

## ASSESSMENT OF THE ANTI-PSORIASIS EFFECT OF *SCROPHULARIA DESERTI* METHANOLIC EXTRACT IN MICE MODEL

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*Psoriasis is an underestimated chronic and autoimmune skin disorder. Topical chemical agents are applied for psoriasis control and treatment, notwithstanding their subordinate efficiency or unsuccessful activities. As an alternative, herbal medicine can also be used in its treatment.*

**The aim** of the present study was performed to assess the anti-psoriasis effect of *Scrophularia deserti* in mice model.

**Materials and methods:** *S. deserti* was purchased and used for methanolic extraction. Extract DPPH radical scavenging activity, polyphenol and flavonoid contents were examined. Sixty male mice were purchased, and psoriasis was induced using 10 days of topical administration of Imiquimod (62.5 mg). Mice were classified into 6 groups: non-psoriasis control (only received distilled water), psoriasis control (only received topical Imiquimod), two *S. deserti* treatments (topical 300 and 500 mg/kg), topical Betamethasone, and topical  $\alpha$ -pinene 9 %. Cytokine distribution and histopathological properties were also determined.

**Results:** the value at which the *S. deserti* methanolic extract scavenges 50 % of free radicals (IC<sub>50</sub>) was 602.71±15.33 µg/mL. The total *S. deserti* methanolic extract flavonoid and polyphenol contents were 16.85±1.12 mg QE/g and 58.47±3.25 mg GAE/g, respectively. IL-22, TNF- $\alpha$ , and IL-17A concentrations increased after psoriasis induction compared to the control group ( $P<0.05$ ). Mice treated with Betamethasone harboured the lowest concentrations of IL-22, TNF- $\alpha$ , and IL-17A ( $P<0.05$ ).

**Conclusions:** Mice treated with *S. deserti* methanolic extract (500 mg/kg) also harboured significantly lower IL-22, TNF- $\alpha$ , and IL-17A ( $P<0.05$ ) compared to  $\alpha$ -pinene and *S. deserti* methanolic extract (300 mg/kg). Mice of the psoriasis control group showed significant epidermis hyperkeratosis, acanthosis, and crust with plentiful inflammatory cells. At the same time, mice treated with *S. deserti* methanolic extract (500 mg/kg) showed significant recovered tissue with normal skin epidermis and dermis, sebaceous glands, and follicles of the hair, besides the lowest rate of inflammatory reactions. Findings showed that the *S. deserti* methanolic extract (500 mg/kg) can efficiently be used as a practical substitute for psoriasis treatment. However, some supplementary research should be performed

**Keywords:** anti-psoriasis, *Scrophularia deserti*, methanolic, extract, antioxidant, phenols, flavonoids, cytokine, Imiquimod, mice model

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### 1. Introduction

Psoriasis is a skin-based disease with immune and inflammatory-mediated reasons characterized by unequal keratinocyte hyperproliferation, giving rise to epidermis inflammatory response, flaking, and thickening [1]. Affected skin may be degenerated to silver, white, or red with scaly plaques [2]. Itching, scaling, burning, erythema, and bleeding are the most routine psoriasis symptoms [3]. About 3 % of the world's population (around 125 million persons) is affected by psoriasis, with up to 30 % frequency of complicated cases [4]. Psoriasis etiology remains indeterminate. Undoubtedly, an initial immune defect gives the impression of being accountable for the cytokines and chemokines-induced cell signalling upsurge, which increases the gene expression and subsequent hyperproliferation in keratinocytes [5].

There were no operative and definitive treatments for psoriasis, and prevailing treatments are accompanied

by mild to severe bad effects, including hepatic and nephron toxicity and skin irritation [5, 6]. Consequently, psoriasis patients prefer to use alternative treatments with less bad side effects, particularly traditional and complementary medicine. Natural medicines appear hopeful in the control and treatment of diverse dermatological diseases [7]. *Scrophularia deserti* (*S. deserti* known as figwort), an important species of the family Scrophulariaceae, is equitably plentiful in desert areas with boosted medicinal effects [7]. It is mostly applied as a healing plant with antifungal, anti-diabetic, antimicrobial, anti-cancer, wound healing, and anti-inflammatory properties [8]. Scropolioside and harpagoside as iroid glycosides are the most substantial *S. deserti* constituents [8]. Traditionally, *S. deserti* was applied to treat diverse kinds of diseases, including inflammatory infections, eczema, scabies, tumours, scrofula, and dermatological disorders [9, 10].

Rendering the boosted importance of psoriasis, its widespread distribution, and the absence of potential and applied work about the anti-psoriasis effect of *S. deserti*, the current investigation was carried out to assess the anti-psoriasis effects of *S. deserti* methanolic extract compared to Betamethasone and  $\alpha$ -pinene in mice model.

**2. Research planning (methodology).**

In order to achieve the desired objectives of this work, the methodology of this, as described in Fig. 1, was followed.

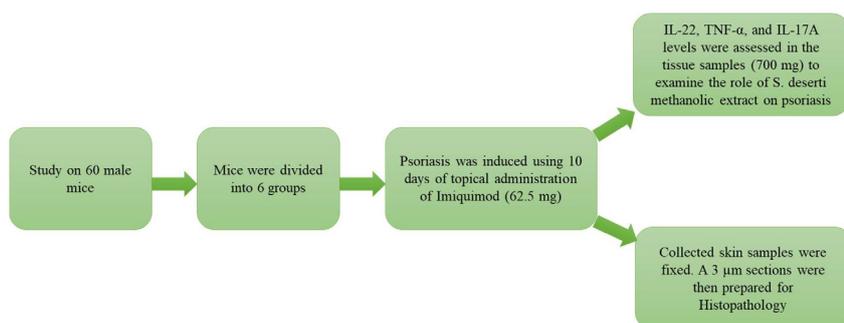


Fig. 1. Ulitrses the steps of this current work

**3. Materials and methods**

This research was carried out in the animal house of the University of Baghdad for the period (05/08/2023–25/12/2023).

*Chemical reagents, diagnostic kits, and devices.*

All chemical reagents,  $\alpha$ -pinene, thiopental sodium, methanol, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, NaOH, and Aluminum chloride (AlCl<sub>3</sub>) with analytical grade were purchased from the Sigma-Aldrich Company (St Louis, MO, USA). Imiquimod (62.5 mg) was purchased from Glen mark Pharmaceuticals, India. Betamethasone ointment (0.1 %) was purchased from Actavis Company, United States. Enzyme-linked immunosorbent assay (ELISA) kit was used for cytokine detection (Legend max, USA). Whatman® cellulose filter paper was also purchased from the Sigma-Aldrich company. For the centrifuge, the Shimadzu, Japan device was used. Mixer was also used for fresh plant powdered (AKP, Iran). The rotary device was purchased from Drgaon company (Dlab, RE100-pro, China).

*Plant materials and extraction.*

The aerial parts of *S. deserti* were purchased from local markets in Baghdad, Iraq, through the spring of 2023. Aerial parts were approved by a Professor of medicinal plants at the Agricultural Research Center. Fresh *S. deserti* aerial parts were stored in the dry shade under suitable conditions and turned into a powder by a mixer. Twenty grams of powdered aerial parts were dissolved in methanol (80 ml), and the extraction procedure was done using 24-hour shaking at room temperature. The achieved extract was

filtered using paper filter No. 3 and then concentrated by a rotary evaporator at 40 °C. The final achievement was kept in the refrigerator for further analysis.

*S. deserti antioxidant activity.*

*S. deserti* extracts at numerous concentrations were added to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution with a reaction mixture volume of 200  $\mu$ L. Then, dark incubation was performed for 30 min at 37 °C. After that, solution absorbance was assessed at 517 nm. Ascorbic acid was applied as a control. The following formula was applied to examine the DPPH radical scavenging activity of *S. deserti* methanolic extract:

$$\text{Scavenging activity} = \frac{1 - \left( \frac{\text{S. deserti absorbance} - \text{blank absorbance}}{\text{Control absorbance}} \right) \times 100}{100}$$

*S. deserti total polyphenol content.*

Folin–Ciocalteu assay [11] was applied to determine *S. deserti* total polyphenols. Seven hundred and fifty microliters of the *S. deserti* methanolic extract and gallic acid were blended with one hundred and fifty microliters of Folin–Ciocalteu reagent. The mixture was then incubated at 22 °C for 5 min. After that, 150  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> (20 %) was added into a mixture, and the solution was incubated at 40 °C for half an hour. Solution absorbance was then checked at 750 nm. Total polyphenol was determined, rendering the gallic acid curve. The following formula was applied to examine the *S. deserti* methanolic extract total polyphenols:

$$\text{S. deserti total polyphenol} = \frac{\text{Gallic acid concentration (mg/ml)} \times \text{S. deserti methanolic extract volume (ml)}}{\text{S. deserti methanolic extract mass (g)}}$$

*S. deserti total flavonoid content.*

Aluminium chloride (AlCl<sub>3</sub>) colourimetric assay was applied [12]. Four hundred microliters of *S. deserti* methanolic extract and quercetin were added to NaNO<sub>2</sub> (5 %, 30 ml) and mixed well for 5 min. Then, 30  $\mu$ L of AlCl<sub>3</sub> (10 %) was added to the achieved solution. The subsequent solution was incubated at 22 °C for 5 min. After that, NaOH (4 %, 400  $\mu$ L) was added to the previous solution. The subsequent solution was incubated at 22 °C for 15 min. Distilled water was applied to increase the mixture volume to 1 mL. After well mixing, solution absorbance was assessed at 510 nm. The total flavonoid was determined, rendering the quercetin curve. The following formula was applied to examine the *S. deserti* methanolic extract total flavonoid:

$$\text{S. deserti total flavonoid} = \frac{\text{Quercetin concentration (mg/ml)} \times \text{S. deserti methanolic extract volume (ml)}}{\text{S. deserti methanolic extract mass (g)}}$$

*Animals.*

In this experimental research, 60 male BALB/c mice weighing 30±2 g were used. These mice were kept in special cages, and the temperature of the animal room was adjusted to 20±2 °C with about 70 % relative humidity. The light program used was 12 hours of light and 12 hours of darkness, with the start of morning lighting at 9 o'clock. Animals had free access to fresh water and food. The mice and food were provided by the animal house of Baghdad University.

*Psoriasis induction and groups.*

Hair removal cream was used to shave the back side of mice (2×3 cm). Then, all mice (except the control group) received topical Imiquimod (62.5 mg) for 10 successive days. After 10 days of imiquimod administration, mice were randomly classified into 6 groups, with 10 mice in each (Table 1). Mice of the non-psoriasis control group only received distilled water. *S. deserti* treatment groups received different concentrations (300 and 500 mg/kg/day) of *S. deserti* methanolic extract topically. Concentrations were prepared using the PBS. Topical betamethasone ointment (0.1 %) was applied for the Betamethasone treatment daily. Topical α-pinene 9 % was also used for the group of these mice.

*Immunological assessment.*

IL-22, TNF-α, and IL-17A levels were assessed in the tissue samples (700 mg) to examine the role of *S. deserti* methanolic extract on psoriasis. Enzyme-linked immunosorbent Assay (ELIS) was applied. The procedure was done after 14 days of the treatment experiment. In total, 700 mg of collected mice skin was frozen using liquid nitrogen (-80 °C). Homogenization was done in RIPA cell lysis solution. Homogenized tissue was centrifuged (12,000 rpm for 10 min). Cytokine detection and quantification were performed on a supernatant solution, rendering the ELIS kit guidelines [13].

*Histopathology.*

Collected skin samples were fixed (10 % formalin). After that, paraffin dehydration and embedment were performed. A 3 μm sections were then prepared. Staining was performed using the Hematoxylin and eosin (H&E).

*Data analysis.*

Collected data were assessed by analysis of variance (ANOVA) test. A post-hoc Scheffe multiple comparison test was also applied to compare the findings of diverse groups. The value of P<0.05 was measured as a statistically significant level. All data were described as standard deviation (SD)

**Research ethics.** The local Institutional Review Board deemed the study exempt from review.

**Informed consent.** Not applicable.

**4. Results**

*S. deserti radical scavenging, total phenol, and flavonoid contents.*

Table 2 shows the *S. deserti* methanolic extract radical scavenging effect and total polyphenol and flavonoid contents. The value at which the *S. deserti* methanolic extract scavenges 50 % of free radicals (IC50) was 602.71±15.33 μg/mL. The total *S. deserti* methanolic extract flavonoid and polyphenol contents were 16.85±1.12 mg QE/g and 58.47±3.25 mg GAE/g, respectively.

*Cytokines analysis.*

Fig. 2 shows the IL-22, TNF-α, and IL-17A concentrations among the collected mice skin samples of diverse groups. Totally, IL-22, TNF-α, and IL-17A concentrations have been increased after psoriasis induction compared to the control group (P<0.05). Amongst the treatment groups, mice treated with Betamethasone harboured the lowest concentrations of IL-22, TNF-α, and IL-17A which had statistically significant differences with those of non-psoriasis control and *S. deserti* methanolic extract (300 mg/kg) (P<0.05). A statistically significant difference was observed for the TNF-α concentrations between all treatment and control groups (P<0.05). However, there were no significant differences in the IL-22 and IL-17A concentrations between mice treated with *S. deserti* methanolic extract (300 mg/kg) and Betamethasone (P>0.05).

*Histopathological findings.*

Fig. 3 shows the H&E staining findings of the anti-psoriasis effects of *S. deserti* methanolic extract in mice models.

Table 1

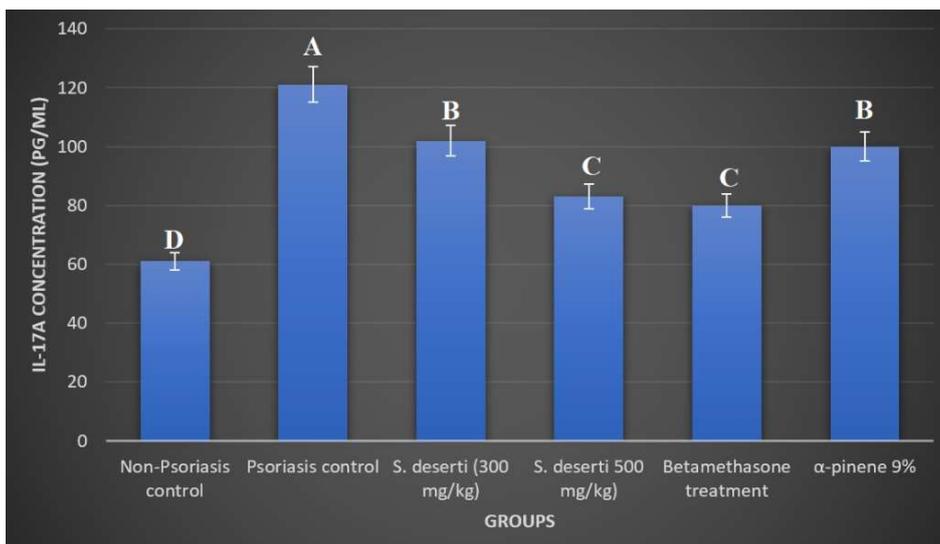
Mice classification

Groups	Topical 5 % Imiquimod cream (62.5 mg)	500 μL of <i>S. deserti</i> extract (300 mg/kg, topical)	500 μL of <i>S. deserti</i> extract (500 mg/kg, topical)	500 μL of topical Betamethasone ointment	500 μL of topical α-pinene 9 %
Non-Psoriasis control	-	-	-	-	-
Psoriasis control	+	-	-	-	-
Treatment I	+	+	-	-	-
Treatment II	+	-	+	-	-
Betamethasone treatment	+	-	-	+	-
α-pinene 9 %	+	-	-	-	+

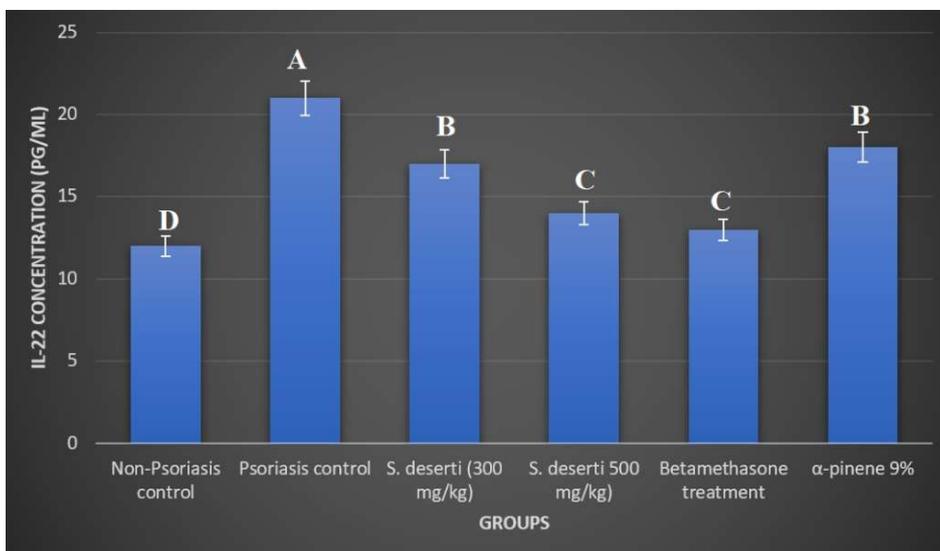
Table 2

*S. deserti* methanolic extract radical scavenging, total polyphenol, and flavonoid contents

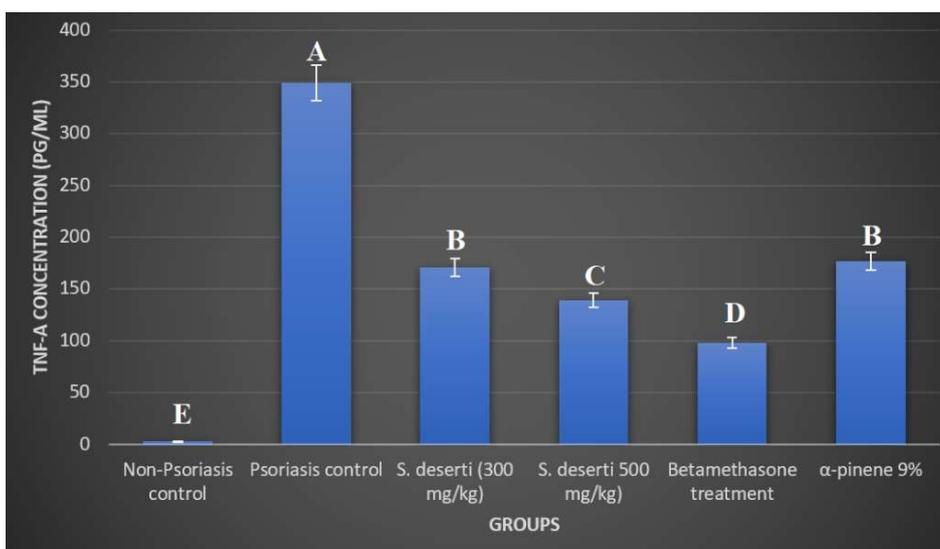
Plant	DPPH IC50 (μg/mL)	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
<i>S. deserti</i> methanolic extract	602.71±15.33	58.47±3.25	16.85±1.12



a



b



c

Fig. 2. Concentrations amongst the collected mice skin samples of diverse groups: a – IL-17A; b – IL-22; c – TNF-α

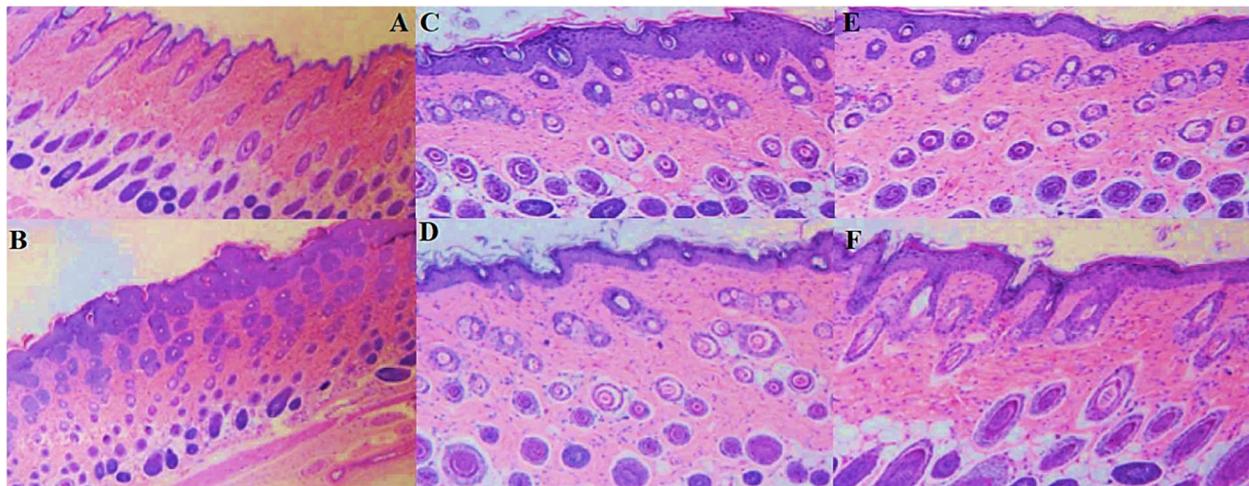


Fig. 3. Mice dorsal skin H&E staining with 100x magnified: A – non-psoriasis control group, normal shapes of skin epidermis and dermis, sebaceous glands, and follicles of the hair; B – psoriasis control group, Significant epidermis hyperkeratosis, acanthosis, and crust with plentiful inflammatory cells; C – *S. deserti* extract (300 mg/kg), somewhat reduction in the cells of the inflammatory infiltrate with a decrease in the epidermis hyperkeratosis; D – *S. deserti* extract (500 mg/kg), significant recovered tissue with normal skin epidermis and dermis, sebaceous glands, and follicles of the hair besides the lowest rate of inflammatory reactions; E – betamethasone treatment group significantly reduced inflammatory reactions and epidermis hyperplasia; F –  $\alpha$ -pinene 9 % treatment, significant reductions in epidermis thickening, inflammatory reactions, and hyperplasia

## 5. Discussion

Psoriasis signifies an immune-based disease with an indistinct reason clear with inflammation activated by the immune dysfunction and subsequent skin inflammation. It has been determined that some specific cells with immunological roles, including TNF- $\alpha$ , IL-17, and IL-22, play a clear portion in the psoriasis pathogenesis [14]. As a result, drugs with anti-inflammatory activities can be used to prevent the spread of disease. Nowadays, adalimumab, efalizumab, alefacept, secukinumab, and ustekinumab have been determined as the most effective anti-psoriasis agents [15]. Nevertheless, it was exposed that they have longstanding side effects, including leukoencephalopathy, brain viral infections, upsurge of anti-drug antibody production, and risk of flushing, pruritus, headache, hypertension, and rash [15]. In this regard, both clinicians and patients prefer to use natural medicinal plants as suitable substitutes for anti-psoriasis drugs. Findings of the previous survey [16] revealed that 54.40 % of patients suffering from psoriasis used medicinal plants with higher application of *Trigonella arabica*, *Aloe vera*, *Anthemis cotula*, and *Catharanthus roseus*. Other medicinal plants, including *Artemisia capillaris*, *Hypericum perforatum*, *Rehmannia Glutinosa*, and *Salvia Miltiorrhiza*, and also plants of the families Fabaceae, Asteraceae, Arecaceae, Myrtaceae, Ericaceae, Ulmaceae, and Compositae were also tested and confirmed to have efficient anti-psoriasis effects [17, 18].

The present survey showed that *S. deserti* extract, particularly with 500 mg/kg concentrations, harboured efficient DPPH-radical scavenging activities with high flavonoids and polyphenols contents. Additionally, *S. deserti* extract harboured high anti-psoriasis activities in H&E histopathology and also IL-17A, IL-22, and TNF- $\alpha$  reduction in skin samples of mice.

As psoriasis pathogenesis is related to the activation of free radication and oxidative procedures and also inflammation, medicinal plants with high antioxidant and anti-inflammatory effects can control the disease development. *S. deserti* extract showed boosted antioxidant and anti-inflammatory effects [19]. Additionally, its suitable antimicrobial effects have also been detected previously [20], which may decrease the risk of microbial contamination of skin affected with psoriasis. Furthermore, the Scrophularia wound healing effects have been described in models of burn and wound [21, 22]. To the best of our knowledge, no research has been carried out to assess the *S. deserti* effect on psoriasis healing. The boosted anti-psoriasis and healing effects of *S. deserti* extract may be due to its effect on fibroblast production and migration in endothelial cells. *S. deserti* mitogenic effect in dermal fibroblasts may clarify its skin wound healing mechanism of action in psoriasis cases [23]. Additionally, presence of diverse chemical components, including Spathulenol, Linalool, Alpha-Terpineol, trans-Caryophyllene, Geraniol, Camphor, 1,8-Cineole, Caryophyllene oxide, virtenal, Terpinene, trans-Sabinene hydrate, and CIS-Linaloloxide, with high antioxidant, wound healing, and anti-inflammatory effects may also cover its anti-psoriasis activity [10]. Previously, 5 diverse phenylpropanoid glycosides, including angoroside A, C, and D, isoacteoside, and acteoside were isolated from Scrophulariaceae. Additionally, their boosted anti-inflammatory effects through macrophage function inhibition were determined [24]. Ghashghaii et al. (2017) [25] reported that rats treated with *S. striata* extract harboured a significant reduction in the wound area with a high decrease in the lymphocytes and a boosted increase in the fibroblast numbers. Moreover, supplementary strictures, including healing tissue arrangement, re-epithelialization, improved collagen

and fibroblast maturity, and angiogenesis were also confirmed in rats treated with *S. striata*, which was similar to the findings of the present research. Similar findings were also reported by Sabahi et al. (2020) [26] (Iran), Zenjin et al. (2018) [27] (Turkey), Jafary et al. (2013) [22] (Iran), and Hadadi et al. (2019) [23] (Iran).

Psoriasis incidence principally involves inflammatory reactions consisting of IFN, TNF- $\alpha$ , and IL-1, 6, 17, 22, and 23 production from macrophages, dendritic cells, and helper cells [28]. Reduction in their production may result in inflammation neutralization and improvement. Here in the survey, mice treated with *S. deserti* methanolic extract harboured lower levels of cytokines compared to the control group. Similarly, *Ficus carica* [29], and *Dysidea avara* [30] extracts showed high anti-psoriasis effects through the reduction in rates of inflammatory cytokines.

**Limitations of the study.** The study was limited to the absence of other supplementary tests, especially phytochemical analysis of the studied medicinal plant, and also a lack of serum-based examinations of liver, kidney, and blood parameters.

**Prospects for further research.** Application of *S. deserti* extract (500 mg/kg) in other animal models and, after reaching affordable findings, its application on human volunteers to treat psoriasis.

## 6. Conclusion

In conclusion, the present survey as the first report revealed that *S. deserti* methanolic extract, particularly with 500 mg/kg concentrations, harboured boosted anti-psoriasis effects through its high antioxidant activities (DPPH radical scavenging effects and high contents of polyphenols and flavonoids) and its interventional effects on the reduction of the levels of TNF- $\alpha$ , IL-17, and IL-22. Through the histo-

pathological examination, rats treated with *S. deserti* methanolic extract (500 mg/kg) showed a significant presence of recovered tissue with normal skin epidermis and dermis, sebaceous glands, and hair follicles without inflammatory responses. Compared to  $\alpha$ -pinene 9 % treatments, rats treated with *S. deserti* methanolic extract (500 mg/kg) showed lower levels of inflammatory cytokines and also better findings of H&E staining, but the rates of cytokines in rats treated with the extract were higher than those treated with betamethasone ointment. Application of *S. deserti* methanolic extract (500 mg/kg) based on its routine uses in folk medicine as a good choice with anti-psoriasis effect is suggested to be performed on human cases.

## Conflict of Interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

## Funding

The study was performed without financial support.

## Data availability

The manuscript has no associated data.

## Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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