TRENDS IN SCIENCES 2025; 22(12): 10723
https://doi.org/10.48048/tis.2025.10723
RESEARCH ARTICLE

Protective Effects of Crude and Light Fraction Patchouli Oil Against UVB-Induced Dermal Aging in Mice: *In silico* and *In Vivo* Study

Essy Harnelly[1,4,*], Aliya H. Halym[2], Nadia Isnaini[2,4], Khairan Khairan[2,4], Zulkarnain Zulkarnain[3,4], Aigia Syahraini[1], Widya Sari[1], Haya Maghfira[1], Syaifullah Muhammad[4,5], Rinaldi Idroes[2] and Mohamed El-Shazly[6]

[1]*Department of Biology, Faculty of Math and Science, Syiah Kuala University, Banda Aceh, Indonesia*
[2]*Department of Pharmacy, Faculty of Math and Science, Syiah Kuala University, Banda Aceh, Indonesia*
[3]*Department of Physiology, Faculty of Medicine, Syiah Kuala University, Banda Aceh, Indonesia*
[4]*ARC-PUIPT Nilam Aceh, Syiah Kuala University, Banda Aceh, Indonesia*
[5]*Department of Chemistry, Faculty of Chemistry Engineering, Syiah Kuala University, Banda Aceh, Indonesia*
[6]*Pharmacognosy Department, Faculty of Pharmacy,Ain-Shams University, Organization of African Unity Street, Cairo, Egypt*

([*]Corresponding author's e-mail: essy.harnelly@usk.ac.id)

Received: 21 May 2025, Revised: 17 June 2025, Accepted: 27 June 2025, Published: 30 August 2025

Abstract

Patchouli oil has gained significant interest due to its antioxidant and anti-aging properties, making it a potential natural ingredient in skincare formulations. This study investigated the protective effects of crude patchouli oil (CPO) and its light fraction (CPO1) against UVB-induced skin aging in Balb/c mice. Mice were exposed to UVB radiation (498 mJ/cm²) for two weeks (5 days per week), followed by topical treatment with CPO or CPO1. Skin aging parameters, including elasticity, moisture, collagen content, epidermal thickness and fibroblast count, were evaluated. FTIR spectroscopy confirmed the presence of bioactive functional groups, such as hydroxyl, aliphatic and carbonyl groups, in both CPO and CPO1. Results demonstrated that both CPO and CPO1 improved skin elasticity, increased moisture levels, enhanced collagen synthesis and restored fibroblast numbers compared to the untreated UVB-exposed group. Notably, CPO1 exhibited the most significant anti-photoaging effects, with increased collagen fiber content (40.83%), skin elasticity (12.30%), and moisture level (24.73%). Additionally, molecular docking studies revealed that acetic acid, a compound in patchouli oil, showed strong binding affinity to MMP9 (–7.1 kcal/mol), with molecular dynamics simulations confirming stable ligand-protein interactions and the highest number of hydrogen bonds (282). These findings highlight the potential of patchouli oil, particularly its light fraction, as a promising anti-photoaging agent that mitigates UVB-induced skin damage and inhibits MMP9 activity. Further research is recommended to validate its molecular mechanisms and explore its application in human skincare products.

Keywords: Patchouli oil, UVB-induced photoaging, MM9 inhibition, Antioxidant activity, Skin antiaging treatment, Mice, Bioactive compounds, Molecular docking

Introduction

Skin aging is the phenomenon of degenerative changes in the structure and function of skin tissues over time, which manifests itself as a gradual loss of skin flexibility. Skin aging, which affects structure, function and appearance, is a complicated process influenced by both internal and external influences [1]. In regions of the skin that are not exposed to sunlight, intrinsic or chronological skin aging can be observed, demonstrating the impact of genetic factors. However, UV irradiation is the primary cause of photoaging, also known as extrinsic aging, which mainly affects the face and forearms from prolonged exposure to sunlight and

other elements like cigarette smoke and air pollution [2]. The two types of UV light, categorized by their wavelengths, ultraviolet A (UVA) (320 - 400 nm) and ultraviolet B (UVB) (280 - 320 nm), reach the Earth's surface, causing skin damage and initiating photoaging. This process starts when UV light is absorbed by skin chromophores, leading to a cascade of photochemical reactions, serving as visible signs of photoaging [3]. One of the main mechanisms is the accumulation of oxidative stress in the skin due to excessive UVB exposure [4,5]. This leads to an increase in Reactive Oxygen Species (ROS), which initiates inflammation mediated by NF-κB-TNF-α, playing a role in exacerbating skin damage and accelerating the photoaging process [6].

UV light triggers the activation of Mitogen-Activated Protein Kinases (MAPKs) in skin cells. MAPKs are essential for various cellular functions, including regulating proliferation, differentiation, apoptosis and inflammation. Different types of MAPKs mediate this process, namely Extracellular Signal-Regulated Kinase (ERK), c-Jun N-terminal Kinase (JNK) and p38 Mitogen-Activated Protein Kinase (p38 MAPK) [7]. Activation of MAPKs leads to the production of Activator Protein-1 (AP-1), which controls the expression of Matrix Metalloproteinase (MMPs), including MMP-1, MMP-3 and MMP-9 [8]. MMPs play a role in the degradation of extracellular matrix (ECM) proteins such as collagen, fibronectin, elastin and proteoglycans [9]. This is a significant factor in skin aging characterized by a lack of elasticity and the formation of wrinkles on the surface [10]. In addition to breaking down collagen, AP-1 also suppresses collagen production by inhibiting TGF-β signaling, which regulates the synthesis of procollagen type I, leading to collagen fragmentation and decreased collagen biosynthesis [11,12]. TGF-β signaling is crucial for maintaining skin integrity by stimulating fibroblasts to produce procollagen type I. AP-1 inhibits this process by down-regulating TGF-β receptor expression and interfering with Smad3 activation, which is necessary for transcription of collagen-related genes. This disruption in collagen synthesis further contributes to the degradation of the extracellular matrix and the advancement of skin aging [11]. To avoid photoaging, using antioxidant-based cosmetics before sun exposure as a skin pre-treatment can be an effective strategy [13].

One Indonesian plant that contains antioxidants and can overcome skin damage due to photoaging is patchouli (*Pogostemon cablin* Benth.) from the Lamiaceae family. Patchouli oil contains several bioactive compounds, including sesquiterpenes such as patchoulene, norpatchoulenol and pogostone, which contribute to its antioxidant and anti-inflammatory properties. Antioxidant and anti-inflammatory properties found in patchouli oil can significantly enhance the healing of skin damage caused by UVB exposure [14]. Among these bioactive compounds, patchouli alcohol (PA) has been identified as the most active sesquiterpene, playing a crucial role in the herb's pharmacological properties [15]. The previous study by Feng [15] indicated that PA strongly enhanced the recovery of UV-induced skin lesions, primarily through its antioxidant and anti-inflammatory properties, along with the down-regulation of MMP-1 and MMP-3 expression.

This study aimed to evaluate the anti-aging effects of patchouli oil on the skin of mice exposed to UVB light. The findings were expected to provide new insights into organic skincare formulations that protect against sun-induced damage and prevent premature aging. Beyond its biological significance, the study also holds economic and commercial implications as the global demand for natural and sustainable skincare products continues to rise. According to recent market research, the global natural cosmetics market is projected to reach USD 54.5 billion by 2027, growing at a CAGR of 6.6% from 2021 to 2027 [16]. The increasing consumer preference for plant-based skincare ingredients further underscores the relevance of this study. The anti-photoaging potential of patchouli oil could contribute to developing innovative cosmetic formulations, offering a safer and more effective alternative to synthetic skincare products.

Materials and methods

Fourier transform infrared (FTIR) spectroscopy

Crude oil and light fraction patchouli oil were obtained from the Atsiri Research Centre laboratory of Syiah Kuala University, Banda Aceh, Indonesia. Patchouli (*Pogostemon cablin*) herb distillation produced crude patchouli oil (CPO), which was fractionated to obtain light fraction patchouli oil (CPO1)

and heavy fraction patchouli oil (CPO2). A chemical compound analysis of patchouli oil was performed using fourier transform infrared (FTIR) Shimadzu Model IR Prestige 21 (2012) at the Environmental Laboratory of the Department of Chemical Engineering, Syiah Kuala University. The process began by powering on the instrument and initializing the system via the software until all status indicators turned green. A holder was placed in the designated slot and a data storage folder was set up on the computer. The background spectrum (BKG) was measured first to correct for atmospheric interference, including CO_2. For sample analysis, 2 mL of the liquid sample was applied to a glass plate and positioned in the holder. The measurement was carried out within a wavelength range of 4500 - 500 cm^1, and the obtained spectrum was saved in PDF or image format for further examination.

UV light sources

UVB lights originated from Philip PL S 9W/01/2P, while exposure and administration of treatments followed the modified method [17]. The hair on the mice's backs was shaved 2×2 cm^2 using a trimmer to obtain a precise observation without interruption during the experiment. UVB exposure was given at a dose of 498 mJ/cm^2 for 10 days in 2 weeks of treatment (5 days/week). Three UVB lamps were placed 3 cm above the mice's backs for 100 s and the total dose received was 4.980 mJ/cm^2 individually. The UVB dose of 498 mJ/cm^2 was adapted from the previous study that used the same type of lamp, a Kernel KN-4003 UVB lamp with a Philips PL S 9W/01/2P narrow band lamp with a wavelength of 311 nm at a distance of 3 cm for 100 s [18]. The UVB dose of 498 mJ/cm^2 was chosen to induce early signs of photoaging while minimizing overt skin injury. While murine and human skin differ in UV susceptibility, this model allows for controlled evaluation of dermal aging markers that parallel some of the structural changes observed in human photoaged skin.

Experimental animal

The experiment was carried out at the Prof. Dr. Nurjanto Teaching Animal Hospital, Faculty of Veterinary Medicine, Syiah Kuala University. This study used male Balb/c mice (*Mus musculus*) weighing between 20 - 30 g and aged 2 to 3 months. Male mice were selected to minimize the influence of hormonal fluctuations that could affect skin aging responses, as estrogen and other sex hormones play a role in skin physiology and wound healing [19]. The choice of 2 to 3-month-old mice corresponds to young adulthood in human age equivalence, which is an appropriate stage for studying early interventions in aging models [20].

The animal underwent a one-week acclimatization period and was treated under controlled conditions, with a temperature range of 20 - 30 ℃, relative humidity of 45% - 64% and 1 12-h light/dark cycle, maintained using an automated monitoring system. These environmental conditions align with standard animal care and welfare guidelines to ensure physiological stability and minimize external stressors that could impact study outcomes. Following acclimatization, the mice were randomly divided into five treatment groups, as shown in **Table 1**.

The sample size per group (n = 5) was determined based on Federer's formula [21]. With the five treatment groups, this sample size ensures reliable results while following ethical research standards. The mice were exposed to UVB light for five days per week [22], with the UVB lamp placed 3 cm from the mice's skin. Each exposure lasted 100 s over a 2×2 cm^2 shaved area on their backs [18]. After UVB exposure, BioNa anti-aging serum and patchouli oil were applied to a 2×2 cm^2 hairless area on the mice's backs. Each mouse received 100 μL of treatment (K+ = BioNa anti-aging serum, P1 = CPO, P2 = CPO1). This study was approved by the Research Ethics Commission, Faculty of Veterinary Medicine, Syiah Kuala University (permit number 234/KEPH/VII/2023).

Table 1 Test treatment groups.

Groups	Treatment	Quantity
K0	Not exposed to UVB + not sampled	5
K-	UVB exposure	5

Groups	Treatment	Quantity
K+	Exposed to UVB light + treated with 100 µL of BioNa anti-aging serum	5
P1	UVB exposure + 100 µL patchouli crude oil applied	5
P2	Exposed to UVB light + treated with 100 µL of light fraction patchouli oil	5

Collagen fiber, elasticity, and moisture measurement

Antiaging parameters, including skin elasticity, moisture, and collagen content, were measured using the Skin Analyzer tool (Digital Test System EH-900U User Manual). Skin analysis was conducted before the test (day 0), day 6, and day 13 in each group (**Figure 1**). All measurements were taken from the central area of each mouse's shaved dorsal skin (2×2 cm²)

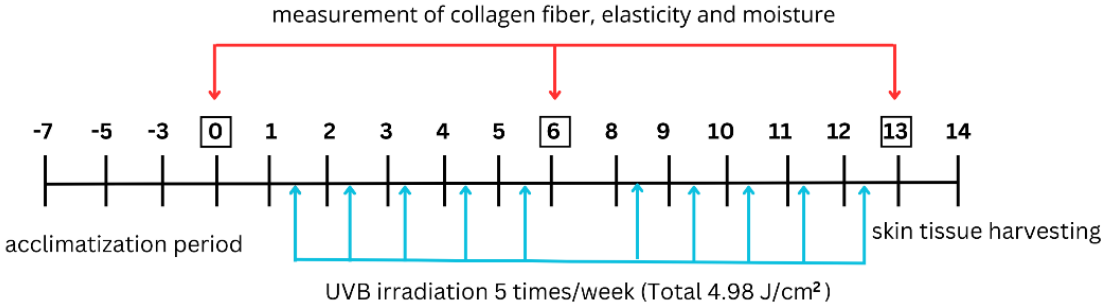

Figure 1 Timing schedule for UVB light and skin treatment of mice.

Statistical analysis for collagen fiber, elasticity and moisture measurement. Data analysis was done using IBM SPSS, followed by Multivariate Analysis of Variance (MANOVA). When the results were considered significant (*p-value* < 0.05), additional post-hoc testing was performed.

Organ collection and histological slide preparations

The treated mice were sacrificed by dislocation, 24 h after the final UVB exposure [18]. The excised tissue was rinsed with physiological NaCl solution and fixed in 10% Buffered Neutral Formalin (BNF) for 24 h. It was then dehydrated in graded alcohol solutions up to absolute alcohol, each step lasting 2 h and repeated twice. Afterward, tissues were cleared in xylene and infiltrated with a xylene-paraffin mixture (1:1) for 1 h, followed by paraffin infiltration for an additional h, repeated twice.

During embedding, the infiltrated tissue was embedded in liquid paraffin in a mold and allowed to harden. Hardened blocks were sectioned with a microtome to obtain 5 µm-thick sections, yielding four optimal sections [15]. For hematoxylin and eosin (H&E) staining, deparaffinization was carried out with xylene and graded alcohol solutions. Sections were stained with hematoxylin for 3 - 5 min, rinsed with distilled water and counterstained with 1% eosin for one min. Final dehydration and clearing were followed by mounting with Entellan [23].

Epidermal thickness and fibroblast count

Epidermal thickness and fibroblast count were observed under a microscope. Epidermal thickness was measured from the basal layer, adjacent to the dermis, to the stratum lucidum, using a 400× magnification lens. Measurements were taken at three fields of view. At the same time, the fibroblast was counted manually at three fields of view.

Statistical analysis for epidermal thickness and fibroblast count

Data were then analyzed using the Statistical Package for Social Science (SPSS). Initially, normality was tested using the Shapiro-Wilk test with a significance level of *p-value* > 0.05. If the data were normally distributed and homogeneous, significance was assessed with a One-Way ANOVA, followed by a

post-hoc test. Results are presented in tables as mean ± standard deviation (SD).

Preparation of patchouli essential oil compound as ligand

Based on previous research that has been done, it is known that the active compounds contained in patchouli essential oil are as many as 68 compounds, but only 66 compounds can be used as raw materials for topical medicines. The number of compounds used as ligands in this study is 8, which refers to the results of network pharmacology analysis in previous studies [24]. It is known that from a total of 66 active compounds of patchouli oil, only 8 compounds can interact with the MMP9 protein. Each of these active compounds was searched for a 3D structure using the PubChem database website (https://pubchem.ncbi.nlm.nih.gov) and then prepared using PyRx software to minimize its energy.

Collection and preparation of the MMP9 target protein

MMP9 protein (PDB ID 4WZV) was determined as the key target protein used in this study. The 3D structure of the protein was obtained from the RCSB PDB database website (https://www.rcsb.org/). The structure was prepared before docking using the BIOVIA Discovery Studio tool, which eliminated H2O molecules and its native ligands previously attached to the protein.

Molecular docking of the ligand and MMP9 protein

The molecular docking was carried out with the Autodock Vina program integrated into the PyRx software. The process was performed on the MMP9 protein's active site. The active site of the MMP9 protein was defined using the BIOVIA Discovery Studio application by selecting "ligand interactions" and all the amino acid residues of the native ligand that bond with the protein were recorded. The amino acid residues of the MMP9 active site as follows: LEU188, ALA189, ALA191, HIS226, HIS230, HIS236, TYR245, PRO246 and MET247. After the molecular docking process was complete, the molecularly docked protein-ligand complexes were studied and visualized with the BIOVIA Discovery Studio software. The binding site was assessed using ligand-residue interaction and 3D structure, which was done by using Pymol software and Ligplot. After the calculation, the docking score with the lowest binding energy value to the target protein was selected.

Molecular dynamics simulation

Molecular Dynamic Simulation was carried out using YASARA (Yet Another Scientific Artificial Reality Application) v.23.5.19 software. The parameters used included temperature 310K, NaCl ion 0.9, pH 7 and program running time 50000 ps (50 ns), which was set in the macro file. After the program was run and completed, the data from the molecular dynamic simulation were visualized in the form of graphical data created using GraphPad Prism 8.

Results and discussion

FTIR spectrum analysis and phytochemical profile of patchouli oil

Two patchouli oil samples in the FTIR spectra (**Figure 2**), the light fraction (CPO1) and the crude oil (CPO), exhibited similar dominant vibrational spectra in their chemical composition (4,000 - 600 cm^{-1}). In the region around 3,400 cm^{-1}, an absorption indicates the presence of hydroxyl groups (-OH), possibly from alcohol compounds or moisture. In addition, in the 3,000 - 2,800 cm^{-1} range, there is a typical absorption for aliphatic C-H bonds commonly found in hydrocarbons. Around 1,700 cm^{-1}, a peak is seen, indicating the presence of carbonyl groups (C=O), which may come from ketones, aldehydes, or carboxylic acids. Meanwhile, in the 1,500 - 800 cm^{-1} region, some absorptions reflect molecular fingerprints, which most likely come from terpenoid compounds in essential oils.

Patchouli plants from local farmers were processed using steam distillation to extract crude patchouli oil (CPO). To enhance its quality, the oil underwent fractional distillation using a vacuum rotary evaporator; following the procedure by a study in this process [25], the crude oil was placed in a round-bottom flask, with the chiller set to 10 °C. The vacuum pump was activated, and the rotation speed was maintained at 65 rpm. The first distillation was conducted at 110 - 120 °C, producing the light fraction collected until no further distillate remained. The second distillation was conducted at 130 - 140 °C, yielding a heavy fraction.

Patchouli oil contains various active compounds such as beta-patchoulene, caryophyllene, alpha-guaine, seychellene, alpha-patchoulene, azulene, patchouli alcohol, globulol, naphthalenol, gamma-gurjune, neoallocimenene, aciphyllene, and delta-guaiene, as identified through GC-MS analysis (**Figure 3**). Many studies have examined the phytochemical bioactivities of these compounds, which exhibit anti-inflammatory, antibacterial and antioxidant properties. As an anti-aging agent, sesquiterpenes in patchouli oil can suppress photoaging-induced ROS production triggered by UVB exposure, thereby helping to prevent collagen and elastin degradation and reduce signs of aging such as skin sagging and wrinkles [26].

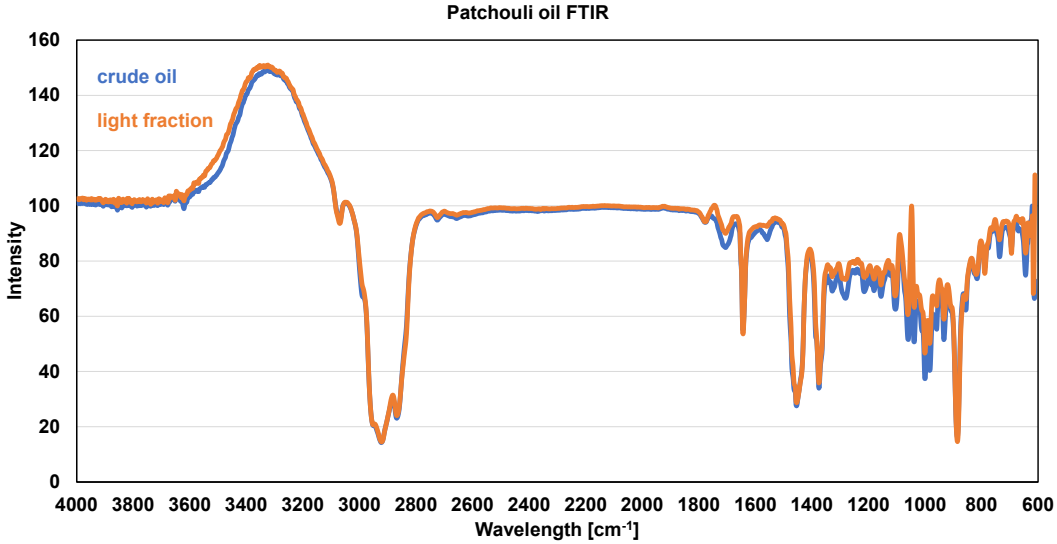

Figure 2 FTIR spectrum for both crude oil and light fraction of patchouli oil.

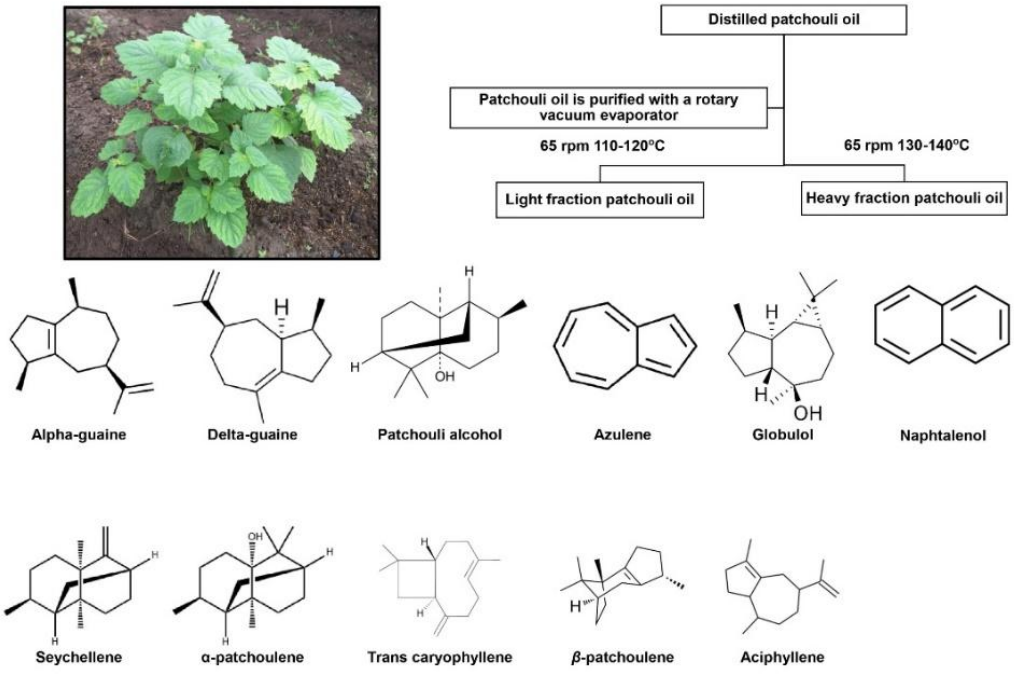

Figure 3 Patchouli oil refinement graphic process and the content of common chemical compounds found in patchouli oil.

Effect of UVB on collagen fiber, elasticity and moisture

Figure 4 presents macroscopic images of mouse skin after 10 days of UVB exposure and treatment with patchouli oil fractions. The negative control group (K-), which only received UVB exposure, showed visible wrinkles, rough texture and erythema from day 5, with the condition worsening over time. However, the use of 100 µL patchouli crude oil (group P1) and 100 µL light fraction patchouli oil (group P2) showed a smoother appearance than group K, both of which showed a tendency to reduce UV-induced erythema and deep wrinkles after nine days of treatment. In photoaging, the collagen structure of the skin becomes hard and stiff.

After day 7, there were residues in the CPO group (P1), such as unabsorbed oil and possible oxidative by-products, left on the mice, indicating potential irritation to the skin if used long-term. In the CPO1 (P2) group, no wrinkles were found and changes were limited to the skin on day 3, followed by significant improvement. This suggests that the light fraction of patchouli oil has the best effect in minimizing the damage caused by photoaging.

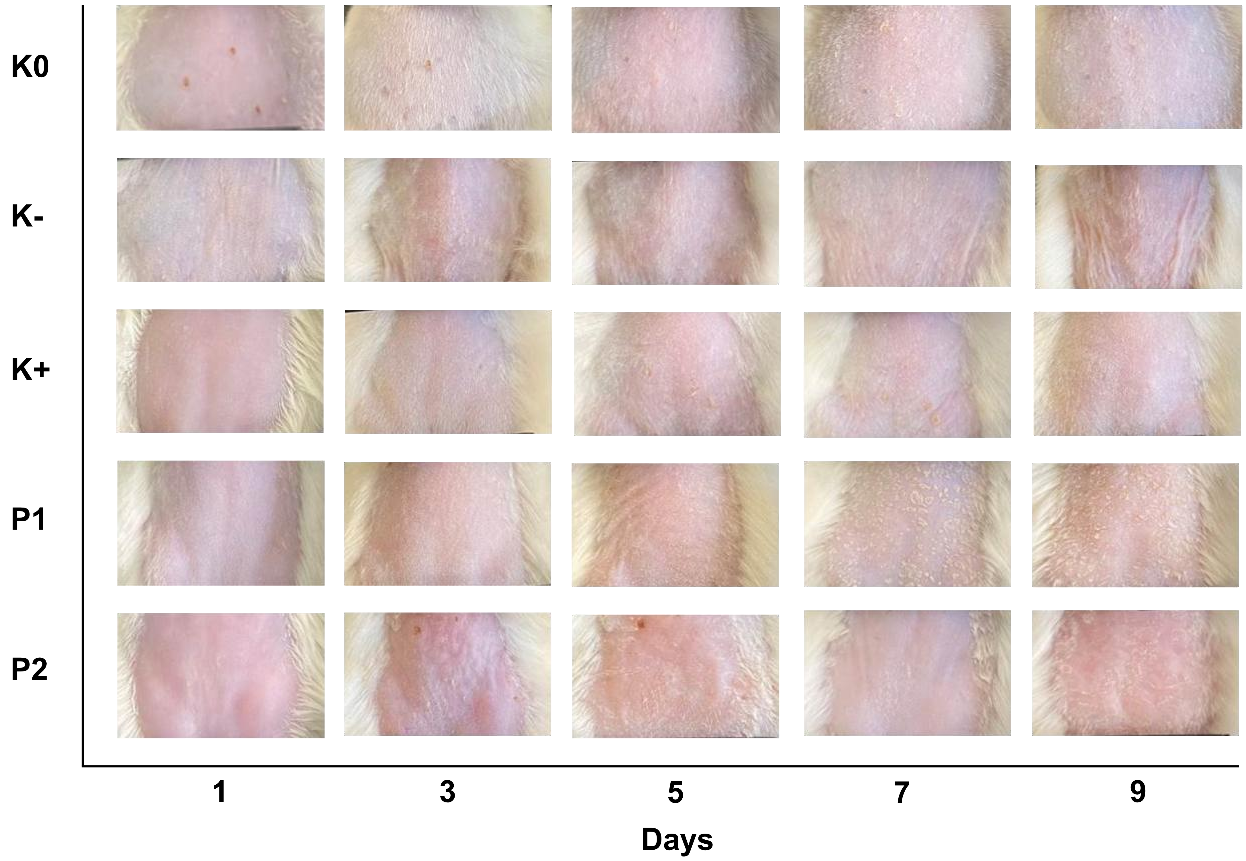

Figure 4: Condition of the mice's skin in each group before the treatment.

Patchouli oil anti-aging activity test

Quantitative analysis of collagen fiber density, skin elasticity, and moisture level showed significant differences among the treatment groups (**Figure 5**). Collagen fiber content in K- decreased to 49%, while P1, P2 and K+ showed an increase of 45.33%, 40.83% and 32.14%, respectively. Skin elasticity also decreased to 40.60% in K-, while P1, P2 and K+ showed an increase of 18.08%, 12.30% and 3.91%, respectively. Similarly, the moisture level in K- decreased significantly to 79.05%, while P1, P2 and K+ showed an increase of 56.72%, 24.73% and 18.89%, respectively.

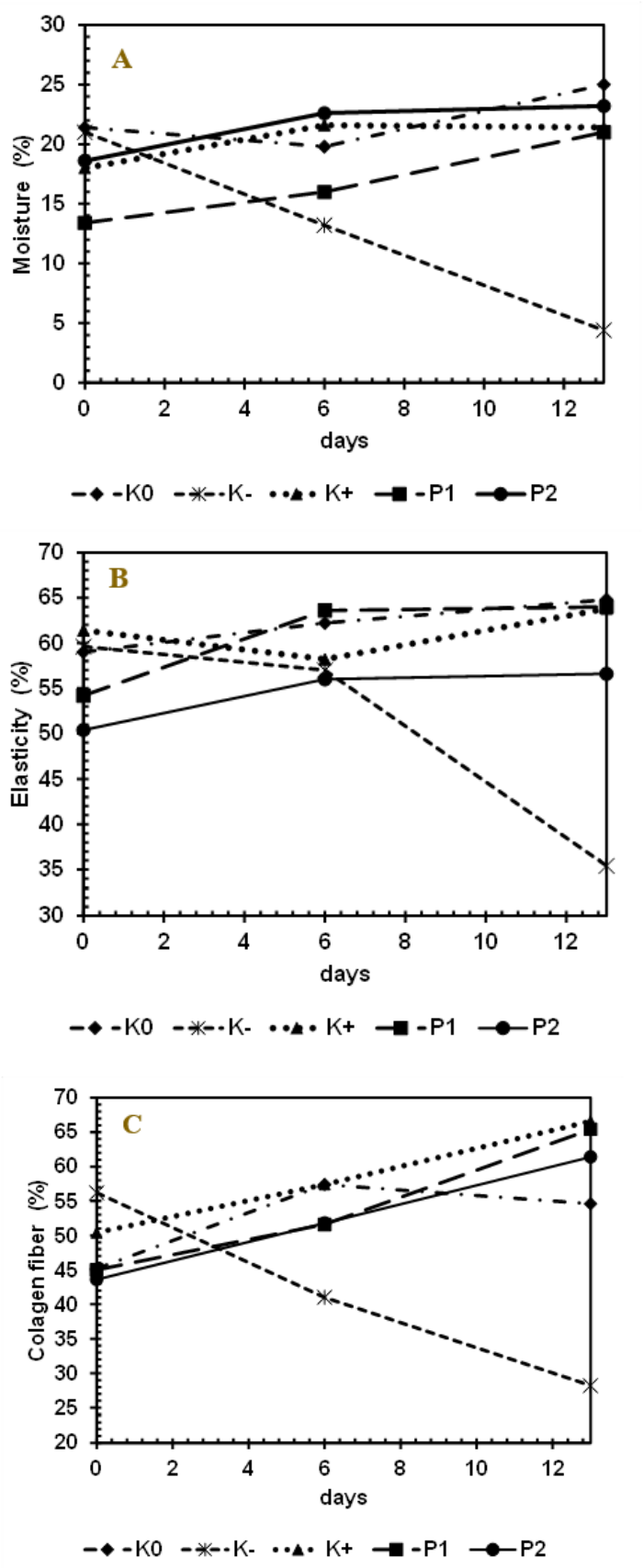

Figure 5 Graph showing the changes in mean skin moisture (A), elasticity (B) and collagen fibers (C) in each group after 13 days of UVB exposure.

On day 13, statistical analysis showed significant differences in collagen fiber content ($p < 0.05$), with post-hoc tests revealing differences between K- and K0, K+, P1 and P2 (**Figure 6**). However, there was no significant difference between K+, P1, and P2. Significant differences were also found in skin elasticity between K- and K0, K+, P1 and P2. Measurement of moisture content showed significant differences between K- and K0, P1 and P2, while K+ did not differ significantly from the other groups

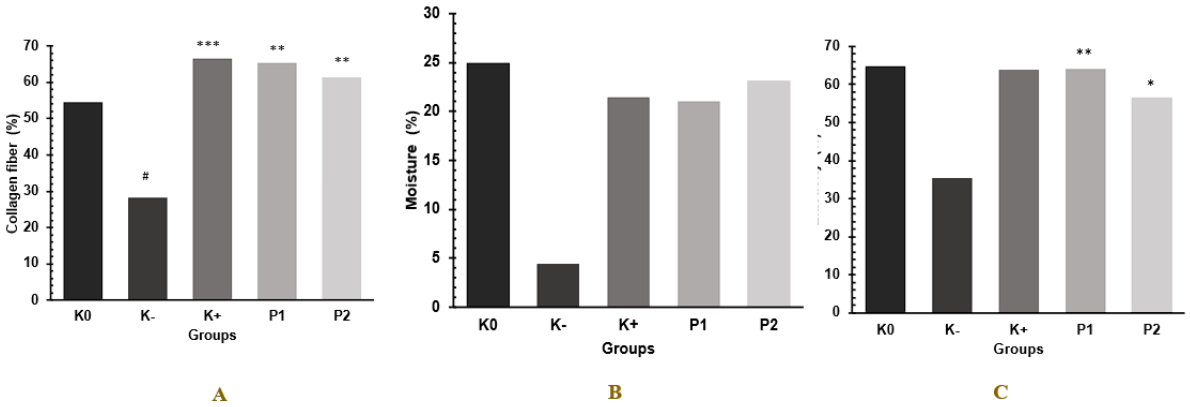

Figure 6 Mean. Changes in collagen fiber (A), elasticity (B) and skin hydration (C) were measured in each group in comparison to the effect of UVB on day 13. The parameters were measured with a skin analyzer. # Versus the regular control group. * Vs. negative control. # *p-value* < 0.05, ## *p-value* < 0.01, ### *p-value* < 0.001, * *p-value* < 0.05, ** *p-value* < 0.01 and *** *p-value* < 0.001.

Epidermal thickness and fibroblast count

Observations of UVB-exposed mouse skin (K-) in **Table 2** and **Figure 7** suggest that excessive UVB exposure reduces the thickness of the epidermis. The negative control group showed a thinner epidermis compared to other treatments, indicating that without protection, UVB can damage the epidermis. The outer living layer of the epidermis, the stratum lucidum, was reduced, with only one to two cell layers remaining, while the stratum corneum thickened. The highest average epidermal thickness in **Table 2** resulted from light fraction patchouli oil application (P2) at 27.29 µm, suggesting effective UVB protection and free radical scavenging. However, the champaca treatment increased epidermal thickness beyond the normal control, potentially causing skin complications and requiring concentration adjustments for the formulation.

Data presented in **Table 2** indicate a reduction in the average fibroblast count following UVB exposure compared to the normal control group. This observation suggests that UVB radiation adversely impacts the dermal layer, specifically affecting the quantity of fibroblasts which can be seen in **Figure 7**. The application of biona serum and test oils, particularly crude (P1) and light fraction patchouli oil (P2), resulted in an increased fibroblast count after UVB exposure, as depicted in **Table 2**. This suggests that the antioxidant properties of these oils may enhance fibroblast proliferation

Table 2 Test treatment groups.

Groups	Treatment	Epidermal Thickness ($\bar{x}$ ± SD) µm	Fibroblast count ($\bar{x}$ ± SD)
K0	Not exposed to UVB + not sampled	15.33^{ab} ± 1.34	143.58^{bcd} ± 11.94
K-	UVB exposure	9.84^{a} ± 0.59	87.2^{a} ± 17.52

Groups	Treatment	Epidermal Thickness ($\bar{x}$ ± SD) µm	Fibroblast count ($\bar{x}$ ± SD)
K+	Exposed to UVB light + treated with 100 µL of BioNa anti-aging serum	16.02^{abc} ± 1.38	103.85^{ab} ± 27.54
P1	UVB exposure + 100 µL patchouli crude oil applied	26.20^{bc} ± 4.50	180.5^{d} ±12.99
P2	Exposed to UVB light + treated with 100 µL of light fraction patchouli oil	27.29^{c} ± 11.82	176.55^{cd} ± 54.39

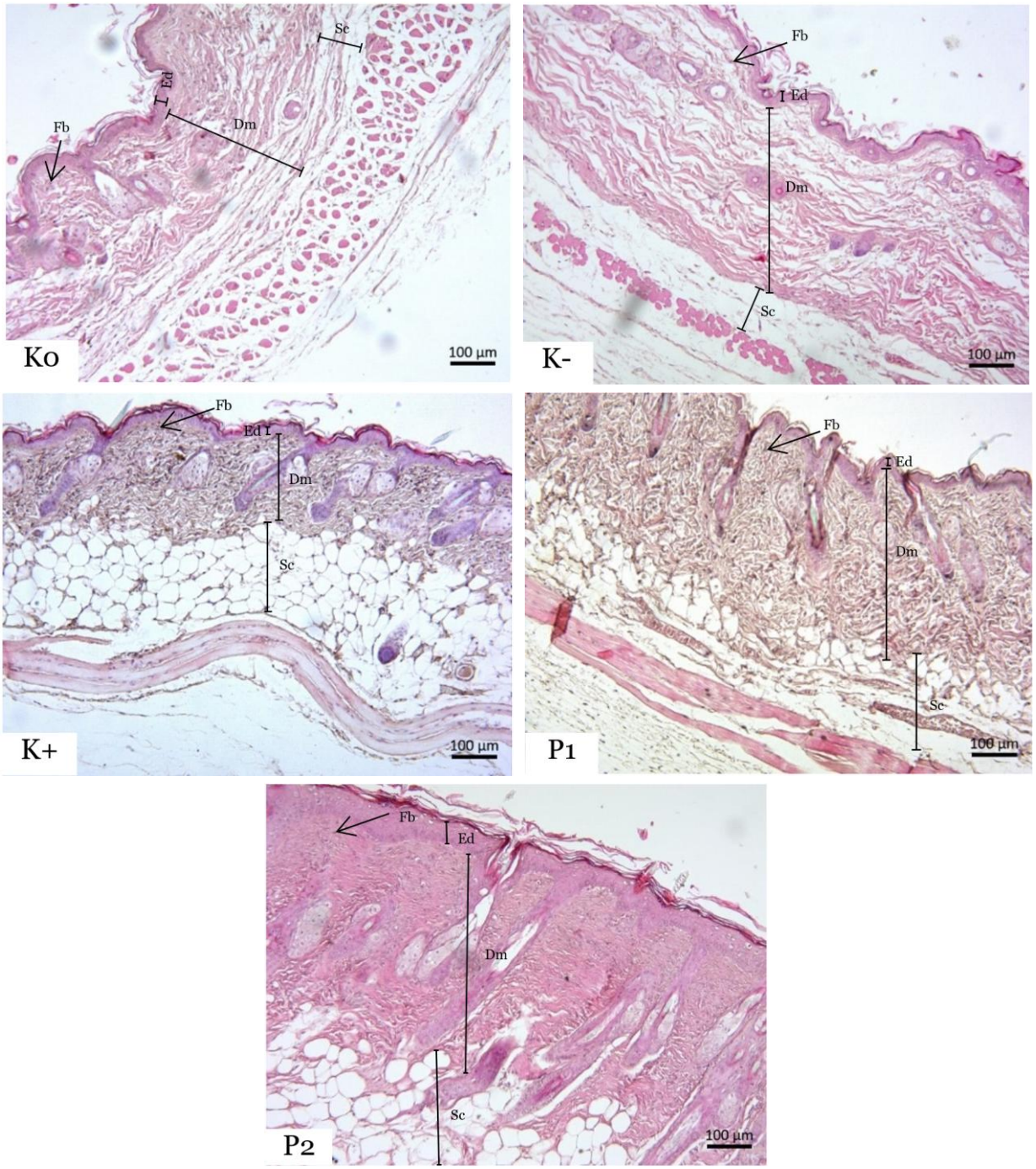

Figure 7 Histological results of mice skin after treatment. Epidermal (Ep); Dermal (Dm); Subcutis (Sc); Fibroblast (Fb).

Molecular docking results of patchouli oil compounds as MMP9 Inhibitor.

The average binding energy value between protein complexes with native ligands and activator/inhibitor controls is low. Binding energy value of patchouli compounds with MMP9 protein can be seen in the following **Table 3**

Table 3 Binding energy value of Patchouli oil active compound complex against MMP9 protein.

No	Protein	Compounds	*Binding Energy*
1	MMP9	EN140 (native ligand)	–10,5 kcal/mol
2		BPHA (Inhibitor)	–10,2 kcal/mol
3		Beta-elemene	–6,7 kcal/mol
4		Bicyclo [5.2.0] nonane, 4-methylene-2,8,8-tr	–6,4 kcal/mol
5		But-3-enal, 2-methyl-4-(2,6,6-trimethyl-1-c	–6,1 kcal/mol
6		Cyclohexanol	–6,1 kcal/mol
7		Acetic acid	–7,1 kcal/mol
8		Caryophyllene oxide	–6,7 kcal/mol
9		Cis-Z-a-Bisabolene epoxide	–7,4 kcal/mol
10		Humulene epoxide I	–6,5 kcal/mol

The EN140 compound, which is a native ligand of the MMP9 protein, forms hydrogen bonds with the MMP9 protein at residues LEU188, ALA189, HIS226, GLU227, HIS230, ASP235 and HIS236 (**Table 4**). This compound also interacts hydrophobically at residues TYR179, LEU187, ALA190, PHE191, HIS192, and LEU222. BPHA compounds, which are inhibitor compounds of MMP proteins, form hydrogen bonds with MMP9 proteins through residues ALA189, HIS226, GLU227, ASP235, and HIS236. In addition to hydrogen bonds, this compound also interacts hydrophobically with MMP9 proteins through residues GLY186, ALA190, LEU222, VAL223, HIS226 and GLU227.

The results of molecular docking between active compounds contained in patchouli oil with MMP9 protein show that most patchouli oil compounds do not form hydrogen bonds with MMP9 protein except Acetic acid compounds, which bind to MMP9 protein at residue TYR248. The hydrophobic interaction is through residues LEU188, ALA189, TYR218, VAL223, HIS226 and GLU227 (**Figure 8**). The native ligand and inhibitor compounds have many residue similarities, such as in hydrogen bonding; the same residues are ALA189, HIS226, GLU227, ASP235 and HIS236. In hydrophobic interactions, the same residues are ALA190 and LEU222. Comparison of amino acid residues on the binding side of the MMP9 protein of each native ligand and inhibitor compound with patchouli oil compounds can be seen in **Table 4**.

When compared to Acetic acid, the hydrogen bond between this compound and MMP9 has no similarities with native ligands or inhibitors. However, in hydrophobic interactions, Acetic acid compound has one residue in common with the inhibitor compound, namely residue GLU227. The existence of hydrogen bonds is the basis for the selection of Acetic acid compounds as candidate MMP9 inhibitors. 3D and 2D visualization of the interaction of the Acetic acid compound with the MMP9 protein can be seen in **Figure 8**.

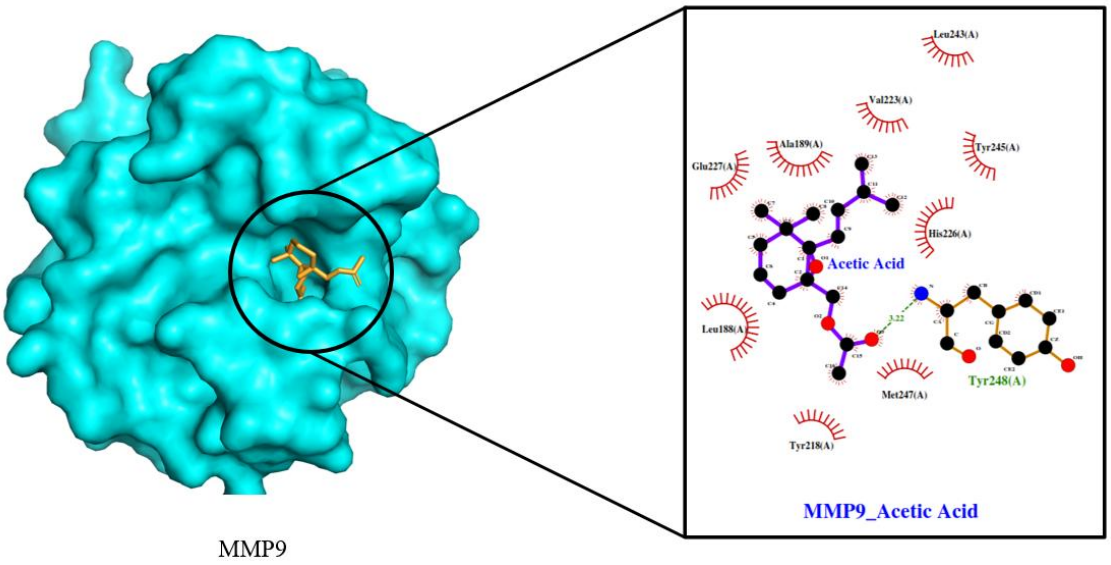

Figure 8 3D and 2D visualization of Acetic acid interaction with MMP9 protein.

Table 4 Amino acid residues of ligand compound and MMP9 protein interaction.

No	Protein	Compounds	Hydrogen Bonds	Hydrophobic Interactions
1	MMP9	EN140 (native ligand)	LEU188, ALA189, HIS226, GLU227, HIS230, ASP235, HIS236	TYR179, LEU187, ALA190, PHE191, HIS192, LEU222
2		BPHA (Inhibitor)	ALA189, HIS226, GLU227, ASP235, HIS236	GLY186, ALA190, LEU222, VAL223, HIS226, GLU227
3		Beta-elemene	N/A	LEU222, VAL223, HIS226, PRO240, ALA242, LEU243, TYR245, MET247, TYR248, ARG249, THR251
4		Bicyclo [5.2.0] nonane, 4-methylene-2,8,8-tr	N/A	PRO240, GLU241, ALA242, LEU243, ARG249, PHE250, THR251, PRO255
5		But-3-enal, 2-methyl-4-(2,6,6-trimethyl-1-c	N/A	GLY186, LEU187, LEU188, ALA189, HIS226, PRO246, MET247
6		Cyclohexanol	N/A	LEU222, GLU241, ALA242, LEU243, ARG249, PHE250, THR251, PRO255
7		Acetic acid	TYR248	LEU188, ALA189, TYR218, VAL223, HIS226, GLU227
8		Caryophyllene oxide	N/A	GLY186, LEU187, LEU188, ALA189, VAL223, HIS226, GLU227, LEU243, TYR245, MET247
9		Cis-Z-a-Bisabolene epoxide	N/A	LEU188, TYR218, LEU222, VAL223, HIS226, TYR245, PRO246, MET247, TYR248
10		Humulene epoxide I	N/A	PRO240, GLU241, ALA242, LEU243, ARG249, THR251, PRO255

Notes: Bolded amino acids represent the same ligand binding site as the control ligand and native ligand. N/A means not available or no binding or interaction.

Molecular dynamic simulation results of complex MMP9 with ligands

The results of molecular dynamics simulation (MDS) analysis of the native MMP9 protein ligand compound (EN140/(2R)-4-(1,3-dioxoisoindol-2-yl)-N-hydroxy-2-[[4-(4-methoxyphenyl) phenyl] sulfonyl-propan-2-yloxyamino]butanamide), MMP9 inhibitor compound (BPHA/(2R)-N-hydroxy-3-phenyl-2-[(4-phenylphenyl)sulfonylamino]propanamide) and Acetic acid compound from patchouli oil can be seen in **Table 5** below. Based on **Table 5,** the ligand RMSD parameter with the lowest value is the Acetic acid compound (1.542 Å), which indicates that the compound does not change its shape too often when it binds to the receptor protein. However, in the RMSD ligand movement parameter, the Acetic acid compound has the highest value (13.569 Å). This is evidenced by the graph in **Figure 6B** which shows a change in protein movement fluctuations, where in the time of 30 ns, the three ligand compounds have the same stability, marked by parallel lines on the graph. However, at 40 ns, there is a fairly high spike indicating the movement of the compound moving away from the protein (yellow line). The high RMSD value indicates the movement of the ligand away from the binding site of the receptor protein

Table 5 MDS Analysis Results of MMP9 Protein Complex with Ligand

No.	Parameters	Native Ligand	Inhibitor	Acetic acid
1	RMSD *Ligand*	2.169 Å	2.26 Å	**1.542 Å**
2	RMSD *Ligand Movement*	**3.529 Å**	5.705 Å	13.569 Å
4	*Binding Energy*	**–6.213 KJ/mol**	–12.792 KJ/mol	–28.098 KJ/mol
5	*No. of H Bond*	268.632 Å	276.249 Å	**282.443 Å**

Description: Bolded values are the best results

The binding energy value parameter of each ligand shows negative results, but one of the ligand compounds, namely the native ligand, has a binding energy value that tends to be positive (–6.213 KJ/mol). The inhibitor ligand compound and Acetic acid showed lower binding energy values compared to the native ligand compound (–12.792 KJ/mol and –28.098 KJ/mol), with the binding energy value of the Acetic acid compound being the lowest compared to the native ligand compound and its inhibitor. The more positive binding energy value represents good binding stability between the ligand and protein. Therefore, based on **Table 5** above, the binding energy between the Acetic acid ligand is not good when compared to the native ligand and its inhibitor.

The parameters of the number of hydrogen bonds between the MMP9 protein and the three compounds namely native ligand, inhibitor and Acetic acid show that the Acetic acid compound has the highest number of hydrogen bonds (282.443 Å) which indicates that the interaction between the MMP9 protein and Acetic acid tends to be stable and strong during the simulation, this is evidenced by the graph in **Figure 9D** which shows the movement of the Acetic acid compound which tends to be constant during the simulation (yellow line)

.

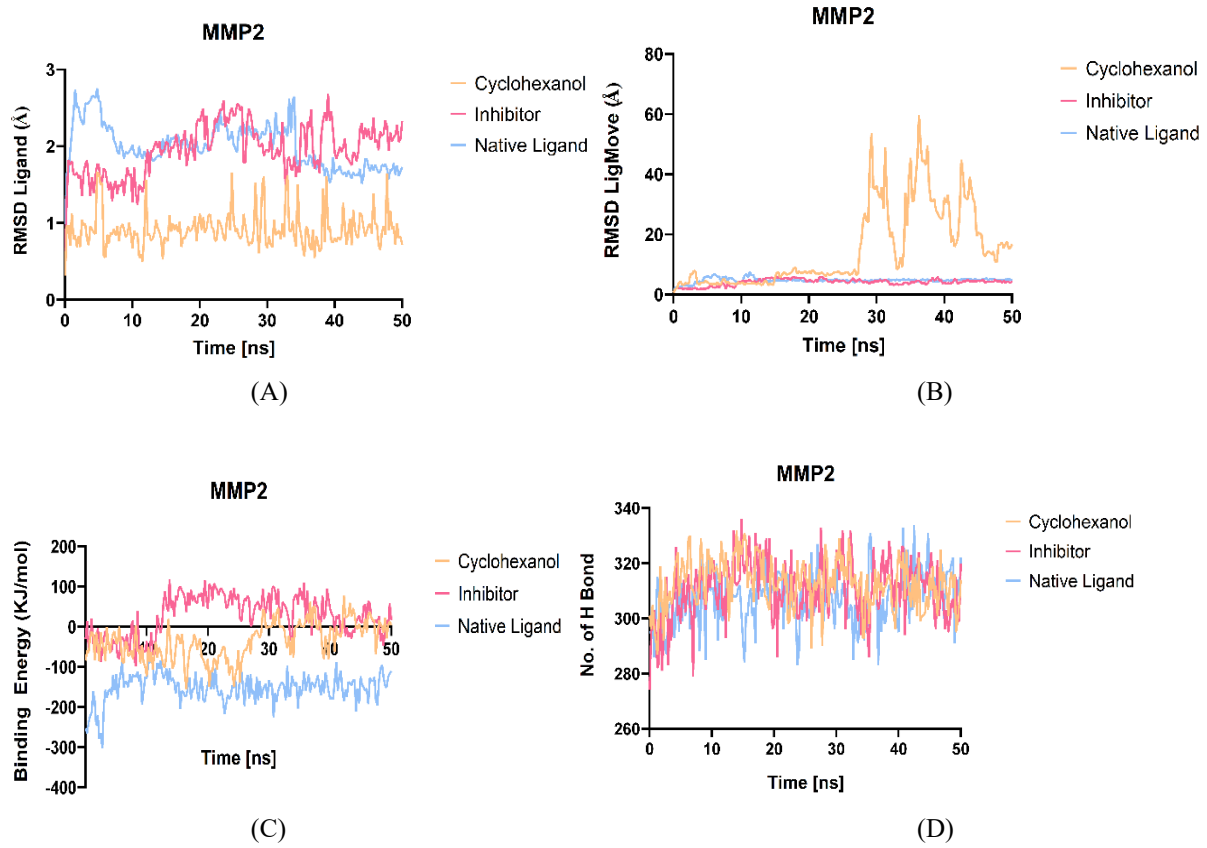

Figure 9 MDS result graph of interaction between MMP9 protein and Acetic acid compound. Graphs of (A) RMSD ligand, (B) RMSD ligand movement, (C) Binding energy and (D) Number of hydrogen bonds.

Discussion

Prolonged exposure to UVB rays causes premature aging of the skin (photoaging), which is characterized by skin thickening, wrinkle formation and decreased elasticity due to the degradation of collagen and elastin fibers. UVB radiation primarily damages these fibers by increasing the production of reactive oxygen species (ROS), which then triggers oxidative stress and inflammation. Antioxidant-based approaches are effective in preventing premature skin aging [27]. Therefore, in this study, the anti-aging effects of crude patchouli oil (CPO) and CPO1 were evaluated by observing macroscopic changes in the skin, including hydration, elasticity, and collagen fiber content.

Patchouli alcohol (PA) is the main compound in patchouli oil that exhibits high antioxidant activity. CPO contains 31.2% patchouli alcohol, while CPO1 contains 23.99%. Research in 2021 by Citrawan *et al.* [18] showed that patchouli oil has an IC_{50} value of 34.12 ppm, which indicates very strong antioxidant activity. A previous study reported that patchouli oil (PO) can significantly inhibit wrinkle formation, improve skin elasticity and increase collagen content [15]. These protective effects of PO are most likely due to its ability to counteract free radicals. Furthermore, PO can significantly and dose-dependently inhibit forskolin-induced melanin production, reduce cellular tyrosinase activity and suppress protein tyrosinase (TYR) expression [26]. This suggests that patchouli essential oil also has potential as a therapy for hyperpigmentation caused by UV exposure.

The research found that the light fraction of patchouli contained a lower concentration of patchouli alcohol, while the heavy fraction had a higher concentration [25]. However, a study in 2023 reported that the heavy fraction had a higher acid value, making it more susceptible to oxidation [26]. This instability makes the heavy fraction less appropriate as a raw material for cosmetic compositions; hence, it was not included in this work.

The main mechanisms of PA in skin protection include the prevention of UVB-induced DNA damage, reduction of oxidative stress, inhibition of signaling pathways that decrease skin integrity and minimization of cytotoxicity. This study showed that the administration of CPO and CPO1 was able to significantly increase collagen fibers in UVB-exposed skin. The average collagen fibers in the P1 (crude patchouli oil) group increased by 45.33%, followed by P2 (light fraction patchouli oil) and K+ (BioNa anti-aging serum). In contrast, in the group that was only exposed to UVB light without treatment (K-), collagen fibers decreased by 49%. Statistical analysis on day 13 showed significant differences between groups (*p-value* < 0.05), so a post-hoc test was performed which showed significant differences between K- and K0 ($p = 0.035$), K+ ($p = 0.001$), P1 ($p = 0.002$) and P2 ($p = 0.005$), as shown in **Figure 7**. However, there was no significant difference (*p-value* > 0.05) between the K+, P1 and P2 groups, indicating that the effectiveness of CPO and CPO1 in maintaining collagen fibers was comparable to that of BioNa anti-aging serum.

In terms of skin elasticity, the P1 group experienced an increase of 18.08%, followed by P2 (12.30%) and K+ (3.91%), while the K- group experienced a drastic decrease of 40.60%. Statistical analysis on day 13 showed significant differences (*p-value* < 0.05) between K- and K+, P1 and P2. Further testing also confirmed significant differences in mean elasticity between K- and K0, K+, P1 and P2, as shown in **Figure 5**.

In terms of skin hydration, the K- group experienced a 79.05% decrease in moisture content, indicating severe UVB damage to the skin. In contrast, the treated groups showed an increase in moisture, namely P1 by 56.72%, P2 by 24.73%, and K+ by 18.89%, as shown in **Figure 5**. Statistical analysis on day 13 showed a significant difference (*p-value* < 0.05) between K- and K0, P1 and P2, indicating that the application of patchouli oil was able to restore skin moisture content lost due to UVB exposure. However, the K+ group showed no significant difference compared to K0, P1 and P2, despite the increase in moisture. Overall, the results of this study indicate that patchouli oil (CPO and CPO1) has great potential in inhibiting photoaging due to UVB exposure, by increasing collagen content, elasticity and skin hydration. These effects are thought to stem from the high antioxidant activity, particularly of patchouli alcohol and other sesquiterpene compounds, which are able to suppress oxidative stress and degradation of skin structure due to UVB exposure.

FTIR analysis showed that the alcohol functional group (-OH) appeared at a wavelength of 3,400 cm^{-1}, indicating the presence of patchouli alcohol. Several studies have shown that patchouli alcohol is responsible for the characteristic aroma of patchouli oil and belongs to the group of oxygenated sesquiterpenes that can function as antioxidants [27]. Furthermore, the difference between the light fraction and the crude oil was clarified through FTIR spectral analysis. The main difference was observed in the absorption intensity at 3,400 cm-¹, where the light fraction showed a stronger absorption band, indicating a higher content of polar compounds or compounds containing hydroxyl groups compared to the crude oil. In addition, the intensity variation at around 1,700 cm^{-1} indicates differences in carbonyl or ketone functional groups, which may contribute to the different bioactivities of each fraction.

More analysis showed that the crude oil displayed multiple peaks in the fingerprint region (~1000 - 600 cm^{-1}), indicating that the crude oil has a more complex molecular composition than the light fraction. This complexity could potentially affect the mechanism of action and bioactivity effectiveness of each fraction in dermatological and cosmetic applications. Macroscopically, the light fraction of patchouli oil was more effective in ameliorating the effects of UVB on mice's skin than the crude oil. This is consistent with the FTIR results, which show that the smaller molecules in the light fraction are more easily absorbed by the skin. Smaller molecules have better penetration because they can cross the stratum corneum and reach the dermis, thus increasing their effectiveness in skin care.

In addition, light fractions have a lower viscosity and are more easily absorbed than thicker crude oils. Crude oils tend to leave residues on the skin’s surface, which may cause irritation or inhibit the absorption of active compounds. These residues may limit the effectiveness of crude oils in dermatological applications. Although, quantitatively, crude oil showed better improvement. Therefore, these findings suggest that light fractions, with simpler composition and better

absorption properties, are more suitable for use in cosmetics and skincare products.

Ultraviolet B (UVB) radiation is a major cause of premature skin aging (photoaging) by increasing the production of reactive oxygen species (ROS). ROS are short-lived reactive molecules that contain unpaired electrons and can be generated from intrinsic or extrinsic sources, such as cellular metabolism and UV exposure [12,28]. In the mechanism of photoaging, ROS play a role in causing DNA damage, disruption of cellular signalling pathways, and imbalance of oxidants and antioxidants, which accelerate skin damage. **Figure 10** shows the reduction in collagen and elastin caused by UVB exposure.

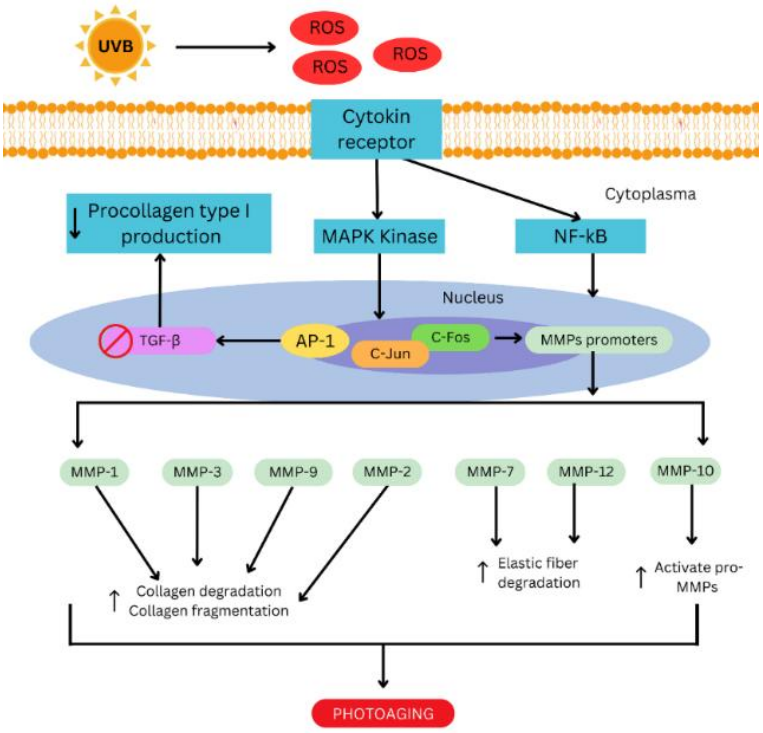

Figure 10 A diagram illustrating how collagen and elasticity vary as we age. The figure is adapted from several studies.

ROS also activates the mitogen-activated protein kinase (MAPK) pathway, consisting of ERK, p38 and JNK, as well as transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). Activation of AP-1 (c-Fos/c-Jun) and NF-κB increases the expression of matrix metalloproteinases (MMPs), such as MMP-1, MMP-3, MMP-9 and MMP-12, which play a role in collagen and elastin degradation [12,28,29]. MMP-1 is the main enzyme that breaks down collagen types I and III, which are further degraded by MMP-3 and MMP-9. Meanwhile, MMP-12, secreted by fibroblasts and macrophages, destroys elastin fibers, causing solar elastosis, which is a decrease in skin elasticity due to the accumulation of damaged elastotic material in the dermis. In addition, AP-1 activation also inhibits the transforming growth factor-beta (TGF-β) pathway, which plays a role in type I procollagen synthesis. As a result, the production of new collagen decreases, thus accelerating skin aging characterized by wrinkles, sagging and loss of suppleness [12].

In addition to collagen and elastin degradation, free radicals can also damage hydrolytic enzymes that regulate the breakdown of filaggrin, which is essential for the formation of a natural moisturizing factor (NMF) in the stratum corneum. NMF is an essential component in maintaining the hydration and barrier function of the skin's epidermis [30]. The main components of NMF come from filaggrin degradation products, such as pyrrolidone carboxylic acid (PCA) and urocanic acid (UCA), which play a role in retaining skin moisture and maintaining optimal epidermal pH. Filaggrin itself is processed from profilaggrin, a major structural protein

in the stratum corneum (SC), which is dephosphorylated and broken down by proteases such as caspase-14, matripase and kallikrein into filaggrin monomers. Once formed, filaggrin monomers undergo deamination or citrulination, which promotes their release from keratin aggregates, before they undergo further degradation into free amino acids, including histidine and glutamine. Histidine is then converted to trans-urocanic acid (UCA), while glutamine is converted to pyrrolidone carboxylic acid (PCA), both of which function as natural moisturizers [31-33]. **Figure 11** shows how UVB light causes a reduction in epidermal hydration.

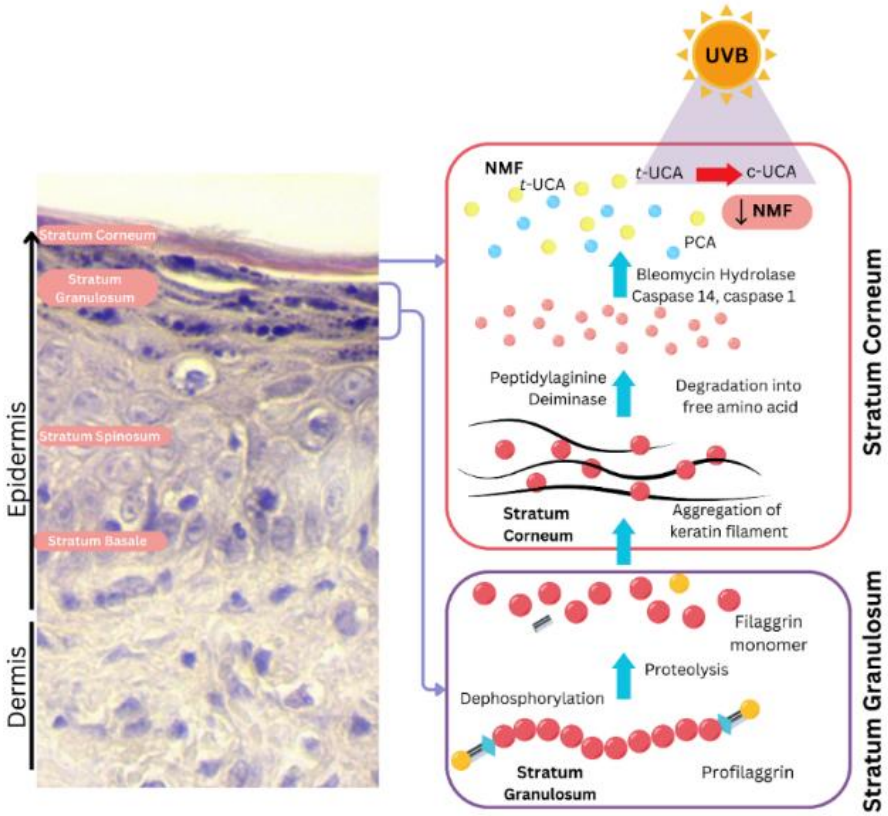

Figure 11 A schematic showing UVB-induced skin moisture loss. Figure obtained from several studies.

UVB exposure contributes to decreased NMF levels in the skin through various mechanisms. One of the main mechanisms is increased oxidative stress due to ROS production, which causes DNA damage and suppresses filaggrin expression in the epidermis. In addition, UVB also increases the activity of proteases such as caspase-14 and kallikrein, which are responsible for filaggrin degradation, leading to uncontrolled breakdown and a decrease in the amount of NMF available in the stratum corneum. This results in decreased skin hydration, impaired epidermal barrier function and increased risk of inflammation and premature aging [34]. The degradation of filaggrin produces hygroscopic amino acids such as pyrocarboxylate and trans-uroccanic acid, which is facilitated by enzymes such as peptidyl arginine deiminase 3 (PAD3). UVB exposure converts trans-uroccanic acid to cis-uroccanic acid (cis-UCA), which reduces NMF production and epidermal hydration [35].

To repair the negative impact of UVB on NMF, antioxidants act as the main protective mechanism. Antioxidants can neutralize ROS generated by UVB exposure, thus preventing oxidative damage to filaggrin and other skin components. In addition, antioxidants also reduce lipid peroxidation in cell membranes, which contributes to the protection of cellular integrity and maintains NMF levels [36].

Patchouli alcohol is a sesquiterpene alcohol found in patchouli oil and has antioxidant properties. The oil also contains phenolic and terpene compounds that contribute to its antioxidant effects. Organic compounds contained in essential oils play an important role in

counteracting free radicals and reducing oxidative stress through the presence of conjugated double bonds and hydroxyl groups in their structure, which are capable of donating hydrogen. The antioxidant effect of patchouli oil mainly works through decreasing ROS as well as inhibiting the NF-κB pathway, which in turn reduces the expression of proinflammatory cytokines [37].

Essential oils and their active constituents penetrate the epidermis through three main pathways: Transcellular (intracellularly through corneocytes), intercellular (through the lipid matrix between corneocytes) and trans appendageal (through hair follicles, sebaceous glands, or sweat ducts). Hydrophilic compounds tend to fall into the intracellular domain, whereas lipophilic compounds traverse the intercellular route. However, the intercellular route is generally considered the main pathway for most molecules. Factors that influence skin penetration include molecular polarity, molecular weight (< 500 Da), concentration of active compounds, oil and water solubility and formulation composition. Essential oils, due to their specific physicochemical properties, can penetrate the skin effectively [38].

In the context of photoaging, this study shows that patchouli oil has potential as a natural anti-photoaging agent that can reduce the negative effects of UVB on the skin. With the increasing demand for natural-based skincare products, these results provide a strong scientific basis for developing essential oil-based anti-aging products. This research is one of the first studies to compare crude oil with the light fraction of patchouli oil in the context of photoaging. The results show that the light fraction has a better penetrating effect and leaves no residue, making it a more ideal candidate for cosmetic formulations.

Patchouli oil, particularly the light fraction, was found to be effective for enhancing collagen synthesis in wound healing [32]. In addition, patchouli oil also minimized other damage caused by photoaging, such as sunburn and hyperpigmentation [26,39,40]. These effects suggest that patchouli oil not only acts as a photoaging prevention agent but also has the potential to improve the condition of skin that has been damaged by UVB exposure. However, although patchouli oil has an overall protective effect, some unexpected results have been observed. For example, P1 (CPO) showed a significant increase in collagen, elasticity and moisture but left an oily residue after seven days. This suggests potential irritation or slower absorption compared to P2, which had a better penetrating effect and left no residue.

Observations of UVB-exposed mouse skin (K−) in **Table 1** and **Figure 1** show that excessive UVB significantly reduces epidermal thickness. The negative control group exhibited a thinner epidermis, particularly in the stratum lucidum, which was reduced to one to two cell layers, while the stratum corneum thickened. These results align with previous study, who reported that UVB-induced ROS increases apoptosis, leading to impaired cell turnover and epidermal thinning. Chronic UVB exposure also induces inflammation, further compromising epidermal integrity and increasing susceptibility to damage and infection.

Application of BioNa serum (K+) post-UVB exposure resulted in an average epidermal thickness of 16.02 µm, comparable to the normal control (K0). BioNa serum, formulated by ARC USK with light fraction patchouli oil and hyaluronic acid, appears effective in restoring skin structure. Hyaluronic acid supports skin hydration and extracellular matrix formation[41], while its degradation by hyaluronidase highlights the need for antioxidant inhibitors to maintain skin integrity.

The highest epidermal thickness (27.29 µm) was observed with light fraction patchouli oil (P2), suggesting superior UVB protection and antioxidant activity. Previous study by Simonsen [42]noted that epidermal thinning correlates with aging and UV exposure, and essential oils can mitigate this by enhancing epidermal health. However, champaca oil treatment increased epidermal thickness beyond normal levels, potentially causing complications and indicating the need for formulation adjustments. Previous research by Zhu [43] associated excessive thickening with abnormal keratinocyte differentiation, emphasizing careful dosing in future formulations.

Essential oils likely promote epidermal thickness through antioxidant action that reduces ROS, preventing mitochondrial DNA damage and keratinocyte apoptosis [44]. Antioxidants inhibit enzymes like NADPH oxidase and bind metal ions to prevent radical formation, enhancing the body's defense system via endogenous antioxidants such as SOD, catalase and glutathione peroxidase [45].

Table 1 and **Figure 1** show a reduction in fibroblast numbers after UVB exposure, consistent with previous result [46], who reported diminished fibroblast activity and collagen production due to UVB-induced ROS. ROS activates MAPK and NF-kB pathways, stimulating AP-1 and increasing MMP expression, which degrades the extracellular matrix [47]. Previous study by Maretta *et al*. [48] further noted that aging fibroblasts impair keratinocyte function and reduce IGF-1 synthesis, contributing to epidermal thinning.

Post-treatment with BioNa serum and patchouli oils (P1 and P2) increased fibroblast numbers, likely due to antioxidant activity promoting proliferation [49]. Patchouli oil's antioxidants support tissue regeneration by improving oxygenation [1] and enhancing collagen expression [50]. Antioxidants also modulate immune responses, stimulate NK cell activity and inhibit carcinogenesis by reducing COX-2 and lipid peroxides [51].

In addition to *in vivo* testing, *in silico* analysis confirmed patchouli oil's potential as an MMP9 inhibitor. MMP9 degrades extracellular matrix components, contributing to skin aging and wrinkle formation. Molecular docking showed that patchouli oil compounds exhibit favorable binding energies (< −7.0 kcal/mol), comparable to the native ligand. Hydrogen bonding further confirmed stable interactions between these compounds and MMP9.

Acetic acid, one of the active compounds, shared hydrophobic interactions (GLU227) with the inhibitor control. Acetic acid's known antimicrobial, antifungal and wound-healing properties support its potential as an anti-aging agent [52]. It lowers skin pH, limits bacterial growth, and accelerates wound healing. Acetic acid also reduces stretch marks and supports gut health by increasing A. muciniphila, which enhances intestinal mucosa and reduces inflammation (inflammaging).

Molecular dynamics simulations (MDS) showed that acetic acid had the lowest RMSD value, indicating stable binding. Although its binding energy was less efficient than the control, its multiple hydrogen bonds and biological activities suggest acetic acid could be a promising anti-aging candidate derived from patchouli oil.

RMSD analysis indicated minimal ligand movement, reflecting stable binding to the MMP9 protein. MM-PBSA calculations confirmed the binding affinity, where more positive values represented stronger interactions. Hydrogen bonds between ligands and proteins were critical for maintaining structural stability and enhancing binding specificity.

This study has several limitations, including the relatively short UVB exposure period (14 days), which may not fully reflect the long-term effects of patchouli oil on photoaging. In addition, using an animal model (mice) suggests metabolic differences from human skin, which could potentially affect the absorption and efficacy of essential oils. The use of BioNa serum as a positive control also presents a limitation, as its full composition is proprietary, making detailed mechanistic comparisons more challenging. Furthermore, while *in silico* analysis suggested potential inhibitory effects of acetic acid on MMP9, direct *in vivo* measurement of MMP9 expression was not performed in this study. In terms of chemical characterization, only FTIR analysis was performed to differentiate the crude and light fractions; a more comprehensive chemical profiling, such as GC-MS analysis of the light fraction, would provide deeper insights into the specific active components contributing to the observed effects. Therefore, future studies should optimize the dosage and frequency of application to ensure long-term safety and effectiveness. Further efforts could include extending the duration of UVB exposure, incorporating human skin models or *in vitro* analysis, and investigating molecular mechanisms such as gene expression related to collagen and elastin synthesis to establish more precise anti-photoaging mechanisms. Additionally, comparative studies with other well-established plant-derived anti-aging agents would provide broader insights into the relative efficacy of patchouli oil in cosmetic applications.

Resolving practical formulation problems is essential for the expanded utilization of patchouli oil in cosmetics. Volatility poses a considerable challenge, since the aromatic chemicals in patchouli oil may evaporate or deteriorate, diminishing efficacy and shelf life; methodologies such as gel or microemulsion systems have demonstrated the capacity to encapsulate the oil and boost stability, hence lowering volatility and facilitating controlled release [53-55]. The stability of pH is a significant concern, as cosmetic products must adhere to a skin-friendly pH range. Research has shown that formulations containing patchouli oil, such as gels,

microemulsions, and creams, can be optimized to sustain stable and suitable pH levels over time, thereby ensuring product safety and user comfort [54-58]. The allergenic potential and likelihood of skin irritation are significant considerations due to the oil's intricate composition; recent studies suggest that properly formulated patchouli oil gels and microemulsions are typically non-irritating and appropriate for topical application, yet thorough testing for irritation and allergenicity is crucial for new products [55,59]. The incorporation of surfactants and stabilizers in microemulsions can augment solubility and stability; however, their selection must account for skin compatibility [54,60]. Advancements in formulation science, including the creation of stable gels, microemulsions and creams, are addressing these problems, enhancing the viability of patchouli oil for safe, effective, and attractive cosmetic uses [53-60].

Conclusions

This study demonstrated the anti-photoaging potential of crude patchouli oil (CPO) and its light fraction (CPO1) against UVB-induced skin damage in Balb/c mice. Topical application of CPO and CPO1 significantly improved skin parameters, including collagen fiber density, elasticity, moisture levels, epidermal thickness, and fibroblast count. Among the two, CPO1 exhibited superior protective effects with minimal skin residue, making it a more promising candidate for anti-aging skincare formulations. FTIR analysis confirmed the presence of key functional groups—hydroxyl, aliphatic, and carbonyl—associated with the bioactive components in patchouli oil. Furthermore, molecular docking and molecular dynamics simulations identified acetic acid from patchouli oil as a potential MMP9 inhibitor, suggesting its role in preventing extracellular matrix degradation and photoaging. These findings highlight the potential of light fraction patchouli oil as a natural anti-aging agent. However, further studies, including extended UVB exposure periods, human skin models and investigations into molecular pathways, are necessary to validate its efficacy and safety for clinical use.

Acknowledgements

The authors are grateful to students and colleagues who contributed to the completion of this study.

Declaration of Generative AI in Scientific Writing

We affirm that all final content, ideas and interpretations presented herein are my own and that the use of AI technologies was conducted in accordance with ethical guidelines and institutional policies. Any AI-generated suggestions were carefully reviewed, edited and integrated under my direct supervision to ensure accuracy, originality, and integrity of the work.

CRediT Author Statement

Essy Harnelly: Conceptualization, Methodology, Supervision, Validation, Funding acquisition, and Writing original draft.

Aliya Hafiya Halym: Data curation and analysis, Investigation, Validation, Visualization, and Writing original draft.

Nadia Isnaini: Data curation, Formal analysis, Investigation, Validation, Visualization, and Writing original draft.

Khairan Khairan: Methodology, Resources, Supervision, and Validation.

Zulkarnain: Methodology, Supervision, and Validation.

Aigia Syahraini: Conceptualization, Software, Data analysis, and Writing original draft.

Widya Sari: Formal analysis, Investigation, and Validation.

Haya Maghfira: Formal analysis, Validation, and Writing original draft.

Syaifullah Muhammad: Methodology, Resources, Supervision, and Validation.

Rinaldi Idroes: Supervision, Writing, and Review Editing.

Mohamed El-Shazly: Supervision, Writing, and Review Editing.

References

[1] H Lee, Y Hong and M Kim. Structural and functional changes and possible molecular mechanisms in aged skin. International *Journal of Molecular Sciences* 2021; **22(22)**,12489.

[2] R Agrawal, A Hu and WB Bollag. The skin and inflamm-aging. *Biology* 2023; **12(11)**, 1396.

[3] MA Tanveer, H Rashid and SA Tasduq. Molecular basis of skin photoaging and therapeutic interventions by plant-derived natural product

ingredients: A comprehensive review. *Heliyon* 2023; **9(3)**, e13580.

[4] C Garg, H Sharma and M Garg. Skin photo-protection with phytochemicals against photo-oxidative stress, photo-carcinogenesis, signal transduction pathways and extracellular matrix remodeling - An overview. *Ageing Research Reviews* 2020; **62**, 101127.

[5] T Tong, R Geng, Kang Seong-Gook, X Li and K Huang. Revitalizing photoaging skin through eugenol in uvb-exposed hairless mice: Mechanistic insights from integrated multi-omics. *Antioxidants* 2024; **13(2)**, 168.

[6] L Hui-Min, C Ming-Yan, X Meng-Han, Z Zhi-Wei, Y Zhang, W Tang, J Cheng, Jia Ni and W Wang. Possible mechanisms of oxidative stress-induced skin cellular senescence, inflammation and cancer and the therapeutic potential of plant polyphenols. *International Journal of Molecular Sciences* 2023; **24(4)**, 3755.

[7] T Xiong, Z Zhang, R Zheng, J Huang and L Guo. N-acetyl cysteine inhibits lipopolysaccha-ride-induced apoptosis of human umbilical vein endothelial cells via the p38MAPK signaling path-way. *Molecular Medicine Reports* 2019; **20(3)**, 2945-2953.

[8] GA Cabral-Pacheco, I Garza-Veloz, CCD Rosa, JM Ramirez-Acuña, BA Perez-Romero, JF Guerrero-Rodriguez, N Martinez-Avila and ML Martinez-Fierro. The roles of matrix metalloproteinases and their inhibitors in human diseases. *International Journal of Molecular Sciences* 2020; **21(24)**, 9739.

[9] N Miekus, C Luise, W Sippl, T Baczek, CEH Schmelzer and A Heinz. MMP-14 degrades tropoelastin and elastin. *Biochimie: A Multidisciplinary Journal for the Rapid Dissemination of Current Research in Biochemistry* 2019; **165**, 32-39.

[10] M Chaudhary, A Khan and M Gupta. Skin ageing: Pathophysiology and current market treatment approaches. *Current Aging Science* 2020; **13(1)**, 22-30.

[11] G Ge, Y Wang, Y Xu, W Pu, Y Tan, P Liu, H Ding, L Yu-Meng, J Wang, W Liu and Y Ma. Induced skin aging by blue-light irradiation in human skin fibroblasts via TGF-β, JNK and EGFR pathways. *Journal of Dermatological Science* 2023; **111(2)**, 52-59.

[12] S Jung-Won, K Soon-Hyo, C Ji-Young, N Jung-Im, H Chang-Hun, C Hye-Ryung and P Kyung-Chan. Molecular mechanisms of dermal aging and antiaging approaches. *International Journal of Molecular Sciences* 2019; **20(9)**, 2126.

[13] X He, F Wan, W Su and W Xie. Research progress on skin aging and active ingredients. *Molecules* 2023; **28(14)**, 5556.

[14] S Srivastava, RK Lal, VR Singh, PK Rout, RC Padalia, AK Yadav, L Bawitlu, D Bhatt, AK Maurya, A Pal, DU Bawankule, A Mishra, P Gupta and CS Chanotiya. Chemical investigation and biological activities of patchouli (pogostemon cablin (Blanco) Benth) essential oil. *Industrial Crops and Products* 2022; **188(A)**, 115504.

[15] F Xue-Xuan, Y Xiu-Ting, L Wen-Jie, K Song-Zhi, L Yu-Hong, X Zhang, X Yan-Fang, Z Xiao-Jun, S Zi-Ren and L Zhi-Xiu. Effects of topical application of patchouli alcohol on the UV-induced skin photoaging in mice. *European Journal of Pharmaceutical Sciences* 2014; **63**, 113-123.

[16] Grand View Research, Available at: https://www.grandviewresearch.com/industry-analysis/natural-cosmetics-market, accessed March 2025.

[17] K Tsukahara, S Moriwaki, T Fujimura and Y Takema. Inhibitory effect of an extract of sanguisorba officinalis l. on Ultraviolet-B-Induced photodamage of rat skin. *Biological and Pharmaceutical Bulletin* 2001; **24(9)**, 998-1003.

[18] A Citrawan, O Suwarsa, H Gunawan, S Adi, R Lesmana, A Achadiyani and SS Adi. Pengaruh krim asam traneksamat terhadap pembentukan keriput dan kadar matriks Metaloproteinase-1 pada mencit (Mus Musculus) jantan galur Balb/c yang dipajan sinar Ultraviolet B. *Indonesian Journal of Clinical Pharmacy* 2019; **8(2)**, 121.

[19] SC Gilliver, JJ Ashworth and GS Ashcroft. The hormonal regulation of cutaneous wound healing. *Clinics in Dermatology* 2007; **25(1)**, 56-62.

[20] K Flurkey, J Currer and D Harrison. *The-mouse-in-biomedical-research*. Volume 3. Academic Press, New York, 2007, p. 637-672.

[21] WT Federer. *Experimental design: Theory and application.* Oxford & IBH Publishing Company, Oxford, 1967.

[22] D Rahmi, R Yunilawati, BN Jati, I Setiawati, A Riyanto, I Batubara and RI Astuti. Antiaging and skin irritation potential of four main Indonesian essential oils. *Cosmetics* 2021; **8(4)**, 94.

[23] S Kandeel and M Balaha. The possible protective effect of simvastatin and pioglitazone separately and in combination on bleomycin-induced changes in mice thin skin. *Tissue and Cell* 2015; **47(2)**, 159-170.

[24] H Maghfira. *Analisis jejaring farmakologi dan kajian pemodelan molekuler anti-photoaging dari minyak nilam (Pogestemon cablin Benth.).* Universitas Syiah Kuala, Banda Aceh, Indonesia, 2024.

[25] LS Desiyana, N Isnaini, V Prajaputa, SS Bilqis and M Ariza. Pelatihan purifikasi minyak nilam aceh secara distilasi molekuler sebagai analgesik topikal pada medicated oil untuk kelompok usaha masyarakat. *Jurnal Pengabdian Magister Pendidikan IPA* 2023; **6(3)**, 838-842.

[26] KJS Kumar, MG Vani, M Chinnasamy, WT Lin and SY Wang. Patchouli alcohol: A Potent tyrosinase inhibitor derived from Patchouli essential oil with potential in the development of a skin-lightening agent. *Cosmetics* 2024; **11(2)**, 38.

[27] W Pei-Wen, H Yu-Chiang, L Tung-Yi, F Jia-You, Y Pei-Ming, C Mu-Hong and P Tai-Long. Comparison of the biological impact of UVA and UVB upon the skin with functional proteomics and immunohistochemistry. *Antioxidants* 2019; **8(12)**, 569.

[28] L Rong-Feng, F Xue-Xuan, L Chu-Wen, Z Xiao-Jun, Y Xiu-Ting, Z Jiu-Yao, X Zhang, X You-Liang, S Zi-Ren, and JYX Zhan. Prevention of UV radiation-induced cutaneous photoaging in mice by topical administration of patchouli oil. *Journal of Ethnopharmacology* 2014; **154(2)**, 408-418.

[29] A Rachmatillah, D Hasni and Y Aisyah. Uji aktivitas antioksidan minyak sereh wangi (*Cymbopogon nardus* (L.) Rendle), minyak nilam (*Pogostemon cablin* Benth.) dan minyak pala (*Myristica fragrans* Houtt). *Jurnal Ilmiah Mahasiswa Pertanian* 2021; **6(4)**, 442-446.

[30] S Muhammad, H Hisbullah, J Rahmi, FN Ritonga and V Prajaputra. Vacuum distillation of aceh patchouli oil into hi-grade and crystal patchouli with rotary vacuum evaporator. *Journal of Patchouli and Essential Oil Products* 2022; **1(2)**, 36-41.

[31] H Kautsarah, J Juanda and M Martunis. Peningkatan kualitas minyak nilam aceh selatan dengan menggunakan rotary vacuum evaporator. *Jurnal Ilmiah Mahasiswa Pertanian* 2023; **8(1)**, 236-247.

[32] M Taufik, S Rizal, E Harnelly, S Muhammad, D Syahrizal, V Prajaputra and N Isnaini. The therapeutic potential of aceh patchouli oil (pogostemon cablin benth.) in enhancing full-thickness wound healing in mice. *Tropical Journal of Natural Product Research* 2024; **8(1)**, 5840-5844.

[33] M Wei, X He, N Liu and H Deng. Role of reactive oxygen species in ultraviolet-induced photodamage of the skin. *Cell Division* 2024; **19(1)**, 1.

[34] P Pittayapruek, J Meephansan, O Prapapan, M Komine, M Ohtsuki. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *International Journal of Molecular Sciences* 2016; **17(6)**, 868.

[35] S Dunaway, R Odin, L Zhou, L Ji, Y Zhang and AL Kadekaro. Atural antioxidants: Multiple mechanisms to protect skin from solar radiation. *Frontiers in Pharmacology* 2018; **9**,392.

[36] MA McAleer and AD Irvine. The multifunctional role of filaggrin in allergic skin disease. *Journal of Allergy and Clinical Immunology* 2013; **131(2)**, 280-291.

[37] G Yang, JK Seok, HC Kang, YY Cho, HS Lee and JY Lee. Skin barrier abnormalities and immune dysfunction in atopic dermatitis. *International Journal of Molecular Sciences* 2020; **21(8)**, 2867.

[38] A Herman and AP Herman. Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: A review. Journal of Pharmacy and Pharmacology. 2015; **67(4)**, 473-485.

[39] TM Ansary, MR Hossain, K Kamiya, M Komine and M Ohtsuki. Inflammatory molecules associated with ultraviolet radiation-mediated skin

aging. *International Journal of Molecular Sciences* 2021; **22(8)**, 3974.

[40] M Michalak. Plant-derived antioxidants: Significance in skin health and the ageing process. *International Journal of Molecular Sciences* 2022; **23(2)**, 585.

[41] V Moosbrugger-Martinz, C Leprince, M Marie-Claire, M Simon, S Blunder, R Gruber and S Dubrac. Revisiting the roles of filaggrin in atopic dermatitis. *International Journal of Molecular Sciences* 2022; **23(10)**, 5318.

[42] S Simonsen, J Thyssen, S Heegaard, S Kezic and L Skov. Expression of filaggrin and its degradation products in human skin following erythemal doses of Ultraviolet B irradiation. *Acta Dermato Venereologica* 2017; **97(7)**, 797-801.

[43] X Zhu, N Li, Y Wang, L Ding, H Chen, Y Yu and X shi. Protective effects of quercetin on UVB irradiation-induced cytotoxicity through ROS clearance in keratinocyte cells. *Oncology Reports* 2017; **37(1)**, 209-218.

[44] F Noor, M Khalid and A Saeed. Computational drug designing: A new paradigm for the treatment of Parkinson's disease. *Biomedical Letters* 2020; **6(2)**, 164-176.

[45] PL Karim, IA Aryani and Nopriyati. Anatomy and histologic of intrinsic aging skin. *Journal of Biomedicine and Translational Research* 2021; **5(11)**, 1165-1177.

[46] GC Limandjaja, LJ Broek, T Waaijman, HA Veen, V Everts, S Monstrey, RJ Scheper, FB Niessen and S Gibbs. Increased epidermal thickness and abnormal epidermal differentiation in keloid scars. *British Journal of Dermatology* 2017; **176(1)**, 116-126.

[47] SR Kim, JW Park, BH Lee, KM Lim and TS Chang. Peroxiredoxin V protects against UVB-Induced damage of keratinocytes. *Antioxidants* 2023; **12(7)**, 1435.

[48] G Maretta, I Fitriya, RE Putra and UKS Ramadhani. Efek pemberian ekstrak kulit pisang kepok (musa acuminata x balbisiana) terhadap kulit mencit (mus musculus) yang terpapar sinar ultraviolet. *Al-Kauniyah: Jurnal Biologi* 2023; **16(2)**, 301-318.

[49] SN Yusharyahya. Mekanisme penuaan kulit sebagai dasar pencegahan dan pengobatan kulit menua. *E-Journal Kedokteran Indonesia* 2021; **9(2)**, 150-159.

[50] SH Shin, YH Lee, NK Rho and KY Park. Skin aging from mechanisms to interventions: Focusing on dermal aging. *Frontiers in Physiology* 2023; **14**, 1195272.

[51] E Pujiastuti and R Islamiyati. Aktivitas antioksidan fraksi etil asetat dan air ranting buah parijoto (medinilla speciosa blume) dengan perendaman radikal bebas dpph. *Cendekia Journal of Pharmacy* 2021; **5(2)**, 135-144.

[52] N Cahaya, E Erfenna and D Rahmawanty. Pengaruh pemberian gel kuersetin terhadap jumlah fibroblas dan re-epitelisasi dalam proses penyembuhan luka bakar derajat IIa pada tikus jantan. *Journal of Current Pharmaceutical Sciences* 2018; **2(1)**, 89-96.

[53] R Guo, W Xu, Y Wang, L Yue, S Huang, Y Xiu, Y Huang and B Wang. A spatially stable crystal-particle gel to trap patchouli oil for efficient colonic drug delivery. *ACS Applied Materials & Interfaces* 2024; **16(22)**, 29198-29209.

[54] B Iskandar, A Lukman, R Tartilla, MDC Surboyo and Leny. Formulasi, karakterisasi dan uji stabilitas mikroemulsi minyak nilam (Pogostemon cablin Benth.). *Jurnal Ilmiah Ibnu Sina* 2021; **6(2)**, 282-291.

[55] T Chen, C Xu, M Wang, Y Cui, R Cheng, W Zhang, X Gao, L Wang, H Qi, S Yu, J Chen, L Ma and H Guo. Preparation of patchouli oil microemulsion gel and its topical application to ameliorate atopic dermatitis in mice. *Gels* 2024; **10(12)**, 796.

[56] H Wahyudi, H Hamzah and I Yudhawan. Formulation and physical quality evaluation of aromatherapy oil combining patchouli (Pogostemon cablin Benth.) and peppermint (Mentha piperita L.) essential oils. *Acta Pharmaceutica Indonesia* 2024; **12(1)**, 11608.

[57] NF Ramadani and P Minerva. Utilization of patchouly oil as facial wash cosmetic for acne-prone skin. *Multiple Sclerosis Journal* 2024; **2(3)**, 207-213.

[58] N Isnaini, V Prajaputra and S Maryam. Formulation and evaluation of O/w body cream containing patchouli oil (Pogostemon cablin Benth.) and drumstick oil (Moringa oleifera) as

potential moisturizing agent. *Journal of Research in Science Education* 2023; **9(10)**, 8001-8007.

[59] N Malik, Y Nikookam, J Hirniak and A Sayedyusef. Poster presentationsH05 Tutankhamun's patchouli slumber: Are we sleeping on it in dermatology? *British Journal of Dermatology* 2024; **191(S1)**, i167-i168.

[60] F Febriyenti, N Suharti and RF Putri. Formulation and evaluation of patchouli oil microemulsion and microemulgel for inhibit the bacterial growth. *Research Journal of Pharmacy and Technology* 2022; **15(1)**, 51-55.