Trop J Nat Prod Res, September 2024; 8(9): 8361 – 8367 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Anti-wrinkle Effectiveness of Clay Stick Mask Formulation of Jackfruit Leaf Ethanol Extract with Scanning Electron Microscopy

Ketut A. Adrianta[1]*, Kadek R. Yustisia[1], Gusti A.S.A Prasanti[1], Ni Ketut D. P. Pratiwi[1], Luh A. W. I. Putri[1], Dwi W. Putri[1]

[1]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, UniversitasMahasaraswati Denpasar, Indonesia*

ARTICLE INFO

Article history:
Received: 07 August 2024
Revised : 15 August 2024
Accepted : 11 September 2024
Published online 01 October 2024

ABSTRACT

Reactive Oxygen Species can reduce collagen density in the skin, causing wrinkles, characterized by indentations on the skin's surface. Preventing wrinkles may be achieved by using a clay stick mask arrangement with jackfruit (*Artocarpusheterophyllus*Lamk.) leaf ethanol extract. The ethanol extract of jackfruit leaves contains alkaloid, flavonoid, tannin, saponin, and quercetin compounds of 6.84 mg/g with moderate antioxidant activity. The clay stick mask has been shown to offer ultra protection that has the potential as an anti-wrinkle. This research aims to evaluate the anti-wrinkle activity of the clay stick mask. An experimental method includes a clay stick mask base, a retinol clay stick mask, and a 5% and 10% concentration clay stick mask. The anti-wrinkle activity of clay stick masks was tested *in vivo* using male white rats. The test results showed that clay stick masks with retinol and 10% concentration were more effective in inhibiting epidermal thickening. Observation made with a 75 mm diameter lup showed that the retinol clay stick mask had the best results. Scanning Electron Microscopy (SEM) was used to study the skin parameters such as wrinkles. Rat with negative, positive control groups, 5% and 10% clay stick mask treatment groups obtained the results of wrinkle scores successively: 0.33 ± 0.57, 1.33 ± 0.57, 0.33 ± 0.57. Based on the statistical analysis, there was a significant contrast between the negative control group and the others. Clay stick masks were able to protect skin damage due to UV exposure which can accelerate aging and wrinkles.

***Keywords*:** Clay stick mask, Anti-wrinkle, *Artocarpusheterophyllus*Lamk.

Introduction

Premature ageing is a physical change in humans caused by age, psychological, and social factors. It is a degenerative process affecting the skin and its underlying support system, composed of the bone, cartilage, and the underlying tissue beneath the skin, leading to structural changes and a loss of skin elasticity, often evidenced by the appearance of wrinkles. Wrinkles are indentations or wrinkles on the surface of the skin caused by coins in the structure of the epidermis, dermis, and hypodermis.[1] In young faces wrinkles are temporary, but wrinkles can also become permanent with varying severity as a form of response to the influence of intrinsic and extrinsic factors.

Intrinsic factors are physiological ageing that occurs as the skin ages, while extrinsic factors are pathological ageing caused by decreased skin function accelerated by excessive UV light exposure. Skin as the outermost organ experiences direct contact with ultraviolet (UV) radiation. When UV radiation comes in contact with the skin, some of it is diffused and reflected on the stratum corneum, while some is transmitted.[2] Generally, UV exposure is beneficial in the synthesis of vitamin D and killing bacteria in the human body, but excessive UV exposure can lead to sunburns, erythema, hyperpigmentation, premature ageing, and even skin cancer.[3,4]

*Corresponding author. E mail:agusaick@unmas.ac.id
Tel: +628199943-543

Citation: Adrianta KA, Yustisia KR, Prasanti GASA, Pratiwi NKDP, Putri LAWI,Putri DW. Trop J Nat Prod Res. 2024; 8(9):8361-8367. https://doi.org/10.26538/tjnpr/v8i9.18

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Ultraviolet (UV) light is categorized into three types: UV A, UV B, and UV C. UV B rays have greater energy than UVA rays and can pierce the earth's external. UV C rays do not reach the earth's external because they are shielded by ozone in the earth's atmosphere.[5] Free radicals are unstable molecules with unpaired atoms or electrons so the establishment of excessive reactive oxygen species (ROS) causes a decrease in collagen density in the skin which causes ageing characterised by the appearance of wrinkles on the skin. ROS formed during UV irradiation can inhibit the Transforming growth factor (TGF)-*β*, thereby inhibiting collagen production and increasing the production of matrix metalloproteinase (MMP)-1, an enzyme that reduces collagen production. Flavonoid compounds can be utilised to counteract free radicals. In wrinkle prevention, an innovation was made in the form of a clay mask stick with natural ingredients (jackfruit leaves). This is due to the continued effort to explore and increase the use of natural ingredients as nutraceuticals, especially in cosmetics for practical applications. The advantages of this clay stick mask include flexibility in usage/application, economical packaging, and being able to maintain the integrity of the active ingredients contained therein. Until now, there has been no research that has developed a clay stick mask of jackfruit leaf ethanol extract with an anti-wrinkle potential. The phytoconstituents of jackfruit leaf extract obtained by High-Performance Liquid Chromatography (HPLC) revealed four compounds (kaempferol, rutin, cholinergic acid, and quercetin).[6] Quercetin is known to exhibit anti-wrinkle activity because it contains hydroxyl groups capable of scavenging free radicals by providing active hydrogen, making it a strong candidate for treating skin damage caused by UVB radiation.[7]

Materials and Methods

Collection and identification of plant material

Fresh jackfruit leaves (*Artocarpus heterophyllus* Lamk.) were harvested in April 2024 from Busungbiu Village, Busungbiu District, Buleleng Regency, Bali, Indonesia (GPS location; Latitude: -8.252174, 114.959206 Longitude: 8°15'07.8"S 114°57'33.1"E). Plant determination was carried out at the MIPA Laboratory Unit, IAIN SyekhNurjati Cirebon with voucher number: 16/In.08/LB.1.1/PP.009/02/2024.

Extraction of plant material

The collected jackfruit leaves were sorted, chopped, and weighed. The jackfruit leaves were then dried on a tray covered with black cloth to avoid direct sunlight exposure until they become brown and brittle. The dried leaves were subsequently sorted and ground into powder using a blender, weighed, and stored in a glass jar. For the extraction, a 70% ethanol solvent was used in a 1:4 ratio with the leaf powder. This mixture was placed in a glass jar, stirred, tightly sealed, and wrapped in black plastic, left to macerate at room temperature for 24 hours, and then filtered through a Buchner funnel.[6] The filtrate was evaporated with a rotary evaporator at 50°C, followed by further evaporation in a water bath to produce a thick extract.

Qualitative phytochemicals screening

A qualitative phytochemical screening was conducted using standard procedures to identify secondary metabolites in Jackfruit Leaf Ethanol Extract, including alkaloids, flavonoids, tannins, steroids, triterpenoids, and saponins.

High-performance liquid chromatography (HPLC) procedure

HPLC Agilent OpenLabEZChrom, USA (Version A.04.10) analysis was performed using a UV-Vis detector. The chromatographic separation was performed using a C18 column with dimensions of 1.8 µm, 15 cm in length, and 4.6 cm in diameter. HPLC analysis was carried out using an isocratic elution with a mobile phase of methanol and aquadest (59:41 v/v), a flow rate of 1.0 ml/min, a column temperature of 35°C, and a detection wavelength of 372 nm. Samples of ethanol extract (1 g) of jackfruit leaves were carefully weighed and transferred into a 25 mL measuring flask and 20 mL methanol was added. The solution was ultrasonicated for 15 minutes then methanol was added up to the 25 mL mark until homogeneous. The solution was filtered with filter paper and then filtered again with a 0.45µm syringe filter. The sample solution with a volume of 20 µL was injected into the HPLC system.

Preparation of the Jackfruit Leaves Natural Clay Mask Stick

The ingredients were first weighed according to the formula in (Table 1) to make 50 g of the product. Part 1 was mixed in the main beaker under a propeller mixer until Trisodium EDTA was dissolved. Part 2 was added and heated to 50 °C. Part 3 was added and mixed until Sodium Stearate was dissolved, and warmed. Part 4 was added to the batch. When pigments were fully dispersed, Part 5 was added. In a separate beaker, Part 6 was premixed. Kaolin was wet completely and then added to the batch under propeller mixing. It was mixed for 10-15 minutes until the batch was smooth. Then Part 7 was added, followed by Part 8 under propeller mixing at low speed. The mixture was poured into components at 70-75° C.[8]

Physical evaluation of the clay mask stick dosage form

The tests conducted included organoleptic, homogeneity, and pH evaluations. The organoleptic test examined the physical properties of the sun stick dosage form, including its shape, colour, and aroma. For the homogeneity test, a sample was spread on a glass slide to ensure uniform composition and to check for the presence of any coarse particles.[1] The pH test was performed using a pH meter. The spreadability test involved placing 1 g of the formulation on a 20 x 20 cm glass slide, covering it with a cover glass, and applying weight until it reached 125 g. The diameter of the spread was measured after 1 minute. The drying time test was conducted by applying 0.7 g of the mask onto a silicone replica skin with an area of 5.0 x 2.5 cm, creating a layer approximately 1 mm thick, and observing the time it took for the formulation to dry. Finally, the ease of cleaning test involved rinsing the silicone replica skin, which had the mask applied, under running water to assess how easily the mask could be removed.[9]

Determination of total flavonoid content

Jackfruit leaf ethanolextract (10 mg) and clay mask stick (5% and 10%) were dissolved with 5 mL of ethanol (high grade) and transferred into a 10 mL volumetric flask and made up to the mark with ethanol. 100 µL was transferred into a volumetric flask, followed by 100 µL of 2% aluminium (III) chloride, 100 µL of 1M sodium acetate, and 2 mL of ethanol pro analysis. The sample was incubated at room temperature. Absorbance measurements were made using UV-visible spectrophotometry (Shimadzu, version 2.70) at a wavelength of 443 nm.[10]

Determination of antioxidant activity (IC_{50})

Jackfruit leaf ethanol extract (10 mg) and clay mask stick (5% and 10%) from each formula were dissolved in ethanol pro analysis to a final volume of 10 mL, resulting in a concentration of 1000 µg/mL. This sample solution was then diluted to achieve concentrations of 100, 250, 500, 750, and 1000 µg/mL. Subsequently, 2 mL of each diluted sample was mixed with 2 mL of 100 µg/mL DPPH, and incubated for 30 minutes, and the absorbance was measured using UV-visible spectrophotometry (Shimadzu, version 2.70) at a wavelength of 516 nm.[9] The IC_{50} (50% Inhibitory concentration) antioxidant activity was determined by measuring the absorbance of the five concentrations and calculating the percent inhibition using Equation 1 below.

$$\%\text{inhibition} = \frac{(\text{control absorbace} - \text{sample absorbace})}{\text{control absorbance}} \text{ x } 100\% \text{----------} 1$$

IC_{50} value is the sample concentration that inhibits parasite growth by 50%. A linear regression line equation is used to calculate IC_{50} values via Probit (probability unit) analysis, which involves plotting a curve representing the relationship between percent inhibition and the logarithm of sample concentration.

Determination of Sun Protection Factor (SPF)

The UV-Vis spectrophotometer was initially calibrated with 96% ethanol. Each sample (clay stick mask samples 5% and 10%) weighing 0.1 g was dissolved in 96% ethanol in a 25 mL volumetric flask using an ultrasonication bath for 5 minutes at room temperature. The absorbance was consistent at wavelengths ranging from 290 to 320 nm, at 5 nm intervals. The absorbance data were reported, and the SPF amount was subsequently calculated.[11] The perseverance of this SPF value uses the Mansur equation (2), namely:

$$\text{SPF} = \text{CF x } \sum_{290}^{320} \text{EE } (\lambda) \text{ x I}(\lambda) \text{ x Abs}(\lambda) \text{……..……..……} 2$$

Epidermal Hyperplasia procedure

The epidermal layer with Hematoxylin Eosin (HE) painting will be observed below a light microscope with 400x magnification. Furthermore, the thickness of the epidermis of male Wistar rats is measured using Image Raster version 3.0 (Miconos, Indonesia) from the basal layer to the granulosum layer with a micrometre scale (µm).[12]

Antiwrinkle testing with scanning electron microscopy procedure

The rat's back was shaved (3x3 cm) and UVB irradiated 1x a day at a light intensity of 65 mJ/cm^2 for 65 seconds for 15 days. After 5 minutes of irradiation, the negative control group was given a clay mask base, the positive control was given a retinol clay mask, and each treatment group was given a 5% and 10% jackfruit clay stick mask on the rat's back for 20 minutes, then cleaned with 0.9% NaCl. On the last day, the comparison of wrinkles on the skin of the rat's back was observed and given a score which includes: A (Deep and permanent coarse wrinkles), B (Shallow coarse wrinkles), C (Coarse wrinkles), and D (No wrinkles).

The rats were then anaesthetized using 10% ketamine before dissection of the back skin to take skin samples. The samples were washed using 0.9% NaCl and soaking the samples using 10% formalin buffer. Afterwards, it was tested using SEM to determine the wrinkle component and score as follows: 0 (No wrinkles), 1 (Coarse wrinkles), 2 (Shallow coarse wrinkles), and 3 (Deep and permanent coarse wrinkles). The smaller the wrinkle score, the more effective the test clay stick mask is to be applied to the skin as an anti-wrinkle[13].

Statistical Analysis

For statistical analysis, the IBM SPSS Statistics for Windows, version 27.0 was used. Kruskal Wallis test was followed by the Mann-Whitney test. P values <0.05 were considered significant.

Table 1: The formula of Jackfruit Leaf Natural Clay Mask Stick

Materials	F0 (0%)	F1 (5%)	F2 (10%)
	Part 1		
Water	18	18	18
Trisodium EDTA	0.075	0.075	0.075
	Part 2		
Butylene glycol	11.25	11.25	11.25
	Part 3		
Stearic acid	3.75	3.75	3.75
	Part 4		
Titanium Dioxide	2.25	2.25	2.25
Butylene Glycol	2.25	2.25	2.25
Water	2.25	2.25	2.25
	Part 5		
Methylparaben	0.1875	0.1875	0.1875
Propylene Glycol	0.1875	0.1875	0.1875
	Part 6		
Jackfruit Leaf Ethanol Extract	0	5	10
Kaolin	11.25	11.25	11.25
Water	9.75	9.75	9.75
Sodium Lauryl Sulphate	3.9375	3.9375	3.9375
Glycerin	3.75	3.75	3.75
	Part 7		
Talcum	3	3	3
Titanium Dioxide	1.5	1.5	1.5
	Part 8		
Simethicone	0.0375	0.0375	0.0375

Results and Discussion

Natural ingredients consist of secondary metabolites that are recognised for their antioxidant activity and ability to quench UV radiation. Jackfruit leaves, in particular, have properties that enable them to absorb or reflect UV rays, making them useful in beauty or cosmetic products designed as anti-wrinkle treatments. The yield of jackfruit leaf extract with the ratio of dry sample weight and the amount of solvent (1:4) was 17.6%. This result met the requirement that the yield of thick extracts should not be less than 10%.[14] The organoleptic test results of jackfruit leaf ethanol extract showed a thick consistency, dark green colour, and aromatic scent.

The phytochemical analysis (Table 2) revealed that jackfruit leaf ethanol extract contains various phytochemicals, including alkaloids, flavonoids, saponins, and tannins. These secondary metabolites, such as flavonoids, have potential antioxidant and antibacterial activity. Plants contain various chemical compounds like alkaloids, saponins, and tannins, which contribute to their pharmacological properties.

HPLC testing was conducted to identify the quercetin content in the ethanol extract of jackfruit leaves (Table 3). The quantitative analysis of the ethanol extract revealed a quercetin content of 6.480 mg/g of extract. The clay stick mask dosage form underwent physical evaluations for organoleptic properties, homogeneity, and pH tests as shown in (Table 4). The organoleptic testing of clay stick masks involved direct consideration of their colour, smell, and texture. The results showed no significant differences in texture and aroma among the different clay mask formulas. Each formula had a unique aromatic odour due to the addition of jackfruit leaf extract and a clay-like texture. The colour differences were minimal, particularly in formulas F2 and F3, which were influenced by the addition of extracts.

The homogeneity test results for the formulated clay stick masks showed that none of the arrangements showed coarse grains when applied to transparent glass, indicating that the formulations were homogeneous. pH stability is an important parameter that determines whether a formulation is stable or not. The pH values of the clay stick mask formulations ranged from 6.41 to 6.93. According to SNI. 16-4399-1996, the permissible pH range is 4.5-8.0. An optimal pH level is

crucial; overdone acidity may lead to skin irritation, whereas overdone alkalinity can result in dry and flaky skin.

The spreadability test was conducted to assess how well the clay stick mask spreads on the skin when applied. The results of the spreadability of clay stick mask arrangements obtained ranged from 5.5 to 6.5. This shows that all arrangements meet the clay mask standard of 5-7 cm.[15] The drying time test was conducted to determine how long it takes for the formulation to form a dry layer on the surface of the skin. The drying time requirement for the formulation is 10 to 20 minutes. The results showed that all clay mask formulations met the standard drying time, which may be due to the quick-drying properties of kaolin. In terms of ease of removal, all formulations were very easy to clean off, as evidenced by how quickly the dried mask could be removed and how clean the silicone skin became, making the mask formulation easy to clean.[9]

Table 2: Phytochemical constituents of the jackfruit leaf ethanol extract

Phytochemical	Result	Chemical reaction
Alkaloids	+	Precipitate
Flavonoids	+	Yellow color
Saponin	+	Stable foam
Tannins	+	Green violet color
Steroid	-	Greenish coloration
Triterpenoid	-	Purplish-red color

+: Present; -: Asent

Table 3: HPLC analysis of quercetin compounds

Sample	Weight of extract (g)	Area (y)	Quercetin (mg/g)
Jackfruit Leaf Ethanol Extract	1	3288389	6.840

Table 4: The physical evaluation result of the clay mask stick

Sample	Observation Description					
	Organoleptic	Homogeneity	pH	Spreadability	Dry time	Cleaning
F0	White, clay texture, and characteristic odour	Homogeneous	6.93	5.5 cm	< 15 minutes	Very easy
F1	Light green, clay texture, and characteristic odour	Homogeneous	6.79	6 cm	< 15 minutes	Very easy
F2	Light green, clay texture, and characteristic odour	Homogeneous	6.41	6.5 cm	< 15 minutes	Very easy

Flavonoid content analysis and antioxidant activity were evaluated using UV-Vis spectrophotometry. This method is suitable because flavonoids possess a conjugated aromatic system, which results in strong absorption bands in both the ultraviolet and visible light spectra. The results are shown in (Table 5). The results of flavonoids in ethanol extract of jackfruit leaves, 5% clay stick mask, and 10% clay stick mask, respectively were 14.63; 7.15; 7.87 mgQE/g. Flavonoids are found in almost all parts of the plant and are reported to have antioxidant properties that can help eliminate reactive oxygen species (ROS).[16] Antioxidant activity testing on clay stick masks is carried out using the DPPH method represented by the IC_{50} value which shows the concentration of the sample that can inhibit 50% of free radicals.[17] The clay stick mask of jackfruit leaf ethanol extract at 5% and 10% concentration obtained IC_{50} values of 414.638 µg/ml and 265.394 µg/ml, demonstrating their ability to reduce free radical activity.

The effectiveness of a sunscreen is indicated by its Sun Protection Factor (SPF) value. This value accurately represents the total of UV energy necessary to produce a minimal erythema dose (MED) on skin protected by the sunscreen, detached by the quantity of UV energy needed to produce MED on defenceless skin. The SPF value serves as an indicator of the product's capability as a UV protector. The results of the sunscreen protection categories are presented in Table 6. Based on the effectiveness of sunscreen measured by SPF value, F1 and F2 are classified under the ultra-protection category. Jackfruit contains a wealth of beneficial secondary metabolites, particularly in its leaves which are rich in phenolic compounds like flavonoids. These compounds are well-known for their powerful antioxidant properties.[18] The characteristic UV absorbent properties of flavonoids have long been recognised as proof of their role in UV protection.[19] Sunscreens function by absorbing UV energy and transforming it into heat, thereby decreasing the harmful effects and penetration of UV rays into the skin.[20] Consequently, the SPF value indicates that the jackfruit leaf ethanol extract clay mask stick can effectively protect the skin from the damaging effects of UV rays.

Epidermal hyperplasia occurs due to reactive oxygen species (ROS) from increased free radicals due to excessive UVB rays. These free radicals induce keratinocytes, leading to increased keratinocyte proliferation, which causes the accumulation of keratin in the stratum corneum and results in epidermal hyperplasia. Generally, normal Wistar rats have an epidermal thickness of 10-20 µm.[12] Based on the observation (Figure 1), the administration of the retinol clay stick mask and the 10% clay stick mask was more effective in inhibiting epidermal thickening because it has a thinner epidermal thickness and meets the range compared to the 5% clay stick mask and the clay stick mask base group.

A clay stick mask is declared effective as a good anti-wrinkle if there are few wrinkles on the skin of the rat's back. Based on the results obtained (Figure 2), the best results were observed by rats in the positive control treatment group that had retinol clay stick mask application with the observation of only coarse wrinkles, followed by 10% clay stick mask which had coarse wrinkles and shallow coarse wrinkles, compared to other controls which had deep and permanent coarse wrinkles, and shallow coarse wrinkles.

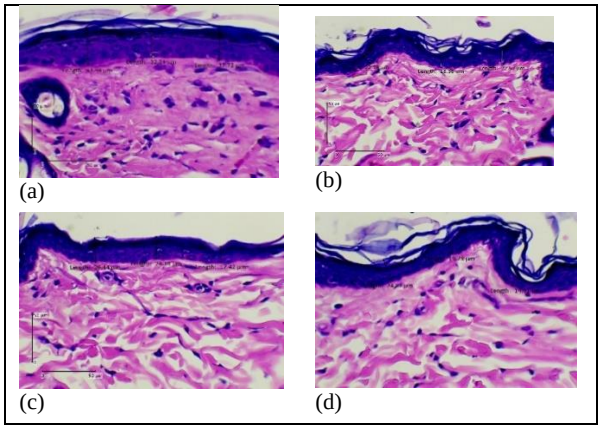


Figure 1: Epidermal Hyperplasia Testing Results; (a) Hyperplasia Test P1 (Negative Control) with an average thickness of 33.38 µm; (b) Hyperplasia Test P2 (Positive Control) with an average thickness of 18.21 µm; (c) Hyperplasia Test P3 (Clay stick Mask of 5% Jackfruit Leaf Extract) with an average thickness of 23.38 µm; (d) Hyperplasia Test P4 (Clay stick Mask of 10% Jackfruit Leaf Extract) with an average thickness of 20.23 µm.

Table 5: Total Flavonoid Content and Antioxidant Activity

Sample	Flavonoid Total Content (mgQE/g)	Antioxidant activity (IC_{50})µg/ml
Extract	14.63	138.168
F1	7.15	414.638
F2	7.87	265.394

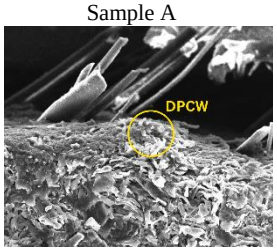
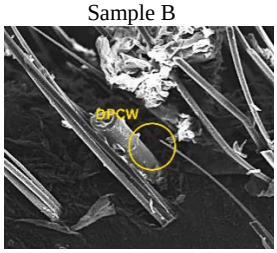
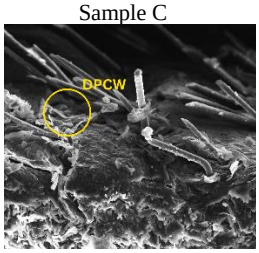
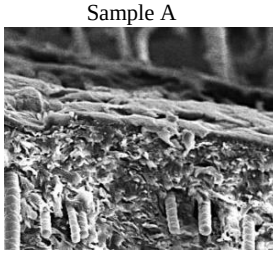
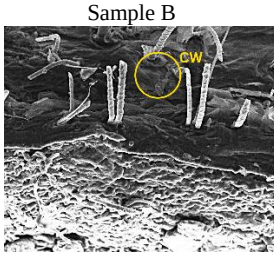
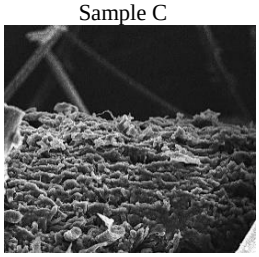
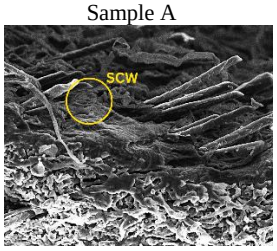
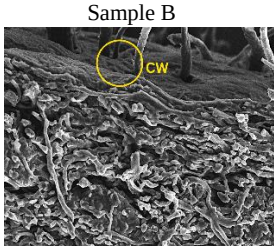
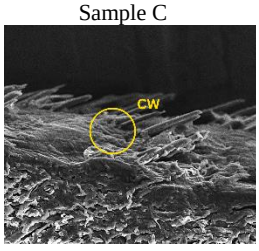
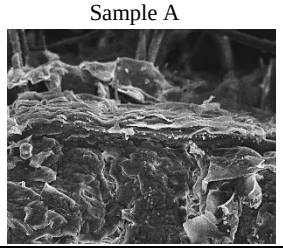
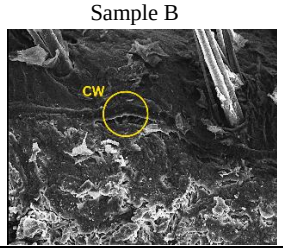
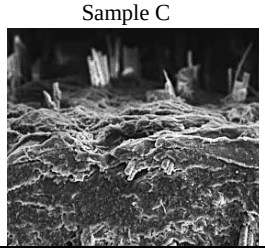
Table 6: SPF value Measurement Results

Sample	SPF value	SPF categories
F1	17.60	Ultra-protection
F2	21.84	Ultra-protection

The results of the SEM method (Table 7) showed various wrinkle scores, including Deep and Permanent Coarse Wrinkles (3), characterised by the loss of fine striations in the rat and there are perpendicular and permanent coarse wrinkles; Shallow Coarse Wrinkles (2), characterised by the loss of fine striations in the skin and there are some deep wrinkles; Coarse Wrinkles (1), characterised by fine scratches on the back that can appear and disappear when moved by the body; and No Wrinkles (0).

Based on the results of the normality test using the Shapiro-Wilk test (Table 8), it was found that the data are not normally distributed, as indicated by a p-value < 0.05.

Table 7: Results of Rat Skin Observation with SEM Method

Description	Result Test (x120)			
	Sample A	Sample B	Sample C	Score
P1				A : 3 B : 3 C : 3 Average: 3
	Sample A	Sample B	Sample C	Score
P2				A : 0 B : 1 C : 0 Average: 0.33±0.57
	Sample A	Sample B	Sample C	Score
P3				A : 2 B : 1 C : 1 Average: 1.33±0.57
	Sample A	Sample B	Sample C	Score
P4				A : 0 B : 1 C : 0 Average: 0.33±0.57

Accordingly, a non-parametric test, the Kruskal-Wallis test was used. The results of non-parametric testing obtained a significant value of 0.032, which differs from $p<0.05$. Subsequently, further non-parametric tests, specifically the Mann-Whitney post hoc test, were performed to assess the contrast between each treatment group.

The outcome of the Mann-Whitney post hoc test (Table 9) indicates significant differences between the P1 group and the P2, P3, and P4 groups, as the p-value < 0.05. These results suggest a significant contrast between the negative control group and the positive control group, the 5% clay stick mask control group, and the 10% clay stick mask control group have differences in wrinkle results, while in other groups it does not appear to be different in meaning because it gets a P-value > 0.05 and shows that there is no difference in wrinkle results in the test group. This is likely due to the quercetin content in the jackfruit leaf ethanol extract clay stick mask having a hydroxyl group (OH), which plays a role in scavenging free radicals. The hydroxyl groups inactivate free radicals by providing active hydrogen, leading to the oxidation and stabilization of free radicals, thereby preventing the formation of ROS.[21] Based on this, the jackfruit leaf ethanol extract clay stick mask is capable of protecting the skin from damage caused by UV exposure capable of accelerating the ageing process and wrinkle formation.

Table 8: Normality Test Result

Group	Normality Test P Value	Description
P1	0.000	Not normally distributed
P2	0.000	
P3	0.000	
P4	0.000	

Table 9: Comparative Test Between Treatment Groups to Wrinkle Score

Group		Significant Value	Description
P1	P2	0.043*	Significant difference
	P3	0.034*	Significant difference
	P4	0.043*	Significant difference
P2	P3	0.068	No Significant difference
	P4	1.000	No Significant difference
P3	P4	0.068	No Significant difference

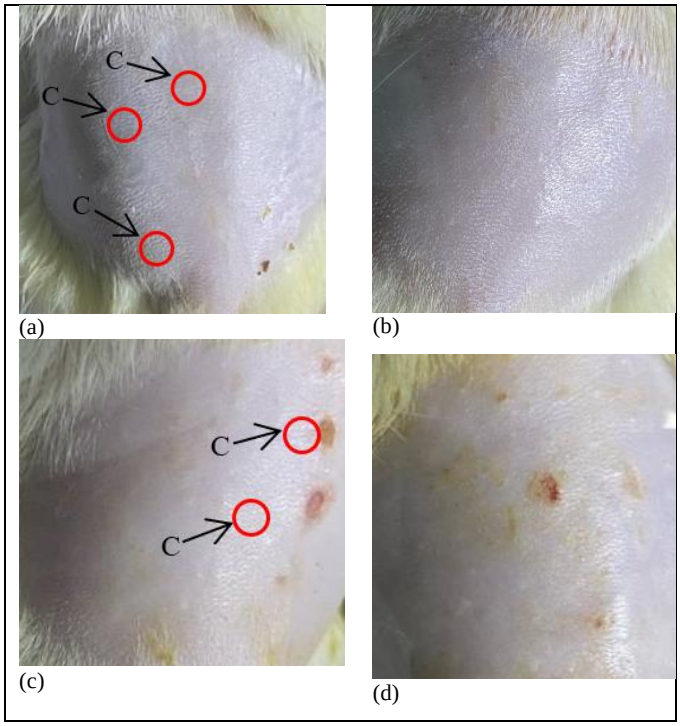


Figure 2: 75 mm Diameter Lup Observation Results on Rat Skin; (a) 75 mm Lup observation results P1 (Negative Control); (b) 75 mm Lup observation results P2 (Positive Control); (c) 75 mm Lup observation results P3 (Clay stick mask 5% jackfruit leaf ethanol extract); (d) 75 mm Lup observation results P4 (Clay stick mask 10% jackfruit leaf ethanol extract).

Conclusion

The clay stick mask made from ethanol extract of jackfruit leaves shows potential as an anti-wrinkle treatment in mice exposed to UV-B radiation. Rats in the negative control group, positive control, 5% and 10% clay stick mask treatment groups showed various wrinkle scores. The best results were shown by the 10% clay stick mask group due to its antioxidant activity with an ability to inhibit free radicals and its potential as a photoprotector with an ultra-protection category equivalent to the positive control group.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors thank the Faculty of Pharmacy Universitas Mahasaraswati Denpasar for the facilities in completing this research.

References

1. Hikmawati D, Maedasari D, Prasetya PR. Smoking and Premature Aging in Form of Facial Wrinkles on Universitas Islam Bandung Security Staff. Artik Penelit. 2017; 5(22):140-143.
2. Maulina D. Role of Mangiferin as an Inhibitor of Collagenase and Elastase Enzymes. Indones J Heal Sci. 2021;1(1):4-9. doi:10.54957/ijhs.v1i1.41
3. Verawati V, Almahdy A, Febriyenti F, Putra DP. *In Vitro* and *In Vivo* Evaluation of Photoprotective Effect of *Elephantopus Mollis* Extracts. Trop J Nat Prod Res. 2022; 6(3):365-370.
4. Kusumawati AH, Farhamzah F, Alkandahri MY, Sadino A, Agustina LS, Apriana SD. Antioxidant Activity and Sun Protection Factor of Black Glutinous Rice (*Oryza sativa* var. *T*rop J Nat Prod Res. 2021;5(11):1958-1961.
5. Minerva P. Using Sunscreen for Skin Health.J Pendidik Dan Kel. 2019;11(1):87. doi:10.24036/jpk/vol11-iss1/619
6. Sayuti NH, Kamarudin AA, Razak NAA, Saad N, Dek MSP, Esa NM. Optimized aqueous extraction conditions for maximal phenolics, flavonoids and antioxidant capacity from *Artocarpus heterophyllus* (Jackfruit) leaves by Response Surface Methodology (RSM). Malaysian J Med Heal Sci. 2020; 16(2):135-144.
7. Rivera-Yañez CR, Ruiz-Hurtado PA, Mendoza-Ramos MI, Reyes-Reali J, García-Romo GS, Pozo-Molina G, Resendiz-Albor AA, Nieto-Yanez O, Méndez-Cruz AR, Mendez-Catala CF, Rivera-Yañez N. Flavonoids present in propolis in the battle against photoaging and psoriasis. Antioxidants. 2021; 10(12):1-30. doi:10.3390/antiox10122014
8. Kobo. Watermelon Purifying Clay Stick Mask. Published online 2019.
9. Syamsidi A, Syamsuddin AM, Sulastri E. Formulation and Antioxidant Activity of Mask Clay Extract Lycopene Tomato (*Solanum lycopersicum L.*) with Variation of Concentrate Combination Kaoline and Bentonite Bases. J Farm Galen (Galenika J Pharmacy). 2021;7(1):77-90. doi:10.22487/j24428744.2021.v7.i1.15462
10. Marpaung JK, Nasution Z, Thaib CM, Siringoringo J. Determination of Jackfruit (*Artocarpus heterophyllus*) Straw Ethanol Extract Total Flavonoid Levels Using Spectrophotometry-Visible. J Farmanesia. 2020;7(1):48-52. doi:10.51544/jf.v7i1.2766
11. Wintariani NP, Suwantara IPT. The SPF (Sun Protection Factor) Value Analysis of Gonda Plant (*Sphenocieo reylanice* Gaertner) Extract Sunscreen Cream Using UV-Vis Spectrophotometry Method. J Ilm Medicam. 2023;9(2):90-95.
12. Wibisono EW. Single Garlic Ethanol Extract Cream (*Allium sativum*) Inhibits Epidermal Thickening of Male Wistar Rats (*Rattus norvegicus)* Exposed to Ultraviolet-B Light. J Kesehat Andalas. 2020;9(1):1. doi:10.25077/jka.v9i1.1275
13. Natalia F, Pinatih GNI, Praharsini IGAA. Administration of Sugar Apple Leaf Extract Cream (*Annona squamosa L.*) Increased Superoxide Dismutase (SOD) Activity and Decreased Skin Matrix Metalloproteinase-1 (MMP-1) Activity in Male White Rats (*Rattus norvegicus)* Wistar Strain Exposed to Ultraviolet. Int J Res Rev. 2023;10(1):623-633. doi:10.52403/ijrr.20230169
14. Badriyah L, Farihah D. Optimization of shallot skin (*Allium cepa* L) extraction using maceration method. J Sint Penelit Sains, Terap dan Anal. 2023;3(1):30-37. doi:10.56399/jst.v3i1.32
15. Nurliani R, Aryani R, Darusman F. Activity Test of African Leaf Extract (*Vernonia amygdalina* Del.) against Acne-Causing Bacteria and its Formulation in Clay Mask Formulation. J Pros Farm. 2020;6(1):74-80.
16. Saputri R, Hakim AR. Determination of Total Flavonoid Content and Total Phenolic Content of Pitanak Fruit Peel Extract (*Nephelium sp.*). J f Pharm Care dan Sci. 2022;2(2):92-96.
17. Widyasanti A, Rohdiana D, Ekatama N. Antioxidant activity of white tea extract (*Camellia sinensis*) by DPPH (2,2 Diphenyl-1-Picrylhydrazyl) method. Fortech. 2016;1(1):2016.
18. Bhad PR, Bobde MV, G S. Chemical constituents and biological activities of *Artocarpus heterophyllus* lam (Jackfruit): A review. Int J Clin Microbiol Biochem Technol. 2021;4(1):005-009. doi:10.29328/journal.ijcmbt.1001019
19. Adrianta KA, Yuda PESK, Purnamasari PS, Suena NMDS. Jackfruit (*Artocarpus heterophyllus* Lamk) Leaf as a Natural Sun Stick Protector Through the Inhibition of the MMP-1 Receptor. Trop J Nat Prod Res. 2023;7(5):2952-2958.
20. Geoffrey K, Mwangi AN, Maru SM. Sunscreen products: Rationale for use, formulation development and regulatory considerations. Saudi Pharm J. 2019;27(7):1009-1018. doi:10.1016/j.jsps.2019.08.003
21. Qi W, Qi W, Xiong D, Long M. Quercetin: Its Antioxidant Mechanism, Antibacterial Properties and Potential Application in Prevention and Control of Toxipathy. Molecules. 2022;27(19). doi:10.3390/molecules27196545