

Original Article



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

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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.

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Introduction

Pсориаз 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- α (TNF- α), interleukin-23 (IL23)/IL-17A, interferon- γ (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 $P \leq 0.05$ 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 secrete 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 <i>E. milii</i> Fraction (n=6)	Ethyl Acetate <i>E. milii</i> Fraction (n=6)
Median (range)	0 (0-1)	3 (1-3)	2 (1-2)	2 (1-2)	1 (1-3)
IL-17					
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	
Median (range)	1 (1-1)	2.5 (2-3)	2 (2-3)	2 (1-3)	1.5 (1-2)
VEGF					
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	
Median (range)	1 (1-1)	3 (2-3)	2 (1-2)	1.5 (1-2)	1.5 (1-3)
TGFβ					
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**

^aComparison of healthy with each other group using the Mann-Whitney test, ^bComparison of induced with each treatment group using the Mann-Whitney test, ^cComparison of clobetasol with each *E. milii* group using the Mann-Whitney test, ^dComparison between two *milli* groups using the Mann-Whitney test, ^eComparison 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 para-

keratosis 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

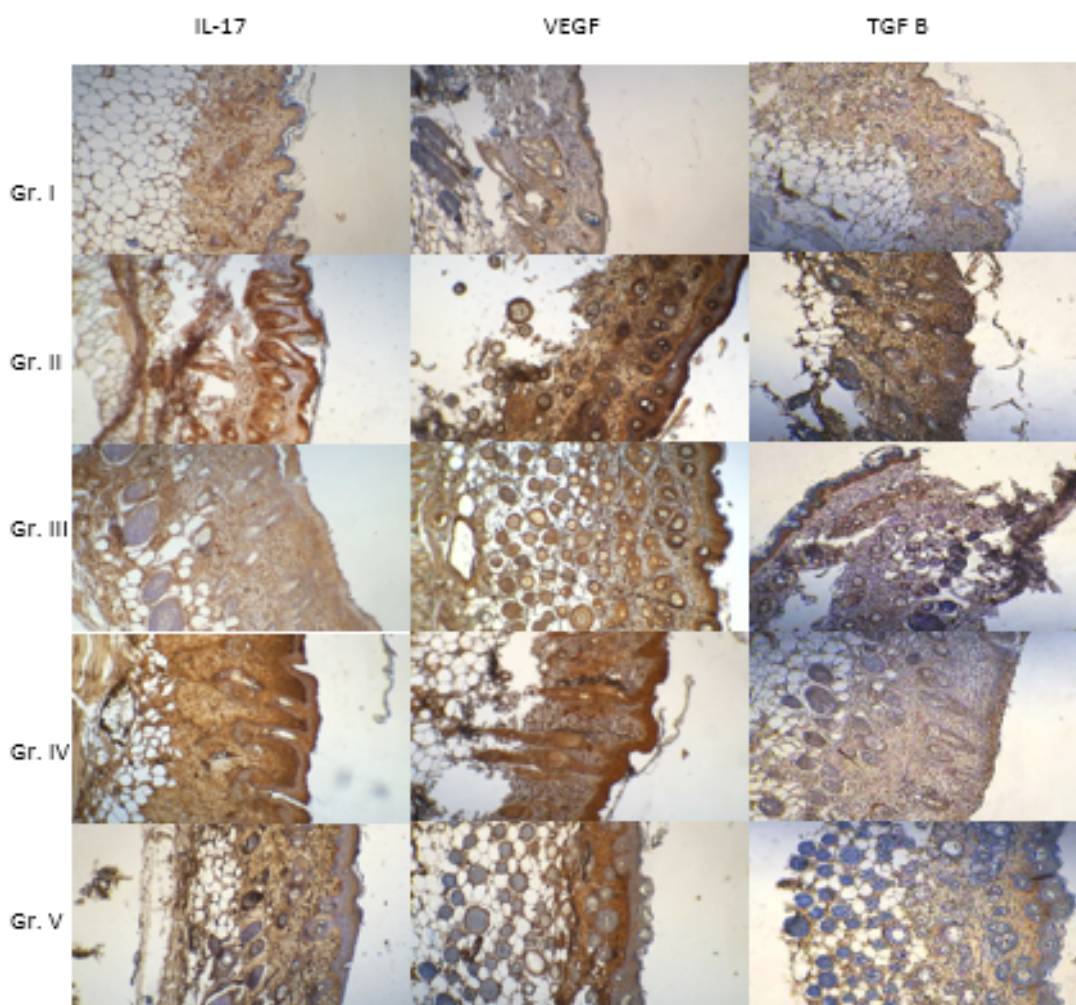


Figure 1. Immunohistochemistry of IL-17, VEGF and TGFβ of the mice skin

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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 <i>E. milii</i> Fraction Group (n=6)	Ethyl Acetate <i>E. milii</i> Fraction Group (n=6)
Median (range)	0 (0-0)	2 (0-2)	0 (0-0)	0 (0-0)	0 (0-2)
<i>p^a</i>		0.008	1.000	1.000	0.628
<i>p^b</i>			0.015	0.015	0.065
<i>p^c</i>				1.000	0.699
<i>p^d</i>					0.699
<i>p^e</i>				0.368	
Median (range)	0 (0-0)	0.5 (0.5-0.5)	0 (0-0)	0.5 (0-0.5)	0 (0-0)
<i>p^a</i>		0.001	1.000	0.628	1.000
<i>p^b</i>			0.002	0.015	0.002
<i>p^c</i>				0.699	1.000
<i>p^d</i>					0.699
<i>p^e</i>				0.368	
Median (range)	0 (0-0)	1 (1-1)	0 (0-1)	0 (0-0)	0 (0-0.5)
<i>p^a</i>		0.001	0.366	1.000	0.628
<i>p^b</i>			0.065	0.002	0.002
<i>p^c</i>				0.394	0.589
<i>p^d</i>					0.699
<i>p^e</i>				0.283	
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)
<i>p^a</i>		0.001	0.051	0.051	0.366
<i>p^b</i>			0.394	0.394	0.065
<i>p^c</i>				1.000	0.394
<i>p^d</i>					0.394
<i>p^e</i>				0.427	
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)
<i>p^a</i>		0.001	0.001	0.001	0.001
<i>p^b</i>			1.000	1.000	1.000
<i>p^c</i>				1.000	1.000
<i>p^d</i>					1.000
<i>p^e</i>				1.000	

Parameter	Healthy (n=7)	Psoriasis Induced Group (n=6)	Clobetasol Group (n=6)	Petroleum Ether <i>E. milii</i> Fraction Group (n=6)	Ethyl Acetate <i>E. milii</i> Fraction Group (n=6)
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)
Papillary congestion	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 (0.5-2)
	p ^a		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	

^aComparison healthy with each other group using the Mann-Whitney test, ^bComparison induced with each treatment group using the Mann-Whitney test, ^c Comparison of clobetasol with each *E. milii* group using the Mann-Whitney test, ^dComparison between two *millii* groups using the Mann-Whitney test, ^eComparison among treatment groups using the Kruskal-Wallis test.

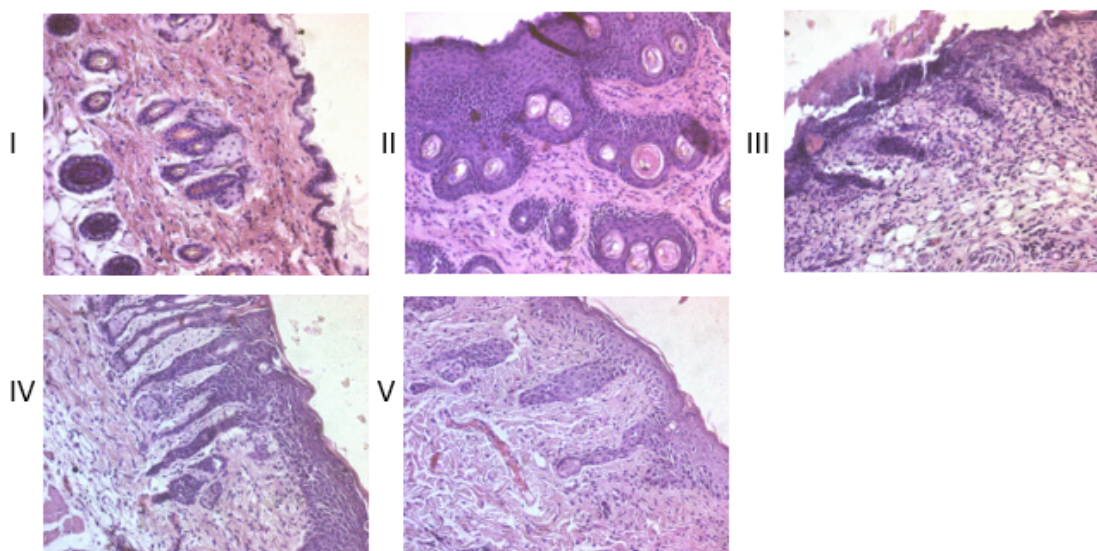


Figure 2. Histopathology slides of the mice skin

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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).

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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.

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