Original Article

# Effect of Topical Petroleum Ether and Ethyl Acetate Fractions From *Euphorbia milii* on Imiquimod-induced Psoriasis-like Skin Inflammation in Mice

**Mohammed Fareed Hameed[1*], Ayah F. Al-Qrimli[2]**

*1. Department of Pharmacology and Toxicology, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.*
*2. Department of Pharmacognosy, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.*

*** Corresponding Author:**
**Mohammed Fareed Hameed, PhD.**
**Address:** *Department of Pharmacology and Toxicology, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.*
**E-mail:** dr.mohammed.fared@nahrainuniv.edu.iq



**Article info:**
**Received:** 03 Jan 2024
**Accepted:** 02 Mar 2024

**Keywords:**
*Euphorbia milii*, Interleukin-17 (IL-17), Imiquimod, Psoriasis, Vascular endothelial growth factor (VEGF)

## ABSTRACT

**Background:** Psoriasis is a chronic, untreatable and disabling disease.

**Objectives:** In this research, we investigated the pharmacological properties of *Euphorbia milii* petroleum ether and ethyl acetate in a psoriasis mouse model.

**Methods:** Thirty-one albino mice were used in this study. They were divided into five groups: group I, healthy animals; group II, inducer group with imiquimod 12.5 mg (5% cream); and groups III, IV and V were imiquimod-induced and then treated with clobetasol 0.05% in group III, *E. milii* petroleum ether fraction in group IV and *E. milii* ethyl acetate fraction in group V. Immunohistochemistry for interleukin-17 (IL-17), vascular endothelial growth factor and transforming growth factor-beta, as well as histopathology were done on the mice skin.

**Results:** *E. milii* petroleum ether group showed a significant decrease in skin immunohistochemistry of IL-17, transforming growth factor-beta, and other histopathology parameters, while ethyl acetate fraction showed a significant reduction in vascular endothelial growth factor and other histopathology parameters

**Conclusion:** *E. milii* petroleum ether and ethyl acetate fractions may have a role in psoriasis treatment in a mice model.

**Citation** Hameed MF, Al-Qrimli AO. Effect of Topical Petroleum Ether and Ethyl Acetate Fractions From *Euphorbia milii* on Imiquimod-induced Psoriasis-like Skin Inflammation in Mice. Pharmaceutical and Biomedical Research. 2024; 10(2):157-166. http://dx.doi.org/10.32598/PBR.10.2.1295.1

http://dx.doi.org/10.32598/PBR.10.2.1295.1

## Introduction

Psoriasis is an inflammatory chronic disease that affects individuals with a genetic predisposition. Psoriasis occurs in about 2% of the population, and the onset of the disease starts at less than thirty years old in more than half of the patients [1]. Keratinocytes are essential in the two phases of initiating and maintaining psoriasis. Many factors can control keratinocytes, like genetics, receptors and cytokines, metabolism, transcription factors, cell signals, non-coding ribonucleic acids, and different peptides and proteins that function like antimicrobial agents. Those modulating factors interact together to change the biological impact of keratinocytes through various mechanisms, leading to the participation of those keratinocytes in psoriasis [2].

The keratinocyte and the immune cells are connected by the cytokines and their action on its receptors. Those cytokines like tissue necrosis factor-alpha (TNF-α), interleukin-23 (IL23)/IL-17A, interferon-gamma (IFN-γ), IL-22 and others produced by the immune cells, activate the keratinocytes leading to different cell signals and pathways. Finally, high keratinocyte proliferation with high production of various antimicrobial proteins, chemokines, cytokines and growth factors occurs with the most important cytokines target therapy are IL-17A, TNF-α and IL-23 [3].

Debilitating psoriasis harms the patient's life and may cause different psychosocial problems. The available therapy is still unsatisfactory for the patients, and the new biological treatments are of limited use because of regulated prescription in many countries and their high costs [4]. Currently, systemic treatments like biologics, immunosuppressant drugs, methotrexate, and retinoids are used. Topical treatments like corticosteroids, coal tars, retinoids, and vitamin D analogs are also available for psoriasis [5]. In addition, clinical practice shows that the topical use of herbal pastes [6] and the use of herbal products with their beneficial and essential therapeutic effects have increased extensively in the present time [7].

People prefer herbal medicine and natural food and practice methods in their daily healthy lives. Herbal cosmetics usage has increased in additional ways for a personal care lifestyle. The causes may be the weighty use of synthetic products, chemicals, dyes, and their derivatives in the past 150 years; those synthetic products cause many side effects, diseases and environmental pollution [8].

*Euphorbia milii* is a shrub related to the Euphorbiaceous family. *E. milii* grows in many tropical regions but is native to Madagascar [9]. Traditionally, the plant *E. milii* has been used to treat hepatitis, cancer and edema [10]. In addition, the leaf is used for swelling relief and ulcer dressing. In some societies, the herb milky latex is also used in cases of injured joints and ligaments and eyelid application [11].

*E. milii* phytochemical analysis has shown different secondary metabolites such as alkaloids, cardiac glycosides, coumarins, steroids, anthocyanins, terpenoids, tannins, flavonoids, and proteins. Those metabolites have many pharmacological properties, such as anti-inflammatory, anticancer, antioxidant, antiviral,and antibacterial [12, 13].

This study investigated the pharmacological properties of *E. milii* petroleum ether and ethyl acetate fractions on imiquimod-induced psoriasis in a mouse model.

## Materials and Methods

### Study design

A randomized study was conducted in the Department of Pharmacology and Toxicology, College of Pharmacy, Al-Nahrain University. Thirty-one albino male mice, six weeks old, were used in this study. The mice were randomly divided into five groups (each with six animals except the healthy group with seven animals). Group I included healthy animals. Group II was the inducer group, receiving 12.5 mg imiquimod (5% cream) once daily (Glenmark) at the skin of the mice back for five consecutive days [14]. Groups III, IV and V used imiquimod with the same procedure, but different treatments were given after three hours. Group III received a steroid clobetasol 0.05% ointment [15] (the state company for drugs industry and medical appliances SDI), group IV received *E. milii* petroleum ether fraction, and group V received *E. milii* ethyl acetate fraction. In the last two groups, the dose was 200 mg/kg once daily [16] for five days.

### Plant material

*E. milii* as a whole plant was collected in July and supplied from houses in Baghdad Green. *E. milii* was authenticated and identified by Sukaena Abbas (Assistant Professor at the University of Baghdad, College of Science, Department of Biology). The roots, thorns, stems, flowers, and leaves were washed and then 14 days air

dried. After that, the whole dried plant was crushed and grinded using an electric grinder to get a coarse powder.

To extract *E. milii* crude material, 400 g coarse powder of plant was mixed with hexane for 24 hours to separate fat and wax. Then, it was filtered and the product was extracted using a soxhlet instrument with 5.3 L of 85% methanol for 12 hours (until it was exhausted). The produced extract was then filtered to take out boiling chips from the extract [17].

### Crude extract fractionation of *E. milii*

Forty grams of the extract was suspended using distilled water (200 mL) and partitioned with petroleum ether (200 mL, B. P 40-60°C), ethyl acetate, chloroform and butanol sequentially repeated three times (200 mL×3 times). Each fraction was left overnight using the separatory funnel to allow solvent extraction completely. Anhydrous sodium sulfate was then added to the different fractions except the butanol. Then, it was filtered and evaporated. The petroleum ether and ethyl acetate fractions were used in the pharmacology part of this research [18, 19].

### Immunohistochemistry (IHC) measurement

IHC measurement is done using IHC kits of vascular endothelial growth factor (VEGF), interleukin (IL)-17, and transforming growth factor-beta (TGFβ). The catalog numbers and companies were ab1316, Abcam, USA; ab214588, Abcam, USA; SL0086R, Sunlong Biotech, China, respectively. The IHC method was evaluated and done by the Department of Pathology, College of Medicine, AL-Nahrain University. The technique is done using the catalog instructions.

### Histopathology procedure

After five days, mice were sacrificed by chloroform anesthetic. Skin tissue was harvested from the back of the mice and stored in buffered formaldehyde [20].

The scoring calculation was used to calculate mice model histopathology score as the following: Munro abscesses (1.5), parakeratosis (1), hyperkeratosis (0.5), acanthosis (1), length of rete ridges (0.5–1.5), papillary papillae congestion (1), dermis lymphocytic infiltrate (0.5–1.5) [21].

### Statistical analysis

Statistical analysis was done using SPSS software, version 26 to calculate the range and median. The Kruskal-Wallis and Mann-Whitney tests were also done. The tests would be significant if P≤0.05 and highly significant ≤0.001 [22].

## Results

## Discussion

Psoriasis is a disease that involves many immune cells that abnormally activate the proliferation and differentiation of keratinocytes [23]. One of the valuable aspects of medicinal plants is using them as an adjuvant therapy to treat disease and prophylaxis in health systems [24].

When 5% imiquimod cream is applied topically, immune cell reactions occur where many immune cells secret inflammatory cytokines, activate dendritic cells, and epidermis hyperplasia [25, 26]. Those reactions and symptoms are almost like the human psoriasis symptoms [27], which indicates the suitability of this animal model for the study of psoriasis disease. In this research, when comparing the healthy and the imiquimod-induced groups, there was a significant increase in readings of skin IHC of IL-17, VEGF and TGFβ (Table 1, Figure 1). This finding supports the idea that T helper 17 cells (Th17)/IL-23 pathway had a primary role in psoriasis [28], where the IL-17A binding to IL-17RA receptor results in activation of nuclear factor kappa B with signal transducer and activator of transcription cascades. Gene expression is responsible for inflammation-activated and sequential lesions of psoriasis developed [29]. Again, IL-17 can also be affected through the IL-23/Th17 axis. Krueger et al. found that IL-23 inhibitors can significantly decrease the expression of IL-22 and IL-17 significantly in lesions of psoriasis [30, 31]. Regarding histopathology parameters, a highly significant increase in hyperkeratosis, parakeratosis, lengthening of rete ridges, acanthosis, papillary papillae congestion and dermis lymphocytic infiltrate, while a significant increase in Munro abscess was found (Table 2 and Figure 2).

Di Cesare et al. proposed that the psoriasis phenotype is fundamentally related to Th17 and IL-23. The dendritic cells in the dermis secrete IL-23, which induces Th17 differentiation and the secretion of pro-inflammatory cytokines later that can act on keratinocytes. Subsequently, parakeratosis and epidermal hyperplasia will occur [32, 33].

As clobetasol is compared to the imiquimod-induced group, a significant decrease in skin IL-17 and TGFβ

**Table 1.** Comparing immunohistochemistry parameters between animal groups

| Parameter | | Healthy (n=7) | Psoriasis-induced (n=6) | Clobetasol (n=6) | Petroleum Ether *E. milii* Fraction (n=6) | Ethyl Acetate *E. milii* Fraction (n=6) |
|---|---|---|---|---|---|---|
| IL-17 | Median (range) | 0 (0-1) | 3 (1-3) | 2 (1-2) | 2 (1-2) | 1 (1-3) |
| | P[a] | | 0.002 | 0.008 | 0.008 | 0.035 |
| | P[b] | | | 0.041 | 0.041 | 0.065 |
| | P[c] | | | | 1.000 | 0.589 |
| | P[d] | | | | | 0.589 |
| | P[e] | | | | 0.701 | |
| VEGF | Median (range) | 1 (1-1) | 2.5 (2-3) | 2 (2-3) | 2 (1-3) | 1.5 (1-2) |
| | P[a] | | 0.001 | 0.001 | 0.051 | 0.138 |
| | P[b] | | | 0.699 | 0.180 | 0.026 |
| | P[c] | | | | 0.310 | 0.065 |
| | P[d] | | | | | 0.485 |
| | P[e] | | | | 0.098 | |
| TGFβ | Median (range) | 1 (1-1) | 3 (2-3) | 2 (1-2) | 1.5 (1-2) | 1.5 (1-3) |
| | P[a] | | 0.001 | 0.051 | 0.138 | 0.138 |
| | P[b] | | | 0.026 | 0.015 | 0.065 |
| | P[c] | | | | 0.699 | 0.937 |
| | P[d] | | | | | 0.818 |
| | P[e] | | | | 0.874 | |

Abbreviations: VEGF: Vascular endothelial growth factor; IL-17: Interleukin-17; TGFβ: Transforming growth factor-beta. PBR

[a]Comparison of healthy with each other group using the Mann-Whitney test, [b]Comparison of induced with each treatment group using the Mann-Whitney test, [c]Comparison of clobetasol with each *E. milii* group using the Mann-Whitney test, [d]Comparison between two milli groups using the Mann-Whitney test, [e]Comparison among treatment groups using the Kruskal-Wallis test.

was found in addition to the Munro abscess, a significant decrease in hyperkeratosis and dermis lymphocytic infiltrate (Tables 1 and 2). A previous study showed that clobetasol could influence the axis of IL-23/IL-17A in the psoriasis inflammatory model in mice, like decreased mRNA concentration of IL-17A and other changes. Clobetasol efficacy may relate to mechanisms like immunosuppressive, anti-proliferative and anti-inflammatory effects [34].

When the petroleum ether *E. milii* group is compared to the imiquimod-induced group, we found a significant decrease in skin IL-17, TGFβ (Table 1 and Figure 1), Munro abscess, hyperkeratosis and papillary papillae congestion, while a high significant decrease in parakeratosis and dermis lymphocytic infiltrate (Table 2 and Figure 2). *E. milii* was studied before, where sitosterol, beta-amyrin acetate and cycloartenol were found in the petroleum ether extraction process. Lupeol and euphol were also found by gas chromatography as minor compounds [12]. The pharmacological effect on the psoriasis model may be related to one or more of those compounds in the petroleum ether fraction. In one study, 9, 19-cycloartenol was used to decrease chronic skin inflammation. The mechanism includes various signaling pathways at different levels due to CD4+ T cell pathogenic suppression of differentiation and its regulatory control [35].

The comparison between ethyl acetate *E. milii* and the imiquimod-induced group showed a significant decrease

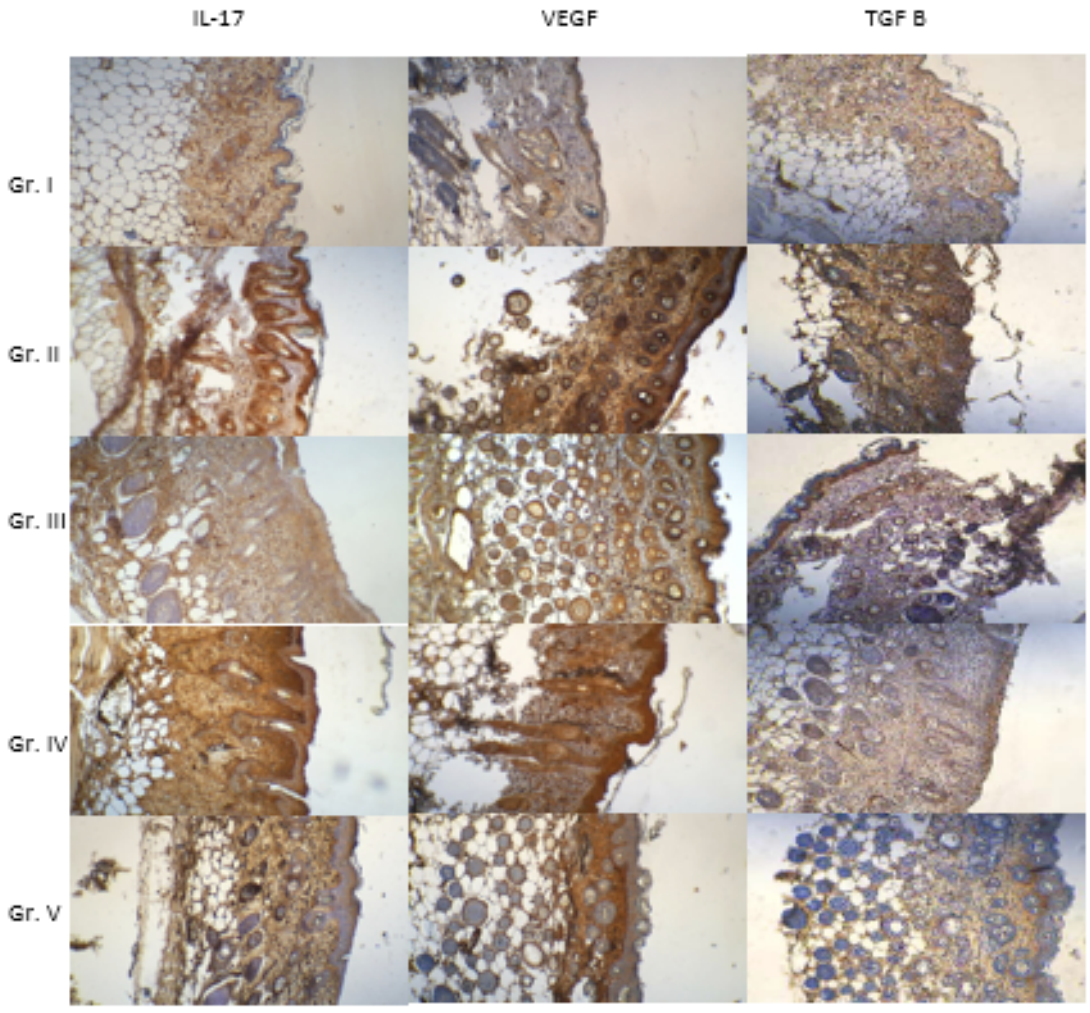


**Figure 1.** Immunohistochemistry of IL-17, VEGF and TGFβ of the mice skin

Abbreviations: VEGF: Vascular endothelial growth factor; IL-17: Interleukin-17; TGFβ: Transforming growth factor-beta.

Note: Group I: Apparently healthy, Group II: Psoriasis induction, Group III: Clobetasol, Group IV: Petroleum ether *E. milii* fraction, Group V: Ethyl acetate *E. milii* fraction (magnification x10).

in VEGF and dermis lymphocytic infiltrate (Table 1) and a significant decrease in hyperkeratosis and parakeratosis (Table 2 and Figure 2). The phytochemical screening of the ethyl acetate fraction showed the presence of flavonoids, anthocyanin, terpenoids, steroids, tannins, anthraquinone and others [18]. The flavonoids show 91.08% of the component, the major constituent containing luteolin, quercitrin, quercetin, naringenin, and kaempferol-3-glucuronide [36].

When the clobetasol group is compared to petroleum ether and ethyl acetate treated groups, no significant difference was found between all parameters except the papillary congestion of petroleum ether fraction. This finding indicates that each tested fraction of *E. milii* is as effective as clobetasol in relation to the parameters used in this model of psoriasis. Also, no significant difference was found between petroleum ether and ethyl acetate fractions for all parameters, indicating that both fractions have the same effect in this model. Finally, no significant difference was found between all treated groups for all parameters except for papillary congestion, indicating that almost the three tested treatment groups had the same efficacy in this mice model (Tables 1 and 2).

## Conclusion

*E. milii* was suggested to have a role in the psoriasis animal model due to the effect of its fractions on different parameters. The petroleum ether fraction affects IL-17 and TGFβ. In contrast, the ethyl acetate fraction on VEGF and other histopathological readings indicate

**Table 2.** Comparing histopathological parameters between the animal groups

| Parameter | | Healthy (n=7) | Psoriasis Induced Group (n=6) | Clobetasol Group (n=6) | Petroleum Ether *E. milii* Fraction Group (n=6) | Ethyl Acetate *E. milii* Fraction Group (n=6) |
|---|---|---|---|---|---|---|
| Munro abscess | Median (range) | 0 (0-0) | 2 (0-2) | 0 (0-0) | 0 (0-0) | 0 (0-2) |
| | P[a] | | 0.008 | 1.000 | 1.000 | 0.628 |
| | P[b] | | | 0.015 | 0.015 | 0.065 |
| | P[c] | | | | 1.000 | 0.699 |
| | P[d] | | | | | 0.699 |
| | P[e] | | | | 0.368 | |
| Hyperkeratosis | Median (range) | 0 (0-0) | 0.5 (0.5-0.5) | 0 (0-0) | 0.5 (0-0.5) | 0 (0-0) |
| | P[a] | | 0.001 | 1.000 | 0.628 | 1.000 |
| | P[b] | | | 0.002 | 0.015 | 0.002 |
| | P[c] | | | | 0.699 | 1.000 |
| | P[d] | | | | | 0.699 |
| | P[e] | | | | 0.368 | |
| Parakeratosis | Median (range) | 0 (0-0) | 1 (1-1) | 0 (0-1) | 0 (0-0) | 0 (0-0.5) |
| | P[a] | | 0.001 | 0.366 | 1.000 | 0.628 |
| | P[b] | | | 0.065 | 0.002 | 0.002 |
| | P[c] | | | | 0.394 | 0.589 |
| | P[d] | | | | | 0.699 |
| | P[e] | | | | 0.283 | |
| Lengthening and clubbing of rete edges | Median (range) | 0 (0-0) | 1.5 (1.5-1.5) | 1.5 (0-1.5) | 1.5 (0-1.5) | 0 (0-1.5) |
| | P[a] | | 0.001 | 0.051 | 0.051 | 0.366 |
| | P[b] | | | 0.394 | 0.394 | 0.065 |
| | P[c] | | | | 1.000 | 0.394 |
| | P[d] | | | | | 0.394 |
| | P[e] | | | | 0.427 | |
| Acanthosis | Median (range) | 0 (0-0) | 0.5 (0.5-0.5) | 0.5 (0.5-0.5) | 0.5 (0.5-0.5) | 0.5 (0.5-0.5) |
| | P[a] | | 0.001 | 0.001 | 0.001 | 0.001 |
| | P[b] | | | 1.000 | 1.000 | 1.000 |
| | P[c] | | | | 1.000 | 1.000 |
| | P[d] | | | | | 1.000 |
| | P[e] | | | | 1.000 | |

| Parameter | | Healthy (n=7) | Psoriasis Induced Group (n=6) | Clobetasol Group (n=6) | Petroleum Ether *E. milii* Fraction Group (n=6) | Ethyl Acetate *E. milii* Fraction Group (n=6) |
|---|---|---|---|---|---|---|
| Papillary congestion | Median (range) | 0 (0-0) | 0.5 (0.5-0.5) | 0.5 (0.5-0.5) | 0 (0-0.5) | 0.5 (0-0.5) |
| | P[a] | | 0.001 | 0.001 | 0.628 | 0.008 |
| | P[b] | | | 1.000 | 0.015 | 0.699 |
| | P[c] | | | | 0.015 | 0.699 |
| | P[d] | | | | | 0.065 |
| | P[e] | | | | 0.007 | |
| Dermis lymph infiltrate | Median (range) | 0 (0-0) | 2 (2-2) | 0.75 (0.5-1) | 0.5 (0.5-0.5) | 0.5 (0.5-2) |
| | P[a] | | 0.001 | 0.001 | 0.001 | 0.001 |
| | P[b] | | | 0.002 | 0.002 | 0.015 |
| | P[c] | | | | 0.180 | 0.818 |
| | P[d] | | | | | 0.394 |
| | P[e] | | | | 0.184 | |

[a]Comparison healthy with each other group using the Mann-Whitney test, [b]Comparison induced with each treatment group using the Mann-Whitney test, [c] Comparison of clobetasol with each *E. milii* group using the Mann-Whitney test, [d]Comparison between two milli groups using the Mann-Whitney test, [e]Comparison among treatment groups using the Kruskal-Wallis test.

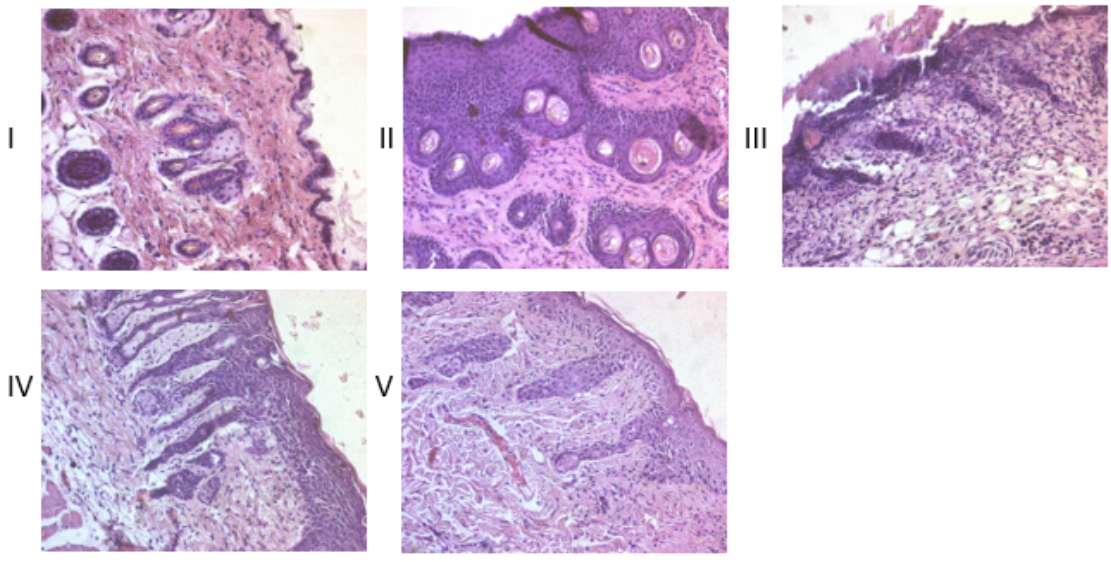


**Figure 2.** Histopathology slides of the mice skin

Group I: Apparently healthy; Group II: Psoriasis induction; Group III: Clobetasol; Group IV: Petroleum ether *E. milii* fraction; Group V: Ethyl acetate *E. milii* fraction (magnification x10).

Notes: Munro abscess, parakeratosis hyperkeratosis, acanthosis, lengthening of rete ridges, dermis lymphocytic infiltrate and papillary papillae congestion were shown.

different pharmacological actions between the fractions that may be related to various chemical compounds.

## Ethical Considerations

### Compliance with ethical guidelines

The study approved by AL- Nahrain University, College of Pharmacy Ethical Committee (Code: 1242, date 25 November 2021).

### Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

### Authors' contributions

Pharmacognosy preparation: Mohammed Fareed Hameed; Pharmacology preparation and data analysis and interpretation: Ayah F. Al-Qrimli; Study design, writing, and final approval: All authors.

### Conflict of interest

The authors declared no conflict of interest.

### Acknowledgments

Special thanks to the Pathology Department, College of Medicine, AL – Nahrain University for cooperation.

### References

[1] Gisondi P, Bellinato F, Girolomoni G, Albanesi C. Pathogenesis of chronic plaque psoriasis and its intersection with cardio-metabolic comorbidities. Front Pharmacol. 2020; 11:117. [DOI:10.3389/fphar.2020.00117] [PMID] [PMCID]

[2] Zhou X, Chen Y, Cui L, Shi Y, Guo C. Advances in the pathogenesis of psoriasis: From keratinocyte perspective. Cell Death Dis. 2022; 13(1):81. [DOI:10.1038/s41419-022-04523-3] [PMID] [PMCID]

[3] Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. Lancet. 2021; 397(10281):1301-15. [DOI:10.1016/S0140-6736(20)32549-6] [PMID]

[4] Tse TW. Use of common Chinese herbs in the treatment of psoriasis. Clin Exp Dermatol. 2003; 28(5):469-75. [DOI:10.1046/j.1365-2230.2003.01322.x] [PMID]

[5] Conrad C, Gilliet M. Psoriasis: from pathogenesis to targeted therapies. Clin Rev Allergy Immunol. 2018; 54(1):102-13. [DOI:10.1007/s12016-018-8668-1] [PMID]

[6] Pettman E. A history of manipulative therapy. J Man Manip Ther. 2007; 15(3):165-74. [DOI:10.1179/106698107790819873] [PMID] [PMCID]

[7] Soulaidopoulos S, Tsiogka A, Chrysohoou C, Lazarou E, Aznaouridis K, Doundoulakis I, et al. Overview of chios mastic gum (Pistacia lentiscus) effects on human health. Nutrients. 2022; 14(3):590. [DOI:10.3390/nu14030590] [PMID] [PMCID]

[8] Kapoor VP. Herbal Cosmetics for Skin and Hair care. Nat Prod Radiance. 2005; 4(4):306-14. [Link]

[9] Ma J, Gilbert MG. Euphorbia, flora of China. St. Louis: Science Press, Beijing, and Missouri Botanical Garden Press. 2008. [Link]

[10] Nellis DW. Poisonous plants and animals of Florida and the Caribbean. Sarasota: Pineapple Press; 1997. [Link]

[11] Joshi S, Sharma B, Bhatia C, Singh R, Thakur R. The wealth of India. Raw materials Ca - Ci. New Delhi; 1992.

[12] Pancorbo S, Hammer RH. Preliminary phytochemical investigation of Euphorbia millii. J Pharm Sci. 1972; 61(6):954-7. [DOI:10.1002/jps.2600610631] [PMID]

[13] Saleem H, Zengin G, Locatelli M, Mollica A, Ahmad I, Mahomoodally FM, et al. In vitro biological propensities and chemical profiling of Euphorbia milii Des Moul (Euphorbiaceae): A novel source for bioactive agents. Ind Crops Prod. 2019; 130:9-15. [DOI:10.1016/j.indcrop.2018.12.062]

[14] Moos S, Mohebiany AN, Waisman A, Kurschus FC. Imiquimod-induced psoriasis in mice depends on the il-17 signaling of keratinocytes. J Invest Dermatol. 2019; 139(5):1110-7. [DOI:10.1016/j.jid.2019.01.006] [PMID]

[15] Pukale SS, Sharma S, Dalela M, Singh AK, Mohanty S, Mittal A, et al. Multi-component clobetasol-loaded monolithic lipid-polymer hybrid nanoparticles ameliorate imiquimod-induced psoriasis-like skin inflammation in Swiss albino mice. Acta Biomater. 2020; 115:393-409. [DOI:10.1016/j.actbio.2020.08.020] [PMID]

[16] Sreenika G, Naga Sravanthi K, Lakshmi B, Thulja P, Sudhakar M. Antioxidant and antitumor activity of Euphorbia milii flower extract against in vivo breast cancer and colon cancer in mice. World J Pharm Pharm Sci. 2015; 4(6):912-34. [Link]

[17] Haleshappa R, Keshamma E, Girija CR, Thanmayi M, Nagesh CG, Fahmeen GHL, et al. Phytochemical study and antioxidant properties of ethanolic extracts of euphorbia milii. Asian J Biol Sci. 2019; 13(1):77-82. [DOI:10.3923/ajbs.2020.77.82]

[18] Hassan AZ, Sweelam H tollah M, Abd-Alla HI, Zohair MM, Ashour WE, et al. Phytochemical analysis and antimicrobial activity of Euphorbia milii. Egypt J Chem. 2023; 66(13):461-473. [DOI:10.21608/ejchem.2023.192476.7575]

[19] Khan TA, Al Nasr IS, Mujawah AH, Koko WS. Assessment of Euphorbia retusa and Pulicaria undulata activity against Leishmania major and Toxoplasma gondii. Trop Biomed. 2021; 38(1):135-41. [DOI:10.47665/tb.38.1.023] [PMID]

[20] Verma SK, Lindsay DS, Grigg ME, Dubey JP. Isolation, culture and cryopreservation of sarcocystis species. Curr Protoc Microbiol. 2017; 45:20D.1.1-1.27. [DOI:10.1002/cpmc.32] [PMID] [PMCID]

[21] Baker BS, Brent L, Valdimarsson H, Powles AV, al-Imara L, Walker M, et al. Is epidermal cell proliferation in psoriatic skin grafts on nude mice driven by T-cell derived cytokines? Br J Dermatol. 1992; 126(2):105-10. [DOI:10.1111/j.1365-2133.1992.tb07805.x] [PMID]

[22] Peacock J, Peacock PJ. Oxford handbook of medical statistics. Oxford: Oxford University Press; 2011. [DOI:10.1093/med/9780199551286.001.0001]

[23] Kamata M, Tada Y. Crosstalk: Keratinocytes and immune cells in psoriasis. Front Immunol. 2023 14:1286344. [DOI:10.3389/fimmu.2023.1286344] [PMID] [PMCID]

[24] Jamshidi-Kia Fatemeh, Lorigooini Zahra, Amini-Khoei Hossein. medicinal plants: Past history and future perspective. J Herbmed Pharmacol. 2018; 7(1):1-7. [DOI:10.15171/jhp.2018.01]

[25] Gilliet M, Conrad C, Geiges M, Cozzio A, Thürlimann W, Burg G, et al. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch Dermatol. 2004; 140(12):1490-5. [DOI:10.1001/archderm.140.12.1490] [PMID]

[26] Palamara F, Meindl S, Holcmann M, Lührs P, Stingl G, Sibilia M. Identification and characterization of pDC-like cells in normal mouse skin and melanomas treated with imiquimod. J Immunol. 2004; 173(5):3051-61. [DOI:10.4049/jimmunol.173.5.3051] [PMID]

[27] van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, Cornelissen F, Mus AM, Florencia E, Prens EP, Lubberts E. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol. 2009; 182(9):5836-45. [DOI:10.4049/jimmunol.0802999] [PMID]

[28] Gauld SB, Jacquet S, Gauvin D, Wallace C, Wang Y, McCarthy R, et al. Inhibition of interleukin-23-mediated inflammation with a novel small molecule inverse agonist of RORγt. J Pharmacol Exp Ther. 2019; 371(1):208-18. [DOI:10.1124/jpet.119.258046] [PMID]

[29] Hawkes JE, Yan BY, Chan TC, Krueger JG. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. J Immunol. 2018; 201(6):1605-13. [DOI:10.4049/jimmunol.1800013] [PMID] [PMCID]

[30] Krueger J, Clark JD, Suárez-Fariñas M, Fuentes-Duculan J, Cueto I, Wang CQ, et al. Tofacitinib attenuates pathologic immune pathways in patients with psoriasis: A randomized phase 2 study. J Allergy Clin Immunol. 2016; 137(4):1079-90. [DOI:10.1016/j.jaci.2015.12.1318] [PMID]

[31] Puig L. The role of IL 23 in the treatment of psoriasis. Expert Rev Clin Immunol. 2017; 13(6):525-34. [DOI:10.1080/1744666X.2017.1292137] [PMID]

[32] Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J Invest Dermatol. 2009; 129(6):1339-50. [DOI:10.1038/jid.2009.59] [PMID]

[33] Toussirot E. The IL23/Th17 pathway as a therapeutic target in chronic inflammatory diseases. Inflamm Allergy Drug Targets. 2012; 11(2):159-68. [DOI:10.2174/187152812800392805] [PMID]

[34] Sun J, Dou W, Zhao Y, Hu J. A comparison of the effects of topical treatment of calcipotriol, camptothecin, clobetasol and tazarotene on an imiquimod-induced psoriasis-like mouse model. Immunopharmacol Immunotoxicol. 2014; 36(1):17-24. [DOI:10.3109/08923973.2013.862542] [PMID]

[35] Su Y, Wu L, Mu G, Wang Q, Yang B, Cheng G, et al. 9,19-Cycloartenol glycoside G3 from Cimicifuga simplex regulates immune responses by modulating Th17/Treg ratio. Bioorg Med Chem. 2017; 25(17):4917-23. [DOI:10.1016/j.bmc.2017.07.042] [PMID]

[36] Rauf A, Khan A, Uddin N, Akram M, Arfan M, Uddin G, et al. Preliminary phytochemical screening, antimicrobial and antioxidant activities of Euphorbia milli. Pak J Pharm Sci. 2014; 27(4):947-51. [Link]

This Page Intentionally Left Blank