Antibacteria Activity and Acute Dermal Irritation Test in Antiacne Facial Wash from Ethanol Extract of Papaya Seeds (*Carica papaya* L.)

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ABSTRACT

Acne is a problem experienced by teenagers or young adults with prevalence of incidence 85%. This high prevalence encourages people to find solutions, one of them is the use of anti-acne cosmetic product. Before the product can be sold, product safety must be considered. The use of unsafe product will decrease health and can cause other effects. Erythema and edema can be used as skin irritation parameter due to side effects of product use. The objective of this research is to determine effectiveness of the anti-acne facial wash product in *P. acnes* and *S. epidermidis* bacteria and to know the safety of antiacne facial wash product which do not cause acute dermal irritation effects. This experimental research use diffusion method that measures its inhibitory zone as an anti-acne activity and use panch *test* method to examine the level of product safety. The results is that antiacne facial wash with ethanol extract of papaya seeds have an antiacne ability in *P. acnes* dan *S. epidemidis* bacteria. The facial wash was observed for 4 weeks of storage. It is found that the shape, smell, color, homogeneity, pH, and the height were stable and in accordance with SNI standard. The result on the safety test shows that the primary irritation index score on the whole formula is 0, so the product of ethanol extract papaya seeds facial wash is safe from an acute dermal irritation effects.

Keywords: Acne, safety product, facial wash, antiacne, acute dermal irritation.

INTRODUCTION

Prevalence of acne problem is very often experienced in adolescents or young adults about 85% with an age range between 15-44 years (Tjekyan, 2008). The high prevalence of acne problems is driving the public to immediate get solutions, one with the use of antiacne cosmetics. The safety of medicines, cosmetics, and foods in Indonesia under BPOM can be known through preclinical testing and clinical trials to be performed before the product is marketed. According to the Regulation of the Head of BPOM RI Number 7 Year 2014 about Non-Clinical Toxicity Test In vivo states that one of the preclinical tests that can be done for cosmetic preparation is an acute irritation test of the skin.

Papaya (*Carica papaya* L.) is a plant that is part of a seeds that contains, among others, phosphatidylcholine, cardiolipin, carpain, benzyl isothiocyanate, benzyl glucosinolate, betasitosterol, karototin, enzyme mirosin, papain, kimopapain, caricature, and glycosinendolpeptidase (Paramesti, 2014). Papaya seeds also contain secondary metabolite compounds class of alkaloids, saponins, flavonoids, and triterpenoid aldehydes that have antibacterial potency (Sukadana et al., 2008).

Acne is a skin disease caused by inflammation of the skin oil glands (follicular polisebasea) which together with clinical changes of blackheads on the face, back, and chest. Some bacteria can cause acne. *Propionibacterium acnes* and *Staphylococcus aureus*, as well as *Staphylococcus epidermidis* bacteria that can cause mild skin infections (Mulyanti, 2015).

The papaya seed ethanol extract has an effective antibacterial activity in acne-causing bacteria. The antibacterial activity is due to secondary terpenoid aldehyde as the main component. The absence of severe erythema and edema in test animals after exposure to facial liquid soap preparations indicates that the preparations are safe against dermal acute irritant effects.

EXPERIMENTAL

2.1 Materials

The tools used are black fabric, jar, stirrer, filter paper, evaporator, water bath, viscometer, pH stick, volumetric, porcelain and petri dish, mortir and stamper, Beaker glass, measuring glass, analytical scale, micropipet, bunsen, tube reaction, tube rack, ose, erlenmeyer, calipers, dropper drip, LAF, autoclave, incubator, heater, aluminum foil, plaster, kassa, vial, wreapping, label. The materials used are papaya seed, rabbit, *P. acnes* and *S. epidermidis*, ethanol 70% and 96%, Na laureth sulphate, cocamid DEA, methyl paraben, HPMC, BHA, propylene glycol, dinatrium EDTA, perfume, citric acid, clindamycin antibiotics, NA, NB, aquades.

2.2 Making extract

The obtained papaya seeds are then washed, sorting, and dried using sunlight. Dry papaya seeds are powdered and macerated for 3 days using 70% ethanol until submerged. The concentration was carried out using an evaporator until the liquid

extract was obtained and evaporated using a water bath until a thickened extract was obtained.

2.3 Phytochemical screening

The papaya seed ethanol extract was tested using color test with several reagents to determine the content of secondary metabolite compounds such as alkaloids, tannins, flavonoids, saponins, phenols, and terpenoids.

2.4 Facial foam formulation

The liquid face soap formulations are prepared according to Table 1.

Table 1. Facial foam formula

Material	Weight Formula (%)			
	KN	F 1	F 2	F 3
Papaya seeds extract	-	5	10	15
Na laureth sulphate	10	10	10	10
Cocamid DEA	5	5	5	5
Methyl paraben	0.1	0.1	0.1	0.1
HPMC	2	2	2	2
BHA	0.01	0.01	0.01	0.01
Propylene glycol	5	5	5	5
Dinatrium EDTA	0.1	0.1	0.1	0.1
Parfum	1	1	1	1
citric acid	±1	±1	±1	±1
Aquades ad	100	100	100	100

2.5 Antibacterial activity test form extract and facial foam

Extract and facial foam containing ethanol extract of papaya seeds were then tested its antibacterial activity on *P. acnes* and *S. epidermidis* bacteria.

2.6 Physical test of facial foam

Observing facial foam on odor, shape, color, pH, viscosity, and foam height for 4 weeks of storage.

2.7 Dermal acute irritation test

Facial foam test for safety on 3 albino rabbit animals who shaved the width of 2x3 cm^2 in every 6 locations. In the body parts that has been shaved feathers are presented doses at 3 minutes, 1 hour, 4 hours, 24 hours, 48 hours, and 72 hours. The appearance of erythema and edema can be used as a parameter of the severity of skin irritation.

RESULTS AND DISCUSSION

3.1 Phytochemical screening

The papaya seed ethanol extract proved to contain secondary metabolite compounds that could potentially be antibacterial according to Table 2.

Table 2. Phytochemical screening

Compound	Reagen	Result
Alkaloids	Dragendorf	Orange sediment
	Meyer	White sediment
	Wagner	Brown sediment
Tannins	$FeCl_3$ 1%	Dark green
Flavonoids	Mg, HCl (p)	Red-orange
Saponins	Aqua, HCl	Foamy
Phenols	$FeCl_3$ 3%	Blue
Terpenoids	Lieberman-Burchard	Brown

3.2 Antibacterial activity test form extract and facial foam

Test results of antibacterial extract and facial foam against *P. acnes* and *S. epidermidis* bacteria, yielding inhibitory zone diameter according to Table 3.

Table 3. Antibacterial activity extract test

Sample	Inhibitory zone diameter (mm)	
	X ± SD (*P. acnes*)	X ± SD (*S. epidermidis*)
Clindamycin	24.3 ± 0.15	25.43 ± 0.65
Ethanol 70%	4.70 ± 0.55	2.83 ± 0.15
Extract 5%	10.8 ± 0.30	12.46 ± 0.37
Extract 10%	14.1 ± 0.47	14.83 ± 0.30
Extract 15%	12.3 ± 0.20	17.00 ± 0.26
Extract 20%	12.4 ± 0.25	15.10 ± 2.02
Extract 25%	8.7 ± 1.56	6.50 ± 0.78
Extract 30%	16.2 ± 0.30	17.43 ± 0.51

Table 4. Antibacterial activity facial foam test

Test group	Inhibitory zone diameter (mm)	
	X ± SD (*P. acnes*)	X ± SD (*S. epidermidis*)
Positive control	20.70 ± 0.26	21.36 ± 0.68
Negative control	6.96 ± 1.30	8.23 ± 0.32
Formula 1	20.33 ± 2.95	22.30 ± 1.25
Formula 2	22.76 ± 2.67	25.40 ± 0.55
Formula 3	25.70 ± 3.12	29.06 ± 1.25

Based on table 3, the data obtained were analyzed statistically. The results analyzed showed that there was a significant difference between positive and negative control with each variation of extract concentration. Therefore, it can be said that the extract of papaya seed ethanol has antibacterial activity against *P. acnes* and *S. epidrmidis* bacteria. Furthermore, based on Table 4, the data obtained were analyzed statistically. The results analyzed show that there is a significant difference between positive and negative controls with each formula. The higher the concentration of the extract on the formula the larger the inhibit zone diameter.

3.3 Physical test of facial foam

Facial foam is liquid, homogeneous, smelling of lemon, yellow color. The color is getting yellow on the formula that contains more extracts. Observed facial foam concerned for 4 weeks, the results can be seen in Table 5.

Table 5. Stability for 4 weeks

	KP	**KP**	**F1**	**F2**	**F3**
Shape	Yes	Yes	Yes	Yes	Yes
Color	Yes	Yes	Yes	Yes	Yes
Odor	Yes	Yes	Yes	Yes	Yes
Homogen	Yes	Yes	Yes	Yes	Yes
pH	Yes	Yes	Yes	Yes	Yes
Viscosity	No	No	No	No	No
Foam height	Yes	Yes	Yes	Yes	Yes

The shape, color, odor, homogeneity, pH, and foam height remained stable during the 4 weeks. pH obtained in accordance with SNI standard of skin pH is 5.0-7.0. At high yield of foam also in accordance with SNI standard that is 2.3-22 cm. However, the viscosity results are unstable and not in accordance with the established SNI standard of 14,000-17,000 cps.

3.4 Dermal acute irritation test

The acute dermal irritation test of the face soap liquid preparation is one of a series of toxicity tests performed to determine the toxic effects of exposure to the substance. Observations made are qualitative observations by looking at whether or not the effects of erythema and edema that arise after the exposure of test preparations on rabbit skin. As well as quantitative observations by classifying the effects of erythema

and edema that arise and are calculated according to the primary irritation index. The results of qualitative observation that is the existence of erythema and edema that almost can not be distinguished from normal skin. Quantitative observation results are in accordance with Table 6.

Table 6. Observation of irritation effect

Time	**Test group (X ± SD)**					
	K Nor	**KP**	**KN**	**F1**	**F2**	**F3**
3 second	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1 hour	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
4 hour	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Primary Irritation Index				0	0	0
Time	Test group (X ± SD)					
	K Nor	KP	KN	F1	F2	F3
24 hour	0.0±0.0	0.0±0.0	0.3±0.5	0.3±0.5	0.0±0.0	0.3±0.5
	0.0±0.0	0.0±0.0	0.3±0.5	0.3±0.5	0.0±0.0	0.3±0.5
48 hour	0.0±0.0	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5
	0.0±0.0	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5
72 hour	0.0±0.0	0.6±0.5	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5
	0.0±0.0	0.6±0.5	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5
Primary Irritation Index				0	0	0

The results of the primary irritant index are the classifieds according to the irritant response category, and it is known that the facial foam of the ethanol papaya seeds extract is safe against the effects of acute dermal irritation. The appearance of erythema and edema in the skin of experimental animals is suspected because the material used in the formula have the potential to cause erythema and edema. Sodium laureth sulphate as anionic surfactant on the facial foam formula table exceeds the usage limit, as stated in the Handbook of Pharmaceutical Excipients of 0.5-2.5%. Use of sodium laureth sulphate that exceeds the limit will cause erythema and edema in the skin. Sodium lauryl sulphate in the Listiani study (2015), proves that the use of 1% sodium lauryl sulphate can cause mild irritation, and in Dewantara's study (2015) also states that the use of 10% sodium lauryl sulfate may cause skin irritation. The occurrence of an inflammatory response due to the use of anionic surfactants is by destroying the epidermal structural proteins, causing hyperhydration and destruction

of the stratum corneum bilayer lipid membrane (Verdy et al., 2013). In the formulation of a DEA cocaine as an ionic surfactant, according to Noor and Nurdyastuti (2009) the DEA cocaine may reduce the irritation caused by anionic surfactants. Therefore, although there is little erythema and edema, the use are still relatively safe in the effects of acute dermal irritation.

CONCLUSIONS

Based on the research, formula 3 (extract 15%) is the most effective formula against bacteria *P. acnes* and *S. epidermidis*. As well as facial foam ethanol extract of papaya seeds is safe against acute dermal irritation because it does not cause severe erythema and edema effects.

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