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Original Article

FORMULATION OF ANDALAS TWIG (*MORUS MACROURA* MIQ.) EXTRACT LOTION AND EVALUATION OF ITS ACTIVITY

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ABSTRACT

Objective: Indonesia has a relatively high UV exposure, which can cause skin damage, including premature aging through oxidative stress. Andalas twig (*Morus macroura* Miq.) has antioxidant and anti-inflammatory compounds that have great potential to protect the skin from UV damage. This study aims to formulate the lotion from Andalas twig extract and observe the antioxidant and anti-inflammatory activity to reduce the impact of premature aging due to extreme UV-B exposure.

Methods: Andalas twigs were macerated with ethanol 70% and tested antioxidant activity using the DPPH method. The extract was then formulated in a lotion dosage form at concentrations of F1 (1%), F2 (3%), and F3 (5%). The lotion was evaluated for its properties for 14 days. Histological examination using the skin of a *Mus musculus l* male balb/c strain under a microscope was also done to determine anti-inflammatory activity on day one and day 21 of treatment. Descriptive analysis was carried out to interpret the histological results.

Results: The extract of Andalas twigs yielded 4% and an IC_{50} value of 339.31 ppm for antioxidant activity using DPPH. Based on the observation, all lotion formulas were yellowish-white in color, homogenous, and with pH in a range of 6-7. The histological assessment didn't show collagen formation as an effect of antioxidant activity. Still, it showed anti-inflammatory activity due to oxidative stress in the form of protective and repair effects on day 21 for lotion F1 and F2. Meanwhile, lotion F3 has cell poly-variation, indicating chronic inflammation.

Conclusion: Lotion of Andalas twig extract did not show antioxidant activity in DPPH but has anti-inflammatory activity at a concentration of 3% (F2) to relieve oxidative stress symptoms. The observed effects may be attributed to bioactive compounds within the extract, highlighting its therapeutic potential. Further research is recommended to confirm the antioxidant activity of Andalas tree twig extracts and validate their potential health benefits.

Keywords: Morus macrora Miq., Lotion, Premature aging, Oxidative stress, UV-B radiation, Antioxidant, Antiinflamation

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INTRODUCTION

Indonesia has a tropical climate, and UV exposure is relatively high. UV exposure that occurs intensively can impact health and may trigger the formation of reactive oxygen species (ROS), which can cause cellular damage and accelerate the skin's premature aging [1]. As a mechanical barrier, skin is a critical defense to protect the human body. The damage to skin cells can have significant impacts, such as redness, pigmentation, and even cancer risk, as skin protects the body from external influences. Moreover, any damage to the skin will interfere with human health and performance; thus, the skin must be maintained and protected. One of the causes that can trigger skin damage is free radicals such as ultraviolet rays. In excessive conditions, UV rays can cause several skin problems, as described previously. The free radicals produced will lead to DNA damage and cause an inflammatory response, impacting continuous cell proliferation and triggering cancer cell formation [2].

These adverse effects arise from oxidative stress after excessive UV exposure, resulting from an imbalance between prooxidants (reactive oxygen species) and antioxidants [2, 3].

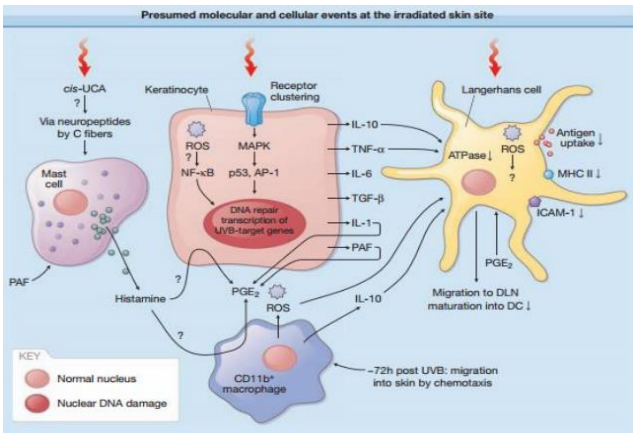

Fig. 1: UV radiation-induced immunosuppression: illustration of molecular and cellular changes in skin subjected to radiation. aTPase =adenosine triphosphatase; DC =dendritic cells; DlN =drainage lymph node; Il =interleukin; ICaM =intermolecular adhesion of molecules; MaPK = mitogen-activated protein kinase; MHC = major histocompatibility complex. NF-κb = nuclear factor κb; PaF = platelet-activating factor; PGE2 = prostaglandin E2; ROS = reactive oxygen species; TGF = transforming growth factor; TNF = tumor necrosis factor; UCa = urocanic acid. Adopted from *Kochevar et al.* [4]

Oxidative stress can be prevented and reduced by providing sufficient antioxidants to the body. Antioxidants have a function of inhibiting cell damage, including oxidation reactions, in which antioxidants will bind to highly reactive molecules such as oxidation reactions. To protect the body from free radical attacks, such as UV rays, antioxidants are needed to stabilize free radicals by complementing the electron deficiency of free radicals to inhibit the occurrence of chain reactions. Antioxidants can also act as hydrogen radical contributors or free radical acceptors. Oxidative stress management is assisted by anti-inflammatories that reduce the body's inflammatory response to damage caused by free radical [5, 6].

The Andalas tree, which grows in Indonesia, including West Sumatra province, is known as a source of phenolic compounds. This plant has also produced various secondary metabolites, including various flavonoid compounds. These compounds have been reported as having broad biological activities, such as anti-inflammation [7, 8]. Therefore, this plant offers benefits, particularly in overcoming skin problems. Previous research reported *M. macroura* twigs to contain bioactive compounds similar to those found in *M. alba,* which have high phenolic, tannin, and flavonoid content, which contribute to antioxidant and anti-inflammatory activities. The selection of Andalas twigs *(Morus macroura* Miq.) as the research object is based on its bioactive potential which has not been widely explored compared to other parts of the plant such as stem and roots [9, 10]. This could be a sustainable raw-material resource from Andalas plants, considering the growth and breeding process of the Andalas tree (*Morus macroura* Miq).

As Indonesia is considered a tropical country with more UV exposure, thus prevention is urgently needed to anticipate skin damage. A study has reported using extract roots murberi (*Morus alba*) in lotion dosage form, which showed that it could protect the skin without irritation [11, 12]. This study introduced an innovation by formulating a lotion dosage form with a variation in the concentration of Andalas twig extract. The lotion dosage form is generally known to have some advantages, including a very light dosage form, and the application can be evenly distributed throughout the body compared to other preparations [13, 14]. Then, the lotion from Andalas twigs was evaluated for its performance, including appearance and pH. Moreover, a histology study was also done to assess its biological effect on the cell skins, which is expected to explore the potential pharmacological effect of the Andalas twig extract.

MATERIALS AND METHODS

Materials

Twigs of Andalas tree, methanol (Merck, Germany), Ethanol 70% (Merck, Germany), DPPH (Himedia, India), ascorbic acid (Merck, Germany), stearic acid (SmartLab, Indonesia), triethanolamine (TEA) (Petronas, Malaysia), glycerin (Merck, Germany), cehtyl alkohol (bratachem indonesia),olive oil (Borges, Spanish), Tween 80 (Himedia India), methylparaben (Himedia, India), fragrance apple (Eastman, America).

Methods

Sampling

Andalas-tree twigs (*M. macroura*) that grow in Bung Hatta Forest Park, Padang City, were collected on soft twigs that did not contain much cellulose and collected in August 2023. Samples were identified at the Herbarium ANDA at Universitas Andalas.

Extraction of andalas-tree twigs (*M. macroura*)

The sample was sliced and dried and then extracted by maceration for 72 h with a ratio of sample and 70% ethanol of 1:5. Then, the filtrate was obtained, and solvent evaporation was performed using a rotary evaporator (Buchi®, Switzerland) to obtain a thick extract.

Phytochemical screening and ash content determination

Phytochemical screening of extracts such as alkaloids, flavonoids, phenolics, saponins, steroids, and triterpenoids, including total ash content, was done according to the Indonesian Herbal Pharmacopeia [15].

Antioxidant activity test of andalas tree twigs (*M. macroura*)

The antioxidant activity of Andalas twig extract was done using the DPPH method, and ascorbic acid was used as the standard. The assay was conducted using 96 well microplates. The standard solution was 10 mg of ascorbic acid and diluted ethanol p. a. with a concentration of 100, 50, 25, 12.5, 6.26, 3.125, and 1.5625 ppm. At the same time, the test solution was 10 mg of sample and diluted ethanol *p. a.* with a concentration of 1000, 800, 600, 400, 200, 100, and 50 ppm.

A 96-well microplate. A 100 µl test and ascorbic acid solution (A_1) was reacted with 100 µl DPPH µl solution and incubated in a dark room at 27 °C for 30 min. Blanko (A_0) A total of 100 µl ethanol p. a. was reacted with 100 µl DPPH 500 µM solution and incubated in a dark room at 27 °C for 30 min; the experiment was carried out in triplicate.

Absorbance was measured using a Microplate Reader ELISA (All Sheng, China) at a wavelength of 517 nm. Absorbance data were tabulated in a table, and the percentage of inhibition was calculated using the equation below.

$$\boldsymbol{Inhibition} = \left(\mathbf{1} - \frac{\boldsymbol{A1}}{\boldsymbol{A0}}\right)\boldsymbol{x}\mathbf{100}\%$$

The concentration relationship data with the percentage of inhibition was then plotted into a linear graph, and the regression equation was obtained. From this regression equation, the IC_{50} value was determined [16].

Formulation of extraction of andalas twigs (*M. macroura*) lotion

The lotion extract from Andalas twigs was formulated at 1%, 3%, and 5% concentrations as F1, F2, and F3 respectively. The formula and the function of each lotion ingredient, as seen in table 1, refer to a previous study [6].

Table 1: Formulation of lotion extract of andalas tree twigs (*M. macroura*) [17]

Ingredients	Concentration (%) b/v			Function
	F1	F2	F3	
Extract of andalas tree twigs (*M. macroura*)	1	3	5	Active ingredient
Stearic acid	5	4	4	Emulsifying agent
Triethanolamine (TEA)	1	0.5	0.5	Alkalizing
Olive oil	20	2	2	Oil Phase
Cetyle alcohol	3	3	3	Co-emulsifer
Glyserin	20	5	5	Humectant
Tween 80	10	10	10	Surfactant
Methylparaben	2	2	2	Preservative
Fragrance apple	qs	qs	qs	Fragrance
Aquadest ml (add)	100	100	100	Solvent

Evaluation of andalas twigs extract lotion (*M. macroura*)

The review of Andalas twig extract in lotion dosage form was done, including:

a. Organoleptic

The organoleptic test was done by direct observation, including the lotion's color, shape, and smell, and was carried out at room temperature until day 14.

b. Homogeneity

A homogeneity test was conducted using two glass objects. The lotion preparation should show a homogeneous composition (evenly mixed) and no visible coarse particles. This evaluation was carried out up to day 14 at room temperature [17].

c. pH

The pH test was carried out using a pH meter (Mettler Toledo, Switzerland). The preparation was taken a little and diluted with distilled water; then, the pH paper was added to the sample to measure the pH. This measurement was carried out until day 14 at room temperature [17].

d. Cycling test

The cycling test is an accelerated method to check the stability of lotion in which one cycle is counted as the lotion was stored at 4 ℃for 24 h, then removed and placed at 40 ℃ for 24 h in a climatic chamber (Memmert, Germany). The experiment was repeated for four cycles; each cycle was observed with several parameters, including organoleptic, homogeneity, and pH, to see whether or not there were changes in the physical properties of the lotion [18].

e. Spreadability test

The spreadability test was carried out by weighing 0.5 g of Andalas tree twig extract lotion and placing it in the middle of a petri dish. Then, another Petri dish weighing gradually (50 and 200 mg) was put above the first petri dish and allowed to stand for 1 min. The diameter of the spread is measured based on the ability of the lotion to spread on the petri dish [17].

Histological assessment of mice skin (*Mus musculus*)

This research was conducted in 48 female mice (*M. musculus*) according to the Federer formula: (t-1) (n-1)≥15. The animal testing was divided into four groups, K0 being a control group that only receives UV-B light exposure. The treatment groups consisted of K1, K2, and K3, applying lotion F1, F2, and F3 consecutively. Before spreading the lotion on the *M. musculus* skin, the UV-B light was exposed for 120 min on mouse skin with a skin area of 3x3 cm that had been haired off. The lotion was then spread about one g twice daily on the backs of the marked mice. During the treatment, mice lived in the animal house of the Faculty of Pharmacy, University of Andalas, with facilities according to the standards set by the Faculty of Pharmacy, University of Andalas; mice lived in cages with a caged area of 40x30x16 cm and received commercial feed which was monitored 2-3 times a day. The histological examination was carried out by overall observation using the HE (Hematoxylin and Eosin) staining technique from the lowest magnification (40x) to the highest magnification (400x) using a microscope (Olympus BX51, Japan) and camera (Olympus DP20, Japan). The ethics of this experiment was approved by the Faculty of Pharmacy Universitas Andalas with number 49/UN.16.10. KEPK-FF/2023 and follows the guidelines of the OECD (Organization for Economic Cooperation and Development),

DATA ANALYSIS

The data obtained were analyzed descriptively through the appearance obtained in the histology cross-section and correlated with other results.

RESULTS AND DISCUSSION

Twenty g of Andalas tree twigs (*M. macroura*) extract was obtained from 500 g of dried and mashed Andalas tree twigs. The total ash content of the extract was 5.7%, which met the standard for Indonesian herbal pharmacopeia (less than 10.2%) [15]. The percentage of total ash is expressed by the amount of mineral in the extract.

The phytochemical screening of Andalas tree twig extract qualitatively showed positive results for flavonoid, phenolic, and steroid compounds. These results matched with previous research, in which the roots, stems, and twigs of the Andalas tree are rich in antioxidant compounds derived from the stilbene group and anti-inflammatory compounds that are very useful in the field of health [8, 19, 20]. The species of *M. macroura* produces isoprenylated flavonoids, stilbene chromones, and 2-2-aribenzopyran, which have been biologically proven to be anti-inflammatory agents [10]. This effect can increase the potential for skin cell repair due to oxidative stress.

Antioxidant activity of andalas tree twigs extract (*M. macroura*)

The DPPH test is carried out to determine the antioxidant activity contained in the Andalas tree twig extract. This method offers benefits, including high efficiency in analyzing many samples, due to its simple procedure and short processing time. In addition, this assay is also highly reproducible, so the results obtained are more consistent. According to the results, the Andalas tree twig extract has an IC_{50} value of 339.31 ppm and an SD population value of 8.4, while ascorbic acid was 14.42 ppm. In antioxidant testing using the DPPH method, the smaller the IC_{50} value, the stronger the antioxidant activity. This indicates that the antioxidant activity contained in the Andalas tree twig extract is low [19].

Evaluation of lotion of andalas tree twigs

The evaluation result for the formula of Andalas twig extract in the lotion dosage form is presented in table 2, and the outcome of the cycling test is in table 3. The lotion formulation was evaluated with the following parameters: organoleptic, homogeneity, pH, and spreadability. The organoleptic observed until day 14 showed color degradation from all the lotion formulas, and the aroma that was getting longer lost its apple aroma. That alteration was likely due to the oxidizing process of some compounds containing phenolic groups [21, 22]. The homogeneity of all formulas was homogenous for up to one week, and then there was a phase separation in week two for formulas 2 and 3 (F2 and F3). The separation phase in F2 and F3 was likely due to the inability or insufficiency of the emulsifying agent to act as a stabilizer in the formulation of the oil and water phases. The pH examination showed constant results ranging from 6-7; per SNI 16-4399-1996, lotion is applied to the skin with a pH ranging from 4.0 to 7.0. The lotion's spreadability met the spreadability test's requirements, which showed a diameter of 5-7 cm. This result indicated good spreadability in which the application and use of lotion will be maximized [20].

Table 2: Result of lotion andalas twigs extract evaluation

Evaluation		Organoleptic	Homogeneity	pH
Day-1	FL1	Yellowish white, apple fragrance	Homogeneity	7
	FL2	Yellow apple fragrance	Homogeneity	6
	FL3	Yellow-brown, apple fragrance	Homogeneity	6
Day-7	FL1	Yellowish white, apple fragrance	Homogeneity	7
	FL2	Yellow apple fragrance	Homogeneity	7
	FL3	Yellow-brown, apple fragrance	Homogeneity	7
Day-14	FL1	Yellowish white, apple fragrance	Homogeneity	7
	FL2	Yellow, white fragrance	Not Homogeneity	7
	FL3	Yellow-brown, flavorful	Not Homogeneity	7

The cycling test aims to see the preparation's stability, which implies the sample's physical properties in fluctuating temperature conditions. The lotion of Andalas twig extract had prominent changes every cycle in each formula. Degradation occurs faster and is visible organoleptically; the separation of water and oil phases occurs in the 2nd cycle, and gas is formed, characterized by bubbles appearing in the 3rd and 4th cycles.

Table 3: The result cycling test of lotion andalas twigs

Day	1	2	3	4
FL1	Yellowish white, Apple fragrance, Homogeneous	Two phases formed: apple fragrance	Formed gas, Apple fragrance	Yellow foam and apple fragrance
FL2	Yellow, Apple fragrance, Homogeneous	Two phases formed: apple fragrance	Discoloration to brown and apple fragrance	Brown foam, Odor Foaming
FL3	Yellow-brown, Apple fragrance Homogeneous	Two phases formed: apple fragrance	Discoloration to blackish brown and Formation of gas, Apple fragrance	blackish brown foam, Smell of foamy

Histological cross-section of mice skin (*Mus musculus*)

Intense exposure to UV-B light can trigger the formation of ROS until oxidative stress occurs, which can cause damage at the cellular level. In UV exposure, the first layer exposed is the skin; the effects of oxidative stress on the skin can accelerate premature aging and erythema [23]. Excessive UV exposure can damage collagen and elastin, causing the skin to sag. Some indicators of premature aging that can be seen are sagging skin, wrinkles, and discoloration of the skin to become redder and paler, commonly called erythema [24, 25]. The histology of *M. musculus* skin after applying lotion Andalas twig is shown in fig. 2 and 3.

UV-B light was exposed to mice (*M. musculus*) to form erythema, an indicator of oxidative stress and one of the characteristics of premature aging. Erythema can be categorized as one of the manifestations of the skin inflammatory process. The initial phase of erythema occurs within 4-12 h [26]. Skin exposure to UV-B radiation for 30 min can induce oxidative stress, which can significantly affect the oxidation equilibrium in the skin.

The results shows that on day one, after UV-B exposure and day one lotion application, the surface epithelium underwent necrosis with dense clusters of inflammatory cells, very different from the control, which showed intact epithelium. Formula 1 showed detached epithelium with a visible cavity at the dermis-epidermis boundary. In contrast, formula two only formed a slight gap, and formula three did not appear to have a gap between the dermis-the epidermis. This shows the protective effect of the lotion against sloughing of the epithelium from its base. The lotion forms a layer that holds the epithelium so that no wounding occurs but causes the skin surface to harden.

On day 21, there was an improvement in the skin epithelium resembling the control after the administration of formula 1, indicating the protective properties of the lotion in overcoming the inflammation that occurred, while on the administration of formula 2, there was a thickening of the skin epithelium with keratinized layers within normal limits. In contrast, formula 3 appeared to have excessive skin epithelium growth with clumped keratin formation. The growth in Formula 3 showed a proliferative effect. This proliferative growth occurs due to keratinocyte response; UV-B exposure can activate keratinocytes, leading to increased proliferation and differentiation, essential for epidermal repair [16]. However, if the reaction arises excessively due to continuous inflammation, it should be avoided because it may lead to tumor-like formation.

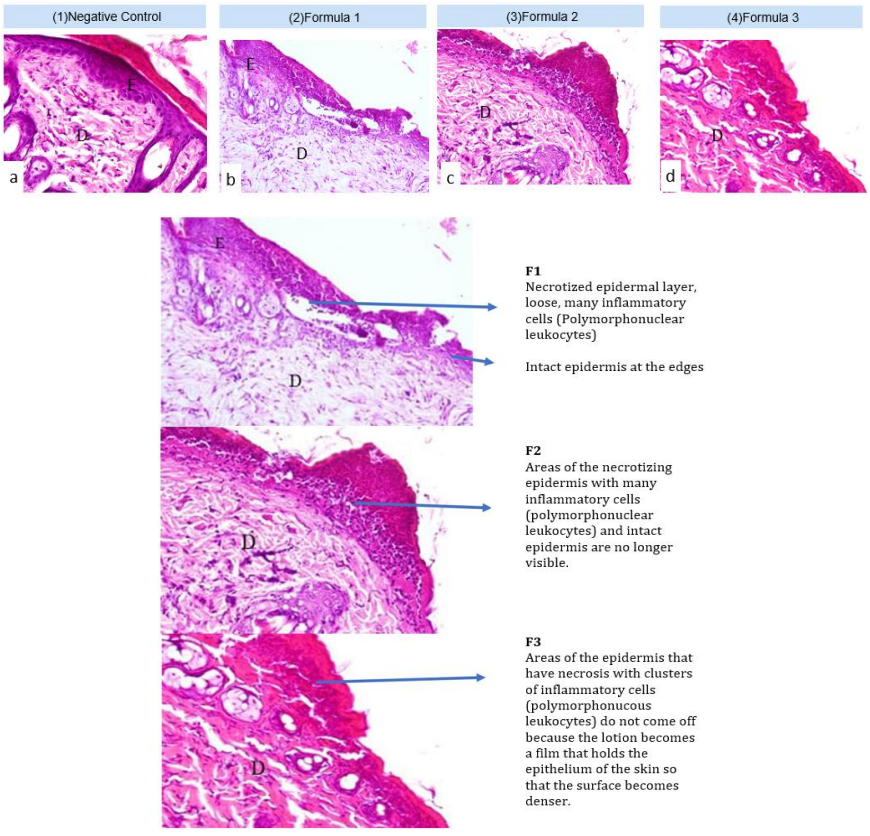

Fig. 2: Histopathological differences of mice skin in control and treatment groups on day one. E is the epidermis layer, and D is the dermis; on day one, after the lotion of Andalas twig extract was applied (Hematoxylin-eosin, 20x objective magnification, scale 1:200µm)

Morus tree twigs contain various bioactive compounds, including flavonoids and stilbenes, which are important in their anti-inflammatory properties [27]. These compounds work synergistically to modulate inflammatory pathways and reduce oxidative stress, which is a major factor in the development of chronic inflammation [28]. Morus twig extract was also shown to significantly reduce the expression of inflammatory mediators such as IL-17A and COX-2, both *in vitro* and *in vivo* [29]. In addition, this extract inhibits the activation of NF-κB, a key transcription factor in the inflammatory process, which in turn reduces the expression of various inflammatory mediators and reduces the overall inflammatory response [28, 29].

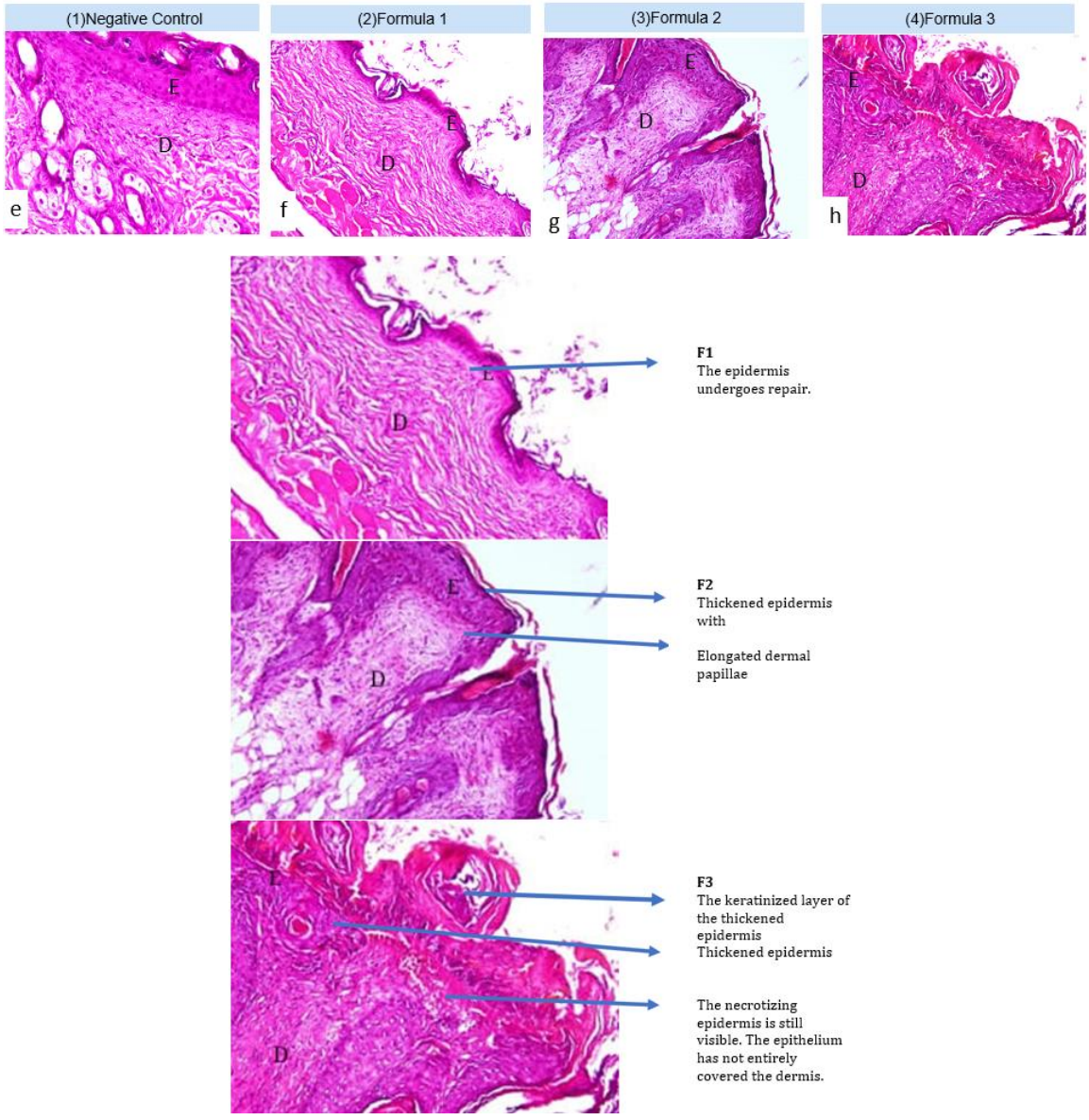

Fig. 3: Histopathological differences of mice skin in control and treatment groups on days 21. E is the epidermis layer, and D is the dermis on day 21 after the applied lotion of Andalas twig extract (Hematoxylin-eosin, 20x objective magnification, scale 1:200µm)

The collagen score cannot be calculated because there is no collagen addition in the assessment results. There were no fibroblasts due to lotion application, but fibroblasts on day 21 emerged from the hypodermis layer, which is thought to be a natural response from the body. Fibroblasts are connective tissues that are also responsible for producing collagen and elastin [30].

With no collagen formed, this follows the antioxidant activity of Andalas tree twig extract, which is very weak, where antioxidants play an essential role in protecting body cells, including fibroblasts, from free radical damage. These free radicals are generated by various factors, such as pollution, UV light, and stress, and can damage existing collagen and inhibit the production of new collagen [31-33].

CONCLUSION

The lotion that contained an extract of Andalas twigs was successfully prepared, but the lotion did not have antioxidant activity as the extract merely had weak activity. The histology study suggests that lotion has anti-inflammatory activity at a concentration of 3% extract. The observed effects may be attributed to bioactive compounds within the extract, highlighting its therapeutic potential. These findings indicate that bioactive compounds in *M. macroura* could be potential candidates for developing skin health-based products, particularly for applications in cosmetics or pharmaceuticals, such as anti-aging and anti-inflammatory lotions.

Formulations with stabilizers must be further tested to increase practical relevance and ensure product stability and shelf life. In addition, the exploration of synergistic effects between *M. macroura* and other antioxidants that have been proven effective may open up opportunities to produce more potent and multifunctional formulations. Further research is recommended to confirm the antioxidant activity of Andalas tree twig extracts and validate their potential health benefits.

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AUTHORS CONTRIBUTIONS

Concept: OMP, MAFF, UMN, NS, LF; Design: OMP, LF; Data Collection or Processing: OMP, MAFF, UMN; Analysis or Interpretation: OMP, MAFF, UMN, NS, LF; Literature Search: OMP, NS, LF Writing: OMP, MAFF, UFN, NS, LF.

CONFLICT OF INTERESTS

All authors have none to declare

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