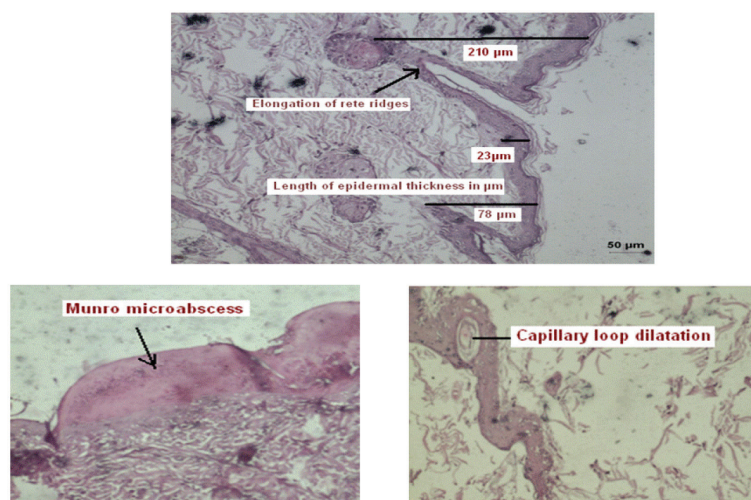


Figure 1 – Photomicrographs of positive control rat skin after UV-B-induced psoriasis.

Table 2

Relative Epidermal thickness and different layers of epidermis on UV-B-induced psoriasis in drug treated rats.

Treatment	% Relative Epidermal thickness (μm)	Thickness of stratum corneum (μm)	Thickness of stratum granulosum (μm)
Positive control	94.86 \pm 6.82	2.95 \pm 0.83	Absent
Standard	22.37 \pm 2.59***	15.62 \pm 1.20	12.83 \pm 0.38
Ethanol extract 200 mg/kg	48.12 \pm 4.39**	5.22 \pm 0.73	1.90 \pm 0.72
Ethanol extract 400 mg/kg	38.57 \pm 2.80**	8.43 \pm 0.69	4.94 \pm 0.80
Compound 1 (50 mg/kg)	41.60 \pm 1.16**	6.35 \pm 1.06	4.24 \pm 0.62
Compound 2 (50 mg/kg)	30.14 \pm 3.10***	8.13 \pm 0.68	5.10 \pm 0.58
Compound 3 (50 mg/kg)	28.92 \pm 1.74***	9.37 \pm 0.92	6.12 \pm 0.72

n = 6; values are expressed as mean \pm SEM. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. The values are * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared against control.

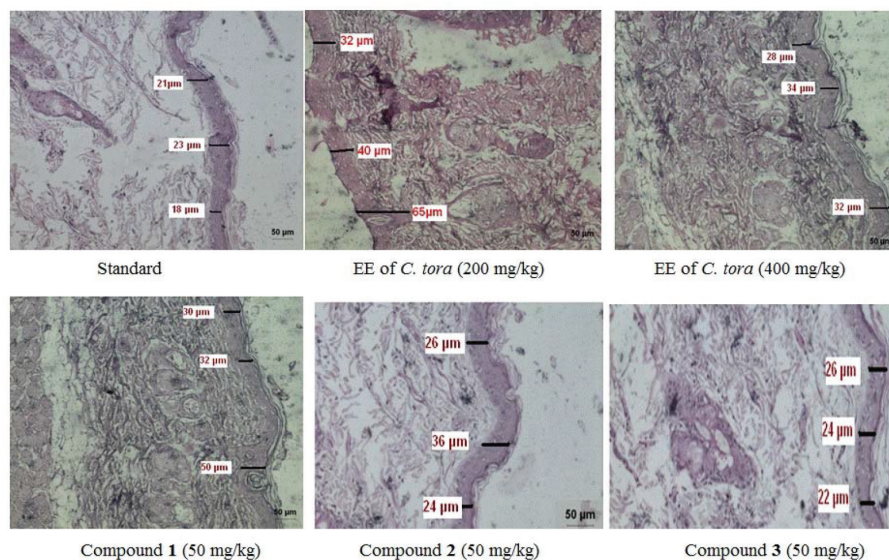


Figure 2 – Histopathological features on UV-B-induced psoriasis in drug treated rats.

kg) and isolated compound 1 treated group, there was a minimal grade lesion of elongation of rete ridges along with capillary loop dilation in the section, as well as an absence of Munro's microabscess. In ethanol extract (400 mg/kg) and isolated compounds 2 and 3 treated groups, there was no lesion of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section of skin of the rats.

The irradiated rat skin treated with ethanol extract of *C. tora* leaves (400 mg/kg), and isolated compounds 1, 2 and 3 showed a significant reduction in the total epidermal thickness, indicating that its presence in the plant has an influence to retard the hyper proliferation of the keratinocytes that occurs when the skin is exposed to UV radiation. The significant retention of the stratum granulosum is probably due to its ability to enhance the keratinisation process which is a protective strategy adopted by the skin when exposed to penetrating radiation. The observed anti-psoriatic activity may be through the inhibition of keratinocyte proliferation.

Conclusion

This study showed that the ethanol extract of *Cassia tora* leaves exhibits a significant antipsoriatic activity, and that it does dependently decreased the relative epidermal thickness of animal skin as well as other histopathological features. The study implies that *C. tora* leaves and its flavonoids could be used as natural therapeutic drugs to prevent psoriatic complications and authenticities the folk claim of the plant in the use of traditional medicine for the treatment of psoriasis. Additional clinical investigation of these compounds is indicated to evaluate the efficacy and safety of their application as dietary supplements with health benefits to psoriatic patients.

Author contributions

VA carried all experiments, interpreted the data and drafted the manuscript and MG designed the study and analyzed the data.

Conflicts of interest

The authors declare no conflicts interest.

Acknowledgement

Authors acknowledge sincere thanks to the management for the facilities granted for the research work.

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