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Research Article

EFFICACY OF LOTION CONTAINING FRACTIONS OF *SELAGINELLA PLANA* LEAVES AND *LAGENARIA SICERARIA* (MOLINA) STANDL. FRUIT FOR RELIEF OF SKIN ERYTHEMA

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ABSTRACT

Objective: The aim of this study is to evaluate the physical stability of two lotion formulations containing *Selaginella plana* and *Lagenaria siceraria* (Molina) Standl. and investigated their safety and efficacy to relieve erythema due to exposure to the sun.

Methods: We conducted a randomized controlled trial consisting of five treatment groups: Negative control, positive control, neutral control, formula A test group (containing a 1% ethanolic fraction of *S. plana* and a 0.5% ethanolic fraction of *L. siceraria* [Molina] Standl.), and formula B test group (containing a 0.5% ethanolic fraction of *S. plana* and a 1% ethanolic fraction of *L. siceraria* [Molina] Standl.). Each group had erythema induced by exposure to sunlight for 30 min between 10:00 and 16:00. The severities of erythema 1, 3, and 24 h after application were assessed.

Results: Formula A was significantly better than formula B ($p<0.05$) at reducing the severity of erythema.

Conclusion: Formula A containing a 1% ethanol fraction of *S. plana* and a 0.5% ethanol fraction of *L. siceraria* (Molina) Standl. showed the greatest reduction in the level of erythema ($p<0.05$). *S. plana* may reduce the prostaglandin synthesis caused by sun exposure.

Keywords: Erythema, *Selaginella* sp., *Lagenaria sp.,* Flavonoids, Amino acids, Fraction of ethanol, Lotion.

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INTRODUCTION

Inflammation is caused by microorganisms, mechanical trauma, chemicals, and the effects of sun exposure. Erythema is one sign of inflammation, and exposure to the sun is the dominant cause in Indonesia, which is a tropical country in which most of the population works outdoors throughout the year. One practical approach to acute sunburn caused by ultraviolet (UV) rays is to treat the symptoms such as erythema (redness), pain (pain), and itching [1-5]. Research and development is active to develop skin care lotions using herbal active ingredients to relieve erythema of the skin due to exposure to the sun [6,7].

In the pathogenesis of erythema, UV light stimulates the release of histamine, kinins, and prostaglandins that cause damage to keratinocytes. The minimal erythema dose (MED) is defined as the minimum amount of radiation that can cause skin erythema and can be observed up to 24 h after exposure to sunlight [8]. Skin exposed to sunlight will produce erythema quickly or slowly and can cause browning of the skin (tanning) [9,10].

Selaginella plana leaves and *Lagenaria siceraria* (Molina) Standl. fruit have been shown in previous studies to contain active compounds that are soothing and useful for the treatment of inflammation. However, the proportions of *S. plana* fractions and *L. siceraria* (Molina) Standl. fractions have not been standardized and required investigation. In addition, assays of flavonoid and amino acid content should be performed before producing lotion formulations. In this study, we evaluated the physical stability of two lotion formulations containing *S. plana* and *L. siceraria* (Molina) Standl. and investigated their safety and efficacy to relieve erythema due to exposure to the sun [11-13].

MATERIALS AND METHODS

This was an experimental study. First, we prepared fractions of *S. plana* and *L. siceraria* (Molina) Standl. in ethanol. Solutions of the extracted fractions were analyzed for organoleptic, moisture content, loss on drying, ash content, water-soluble extract content, and ethanol-soluble extract content. In phytochemical screening, flavonoids in the ethanolic fraction of *S. plana* and amino acids in the fraction of *L. siceraria* (Molina) Standl. were assayed. A lotion containing a fraction of *S. plana* and a fraction of *L. siceraria* (Molina) Standl. was prepared, characterized by physical evaluation, and tested for stability. The safety and efficacy of the lotion *in vivo* was evaluated. Ethics approval was obtained from the Faculty of Medicine, University of Indonesia, Number 419/UN2.F1/ETHICS/2016. The MED was determined in 22 volunteers. MED readings were taken before and immediately after 30-min exposure to UV radiation from the sun. The lotion was applied in a randomized double-blind manner, and MED readings were taken 1, 3, and 24 h after the application of the lotion.

Equipment and materials

The following equipment was used in this study: Analytical balance (Kenko), glass labware, homogenizer (Sower), freeze-dryer (Modulyo), spectrophotometer (UV mini-1240; Shimadzu), centrifuge (Kobuta 5100; Japan), pH Meter (Hanna), Brookfield viscometer (USA), high-performance liquid chromatography (HPLC) (Waters, USA), and a Mexameter® (CK).

The botanical materials were *S. plana* leaves from Bogor, *L. siceraria* fruit, and extract *Chamomilla recutita* (Matricaria).

Procedures

Preparation of S. plana fraction

Fresh leaves of *S. plana* were sorted, washed, chopped, and dried in an oven at 45°C. The dried powder was extracted by maceration in 70% ethanol. N-hexane was added to obtain an n-hexane extract fraction. To ensure the quality of the fraction, the fraction was characterized by

performing phytochemical screening for alkaloids, flavonoids, saponins, tannins, and triterpenoids/steroids.

Determination of quercetin (a flavonoid) levels in the leaf fraction of S. plana

The determination of flavonoid was performed using a Spectrophotometer UV Vis. 0.5 mL of standard or sample solutions was added into 1,5 mL of ethanol followed by 0.1 mL of 10% $AlCl_3$. Afterward, 0.1 mL of 1M CH_3COONa and 2.8 mL of aquadest was added to the mixture. The mixture was kept in a dark room. The absorbance of the standard solution was read at a wavelength of 417 nm. To samples (0.5 mL) with a concentration of 1000 µg/mL was added to 1.5 mL of ethanol, 0.1 mL of 1 M CH3COONa·$3H_2O$, and 2.8 mL of aquabidest. The mixture was kept in a dark room for 30 min. A UV-VIS spectrophotometer (UV-1240; Shimadzu) was used to read the absorbance of the solution fraction was read at a wavelength of 417 nm [14].

Preparation of L. siceraria fraction

Some of the fresh *L. siceraria* was extracted by maceration in 96% ethanol. The next stage was fractionation using ethyl acetate. We performed phytochemical screening of alkaloids, flavonoids, saponins, tannins, triterpenoids/steroids, and amino acids to ensure the quality of the fractions.

Determination of amino acid concentrations in the ethanol fraction of L. siceraria

An HPLC method was used to determine the amino acid concentrations in the ethanol fraction of *L. siceraria* by comparison with a standard solution of amino acids. An HPLC instrument with a type ACCQ Tag Ultra C18 column and a photodiode array detector was used at a UV detection wavelength of 260 nm. A gradient mobile phase system was used at a flow rate of 0.7 mL/min. The injection volume was 1 µL.

Lotion formulations

The lotion was prepared by making an emulsion of oil and water phases with an emulsifier. Two formulas, A and B, were made. Table 1 shows the compositions.

Evaluation of lotion preparations

The lotion formulas were evaluated for organoleptic including color, odor, and homogeneity; pH, viscosity, and particle size.

Physical stability tests of the lotion test preparations

The stability of the lotion preparations was tested for odor, color, and pH and evaluated at temperatures of 40°C±2°C; 28°C±2°C, and 40°C±2°C for 12 weeks, with observations every 2 weeks.

Table 1: Lotion compositions: Formula A (FA) and Formula B (FB)

Compositions	FA (%)	FB (%)
Selaginella plana	1	0.5
Lagenaria siceraria	0.5	1
Glycerin	5	5
Triethylamine	0.2	0.2
Sodium EDTA	1	1
Propylene glycol	1	1
Methylparaben	0.18	0.18
Aquabidest	79.9	79.9
Stearic acid	3	3
Glyceryl monostearate	2	2
Cetyl alcohol	2	2
Isopropyl myristate	2	2
Paraffin liquid	2	2
Butyl hydroxytoluene	0.1	0.1
Propylparaben	0.02	0.02

Safety test

Safety tests of the lotion preparations were performed by observing the skin of the subjects and assessing the degree of irritation or allergic reactions 48 h after lotion application. Possible side effects included redness, swelling, edema, itching, and sores.

Safety and efficacy test procedure

A randomized controlled trial was conducted to test the safety and efficacy of the lotion formulations (formulas A and B). There were five treatment groups, with 22 volunteers in each group: Negative control, neutral control, positive control lotion containing 1% chamomile extract, formula A lotion, and formula B lotion. A Mexameter®18 was used to measure skin erythema [15]. The MED readings were taken before and immediately after 30-min exposure to natural sunlight UV radiation [16]. The subjects were assigned to their groups randomly, and the subjects and evaluators did not know which lotion had been applied (double-blind study). The MED readings were taken at 1, 3, and 24 h after the application of lotion and 30-min exposure to sunlight. The MED values (mJ/cm^2) were statistically analyzed to determine the distribution, homogeneity, and differences in the degree of reduction in erythema after 24 h for each group.

RESULTS AND DISCUSSION

The yield of *S. plana* in the ethanol fraction obtained was 14.92%. The fraction was a dark green viscous liquid with a distinctive odor. Analysis showed that the fraction had a water content of 19.02%, total ash content of 11.17%, acid-insoluble ash content of 0.06%, ethanol-soluble fraction content of 8.37%, and a water-soluble fraction content of 21.86%. Phytochemical screening of the *S. plana* ethanol fraction showed the presence of flavonoids, steroids/triterpenoids, tannins, and saponins [11-13].

The yield of the ethanol fraction was 9.91%. The fraction was a brown viscous liquid with a distinctive odor like that of caramel. Analysis showed that the fraction has a water content of 20.88%, total ash content of 10.94%, acid-insoluble ash content of 0.11%, ethanol-soluble fraction content of 11.37%, and a water-soluble fraction content of 18.86%. Phytochemical screening of the *L. siceraria* ethanol fraction showed the presence of sterols, saponins, and amino acids.

Determination of flavonoids

The total flavonoid equivalent to quercetin was 24.995%. The yield of the ethanol fraction was 9.91%. The fraction was a brown viscous liquid with a distinctive odor like that of caramel. Analysis showed that the faction had a water content of 20.88%, total ash content of 10.94%, acid-insoluble ash content of 0.11%, ethanol-soluble fraction content of 11.37%, and a water-soluble fraction content of 18.86%. Phytochemical screening of the *L. siceraria* ethanol fraction showed the presence of sterols, saponins, and amino acids.

Determination of amino acids

The HPLC analysis of the ethanol fraction of *L. siceraria* showed the presence of 15 essential amino acids. The level of amino acid equivalent to L-glycine was 0.124%.

Evaluation of lotion formulas A and B

The pH of formulas A and B ranged from 6.18 to 6.39, which fell within the intended pH range of 4.5–6.5.

Viscosity tests using spindle 4 showed that the viscosities of formula A and B were 4600 cps and 5100 cps, respectively. Rheograms of formulas A and B showed that the lotions exhibited thixotropic plastic properties.

Physical stability of lotion formulas

Organoleptic testing of formulas A and B was performed at 4°C±2°C, 28°C±2°C, and 40°C±2°C. After a cycling test and centrifugation, the lotion formulas did not change color or show clumping or phase separation, which indicated that the lotions had good stability.

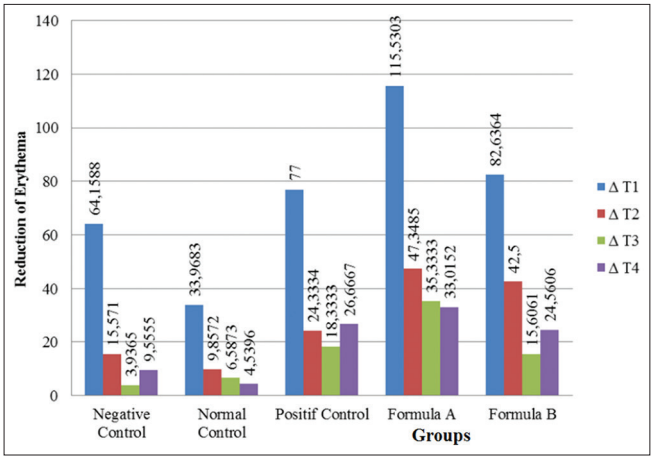

Fig. 1: Reduction of erythema in all groups

Preliminary safety

A safety test in 12 volunteers as preparation for 48-h usage did not cause red skin, itching, burning, heat edema, or raised areas.

Efficacy

The analysis of erythema using one-way ANOVA showed that the formula A group had the highest degree of reduction in erythema among all groups as shown in Fig. 1. The most important therapeutic effect on the reduction of inflammation is the inhibition of the oxidation of arachidonic acid, which leads to the inhibition of lipoxygenase and/or prostaglandin synthesis activity. Therefore, *S. plana* may reduce prostaglandin synthesis caused by exposure to the sun.

CONCLUSION

Among the five groups (positive, negative and neutral control groups, and formula A and B groups), the formula A group, which was treated with formula A containing a 1% ethanol fraction of *S. plana* and a 0.5% ethanol fraction of *L. siceraria* (Molina) Standl., showed the greatest reduction in the level of erythema ($p<0.05$). *S. plana* may reduce the prostaglandin synthesis caused by sun exposure.

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