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Research Article

Irritation Assessment of Herbal Deodorant from Essential Oil of Pomelo (*Citrus maxima*) Peel on Rabbit Skin

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Abstract: A deodorant manages an unpleasant body odor through both reducing and suppressing antibacterial activity and it is formulated generally using antibacterial agents and fragrances. Nowadays, quaternary ammonium compounds like triclosan, aluminum salts, and odor eliminators are used as antibacterial agents and fragrance in deodorant products. A nature-based deodorant potentially replace the synthetic deodorant with less irritating effect. Pomelo (*Citrus maxima*) is one of the Indonesian plants which has some bioactive compounds, including limonene. Essential oil of Pomelo peel (EOPP) exhibits an antibacterial activity as well as fragrance. The aim of this research is to observe the irritation effect of EOPP deodorant on rabbit skin. The irritation assay of herbal deodorant of EOPP with various concentrations (F1: 1.25%; F2: 2.5% and F3: 5%) was carried out based on protocol for dermal acute irritation test on rabbit skin. All the formulations performed the same grades (negligible criteria) with the irritation index of F1, F2 and F3: 0; 0.037 and 0.148, respectively. The herbal deodorants with various concentrations of EOPP did not show an irritating effect on rabbit skin.

Keywords: herbal deodorant; essential oil; skin irritation; Pomelo peel; Citrus maxima

1. INTRODUCTION

Perspiration induces the interaction between bacteria, (e.g. *Staphylococcus epidermidis*) and the dead cells in the skin and lead to bromhidrosis axilla (BA) [1], [2], [3], [4]. Additionally, BA is possibly a result of the activity of microbial enzymes [5], [6]. This body odor may cause discomfort [1], [7]. A deodorant manages an unpleasant body odor through both reducing and suppressing antibacterial activity [8]. Deodorant is formulated generally using antibacterial agents and fragrances [9], [10]. Quaternary ammonium compounds like triclosan (TCS), aluminium salts, and aromatic odor-masking agents are used as antibacterial agents in deodorant products [11]. Although some of these agents have bad effects, not only irritating or sensitizing the skin, but also may improve Alzheimer's disease condition, breast and prostate cancers. Also, the risk of antibiotic resistance may occur. Thus, a herbal deodorant is an alternative for treatment of BA due to its potency against *Staphylococcus sp*. [12], [13].

Pomelo or *Citrus maxima* is one of the Indonesian plants which has some bioactive compounds. For instance, lycopene; pectin; flavonoids; vitamin C; limonene; 9,10-anthracenedione;

and 1,8-diethoxy-antraquinon [14]. Furthermore, the essential oil of Pomelo peel (EOPP) exhibits the MIC and MBC against *Staphylococcus epidermidis* were 0.3125 % v/v and 1.25 % v/v, respectively. In addition, EOPP may also act as a fragrance and thus EOPP has functions both as an antibacterial agent and fragrance in deodorant formulations [15]. Therefore, EOPP was formulated as a deodorant product in stick form whereas till date, there is no deodorant product developed using EOPP.

Toxicity study needs to be conducted in order to observe the safety of new products, including cosmetics before used. In this study, we performed a nonclinical toxicity assay, particularly an acute irritation test on rabbit skin [16].

2. MATERIALS AND METHODS

2.1. Materials

Pomelo fruit (*Citrus maxima*) obtained from local market and determination number conducted in Pharmacognosy and Phytochemistry Laboratory with voucher number 0126/C/UD-FF/UMI/VII/2024, sodium sulfate anhydrous (PA, CP), distilled water, sodium hydroxide (PA, Merck), propylene glycol (Technical), stearic acid (cosmetic grade), cetyl alcohol (cosmetic grade), and litmus paper (NESCO®).

2.2. Methods

2.2.1. Sample Preparation

The essential oil of Pomelo peel was extracted by hydro-distillation. Three hundreds mililitres (300 mL) of distilled water was added to 200 g of fresh pomelo peel. The extraction process was done for ± 3 hours and it was repeated until 500 g of pomelo peel was extracted. The pure essential oils were collected by separating the water using sodium sulfate anhydrous.

2.2.2. Stick Deodorant Formulation

The essential oils were formulated to make herbal deodorants using various concentrations with propylene glycol as a solvent (F1 = 1.25%, F2 = 2.5 %, and F3 = 5 %). The basis formulations (stearic acid 25%; sodium hydroxide 2.5%; cetyl alcohol 19%, and Propylene glycol 50%) were then added with the essential oils (F1, F2 and F3).

2.2.3. The Characteristic Assay of Stick Deodorant

a. Organoleptic Test

   The color, shape and smell changes of stick deodorants were observed on day 0 and 14.

b. pH Test

   The pH test was carried out once all the materials of stick deodorant were put in a roll up case (before hardening).

c. Homogeneity test

   The stick deodorants were spread onto a transparent glass to see whether all the ingredients were homogenous once the glass looked clear without coarse particles.

2.2.4. Animal Preparation

We used 3 male albino rabbits in this study. The rabbit hair was cut in the back area about 10 x 15 cm for the testing area, including the control. Before using the animal for this research, it was

approved by the Ethical Committee of Universitas Muslim Indonesia, Makassar (No. 105A.1/KEPK-UMI/IV/2023).

2.2.5. Application and Observation of the Herbal Deodorant

Each of the deodorant formulas (F1, F2, F3) was applied in the testing area (on skin surface at the back area) of rabbits at about ± 6 (2 x 3) cm^2 and a control area, which was an empty area (without applying the deodorant formula). After spreading the formula, all the area, including the controls, were covered with non-irritant gauze and bandage for 4 hours. In the next 4 hours, the gauze and bandage were removed, all the testing area, including the control area, was removed with water. The skin reaction (erythema and edema) was done in 1 hour after the application, and continued in hour 24, 48 and 72 (Table 1).

Table 1. Skin reaction scoring [16]

Erythema reaction	**Score**
None erythema	0
Very little erythema	1
Clear erythema	2
Moderate erythema	3
Heavy erythema (Flesh red)	4
Edema reaction	**Score**
Non edema	0
Very little edema	1
Mild edema (border area looks clear)	2
Moderate edema (the edema area become wider about 1 mm)	3
Heavy edema (the edema area become wider more than 1 mm until out of the application area)	4

2.3. Data Analysis

The Index of Primary Irritation was calculated based on the skin reaction score using the following formula:

$$\text{Index of Primary Irritation} = \frac{A - B}{\text{C}}$$

A: the average score of erythema and edema in all observation time (sample)
B: the average score of erythema and edema in all observation time (control)
C: the total number of tested animal

3. RESULTS AND DISCUSSION

Pomelo (*Citrus maxima*) is an Indonesian plant with numerous bioactive components, including essential oil [14]. EOPP has an excellent antibacterial effect to inhibit the bacteria causing body odor [15]. Furthermore, it also has a pleasant aroma and therefore is suitable to be developed as an herbal deodorant.

EOPP was extracted from Pomelo peel by hydro-distillation and formulated to stick deodorants in different concentrations, F1, F2, and F3 and made triplicate. All herbal formulations with various concentrations (F1, F2, and F3) exhibit good stability both before and after storage until day 14 based on the organoleptic result (Table 2). Organoleptic test was aimed to identify the

stability of the formulations, whether alterations occur, like the physical appearance (solid to liquid), color, and the smell after being stored[17], [18].

Table 2. Results of physical examination of EOPP deodorant

Parameters	**F1**			**F2**			**F3**		
pH	7	7	7	7	7	7	7	7	7
Homogeneity	H	H	H	H	H	H	H	H	H
Organoleptic result									
• **Physical appearance**									
Day 0	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid
Day 14	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid
• **Color**									
Day 0	White	White	White	White	White	White	White	White	White
Day 14	White	White	White	White	White	White	White	White	White
• **Smell**									
Day 0	Weak	Weak	Weak	Moderate	Moderate	Moderate	Strong	Strong	Strong
Day 14	Weak	Weak	Weak	Moderate	Moderate	Moderate	Strong	Strong	Strong

Note: H: Homogeneous

Table 3. Irritation response category of herbal deodorant formula

Formulation	**Index of Primary Irritation**	**Response criteria**
F1	0	Negligible
F2	0.037	Negligible
F3	0.148	Negligible

Aligned with the homogeneous test, all the deodorant formulations perform a satisfactory result (Table 2). Homogeneity test was also done to observe the stability of deodorants [13], [17], [18], [19]. Moreover, pH results showed a non-irritant result and thus a further irritation assay can be conducted using rabbit skin (Table 2), not extreme (pH or pH $\geqslant$ 11.5) and also aligned with the criteria for armpit skin (pH 4-8) [13], [20].

control
erythema score: 0
edema score: 0

F3 (h-24)
erythema score: 0
edema score: 0

F3 (h-48)
erythema score: 0
edema score: 0

F3 (h-72)
erythema score: 0
edema score: 0

Figure 1. Skin reaction score of herbal deodorant on rabbit skin. Notes: h-24: observation in 24 hour after deodorant application; h-48: observation in 24 hour after deodorant application; h-72: observation in 24 hour after deodorant application

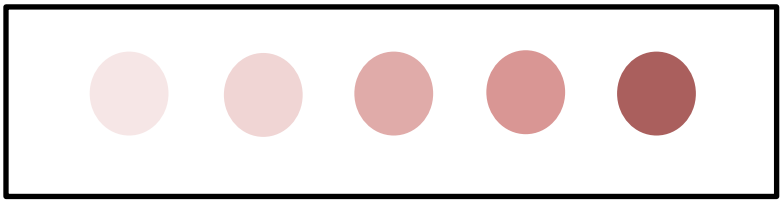

Figure 2. Kit Scoring for erythema in irritation assay on rabbit skin (EC00202305017, 2023; BPOM, 2014)

Since the pH result for all the formulations is 7, it meets the protocol standard for further irritation assay using rabbit skin. This assay was carried out based on an in vivo toxicity guide for acute dermal irritation [16]. The irritation index was determined by scoring the erythema and edema reaction (0-4) of each formulation on rabbit skin (Figure 1). The erythema score was decided using kit scoring due to the possibly subjective decision. This kit scoring (Figure 2) was made from an interpretation of the guide description from an in vivo toxicity guide for acute dermal irritation (Table 2). All the formulations performed the same grades (negligible criteria) with the irritation index of F1, F2 and F3: 0; 0.037 and 0.148, respectively (Table 3). Hence, all deodorant formulations did not show irritating effect on rabbit skin.

4. CONCLUSION

F1 (EOPP 5%), F2 (EOPP 10%), and F3 (EOPP 15%) have the same results for acute dermal irritation test on rabbit skin (negligible). Therefore, the herbal deodorant with various concentrations of essential oils from pomelo peel did not exhibit an irritation effect. Based on organoleptic result, F3 gave the best result due to the good smell. Thus, F3 (EOPP 15%) is the best formulation to be developed as a herbal deodorant.

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