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IDENTIFICATION AND ANTIBACTERIAL ACTIVITY OF PROPIONIBACTERIUM ACNES OF ETHYL P-METHOXY CINNAMATE ISOLATED FROM KAEMPFERIA GALANGA LINN

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ABSTRACT

Kaempferia galanga Linn is a typical Indonesian plant that contains the compound Ethyl P-Methoxy Sinnamate which has antibacterial activity. Acne is a common skin condition characterized by inflammation of the hair follicles and oil glands in the skin. The most effective acne treatment is the use of antibiotics. This research is an experimental study and aims to identify galangal rhizome isolates (Kaemperia galangan L) obtained from N-Hexane extract. using Thin Layer Chromatography (TLC) and Densitometry TLC as well as testing the antibacterial activity of the acne-causing Propionibacterium acnes ATCC 6919 using Complete Tool Design (RAL) with SPSS analysis, confidence level ($p<0.05$) and continued with the ANOVA test using the disc diffusion and liquid dilution methods for MIC. On In Vitro test Kaempferia galanga Linn contains antibacterial compounds that cause acne, namely Ethyl P-Methoxy Sinnamate with medium-strong strength at a concentration of 0.6%; 1.2%, and 2.4% with an inhibition zone of 8.70 mm; 10,10 mm and 11.70 mm. The clear zone for Clindamycin is 27.30 mm. Ethyl P-Methoxy Cinnamate (EPMS) isolated from Kaempferia galanga Linn N-Hexane extract Kaempferia galanga Linn has a concentration of 1.572 g/mL and has activity against Propionibacterium acnes bacteria at a concentration of 0.6%; 1.2% and 2.4%

Keywords: *Antibacterial, Ethyl P-Methoxy cinnamate, propionibacterium acnes and Diffusion, Dilution*

INTRODUCTION

Acne on facial skin is quite disturbing to the appearance, many people try to use skincare products to eliminate and even prevent acne. Acne is a skin disorder caused by chronic inflammation of the pilosebaceous unit which consists of non-inflammatory lesions such as open comedones and closed comedones as well as inflammatory lesions in the form of papules, pustules, and nodules. Generally, acne first appears during adolescent puberty when puberty hormones increase around the age of 8 - 12 years, acne appears like blackheads around the back and cheeks (Afriyanti, 2015).

As many as 85% of acne is found in adolescents However Acne is also found in 20-40% of ages adults and is found most often in women. Reason Certain pimples mature Not yet confirmed (Handayani & Wijayanti, 2015). Some contributing factors in the appearance of pimples among other consequences hypersecretion of androgen hormone, increases sebum secretion, exposure/infection to acne bacteria such as *Propionibacterium acnes*, hyperkeratosisform microcomedonesand increased response inflammation. Exposure to rays of the sun also becomes the reason appearance of pimples Because radiation ultraviolet light will cause peroxidation comedogenic and reactionary inflammation (James, 2016).

Lowering sebum production, and decreasing amount of colonies of acne-causing bacteria such as *Propionibacterium acnes* can be reduced by administering an antibacterial agent such

as erythromycin, clindamycin, and tetracycline (James, 2016). Antibiotic resistance is a growing concern and has prompted efforts to limit the duration of antibiotic courses and emphasize combination treatment regimens. The resistance pattern *of Propionibacterium acnes* corresponds to trends in antibiotic use. Treatment outcomes worsen as resistance emerges (Madelina & Sulistiyaningsih, 2018).

Kaempferia galanga belongs to the Zingiberaceae family and owns 53 genus And more than 1,200 species, Wrong One the genus is *Kaempferia. Kaempferia* has around 50-60 species of medium size in the family Zingiberaceae (KEMENKES, 2017). Rhizome aromatic ginger mostly contains alkaloids And oil essentials, which consist of cineol, sour cinnamate, ethyl ester, camphene, paraeumarin, and anisic acid (Kemenkes RI, 2012). EPMS is included in the class of ester compounds that contain a benzene ring and a methoxy group which is non-polar and also a carbonyl group that binds ethyl which is slightly polar so that in its extraction it can use solvents that have varying polarities, namely ethanol, ethyl acetate, methanol, water, and hexane. The solvents used for extraction must have different polarities (Indonesia, 2017). Extraction of EPMS from aromatic ginger uses a temperature less than its melting point, namely 48 – 50 ^{o}C (Hydrodistillation, 2015a). Therefore, research was carried out to identify isolates of *Kaempferia galangan Linn* and the antibacterial activity of *Propionibacterium acnes*, which is expected to be used as an active substance that can reduce acne on the face and not cause resistance.

METHOD

In this research, various materials and tools were employed to investigate the antibacterial properties of Kaempferia galanga Linn extract. The materials included galangal rhizomes, solvents like Toluene and N-Hexane, and laboratory-grade chemicals (Arikunto, 1993). The tools used encompassed digital scales, an oven, a pH meter, glassware, a magnetic stirrer, and a densitometer. The method involved population and sample collection, extraction of galangal rhizomes, testing the quality of the extract, preparation of the isolate, identification of the isolate using Thin Layer Chromatography (TLC), and an antibacterial activity test against Propionibacterium acnes.

The extraction process consisted of maceration and concentration, yielding an N-Hexane extract. Quality testing involves specific and non-specific parameters. The isolate preparation involved crystallization and purification. Identification of the isolate was conducted using Thin Layer Chromatography (TLC) and densitometry (Fortuna Maudy Sintya et al., 2023). The antibacterial activity test utilized sterilization, media preparation, bacterial screening, bacterial suspension preparation, disc diffusion method, and determination of Minimum Inhibitory Concentration (MIC) (Astrilia Damayanti dan Endah Ayu Fitriana Program, 2015). Data analysis employed a completely randomized design with SPSS analysis and ANOVA at a confidence level of ($p<0.05$). This comprehensive methodological approach aimed to assess the antibacterial efficacy of the Kaempferia galanga Linn extract against Propionibacterium acnes.

RESULTS AND DISCUSSION

Extraction of Kaempferia galanga linn rhizome

Kaempferia *galanga linn* rhizome extract is made by maceration using N-Hexane solvent. The extract is made from one part of 9.20 kg of Simplicia powder into the macerator, then the solvent is added. *Kaempferia galanga linn* rhizomes were extracted using N-Hexane solvent in a ratio of 1:5. Simplicia 9.20 kg of dried galangal rhizomes were extracted using 50 L of N-Hexane. The resulting N-Hexane Extract was 850 g.

Table 1. Yield results of N-Hexane Extract of Galangal Rhizome

Sample weight (g)	Extract Weight (g)	Yield (%)
9200	850	8.56

A yield examination was carried out to determine the % of extract obtained from the amount of simplicia used. *Kaempferia galanga linn* rhizome used with a simplicia weight of 9.20 kg had a yield of 8.56%. The extract obtained was weighed compared to the initial weight of the simplicia during extraction. The size of the yield shows the effectiveness of the extraction process. The effectiveness of extraction is influenced by the type of solvent used in the filter.

Extract Quality Test

The identity of the extract, rhizome used for the extract is *Kaempferia galanga L*

Table 2. Quality Test of *Kaempferia galanga L* rhizoma Extract

Parameter	Results
Organoleptic	Form: Liquid like oil Color: Yellowish brown Smell: Typical *Kaempferia galanga L* rhizoma Taste: Typical *Kaempferia galanga L* rhizoma
compounds that dissolve in water	12.70%
Compounds that dissolve in ethanol	9.08%
Loss on Drying	9.33%
Water content	8.67%
Ash content	0.41%
Ash content (not soluble in acid)	0.15%

Organoleptic testing aims to observe important changes in the implementation of the quality of pharmaceutical preparations. From the results of observations of the N-Hexane Extract of *Kaempferia galanga L* rhizoma, the results were obtained as an oil-like liquid with a distinctive color and taste of *Kaempferia galanga L* rhizoma, the extraction results were like oil because aromatic ginger rhizomes were high in essential oil content (Setyawan Eko, Putratama Pandhu, 2015).

The compound levels in the galangal rhizome extract were found to be 12.07 for water-soluble compounds and 9.08% for ethanol-soluble compounds. This shows that the compounds

in aromatic ginger extract contain organic compounds that are pollic (soluble in water and alcohol).

Ethyl P-Methoxy Cinnamate Isolate

Ethyl P-Methoxy Cinnamate (EPMC) isolate is obtained by leaving the extract resulting from the evaporator in an open room at room temperature 1x24 until saturated or until EPMS crystals form. The EPMC crystals formed were filtered using filter paper. Total EPMS formed: 200.169 grams

Table 3. Yield results of EPMC isolate from *Kaempferia galanga L* rhizoma

Galangal rhizome extract (g)	Isolate (g)	Yield (%)
850	200,169	23.54

Characteristics Test of Ethyl P-Methoxy Cinnamate (EPMC) Compound

Characteristics of Ethyl P-Methoxy Cinnamate Compound (EPMC) were tested using Thin Layer Chromatography (TLC) and Thin Layer Chromatography (TLC) Densitometry. The eluent used in the TLC test was Toluene: ethyl acetate P with a ratio of 95:5 compared between the sample and the EPMS standard from the results of observations under a UV lamp with a wavelength of 254 nm, the penotolon spot produced between the standard and the isolated sample was found to be the same spot (Figure 1) (Gangaram et al., 2022). This shows that the Rf value of sample (c) shows the same retention value (Rf), namely 0.63, which is the same as the EPMS standard spot (a) and the spot formed. The EPMS isolate obtained looks the same as the standard spot and it is suspected that the compound is ethyl p-methoxy cinnamate. However, when compared with the spots produced by the aromatic ginger extract spot (b), there are several spots formed which indicate that there are still other compounds in the aromatic ginger extract (Hydrodistillation, 2015b).

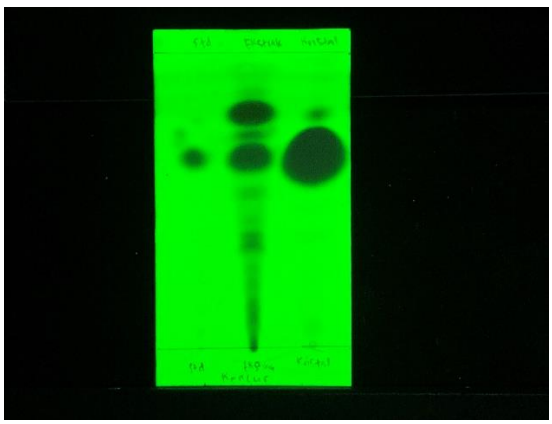

Figure 1. Thin layer chromatography profile: a. EPMC Standard, b. *Kaempferia galanga L* Extract, b. *Kaempferia galanga L* isolate

Characteristics using TLC Densitometry were carried out using TLC Densitometry. The isolated EPMS levels were compared with Standard EPMS. The measurements used the mobile phase Toluene: ethyl acetate P with a ratio of 95:5, using silica gel 60 F254 as the stationary phase at a wavelength of 254 nm. The standard solution is made into a series of dilutions to

obtain levels that are close to the dilution series. The Ethyl P-Methoxy Cinnamate content was obtained at 15.72%, and the results of the EPMS content were obtained by the requirements stated in the Indonesian Herbal Pharmacopoeia, which stated that the EPMS content was not less than 4.30% (Jala et al., 2022).

Table 4. Results of measuring EPMS levels in *Kaempferia galanga L* isolates

No.	EPMC samples	Area Size (AUC)	Concentration (mg/mL)	Content (%)
1	Replication 1	0.01930	1,636	16.36
2	Replication 2	0.01727	1,442	14.42
3	Replication 3	0.01963	1,639	16.39
	Average		1,572	15.72

Propionibacterium acnes antibacterial activity test

The antibacterial activity of EPMC compounds isolated from *Kaempferia galanga L* rhizomes against *Propionibacterium acnes* was carried out using disc diffusion and minimum inhibitory concentration (MIC) using the liquid dilution method.

Disc diffusion method

The antibacterial activity of Ethyl Para Methoxy Cinnamate (EPMC) crystals against *Propionibacterium acnes* bacteria in vitro showed antibacterial activity with the formation of a clear zone around the disc. Based on the results of testing the antibacterial activity of EPMS crystals with a concentration of 0.3%; 0.6%; 1.2%; and 2.4% in vitro which has inhibitory activity against Propionibacterium acnes bacteria, namely concentrations of 1.2% and 2.4%. Respectively 8.7 mm; 9.73mm and 10.03mm. The inhibition zone formed on the clindamycin (oxoid) disc as a positive control still had the greatest inhibitory power, namely 27.30 mm (table 5). The results of the SPSS analysis continued with the ANOVA test using RAL for each concentration tested against *P. acnes bacteria* with a concentration of 0.6%; 1.2 and 2.4% obtained results that were significantly different ($p<0.05$).

Table 5. Diameter Inhibition Test Results against Propionibacterium Acnes

Test Sample	Barrier Diameter (mm)			
	Rep 1	Rep 2	Rep 3	Average
Negative Control (DMSO 30% + Aq. Dest)	-	-	-	-
Positive Control (Clindamycin Disc Oxoid)	29.6	26.2	25.7	27.3
EPMS 0.3%	-	-	-	-
EPMS 0.6%	8.6	8.5	9.0	8.70
EPMS 1.2%	10.4	9.8	10.1	10,10
EPMS 2.4%	11.6	11.7	11.8	11.70

Liquid Dilution Method

Testing using the dilution method was carried out to determine the minimum inhibitory concentration (MIC), to strengthen the antibacterial activity data. In the liquid dilution method, the concentration tested is the same as the disk diffusion test, namely a concentration of 0.3%;

0.6%; 1.2%, and 2.4% EPMS crystals compared with 3 types of controls, namely media, bacteria, and solvent (Kusuma, 2016). The 1.2% and 2.4% concentrations still look clearer when compared to the 0.3% concentration which looks clearer when compared to the 0.6% concentration which still looks a little more cloudy, which means there is still a little growth of Propionibacterium acnes (Table 6).

Table 6. EPMS Minimum Inhibitory Concentration (MIC) Test Results against *Propionibacterium acnes*

No.	Test Sample	Growth of *Propionibacterium acnes bacteria*
1.	EPMS 0.3%	+
2.	EPMS 0.6%	+-
3.	EPMS 1.2%	-
4.	EPMS 2.4%	-
5.	Media control	-
6.	Bacteria control	+
7.	Solvent control	-

CONCLUSION

The compound E-Para Methoxy Cinnamate (EPMS) from N-Hexane Extract of Galangal Rhizome has antibacterial activity against Propionibacterium acnes, the bacteria that causes acne, with a Completely Randomized Design (CRD) analyzed using SPSS and followed by the ANOVA test which has a significant value (p<0, 05) at concentrations of 1.2% and 2.4% respectively were 8.7 mm; 10.10 mm and 11.70 mm with moderate-strong criteria. EPMS compound with a concentration of 0.6%; 1.2% and 2.4% were shown to have antibacterial activity against *Propionibacterium acnes*.

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