Moisturized and non-irritating hand gel based on sappan wood (*Caesalpinia sappan* l.) and limau citrus peel (*Citrus amblycarpa* (hassk.) ochse) extracts

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Abstract

Background: Hand gel is a preparation used to protect hand skin from dryness. The antioxidant compounds in sappan wood and limau citrus peel can moisturize hands by preventing the oxidation of oils and fats on the surface of the (sebaceous glands).

Objective: This study aims to formulate hand gel from ethanol extract of sappan wood with a combination of limau citrus peel extract that can moisturize hands and not to cause irritation.

Methods: Sappan wood and limau citrus peel simplicia were macerated using 96% ethanol. The ethanolic extracts were then formulated into hand gel by varying the concentration of the ethanol extracts. The evaluation of the hand gel involved an organoleptic test, homogeneity test, pH test, spreadability test, viscosity test, and skin moisture test. A primary irritation test was carried out to ensure the hand gel's safety.

Results: The results shows that the three hand gel formulas produce an orange-red color and soft scent of roses and citrus, with a pH of 7.8 and with good homogeneity. The formulas also have a spreading level of FI (5 cm), FII (5.2 cm), and FIII (5, 1 cm), and viscosity values were FI 12000 mPas, F II 12500 mPas, and F III 12400 mPas. The humidity test results on the three formulas shows different moisture values of 45.16%, 46.17%, and 45.09%, respectively (category of normal or moisture). The hand gel formulas have an irritation index of 0 (no irritation category).

Conclusion: The hand gel formulas meet the quality requirements of hand gel, have moisturizing agent, and are not irritating.

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Introduction

During the COVID-19 pandemic, we must maintain hand hygiene to prevent the spread of the COVID-19 virus; therefore, we must wash our hands frequently. The use of soap or detergent and hand sanitizer can cause skin surface fat to be emulsified and hydrophilic materials in the stratum corneum dissolved. Skin moisturizing cosmetics are needed to protect the skin from excessive water evaporation and damage to the stratum corneum that causes skin dehydration.[1]

Advances in science and technology in the development of research tools for skin biological function change our understanding of the effects of cosmetics, including the top layer of skin hydration and its barrier effect.[2] In addition, sun exposure, air pollution, and free radicals can also cause dry and damaged skin.[3] Hand gel is one of hand skin moisturizer preparation. Hand gel is a semisolid preparation to soften and protect hand skin.[4]

In recent years, there has been an increase in the acceptance of herbal medicines. Many researchers are investigating the potential use of plant species to develop new products, including cosmetics.[5] Among these plants are sappan wood (*Caesalpinia sappan* L.) and limau citrus (*Citrus amblycarpa* - Hassk.) which have widely popular medicinal uses and stand out as sources of secondary metabolites. The stems of sappan trees have various secondary metabolite compounds, including flavonoids, alkaloids, saponins, tannins, terpenoids, phenyl propane, and brazilin.[6] Sappan wood extract and its secondary metabolite components may induce oxidative stress.[7] The antioxidant activity of sappan wood is evidenced by the IC_{50} value of sappan wood ethanol extract of 60.5 g/mL;[8] IC_{50} value of

ethanol extract of sappan wood was 1.44 g/mL (strong category);[9] The ethanol extract of sappan wood using the DPPH, ABTS and FRAP methods has IC_{50} value of 101.47 ppm (weak category), 26.70 ppm (medium category) and 11.37 ppm (strong category).[10]

The use of limau is limited to the use of its fruit.[11] The production of limau citrus in Indonesia reaches 120,000 kg per year, but the fruit yield is not maximized, especially the peel.[12] To increase the economic value of limau citrus peel, it is necessary to harness its economic value through various activities including research, one of which is in the pharmaceutical field. Several scientific studies have used parts of this citrus plant, including leaves, stems, fruit, and skin. Based on the research on limau citrus peel, compounds found in the fruit include flavonoid and and polyphenols.[13] Wulandari *et al*. (2013) tested the antioxidant activity of n-hexane, ethyl acetate, and methanol extracts of limau citrus peel using the DPPH method.[14] The IC_{50} value of the three extracts was 162.16 g/mL (weak category antioxidant), 134.02 g/mL (medium category antioxidant) and 94.01 g/mL (strong category antioxidant). Limau citrus peel contains citric acid which is known to lighten the color of composite resins because it contains OH groups, the strong oxidants. Cheong (2013) stated that lime fruits contain citric acid, which is found in many ripe oranges. Ripe lime has antioxidant activity. The antioxidant activity of the methanolic extract of limau citrus peel is due to the presence of secondary metabolites, namely flavonoids, alkaloids, and polyphenols.[14] According to Jamal (2000) limau citrus peel also contains about 1.20% essential oil with -citronellol, (R)-(+) citronellal, Pinene, D-limonene, 4-methyl-1-(1-methyl ethyl)- 3-cyclohexene-1-ol, -linalool, -terpineol, -farnesene, -citral, L-Isopulegol, and cis-linalyl oxide as the main components. Essential oil contained in limau citrus is more sensitive to gram-positive bacteria than gram-negative bacteria. The ethanol extract of limau citrus peel contains a total flavonoid of 0.3324 mgQE/g (89% inhibition) and shows antioxidant activity.[15] Based on the literature search, there has been no research on hand gel formulations using sappan wood or limau citrus peel. Moreover, dermatotoxicity data from hand gel products are also very few. According to the chemotaxonomic approach, because of the presence of the same compound in limau citrus peel, which has the potential as an antioxidant, it is possible that the two plants, sappan wood, and limau citrus peel, can be combined as hand gel preparations. Natural antioxidants combination from sappan wood and limau citrus peel is synergistic in protecting human skin from oxidative stresses.[16] The limau citrus peel extract in the formula serves to stabilize the content of brazilin as an antioxidant compound. Physico-chemical characterization was performed, including skin moisture testing after hand gel application. To ensure the safety of cosmetics, especially hand gel, it is necessary to test for skin irritation. Skin irritation is defined as reversible damage to the skin caused by the application of a substance, one of which is cosmetic, which is usually characterized by erythema and edema.[17] The skin irritation test method that can be done is the primary irritation test method.[18] Therefore, the overall objective of this study is to make hand gel using the ethanol extracts of sappan wood and limau citrus peel, to test the activity of this product as a moisturizer, and to evaluate skin irritation in mice.

Materials and Methods

Materials and tools

Sappan wood and limau citrus peel were purchased from Sungai Pangkalan, Bengkayang district, West Kalimantan, Indonesia, and identified in the Indonesia Institute of Sciences (LIPI) Bogor (1333/IPH.1.01/If.07/V/2017 for sappan wood and B-957/V/DI.05.07/12/2021 for limau citrus). Technical ethanol (96%), technical n-hexane, glycerin, HPMC, sodium benzoate, rose water, and distilled water were purchased from Alkamid Co. (St. Tehran, Iran). NaCl (0.9%) were obtained from PT. Sanbe Farma (Jakarta, Indonesia). Filter paper, sterile gauze, dressing retention sheet, and plaster (OneMed) were also used. The test animals were male white Spraque-Dawley mice.

Sample collection and processing

Sappan wood and lime were obtained from Sungai Pangkalan, Bengkayang district, West Kalimantan, Indonesia. Samples were wet sorted to remove dirt or impurities, then dried using an oven at 40°C (Gemmyco Digital #YCO-N01) to obtain dry simplicial with a moisture content of 6.43%, and 7,55%, respectively. The dried simplicia was then chopped and mashed using a blender (Miyako®). Simplicias were macerated in maceration vessel using 96% of ethanol. The extract's color of the brazilin pigment in sappan wood will produce a fairly sharp orange-red to brown and is stable.[19] The extracts were thickened using a rotary evaporator (Dragon LAB RE-10 Pro) and obtained a viscous fraction with a yield of 1.62%, and 22,73%, respectively.[20]

Formulation and evaluation of hand gel

Hand gel formulations were based on the basic formula and pre-formulation studies as shown in Table 1. The hand gel was made by dispersing HPMC with hot water (temperature of 80-90ºC) in a ratio of 1:5 (Mixture A). The ethanol extract of sappan wood and that of limau citrus peel were dissolved in glycerin, then

Table 1. Hand gel formula from sappan wood and limau citrus peel ethanol extracts.

Composition	Concentration (%)			Range (%)	Function
	FI	FII	FIII		
Sappan wood ethanol extract	0.01	0.02	0.03	-	Active substance
Limau citrus peel ethanol extract	0.03	0.02	0.01	-	Active substance
Glycerin	5	5	5	≤50	Cosolvent
Hydroxypropyl methylcellulose (HPMC)	0.25	0.25	0.25	0.25-5.0	Gelling agent
Sodium benzoate	0.1	0.1	0.1	0.1-0.5	Preservative
Ethanol (96%)	5	5	5	≈30	Solvent
Rose water	0.1	0.1	0.1	-	Perfume
Aquadest	Until 100	Until 100	Until 100	-	Solvent

*FI: Formula I; FII: Formula II; FIII: Formula III.

ethanol 96% was added. Next, sodium benzoate which had been dissolved in water was added and followed by rose water (Mixture B). Mixture B was added into mixture A until homogeneous.[4,21]

The evaluation of the hand gel involved the organoleptic test for the assessment of color, odor, and texture),[22] pH value test using pH-meter (PH-108 ATC),[23] homogeneity test,[24] spreadability test, and viscosity test using Brookfield viscometer (Ntech®)[25] at room temperature using spindle number RV-7 at 20 rpm.[26]

Tasters in organoleptic tests must have an interest in sensory organoleptic testing and are willing to participate. They must be consistent in making decisions, in good health, free from ENT diseases, are not color blind, and must not have psychological disorders. They shall not averse to the food to be tested (not allergic) and shall fast at least one hour before the test hour. They shall not smoke, chew gums, and consume food and soft drinks at least 20 minutes before the test. Should they have influenza and sore eyes, they shall refrain from tasting. They shall not eat very spicy food at lunch if the test is carried out during the day. The tasters must not have used cosmetics such as perfume and lipstick and must wash their hands with unperfumed soap during the test.[22]

Skin humidity test

The skin humidity test using skin moisture analyzer (OEM MTA-8752633) uses human volunteers who must be in good health with no history of diseases related to skin allergy, and are willing to be a panel member. The test was carried out by applying the hand gel preparation on the left forearm.[27] The tested skin is divided and marked into 3 (three) parts. The standard human skin moisture scale is dry (0%-45%), normal or moist (46%-55%), and very moist (56%-100%).[28]

Primary irritation test

The test animals were male white Spraque-Dawley rats and the primary irritation test used closed patch test method.[18,29] Observations were made for 72 hours. For each treatment, three rats were used. The five treatments are three hand gel test groups, positive control using 30% sodium lauryl sulfate, and negative control group using 0.9% NaCl. Twenty-four hours before the treatment, the hair on the back was shaved[30] with a size of 2 x 2 cm. A dose of 0.5 mL of the test material was applied to the test site. The smeared area is covered with sterile gauze pad, then covered again with dressing retention sheet and plastered.[31] After 24 hours, they were removed and the test area was cleaned with water to remove any remaining test material. The test area was examined at 24, 48, and 72 hours. It was observed for changes in skin caused by reaction to the test material and assessed by giving a score of 0 to 4 depending on the severity of the skin reaction (erythema and edema).[30] It was then matched on the score table irritation (Table 2). Next, the results are calculated to get primary irritation index (PII) of the skin with the following calculation:

$$PII = \frac{(A - B)}{C}$$

where A is the score of erythema and edema in all samples (at 24, 48, and 72 h) which is divided by the number of observations, B is the value of erythema and edema in all controls (at 24, 48 and 72 hours) which is divided by the number of observations, and C is the number of animals. PII at that time was based on ISO 10993-1013 categories and BPOM standard (Table 3).[32]

Ethical considerations

The study was reviewed and approved by the ethics committee of Muhammadiyah School of Nursing and Sciences, Pontianak, Indonesia (reference number 32.A/II.1.AU/KET.ETIK/I/2022). Participants were informed about the aims of the study, and the study protocol was explained to them. All the participants provided signed written informed consent. They were also informed of their right to withdraw from the study at any time.

Table 2. Skin irritation scores.

Formation of erythema and edema	Score
Erythema	
No erythema is formed	0
Very slight erythema	1
Erythema is clearly visible	2
Moderate to severe erythema	3
Severe erythema to eschar formation that hinders the assessment of erythema	4
Edema	
No edema formed	0
Very small edema	1
Minor edema (border area visible, clear)	2
Moderate edema (area increases by about 1mm)	3
Severe edema (area increases by more than 1mm and widens)	4

Table 3. Irritation response category.

Irritation Index	Response category
0.0-0.4	No irritation occurs; mild irritation
0.5-1.9	Medium irritation
2.0-4.9	Irritation
5.0-8.0	Strong irritation

Results

The study was conducted in four stages: preparation of samples,[1] hand gel formulation,[2] skin moisturizer test,[3] and irritation potency[4] test using the primary irritation test method.

Hand gel formulation

The hand gel was made into three formulas, using the ethanol extract of sappan wood and the ethanol extract of limau citrus peel as the active substance. Glycerin was used as a cosolvent to increase the solubility of the sample and as a humectant. We also used HPMC as a gelling agent, sodium benzoate as a preservative, 96% ethanol as a solvent and preservative, rose water as a perfume, and aqua dest as a solvent.[21] The resulting hand gel preparations were evaluated to examine their physical quality, including organoleptic, homogeneity, pH, spreadability, and viscosity tests.

Three formulas showed orange-red color, the scent of roses and citrus, and produced a soft texture. The pH test aims to determine the acidity and basicity levels of the hand gel preparation so as not to irritate the skin. The results showed that the three hand gel formulas had a pH of 7.8. Homogenous visualization glass plate model was used to observe the presence or absence of parts that were not completely mixed in the three formulas.[24] The results showed that the three hand gel formulas were homogeneous (Table 4). The spreadability test aims to determine the ability of the hand gel to spread evenly on the skin and to facilitate easy application. A good standard of dispersion in gel preparations is 5-7 cm.[33] The average results of FI was 5 cm, F II was 5.2 cm, and F III was 5.1 cm. The viscosity test determines the viscosity of a hand gel preparation.[34] The results showed that the viscosity values were FI 12000 mPas, F II 12500 mPas, and F III 12400 mPas.

Skin moisture test

The humidity test was carried out to determine the moisture content of the skin after given each formula. Table 5 shows the results of the gel moisture test in which the three formulas showed different moisture values of 45.16%, 46.17%, and 45.09%, respectively.

Table 4. Organoleptic and homogeneity test results of hand gel.

Formula	Organoleptic test	Homogeneity	
		Test results	Description
FI			Homogeneous
FII			Homogeneous
FIII			Homogeneous

Table 5. Skin humidity test results.

Initial humidity (%)		Formula	Average moisture test results (%)	
O	W		O	W
19.30	18.00	FI	26.66	45.16
		FII	30.76	46.17
		FIII	25.94	45.09

*FI: Formula I; FII: Formula III; FIII: Formula III; O: oil content; and W: water content

Table 6. Primary irritation test results.

Tests Group		Irritation observation 24 h	48 h	72 h	Irritation description Erythema/ Edema 24 h	48 h	72 h	Irritation score	Irritation category
K-	R1				0/0	0/0	0/0	0	No irritation occurs
	R2				0/0	0/0	0/0		
	R3				0/0	0/0	0/0		
Average					0/0	0/0	0/0		
Stdev					0	0	0		
K+	R1				0/0	0/0	0/0	2.11	Irritation
	R2				0/0	3/3	3/3		
	R3				0/0	1/0	3/3		
Average					0/0	1.33/1	2/2		
Stdev					0	1.47	1.55		
FI	R1				0/0	0/0	0/0	0	No irritation occurs
	R2				0/0	0/0	0/0		
	R3				0/0	0/0	0/0		
Average					0/0	0/0	0/0		
Stdev					0	0	0		
FII	R1				0/0	0/0	0/0	0	No irritation occurs
	R2				0/0	0/0	0/0		
	R3				0/0	0/0	0/0		
Average					0/0	0/0	0/0		
Stdev					0	0	0		
FIII	R1				0/0	0/0	0/0	0	No irritation occurs
	R2				0/0	0/0	0/0		
	R3				0/0	0/0	0/0		
Average					0/0	0/0	0/0		
Stdev					0	0	0		

Primary irritation test

The results showed that the negative control had an irritation score of 0 (no irritation). As shown in Table 6, the positive control had an irritation score of 2.11 (irritation), while FI, F2, and F3 each with an irritation score of 0 (no irritation).

Discussion

Hand gel formulation

The orange color in the hand gel is brazilin, which is influenced by acidity or pH values.[35] The stability of the pigment is influenced by several factors, including pH, oxidizing agents, sunlight, and storage conditions. At acidic pH, sappan wood extract is yellow, while at alkaline pH it is red to purplish red.[36] The addition of acids and bases will affect the color of the sappan extract. The citric acid, an intermediate of the tricarboxylic acid cycle, is found in various acidic fruit juices,[37] including the limau citrus peel. The citric acid in orange peel can stabilize the pH of the preparation. The skin has an acid mantle with a pH of 4.5-7.5. The function of the skin's acid mantle is as the skin's first protection and can neutralize materials with a pH that is not too far from the skin's pH.[1] Thus, the pH of the three hand gel formulas is still safe to use, including weak bases, and can still be neutralized by the skin's acid mantle. Homogeneous hand gels allow homogeneity of the active substance and an even distribution of the preparation when applied to the skin. Good homogeneity is achieved if there is no presence of coalescen, agglomerate, and sendiment.[38] Gelling agents have a strong influence on homogeneity. In this study, we use HPMC, which acts as a colloid protective agent by preventing the formation of droplets and particles from coalescence and agglomeration, thereby preventing the formation of sediments.[21] The three hand gel formulas meet the requirements for good spreadability. The viscosity test determines the viscosity of a hand gel preparation.[34] Gelling agents have an influence on the viscosity. HPMC is used as a viscosity-forming agent and gel preparation in topical products, where the colloid formation event that occurs can prevent droplets and the occurrence of coalescence, agglomeration, and sedimentation.[21]

Skin moisture test

Based on the results of the study, the three formulas are in the category of normal or moisture.[28] The moisture content produced by the hand gel is caused by the water content and the gelling agent. The fat layer on the surface of the skin and the ingredients in the stratum corneum can bind water resulting in skin flexibility.[1] Meanwhile, HPMC forms a protective colloid on the skin surface to prevent excessive evaporation of water on the skin, especially the stratum corneum.[21]

Based on previous studies, the results of the phytochemical screening of limau citrus extract positively contained flavonoids[13] and sappan wood extract positively contained brazilin compounds as one of the phenolic compounds.[36] In addition, the antioxidant in the ethanol extract of sappan wood and the ethanol extract of limau citrus peel can prevent the oxidation of oils and fats.[39] The surface of the skin is a layer of fat that comes from the sebaceous glands and referred to as the fat coat of the skin. Sebum is important for the skin's surface because it contains a lot of fat that collects a lot of moisture.[1]

Primary irritation test

Using a closed patch method, the primary irritation test aims to compare the presence of erythema and edema in the skin. The test results are then calculated and categorized to obtain the Primary Irritation Index (PII).[40,41] Irritation is a symptom of inflammation that occurs on the skin or mucous membranes after prolonged treatment using chemicals or other materials.[30] The skin irritation is caused by an inflammatory reaction due to the release of proinflammatory cytokines from keratinocytes. The resulting response causes disruption to the skin barrier, changes in epidermal cells, and the release of cytokines. Symptoms in the acute phase are erythema, edema, crusting, tenderness, vesicles, or pustules.[32]

The ingredients in the hand gel are glycerin, HPMC, sodium benzoate, 96% ethanol, rose water, and aqua dest. The following materials were used in safety in this study. HPMC is non-toxic and non-irritant. Sodium benzoate can cause urticaria and non-immunological reactions on the skin when used at a concentration above 5%, but in this research, we only used 0.1%. The concentration of ethanol used was only 5% because a concentration of more than 50% can cause skin irritation if applied topically.[21] Based on the results of the primary irritation test and the literature research, it can be concluded that the three hand gel formulas are not irritating. SLS as a positive control with a concentration of 30% had moderate toxicity effects in the form of acute effects such as skin irritation, membrane irritation, and eye irritation.[30,40] SLS with concentrations of 10% and 30% cause moderate irritation.[42]

The presence of flavonoid compounds in limau citrus peel extract[13] and phenolic compounds, including brazilin in the polar extract of sappan wood[36] which has antioxidant,[43,44] can protect skin tissue from damage, one of which is lipid peroxidation. Lipid peroxidation plays an important role in pathophysiological events associated with various diseases, including damage to skin tissue.[45] In addition, based on the analysis of the oil in lime peels using GC-MS, the components are β-pinene, cimena, and limonene.[46] Limonene can have anti-inflammatory effects by inhibiting pro-inflammatory cytokines and the production of oxygen species radicals (ROS), and by inactivating eosinophil migration so that it is related to the ability of limonene to be considered inflammation related to the irritation of the skin.[47] This study has some limitations. The first limitation is the lack of information and previous research studies on hand gel formulation from a natural source. Another limitation is time constraints because this is basic research that requires reporting of results data in less than one year.

Conclusions

To conclude, the hand gel formulated from the combination of ethanol extract of sappan wood (*Caesalpinia sappan* L.) and ethanol extract of limau citrus peel (*Citrus amblycarpa* (Hassk.) Ochse produced an orange-red color and had good homogeneity, with a pH value of 7.8, spreadability of 5 cm, 5.2 cm and 5.1 cm, and a viscosity of 12000 mPas, F II 12500 mPas, and F III 12400 mPas, respectively. Based on the humidity test results, the three formulas showed different moisture levels: 45.16%, 46.17%, and 45.09%, respectively (category of normal or moisture). The primary irritation index (PII) of erythema and edema was 0 (no irritation) for the negative control, 2.11 (irritation) for the positive control, and 0 (no irritation) for FI, F2, and F3. This study suggests that the hand gel from the ethanol extract of sappan wood and the ethanol extract of limau citrus peel can moisturize hands, is not irritating, and is safe to use.

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