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Research Article
Antibacterial Tests Against Acne *in vitro*, the Physical Stability and Patch Test using Cream Containing Ethyl p-methoxycinnamate Extracted from *Kaempferia galanga* L., Rhizoma

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Abstract

Background and Objective: An activity of certain bacterias is one of the causes of acne. Rimpang kencur (*Kaempferia galanga* L.) has an antibacterial agent from compound ethyl p-methoxycinnamate (EPMC). The purpose of this study is to find out the antibacterial activity using EPMC against *P. acne*, *S. aureus* and *S. epidermidis* with the physical stability of the cream and its safety use as an antiacne cream. **Materials and Methods:** The antibacterial activity and minimum inhibitory concentration of EPMC are 0.3, 0.6, 1.2 and 2.4% was done using disc diffusion method and broth dilution test. Data obtained from *in vitro* test of bacterial activity was analyzed using descriptive analysis and Complete Randomized Design (CRD) with 99% ($p<0.01$) level of confidence. **Results:** The result shows that all EPMC concentration has significant antibanterial activity ($p<0,01$) respectively gaining clear zone against *P. acne* (9.00, 11.50, 14.50 and 16.00 mm), *S. aureus* (9.00, 11.50, 16.50 and 22.00 mm) and *S. epidermidis* (10.50, 12.50, 20.50 and 27.00 mm). **Conclusion:** The EPMC compound with the 0.6, 1.2 and 2.4% concentration is proven to have MIC against *P. acne* bacterias, while on the *S. aureus* and *S. epidermidis* reaches up to 1.2 and 2.4% concentration. From the results of safety use (pacth test) on 12 subjects there were no evidence of allergic irritation, therefore cream EPMC 1.2% is safe to be used in topical preparation.

Key words: Ethyl p-methoxycinnamate, *Kaempferia galanga*, antibacterial activity, cream stability

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acne is the most common skin disorder occurring universally with an estimated prevalence of 70-87%[1], caused by opened pores of the skin being blocked by oil, dead skin cells, bacterial activity, cosmetics, food and chemical substances. Acne vulgaris is a common acne, affecting about 70-80% of adolescents and young adults. It is a multifactorial disease of the pilosebaceous unit[2]. Although there is virtually no mortality associated with this disease, there is often significant physical and psychologic morbidity[3]. Acne vulgaris is a chronic inflammatory dermatosis which is notable for open and/or closed comedones (blackheads and whiteheads) and inflammatory lesions including papules, pustules or nodules[4].

Acne frequently begins in the prepubertal period when adrenal androgens stimulate the sebaceous glands and possibly, the follicular epithelium. Later, around puberty, ovarian and testicular androgens play a role and the amount of acne increases in susceptible individuals, but the fundamental process remains the same according to Shalita[5].

Some bacterias can stimulate acne formation and imflammation. Bacterial activity which stimulates acne inflammation is caused by *Propionibacterium acne*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. A substance which has antibacterial activity such as antibiotics which are often prescribed in beauty clinics is needed to cure acne[6].

One of the natural substances which have been studied for its antibacterial activity derives from rimpang family Zingiberaceae, one of them is *Kaempferia galanga* L., rhizome. The compound which is derived from (*Kaempferia galanga* L.). The rhizomes of *K. galanga* have been used by indigenous medical practitioners to treat bacterial infections[7] and the suspectable compound for its antibacterial activity is ethyl p-methoxycinnamate (EPMC). Ninety five percent ethanol extract of this plant possessed antibacterial activity against *Staphylococcus aureus* and hot water extract against *Escherichia coli*[8]. The EPMC also exhibits significant anti-inflammatory potential by inhibiting proinflammatory cytokines[9]. The antibacterial activity of *Kaempferia galanga* L., extract have been proven by testing it on bacteria *S. aureus*, *S. pyogenes*, *E. coli*, *M. tuberculosis*, *V. cholera*, *V. parahaemolyticus*, *K. pneumonia*, *P. aeruginosa* and *S. typhi*[10]. Based on the literature study, there has been no study about the antibacterial activity for the treatment of acne from EPMC compound in *Kaempferia galanga* L., rhizome.

Therefore, this study will examine the antibacterial activity of EPMC from *Kaempferia galanga* L., rhizome towards *P. acne*, *S. aureus* and *S. epidermidis*. The EPMC compound was applied in the form of cream product in order for it to be stable and not cause allergic reactions or irritations towards the consumer and also to mask the odor from the extract which could cause discomfort for the consumer.

MATERIALS AND METHODS

Research materials: Research materials consist of *Kaempferia galanga* L., rhizome, hexane, bacteria *P. acnes*, *S. epidermidis* and *S. aureus* ATCC 33862. Bacterial media used: Blood agar *Brucella*, Nutrient Broth (NB), Brain Heart Infusion (BHI) and Mueller-Hinto agar. Reference antibacterial substance is clindamycin (Oxoid). Solvent for EPMC crystal which was used for bacterial testing was DMSO 30% and aquades. Cream materials: Aquades, stearic acid (5%), cetyl alcohol (3%), isopropyl myristate (3%), gliseril monostearate (2.08%), propylparaben (0.02%), methylparaben (0.18%), TEA (0.42%), BHT (0.1%) and propylene glikol (10%).

Research tools: The tools which were used are: Extraction set, rotatory vacuum evaporator, paper disc, generating kit (Oxoid), shaker, anaerob jar (Merck), autoclave (Hiramaya, Japan), incubator 37°C (Memmert), capillary pipe, TLC plate $G_{60}F_{254}$, chamber, Differential Scanning Calorimetry (DSC-60 Shimadzu), spectrophotometer IR (Shimadzu).

Research method: In this study, to obtain EPMC crystals from *Kaempferia galanga* L., rhizome, a non-polar solvent was chosen and then maceration method was used. The EPMC crystals were then obtained and identified for its characteristics through several steps; TLC by comparing the retention time (Rf) between EPMC from isolate and EPMC standard, melting temperature and impurity assay was done using Differential Scanning Calorimetry (DSC) and analysis of chemical structure between the EPMC isolate and EPMC standard was done using IR spectrophotometer. Antibacterial activity of EPMC crystal compound was tested using disc diffusion method[11] towards *P. acne*, *S. aureus* and *S. epidermidis* bacteria. The *in vitro* test was done using 4 different concentrations, positive control and negative control. The concentration of *Kaempferia galanga* L., rhizome extract was 0.3, 0.6, 1.2 and 2.4%. The positive control was clindamicyn disc and negative control was aquades and 30% DMSO. The bacterial activity was measured using a ruler (Oxoid) from the diameter of the clear zone formed in the agar of the petri disc. The criteria of antibacterial activity refers to Davis and Stout[12] and Ambarwati[13] who classified it into: low (<5 mm), moderate (5-10 mm), strong (10-19 mm) and

very strong (>20 mm). Minimum Inhibitory Concentration (MIC) from EPMC compound was analyzed using dilution method, according to the level of turbidity from the liquid media in the tube. The turbidity of al test solutions with different concentrations was then compared to the turbidity of the control (bacteria and solvent) using oxoid standard (1.5×10^8 bacteria cells mL^{-1}). If the concentration of the test solution with the lowest concentration is less turbid than the control (bacteria and solvent) then that value is considered as the MIC. The cream was made using EPMC from *Kaempferia galanga* L., rhizome with *in vitro* antibacterial test of 1.2% concentration and cream base as the negative control. Cream was evaluated for its physical evaluation which comprised of organoleptic, homogenity, pH, globule diameter, viscosity, flow rate and consistency. Evaluation of physical stability was done by conducting a stability test at temperatures 4±2, 25±2 and 40±2°C, cycling test, mechanical (centrifugal test). Patch test was done by spreading ±0.8 mg cream on the skin of upper back and then covered with a patch test tool, after that the symptoms which arise after 48 h of use were observed[6].

Data analysis: Data obtained from *in vitro* test of bacterial activity was analyzed using descriptive analysis and complete randomized design with 99% ($p<0.01$) level of confidence.

RESULTS

Extraction of *Kaempferia galanga* L., rhizome: As much as 8 kg fresh *Kaempferia galanga* L., rhizomes produced 2000 g of dry powder *Kaempferia galanga* L., rhizome or as much as 25% rendement from fresh weight. Results of maceration process which was obtained 37.52 g from hexane solution. The EPMC crystals which have been purified by recrystalization obtain was 11.04 g.

Two characterization tests of crystal

Thin Layer Chromatography (TLC)

Structure analysis using IR spectrophotometer: Based on the measurement and EPMC structure analysis using IR spectrophotometer, compared to EPMC standard from literature, there were similarities in the chemical structure including the same functional groups and wave absorbance which were very similar.

Antibacterial activity response: Determination of antibacterial activity response towards hexane extract containing ethyl p-methoxycinnamate (EPMC) was done by using disc diffusion and MIC using liquid dilution method. Based on the results of antibacterial activity test EPMC crystals isolated from *Kaempferia galanga* L., rhizome extract at a concentrations of 0.3, 0.6, 1.2 and 2.4% *in vitro*, suggests that each concentration of EPMC has inhibitory activity against *P. acne.* From the results of ANOVA each concentration tested against bacteria *P. acne* with a concentration of 0.3, 0.6, 1.2 and 2.4% gave significantly different results ($p<0.01$). Clear zone formed at concentrations of 0.3, 0.6, 1.2 and 2.4% respectively 9.00, 11.50, 14.50 and 16.00 mm. Clear zone formed on a standard drug clindamycin as an antibacterial acne still has the biggest clear zone of 33.00 mm.

Clear zone formed from the test results towards *S. aureus*, according to the criteria shows antibacterial activity from concentrations of 0.3% medium, 0.6% strong, 1.2% strong and very strong 2.4%. From the concentrations, the lowest concentration was still able to form a clear zone which indicates antibacterial activity.

Liquid dilution method: Testing using liquid dilution method was done to determine the MIC, in order to strengthen antibacterial activity data. In this method, the concentrations which were tested were 0.3, 0.6, 1.2 and 2.4%. The EPMC crystal compared to 3 controls: media, bacteia and solvent. Results of MIC test using dilution method towards *S. aureus* and *S. epidermidis* shows that at concentrations of 1.2 and 2.4% the liquid is less turbid compared to the liquid with concentrations of 0.3 and 0.6%. There is not much difference in the results when compared to results from testing towards *P. acne*, *S. aureus* and *S. epidermidis* toward *P. acne* at concentrations of 0.6, 1.2 and 2.4% it was visibly less turbid than the one with aconcentration of 0.3% which was turbid.

Formulation and evaluation on cream physical stability: Anti-acne cream was formulated using ethyl p-methoxycinnamate (EPMC) as the active ingredient at a concentration of 1.2%. Oil phase including stearic acid, cetyl alcohol, propylparaben, gliseril monostearate and isopropyl myristate were heated at 70°C until melted, then was cooled to 50°C. Afterwards, BHT was added into the oil phase and homogenously mixed.

Materials soluble in water are propylene glycol, TEA and methylparaben. TEA was previously dissolved in 10 mL of aquades, methylparaben dissolved in propylene glycol and then TEA was added and poured in to aquades and then homogenized. The EPMC was dissolved in ethanol and poured I to the water phase. The oil phase was then pour and mixed in to the water phase at the same temperature until homogenized using a homogenizer at 3500 rpm. The

combination of preservatives between methylparaben and propylparaben was used together to broaden the spectrum of both substances[15].

pH test: The storage of cream at different pH affects the pH of cream.

Measuring of consistency: Consistency of semisolid preparation was done using a penetrometer. From the measurement at week-0 the value was 349 1/10 mm from cream base and 355 1/10 mm for EPMC 1.2% cream. Whereas at week-12 a decrease in consistency occurred to 338 1/10 mm for cream base and 342 1/10 mm for EPMC 1.2% cream.

Mechanical test: Based on the mechanical centrifugation test at 5000-10.000 rpm for 30 min both creams did not experience phase changes. Therefore it can be stated that both creams have good stability.

Safety test of cream: Before safety test of the cream was done (patch test) EPMC crystals from hexane extract was analyzed for its residual hexane content. The analysis gave results of hexane content below dibawah 1 ppm (<0.0001%) or undetectable which meant that EPMC crystals were safe to be used in a topical preparation. Patch test was done for 48 h (2 days) towards 15 subjects. The cream was spread on the upper back skin as much as ±80 mg using 2 different creams; they were the cream base and EPMC 1.2% cream. Afterwards, the cream was examined throughout the first 30 min and 48 h to determine whether or not an allergic reaction occurred. From 15 subjects at the beginning of the study, only 12 were successful inclusion criteria until the end of the study, 3 volunteers dropped out. Tests conducted on 12 subjects (12-17 years old), health with normal or dry skin types. Cream containing EPMC 1.2% and a cream base tested topically on the upper back of the subject.

Testing procedure as follow Colipa guidelines:

Type patch : Hypoallergenic tape
Location : Back
Frequency : once
No. of application : ±80 mg

Assessment visual scoring system after 30 min, 24 h and 48 h later. Data obtained is processed to obtain a Primary Irritation Index (PII) using formula:

$$PII = \frac{\text{No. of erythema and edema 24 and 48 h}}{\text{No. of volunteers} \times \text{amount of observation time}}$$

PII = 0

DISCUSSION

The EPMC crystal obtained from this study was 11.04 g with a yield of 0.552%. The obtained EPMC is small when compared with the results from Taufikurohmah and Nurhayati[16] research that is equal to 2.11%. The difference yield of EPMC resulting from such research can occur because the rhizomes used are different. This difference could come from rhizomes age, type of soil and harvest time. Compared with the research Soeratri *et al.*[17] using the same methods (maceration) and same solvent, EPMC produced yield of 0.267%; smaller than the amount of this study. The difference results from both study determined by the length of the immersion process and recrystallization process.

Ethyl p-methoxycinnamate (EPMC) in kencur is the main component of *Kaempferia galanga* L., rhizome, the crystal EPMC is easily isolated and purified[18]. The EPMC compound obtained in this research was compared with the standard compound using Thin Layer Chromatography (TLC), spectrophotometer FT-IR and Differential Scanning Calorimetry (DSC) method.

The mobile phase which was used for TLC as the mobile phase was hexane- ethyl acetate (8:2)[17]. The results of observation under UV light at λ 254 nm, the spot was clearly visible compared to visibility before and after being sprayed with H_2SO_4 10%. Based on the EPMC characterization test and observation under UV lamp 254 nm, all three spots on the TLC plate with silica $G_{60}F_{254}$ as the stationary phase were visible and had similar retention (Rf) of 0.54 from spot 1 on *Kaempferia galanga* L., extract (K), EPMC sample (S) and EPMC standard (Std.) (Fig. 1).

The EPMC compound has an aromatic functional group C-H, carbonyl ester C=O and substitution ring 1.4 disbititution (para). Based on literature, FT-IR results of the three functional groups have absorbance respectively, 2978.09, 1705.07 and 2036.83-1913.39 cm^{-1} which is the same as the absorbance of EPMC sample 2978.19, 1705.13 and 2036.90-1909.59 cm^{-1} (Fig. 2). Besides that, there is also a similarity in functional group owned by EPMC literature (Table 1). This result was strengthened by supposition that the compound was ethyl p-methoxycinnamate (EPMC).

The determination of melting point using DSC shows that the melting peak of EPMC crystal was at 51°C. Whereas the

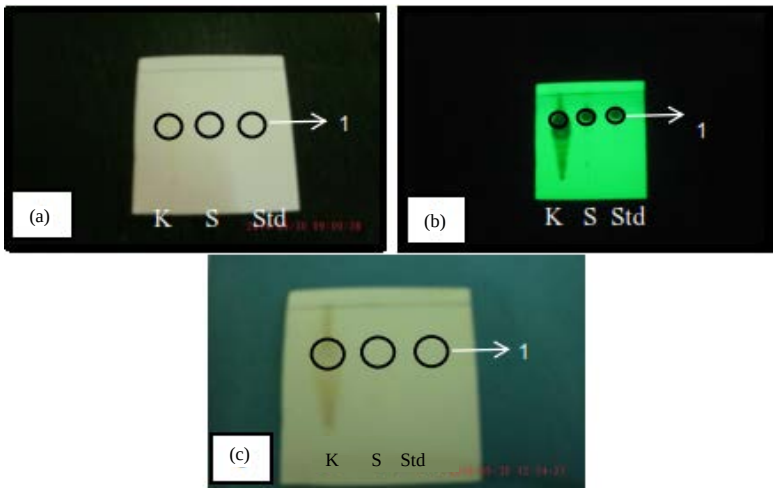

Fig. 1(a-c): Chromatogram (a) Visual, (b) UV λ 254 nm and (c) H_2SO_4 10%, K : *Kaempferia galanga* L., extract, S: EPMC sample and Std: EPMC standard

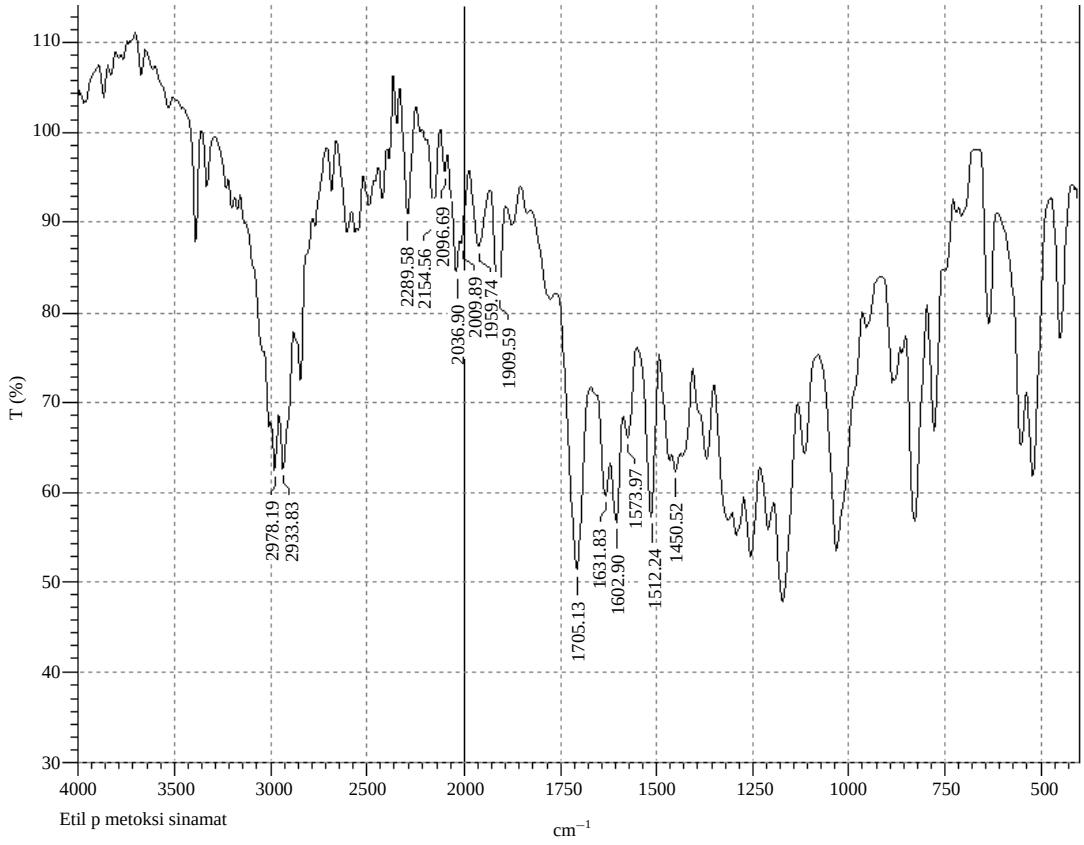

Fig. 2: Result of EPMC crystal measurement using FT-IR

melting point temperature from the reference was 48-50°C[19]. It can be seen that the melting point of EPMC from both sample and standard were similar (Fig. 3).

Kaempferia galanga L., extract has activity against *P. acne* bacteria that cause acne has been investigated by Bahar *et al.*[20]. From the results of ANOVA each concentration

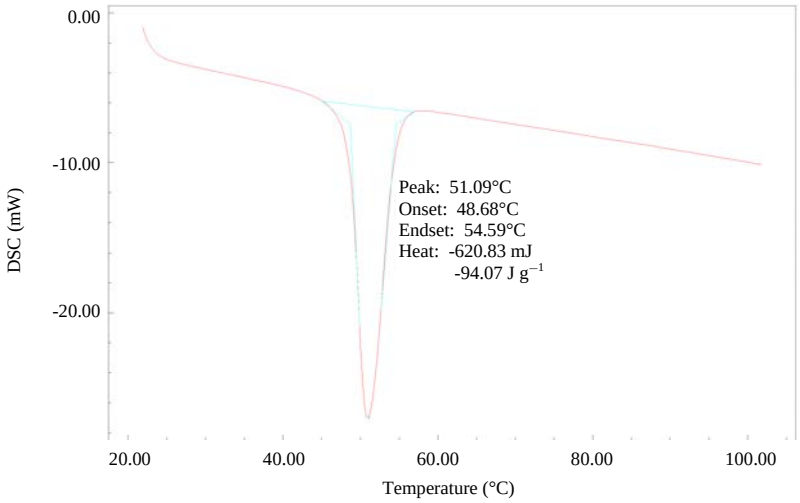

Fig. 3: DSC thermogram of sample EPMC crystal

Table 1: Interpretation of IR spectrum between EPMC sample and EPMC standard

	Wave length (cm^{-1}) EPMC	
Characteristic *FG = Functional group	Sample	Standard literature Nugraha *et al.*[14]
FG = C-H aromatic	2978.19 cm^{-1}	2978.09 cm^{-1}
FG-C-H aliphatic	2933.83 cm^{-1}	2931.80 cm^{-1}
FG ring substitution benzenering1.4 distribution (para)	2036.90-1909.59 cm^{-1}	2036.83-1913.39 cm^{-1}
FG C=O carbonyl ester	1705.13 cm^{-1}	1705.07 cm^{-1}
FG C=C alkenes	1631.83 cm^{-1}	1627.92 cm^{-1}
FG C=C aromatic	1602. 90 cm^{-1}	1604.77 cm^{-1}

Table 2: Mean zone inhibition of crystal EPMC rhizome extract powder against *P. acne*

Treatments	Mean inhibition zone (mm)	Antibacterial activity criteria Davis and Stout[12]
Concentration (0%) (Negative control)	-	-
Concentration (0.3%)	9.00	Moderate
Concentration (0.6%)	11.50	Strong
Concentration (1.2%)	14.50	Strong
Concentration (2.4%)	16.00	Strong
Clindamycin (Positive control)	33.00	Very strong

Antibacterial activity criteria according to Davis and Stout[12] and Ambarwati[13], Weak: <5 mm, Moderate: 5-10 mm, Strong: 10-19 mm and Very strong: >20 mm

Table 3: Mean zone inhibition of crystal EPMC rhizome extract powder against *S. aureus* bacteria

Treatments	Mean inhibition zone (mm)	Antibacterial activity criteria Davis and Stout[12]
Concentration (0%) (Negative control)	-	-
Concentration (0.3%)	9.00	Moderate
Concentration (0.6%)	11.50	Strong
Concentration (1.2%)	16.50	Strong
Concentration (2.4%)	22.00	Very strong
Clindamycin (Positive control)	32.50	Very strong

Antibacterial activity criteria according to Davis and Stout[12], Weak: <5 mm, Moderate: 5-10 mm, Strong: 10-19 mm and Very strong: >20 mm

tested against bacteria *P. acne* with a concentration of 0.3, 0.6, 1.2 and 2.4% gave significantly different results ($p<0.01$). Clear zone formed at concentrations of 0.3, 0.6, 1.2 and 2.4%, respectively 9.00, 11.50, 14.50 and 16.00 mm. Clear zone formed on a standard drug clindamycin as an antibacterial acne still has the biggest clear zone of 33.00 mm (Table 2).

Based on the criteria for antibacterial activity according to the criteria[13] clear zone formed at concentration 0.3% was medium, 0.6% strong, 1.2% strong and 2.4% strong (Table 2). From these criteria show that crystalline EPMC obtained from hexane with a concentration of 0.3%; the lowest concentration still forms a clear zone indicating the presence of antibacterial activity. The results of antibacterial tests against *S. aureus* bacteria showed that the concentration of 0.3 and 0.6% had the same clear zone as the clear zone formed on *P. acne* which were 9.00 and 11.50 mm. While concentrations of 1.2 and 2.4% showed inhibition zone of 16.50 and 22.00 mm greater than the concentration inhibition zone formed on *P. acne* (Table 3). But the inhibition zone formed in the positive control (clindamycin) on *S. aureus* bacteria was 32.50 mm, slightly smaller when compared with clindamycin on *P. acne* bacteria test which was 33.00 mm (Fig. 4).

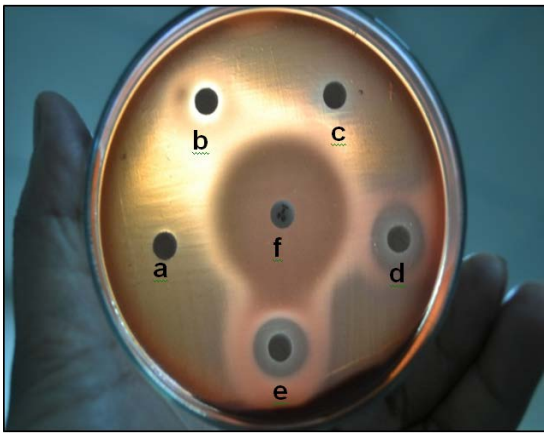

Fig. 4: Antibacterial test results *P. acne* against crystal ethyl p-methoxycinnamate from *Kaempferia galanga* L., rhizome extract powder, a: Concentration 0% (Negative control), b: Concentration 0.3%, c: Concentration 0.6%, d: Concentration 1.2%, e: Concentration 2.4% and f: Clindamycin (Positive control)

Table 4: Mean zone inhibition of crystal EPMC rhizome extract powder against bacteria *S. epidermidis*

Treatments	Mean inhibition zone (mm)	Antibacterial activity criteria Davis and Stout[12]
Concentration (0%) (Negative control)	-	-
Concentration (0.3%)	10.50	Strong
Concentration (0.6%)	12.50	Strong
Concentration (1.2%)	20.50	Very strong
Concentration (2.4%)	27.00	Very strong
Clindamycin (Positive control)	35.00	Very strong

Antibacterial activity criteria according to Davis and Stout[12], Weak: <5 mm, Moderate: 5-10 mm, Strong: 10-19 mm and Very strong: >20 mm

Observation towards *S. epidermidis* shows that at concentrations of 0.3, 0.6, 1.2 and 2.4% each forms a clear zone of 10.50, 12.50, 20.50 and 27.00 mm (Table 4). All concentrations tested on bacteria *S. epidermidis* from concentrations of 0.3-2.4% EPMC have inhibitory compounds against bacteria *S. epidermidis.* This is evident from the clear zone formed on the discs in all concentrations.

Based on the criteria for antibacterial activity, concentrations of 0.3-2.4% towards *S. epidermidis* showed strong and very strong antibacterial activity with a large clear zone of 10-19 and more than 20 mm (10-19 and >20 mm) (Table 4, 5). However, if the antibacterial activity of a clear zone formed from bacteria *S. epidermidis* at concentrations of 0.3 and 0.6% are observed, it has a significant value of 0.045 ($p>0.01$) which means that it was not significantly different when compared with concentrations of 1.2 and 2.4%.

Table 5: Results of minimum inhibitory concentration of EPMC *Kaempferia galanga* L., rhizome towards bacteria

	Bacteria		
Treatments	*P. acne*	*S. aureus*	*S. epidermidis*
Concentration (0.3%)	0	++	++
Concentration (0.6%)	-	+	+
Concentration (1.2%)	-	-	-
Concentration (2.4%)	-	-	-

++: Active (Present growth), +: Active (Few growth) and -: Non active (No growth)

Table 6: pH measurement results of storage at different temperatures

	pH at temperature					
	4°C		25°C		40°C	
Weeks	Base	Cream (1.2%)	Base	Cream (1.2%)	Base	Cream (1.2%)
0	-	-	6.46	5.79	-	-
2	6.25	5.79	6.26	5.80	6.07	5.55
4	6.30	5.82	6.30	5.75	6.18	5.58
6	6.31	5.84	6.26	5.60	5.84	5.43
8	6.28	5.85	6.25	5.56	5.79	5.43
10	6.25	5.93	6.12	5.73	5.77	5.52
12	6.16	5.78	6.17	5.80	5.79	5.50
Average	6.26	5.83	6.23	5.71	5.91	5.50

From the results of antibacterial activity against *P. acne* measurements, *S. aureus* and *S. epidermidis* using disc diffusion method, the greater the EPMC concentration tested, the greater the antibacterial activity is generated. This is indicated by the clear zone formed around the disc.

Kaempferia galanga L., rhizome extract from ethanol fraction at concentration of 0.75% had activity against *P. acne* bacteria. Then the ethanol extract obtained was used in clinical trials on 30 volunteers who suffer from acne with mild to moderate in gel dosage form. From the research Siswanto *et al.*[21] gel containing *Kaempferia galanga* extracts has *Kaempferia galanga* L., dominant odor, so that the EPMC isolation and the formulation in a cream dosage form could reduce the typical odor of *Kaempferia galanga* which can reduce the comfort of the patients.The formulation of 1.2% ethyl p-methoxycinnamate (EPMC) was chosen based on the results of the antibacterial test using criteria[13]. And ANOVA analysis results which was significantly differ significant, as well as MIC from dilution test method.

Based on the storage forcream at 4±2°C for 12 weeks on towards cream base, the data obtained for average pH was almost neutral at 6.26. When compared to 1.2% ethyl p-methoxycinnamate (EPMC) cream, the pH was slightly acidic with an averages pH of 5.83 (Table 6).

The same pH occurred at 25±2 and 40±2°C during 12 weeks storage the average pH of cream base was close to neutral 6.23 and 5.91; compared to the 1.2% EPMC cream which was slightly acidic 5.71 and 5.50). The addition of 1.2%

Table 7: Category erythema and edema (Draize-FHSA scoring system)

Erythema		Edema	
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 mm)	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Table 8: Primary skin irritation index

Category	Primary Irritation Index (PII)
Non irritant	0.0-0.4
Negligible irritant	0.5-1.9
Moderate irritant	2.0-4.9
Severe irritant	5.0-8.0

Table 9: Safety test results

	Score					
	30 min		24 h		48 h	
Subject	Base	EPMC (1.2%)	Base	EPMC (1.2%)	Base	EPMC (1.2%)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0

EPMC concentration towards teh cream affects the pH of the cream where the cream became more acidic than the base. In oil in water creams, the slight decrease in pH of the cream could be affected by oxidation during 12 weeks storage, where the EPMC is hydrolyzed due to the water content in the base becomes para methoxy ceramic acid which causes EPMC 1.2% creamto become acidic[17]. The slight change in pH which occurred in EPMC 1.2% cream for during 12 weeks storage can be said to be safe for the skin as the pH is still within teh acceptable pH range of the skin which is pH 4.5-6.5 (Table 6). The addition of 1.2% EPMC in the cream could maintain teh consistency of the cream. Besides that, the use of stearic acid in oil in water emulsions is able to increase the stability of the cream when reacted with a water soluble emulgator. In the cream making process, strearic acid is reacted with TEA during 12 weeks to increase the stability during 12 weeks storage. According to the cycling test, both creams had good stability. Based on observation of 12 subjects, assisted and consulted by the doctors, all the results have been declared safe since no erythema and edema occured and from calculation of pII in the safety test (according to Draize-FHSA scoring system, explained in Table 7-9), it can be seen that both the cream base and the 1.2% EPMC cream did not cause erythema and edema, therefore, it is safe to be used as a topicalpreparation.

CONCLUSION

Ethyl p-methoxycinnamate from *Kaempferia galanga* L., rhizome extract has been proven to have antibacterial activity against *P. acne*, *S. aureus* and *S. epidermidis* significantly ($p<0.01$) at concentration 0.3, 0.6, 1.2 and 2.4%. The EPMC with concentrations of 0.6, 1.2 and 2.4% has been proven to have MIC towards *P. acne*, whereas towards *S. aureus* and *S. epidermidis* the MIC was 1.2 and 2.4%.

The cream preparation containing 1.2% EPMC, based on the evaluation and physical stability study which includes cycling test, consistency test, mechanical test and cream stability at 4±2, 25±2 and 40±2°C has a good stability. Safety study of the anti-acne cream containing 1.2% EPMC towards 12 subjects via patch test method was confirmed to be safe towards teh skin as a topical preparation.

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