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ANTIBIOFILM DETERMINATION OF *Curcuma longa* L. RHIZOME AS DRUG  
CANDIDATES IN HANDLING *Propionibacterium acnes* RESISTANCE

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## ABSTRACT

*Propionibacterium acnes* is the cause of acne vulgaris which is characterized by comedones, papules, pustules, and nodules. The problem of antibiotic resistance against *P. acnes* is increasing, so we need compounds that can inhibit the formation of biofilms, one of which is *Curcuma longa* L. This study aims to determine the antibiofilm activity from the active fraction of *Curcuma longa* L. against *P. acnes*. The analytical method used is descriptive quantitative. The analysis method included test of extract parameters, determination of curcuminoid levels, antibacterial and antibiofilm activity from the active fraction of *Curcuma longa* L., and GC-MS analysis. The results showed that the extract parameters test consisted of organoleptic, drying shrinkage (9.2%), and water content (5%), appropriate to Indonesian herbal pharmacopoeia standards. Antibacterial activity test of both extract and fraction *Curcuma longa* L., obtained the highest value in the methanol fraction. The activity results test of the methanol fraction obtained bacteriostatic activity in the concentration range of 0.5-0.75%, while at a concentration of 1-5% having bactericidal activity. The *P. acnes* antibiofilm test had an inhibition value of 0.25% with a minimum inhibitory concentration of biofilm (MICB) of 0.5%. The main compound identified in the active methanol fraction was aR-turmerone. In conclusion, the rhizome of *Curcuma longa* L. has potential as an antibiofilm against *P. acnes*, especially the aR-turmerone compound which is the main component of the essential oil of *Curcuma longa* L.

Keyword :*P. acne*, Resistant, *Curcuma longa* L, Antibiofilm

## BACKGROUND

Acne vulgaris is a common inflammatory condition in the polysebaceous unit that occurs in adolescents and young adults characterized by comedones, papules, pustules, and nodules caused by *P. acnes* bacteria (Afriyanti R.N., 2015). Data on *P. acnes* resistance in the world shows that more than 50% of *P. acnes* strains are resistant to topical macrolides (Walsh *et al.*, 2016). In Indonesia, 12.9% cases were found to be resistant to tetracycline, 45.2% were resistant to erythromycin, and 61.3% were resistant to the clindamycin group (Madelina W

&Sulistiyaningsih, 2018). The mechanism of antibiotic resistance in *P. acnes* is mutations in chromosome points, especially in the 23S rRNA gene for macrolide resistance and the 16S rRNA gene for tetracycline resistance (Drenoet *al.*, 2018).

Handling the problem of antibiotic resistance, can be done by looking for new compounds from the antibacterial mechanism of action, by inhibiting the formation of biofilms. The proposed alternative is inhibition of the autoinducer-2 (Ai-2) dependent quorum sensing protein (Linfante, *et al.*, 2018). One of the plants that has this mechanism is *Curcuma longa* L. (Packiavathyet *al.*, 2014; Tamfu, AN, *et al.*, 2020). The problem urgency of resistance to the treatment of *P. acnes* is the focus of this study. The proposed alternative treatment is looking for drug candidates, especially from the rhizome of *Curcuma longa* L., with the aim to knowing the antibiofilm activity of the *Curcuma longa* L. extract fraction against *P. acnes* bacteria.

## RESEARCH METHODS

### Instrument

The main tools used are macerator, incubator (Memmert), autoclave (ALP), separating funnel (Pyrex), biological safety cabinet (Biobase), UV-Ozone sterilizer (Elitech), azeotroph distillation (Pyrex), furnace (Yudian), oven (MRC), 48 and 96-hole microwell plates (Biologix; NEST), TLC vessels, UV-Vis spectrophotometry Cary 60 (Agilent Technology), petri dishes (Anumbra), 300 mm calipers (Tricle brand), digital analytical balance (Sartorius) , mini-15K micro centrifuge (Allsheng), and microscope (Yazumi).

### Materials

The ingredients used are *Propionibacterium acnes* ATCC 6919, turmeric rhizome simplicia (*Curcuma longa* L.) (B2P2TOOT), gram staining reagent (ST.Reagenesia), dimethyl sulfoxide(DMSO) 99.9% (Emsure), methanol (DPH), Thin Layer Chromatography (TLC) plate (Emsure) , ethyl acetate (DPH), hexane (Alfa aesar), chloroform (Smart-lab), Brain Heart Infusion Broth (BHIB) (Oxoid) media, glucose (Emsure), Nutrient Agar (NA) media (Hi Media), acetic acid 30% (DPH), 0.2% crystal violet (Certistain), phytochemical screening kit (DPH; Emsure).

### Data analysis

The analytical technique used in this research is descriptive quantitative. Descriptive statistical analysis is a statistic used to analyze data by describing data that has been collected as it is without intending to make conclusions that apply to the public or generalizations.

### Plant Determination

Plant determination is a process in determining the name or specific type of plant. The plant identification used was *Curcuma longa* L. simplicia which was determined in B2P2TOOT. The identification of *Curcuma longa* L. simplicia fragments was also microscopically to see identifying fragments such as starch, cortical parenchyma containing yellow material, transport bundles with ladder-type thickening, covering hairs, periderm, and stele parenchyma in accordance in Pharmacopoeia Herbal 2nd Edition.

### Extraction and Extract Parameter Test

Extraction using the maceration method, *Curcuma longa* L.simplicia was first grind using a laboratoryblender. The refined simplicia then sieved using Mesh 22. Furthermore, 970 grams of simplicia powder was macerated using 70% ethanol in a ratio of 1:10, stirring occasionally for the first 6 hours and left for 18 hours, in a dark place. Replace the solvent twice with the same amount and type. Then the macerate was filtered, and the filtrate obtained was concentrated with rotary evaporator vacuum at 50°C temperature, speed of 50 RPM, and pressure of -10 mmHg, the concentrated extract was then thickened in water bath with 70°C temperature (Daulay, A.S., & Nadia, S., 2019).

Parameter test of *Curcuma longa* L. extract included drying shrinkage test at 105°C with 2 grams of thick extract; the water content test wastriplicate, with 5 grams of extract put into a flask than added 300 mL toluene; lastly, the ash content test by placing 5 grams of extract into a furnace at a temperature of 400°C, cooled outside the furnace to 100°C temperature, put in a desiccator. The cup and ashes were weighed to obtain a constant weight (RI, Kementerian Kesehatan,2017).

### Phytochemical Screening

Phytochemical screening procedure adapted from Farnsworth, N. R. (1966) with modification.

1. Alkaloids, 0.1 grams of sample added Mayer and Dragendroff reagents in 2 test tubes. The formation of a precipitate indicates a positive result for alkaloids.
2. Flavonoids, 0.1 grams of sample added 0.1 gram of zinc powder and 1 mL of concentrated hydrochloric acid, shaken, and allowed to separate. Flavonoids are positive if red, yellow, orange colors are formed on the amyl alcohol layer.
3. Saponins, 0.1 grams of sample was put into a test tube, added 10 mL of hot water, cooled and then shaken for 10 seconds. Saponins are positive if 1-10 cm foam is formed, is stable for 10 minutes, and does not disappear when 1 drop of 2N hydrochloric acid is added.
4. Tannins, 0.1 grams sample was added with saturated NaCl, shaken and observed then added 1% gelatin, then observed again. Positive results for tannins if a precipitate is formed.
5. Steroids/Triterpenoids, 0.1 grams sample was ground in a mortar with ether, filtered with a cotton-stoppered pipette. The filtrate was stored in a steam cup, allowed to evaporate to dryness, then dripped with anhydrous acetic acid and concentrated H<sub>2</sub>SO<sub>4</sub>. A positive result for steroids is indicated by a color change to green or blue, and if positive for triterpenoids is indicated by the formation of a purple/magenta color.
6. Polyphenols, 0.1 grams sample was added with 3 drops of FeCl<sub>3</sub> 1%, producing a blueish-black or greenish-black color, which indicates a positive result containing polyphenolic compounds.

### Fractionation

Fractionation of *Curcuma longa* L. extract using 3 solvents : n-hexane, ethyl acetate, and methanol with a ratio of 2:1, as for the fractionation path, it can be seen in Figure 1.

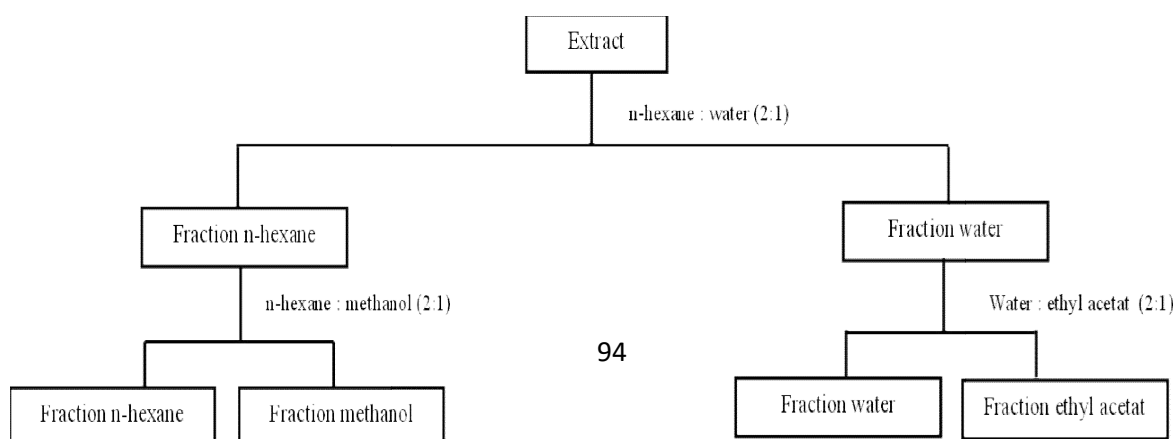


Figure 1. Fractionation Flow (Aji, N. *et al.*, 2022).

### **Thin Layer Chromatography (TLC) Test**

Thin Layer Chromatography (TLC) was performed to separate organic compounds. TLC plate in the oven at 105°C. The chromatography vessel was filled with an eluent mixture of chloroform: methanol (95:5). Spot the obtained fraction into the TLC plate with a capillary tube and allow it to dry. Put it in the vessel and let the eluent rise, marking the eluent limit. The analysis using UV 365 lamps. From the chromatogram results, the methanol fraction and ethyl acetate fraction were separated from the analyte and then proceeded to calculate the Retardation factor (Rf) value (RI, Kementerian Kesehatan, 2017).

### **Determination of Total Curcuminoid**

Perform the assay as indicated on the Spectrophotometry. Weigh approximately 10 mg of sample, put it in a volumetric flask 10 mL, add of ethanol up to the volume limit mark, vortex for 30 minutes and let it for 1 hour. The solution then filtered with filter paper and collected in a test tube. The comparison solution weighed approximately 10 mg of curcumin, put it in a 10 mL volumetric flask, add ethanol to volume. Make a series of dilutions of the reference solution with five dilution levels in mg/L. Ethanol blank solution pipette separately 3 mL. The test solution, the reference solution series and the blank solution each into a suitable container. Measure the absorption at the maximum absorption wavelength of 420 nm. Create a calibration curve and calculate the sample concentration with the equation  $x = y - \text{intercept}/\text{slope}$  (RI, Kementerian Kesehatan, 2017).

### **Antibacterial Test**

Antibacterial activity using disc diffusion method. This method uses a petri dish containing a nutrient agar media. The bacterial suspension was spread with cotton, where the bacteria inoculated using the streak plate method in biological safety. The test solution was dripped as much as 20 µL on a blank paper disc. The solution concentration used is the sample concentration that produced the most optimal inhibitory power against *P. acnes*, with positive control clindamycin and DMSO as negative control. This experiment was repeated three times. The cultures were incubated at 37°C for 24 hours under anaerobic conditions. The results of the inhibition zones obtained were then calculated for the mean and standard deviation (SD). The antibacterial activity of the sample was indicated by the formation of a clear zone around the paper disc on the media and measured using a caliper. The antimicrobial inhibitory zone activity was grouped into four categories, weak activity (<5 mm), moderate (5-10 mm), strong (>10-20 mm), and very strong (>20-30 mm) (Datta, F. *et al.*, 2019).

### **Antibiofilm Test and Biofilm Staining**

Extract fraction using staining method with crystal violet. *P. acnes* biofilm was formed on a 48-hole microplate and three times in succession, 50 µL of cell suspension with a Mc Farland turbidity of 0.5 was inoculated into the microplate up to 400 µL of BHIB media. The solution

concentration used is the sample concentration that produced the most optimal inhibitory power against *P. acnes*, with positive control (clindamycin) and negative control (DMSO). The sample concentrations used were 0.001%, 0.025%, 0.05%, 0.075%, 0.1%, 0.25%, 0.5%, 0.75%, 1%, 2.5%, and 5%. The microplate then incubated for 48 hours at 37°C. Biofilm precipitate that had been stained by crystal violet, then added with 30% 300 µL of acetic acid to release the crystal violet which was absorbed by the biofilm. The biofilm quantity will be proportional to the absorbance of crystal violet (OD585).

### Analysis of *Curcuma longa* L. Active Fraction using GC-MS

Component screening for volatile active compounds using GC-MS with preparation conditions run time : 58,333 minutes, heater : 270°C, pressure : 7,6522 psi, total flow : 54 mL/min. The GC-MS analysis of the active fraction which has antibiofilm activity.

## RESULTS AND DISCUSSION

### Extraction Results and Extract Parameters

The simplicia *Curcuma longa* L. which has obtained from the “Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT)” with a letter of determination number KM.04.01/2/1291/2022. Identification of *Curcuma longa* L. simplicia fragments was microscopically to confirm the identity of the material used. The results of the observations can be seen in Figure 1. There are identifying fragments such as starch, cortical parenchyma containing yellow material, transport bundles with ladder-type thickening, covering hairs, periderm, and stele parenchyma which are in accordance with the Herbal Pharmacopoeia Edition II (RI, kementerian Kesehatan, 2017).

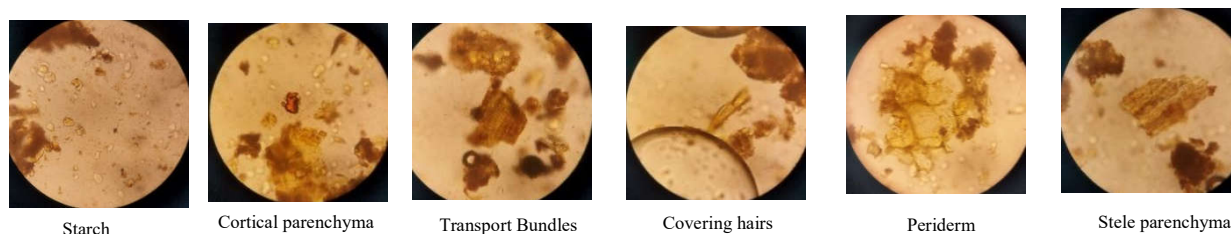


Figure 1. *Curcuma longa* L identification fragments

Table 1. Parameters of *Curcuma longa* L extract

No	Parameter	Result	Requirements*
1	Organoleptic	Dark yellow thick extract, characteristic aroma of <i>Curcuma longa</i> L. rhizome, slightly bitter taste.	Thick extract; yellow; characteristic odor; slightly bitter taste.
2	Drying Shrinkage (% w/w)	9,2 ± 0,01	<10%
3	Water content (% v/w)	5 ± 0,04	<10%
4	Total ash content (% w/w)	2,43 ± 0,01	<8,2%

\*Indonesian Herbal Pharmacopoeia 2nd Edition (RI, Kementerian Kesehatan, 2017)

Simplicia was extracted using maceration method with 70% ethanol as solvent. The results of maceration obtained extract yield as much as 23%. According to the Indonesian herbal pharmacopoeia 2nd edition, the percentage yield of *Curcuma longa* L rhizome extract is not less than 11% of the extraction results appropriate the minimum requirements. As for the quality parameters can be seen in Table 1.

In the flavonoid test, it produces an orange colored solution caused by a reduction reaction with Zn and HCl, producing complex compounds that are orange to red in color (Ningsihet *al.*, 2016). This is in accordance with the statement of Ezeah, C *et al.*, (2020) which states that the resulting orange to red color indicates the presence of flavonoids as a result of reduction by concentrated hydrochloric acid and Zn powder. At the end of the reaction the structure of flavonoids is converted into flavilium salts which can be withdrawn with amyl alcohol. The flavonoid reaction can be seen in Figure 2(i).

Table 3. Phytochemical Screening of *Curcuma longa* L.

Compound Group	Simplicia	Liquid extract
Alkaloids	-	-
Flavonoids	+	+
Polyphenol	+	+
Tannins	+	+
Saponins	-	-
Steroids/triterpenoids	+	+

Description: (+): contains compounds

(-): does not contain compounds

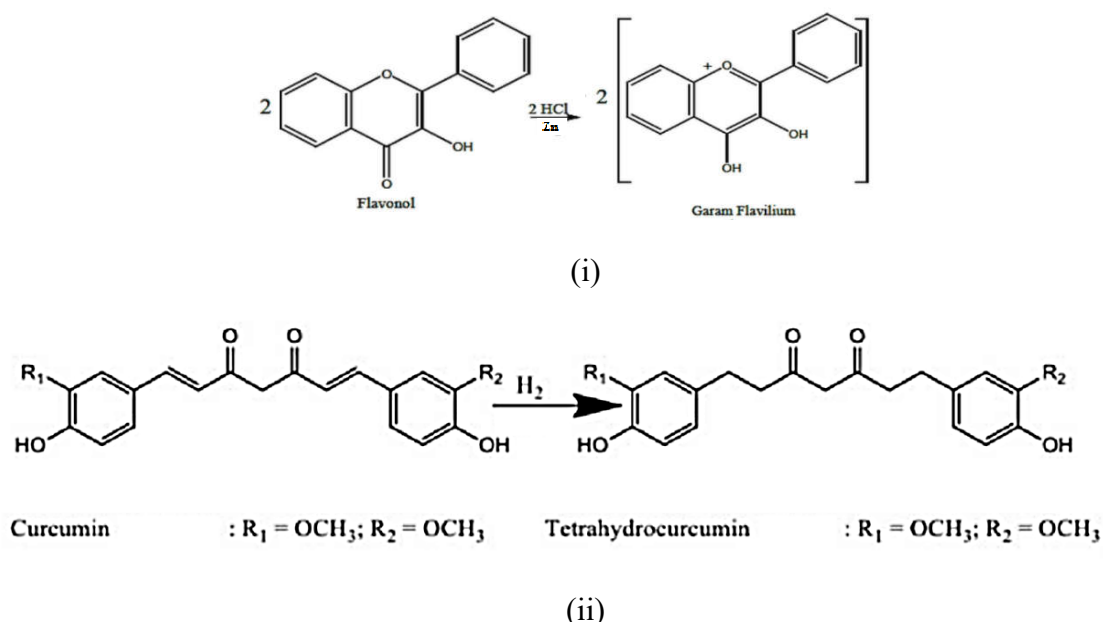


Figure 2. (i) Flavonol reduction reaction to Flavilium, (ii) Addition reaction of curcumin to tetrahydrocurcumin (Ezeah, C *et al.*, 2020; Sudewoet *al.*, 2020).

In the flavonoid test, the possibility of false positive results may occur, this is due to the presence of curcuminoids which are yellow and soluble in amyl alcohol. Therefore, a confirmation test against the standard, which was reacted with concentrated Zn powder and

HCl reagents. The results of the confirmation test showed that the comparison standard for curcumin after being reacted with Zn and HCl powder, the color changed from yellow to transparent, this can be explained based on Figure 2(ii) shows that curcumin will occur an addition reaction in the presence of hydrogen released from the reaction of zinc and HCl. The result of the curcumin reaction is tetrahydrocurcumin which is a colorless derivative of curcumin. So that the curcumin flavonoid test will not cause false positives (Lenny, S. 2006).

## Fractionation Results

Table 4. Yield fraction of *Curcuma longa* L. extract (*Curcuma longa* L.)

Faction Type	n-Hexane	Methanol	Water	Ethyl acetate
Yield (% b/b)	5,00	35,20	34,20	19,50

Fractionation was obtained by liquid-liquid extraction method. The fractionation process uses solvents that do not mix and do not dissolve each other. The fractionation process using the liquid-liquid extraction method, four fractions were obtained, n-hexane fraction, ethyl acetate fraction, methanol fraction, and water. The yield of the fractionation results can be seen in Table 4. The highest yields were obtained in the methanol and water fractions. The total yield of the rhizome fraction was 93.9%, it means that during the fractionation process there were missing components. The results of the fractionation based on the yield showed that the 70% ethanol extract had the most content, polar compounds to semi-polar gradients.

## Thin Layer Chromatography and Total Curcuminoid Contents

After fractionation, chromatographic tests on the methanol and ethyl acetate fractions. The test results show that the ethyl acetate and methanol fractions contain curcumin as seen in Figure 3.

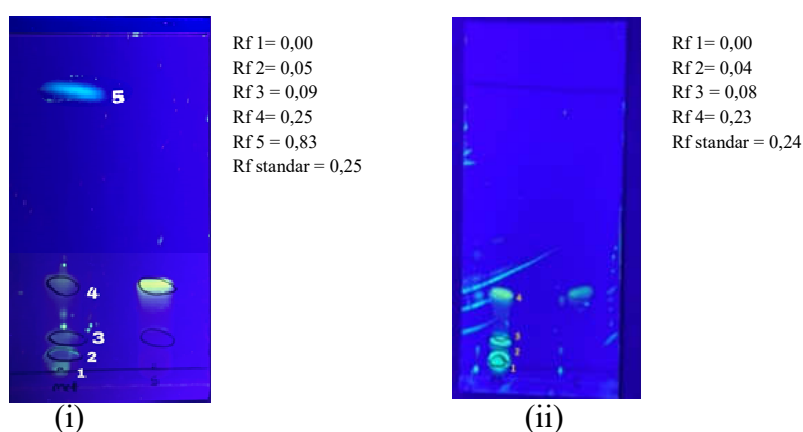


Figure 3. TLC : (i) methanol fraction and (ii) ethyl acetate

Thin Layer Chromatography (TLC) test using chloroform: methanol (95:5) as eluent is a suitable solvent for curcuminoids (Jayaprakasha GK *et al.*, 2005). The initial phenomenon that occurs in the chamber is the balance between the eluent phase and eluent vapor phase in the chamber (Maulana, M. 2018). Evaporation from the eluent in the plate to the chamber

causes the eluent velocity to decrease. After the separation process is complete, the TLC plate is removed and dried. The analysis using aUV lamp at wave length 365 nm. The results of the chromatogram will show the methanol fraction and ethyl acetate fraction which are separated from the analyte. Rf is the midpoint distance of the spot from the starting point / the distance of the solvent front edge from the starting point (Nurgustiyanti, 2021). The Rf value which indicates the curcumin obtained by the methanol fraction is 0.25. The TLC plate of the Ethyl Acetate fraction obtained an Rf value of 0.23.

After being identified qualitatively, the total curcuminoid content was calculated as curcumin. The results of maximum absorbance readings under test conditions, obtained at a wavelength of 420 nm, which is the visible light absorption region. The calibration curve was made at five points in the range of 1-5 mg/L. Absorbance measurements were made and a calibration curve was made to obtain the regression equation  $y=0.939x + 0.2899$ , with a value of  $R=0.9990$ . After knowing the regression equation then calculated the sample concentration. The measurement results showed that the 70% ethanol extract contained  $0.32 \pm 0.003\%$  curcuminoids. This value indicates that it does not meet the quality requirements of the Indonesian Herbal Pharmacopoeia 2nd edition, which cannot be less than 11.17%. In contrast to the extract, the methanol and ethyl acetate fractions had a higher content than the extract, which can be seen in Table 5.

Table 5. Total Curcuminoid

No.	Sample	Concentration (% b/b)
1	Methanol fraction	$0,32 \pm 0,003$
2	Ethyl acetate fraction	$18,51 \pm 0,04$
3	Extract	$7,28 \pm 0,02$

The highest curcuminoid content in the ethyl acetate fraction was  $18.51 \pm 0.04\%$ . This shows that curcuminoids are more soluble in the ethyl acetate fraction compared to other solvents used in the fractionation process.

### Antibacterial Activity Test Results

Antibacterial activity test as a preliminary before entering the antibiofilm activity test stage. The determination of the MIC value was based on the antibacterial activity test. Based on the research of Ibrahim *et al*, (2020) the test of antibiofilm activity was obtain under the minimum inhibitory concentration (MIC).

Table 6. Antibacterial activity of fractions and extracts

No.	Sample	Inhibitory zone (mm)	Description
1	Extract 10%	$7,30 \pm 0,11$	Moderate
2	Water fraction 10%	$0,00 \pm 0,00$	No inhibition
3	Methanol fraction 10%	$14,80 \pm 0,10$	Strong
4	Ethyl acetate fraction 10%	$13,00 \pm 0,14$	Strong
5	n-Hexane fraction 10%	$0,00 \pm 0,00$	No inhibition
6	Clindamycin 1% (+)	$8,70 \pm 0,10$	Moderate

7	DMSO 10% (-)	0,00 ±0,00	No inhibition
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Antibacterial testing on extracts and fractions at a concentration of 10%, the concentration was selected based on the optimization results. Positive control used 1% clindamycin and 10% DMSO negative control. The results of the antibacterial activity (Table 6 and Figure 5) of the fraction and extract with a concentration of 10% showed that the methanol and ethyl acetate fractions had strong category activity (10-20 mm), while the n-hexane fraction and extract had moderate activity (5-10 mm). Based on the test results, the methanol fraction is the highest inhibition zone diameter. So that the methanol fraction by further testing, screening compounds use the GC-MS method and biofilm testing.

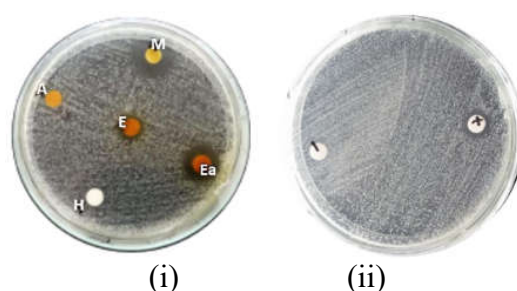


Figure 5. (i) antibacterial activity test results against H (n-Hexane fraction), A (water fraction), Ea (ethyl acetate fraction), M (methanol fraction) and E (extract) antibacterial activity results; (ii) positive control (Clindamycin 1%) and negative DMSO 10%

Antibiofilm assays on *P. acnes* with liquid BHI media added with glucose. Based on the test procedure by Holmberg A, *et al.*, 2009 the use of liquid media BHI (Brain Heart Infusion) supplemented with glucose can propagate biofilm formation in *P. acnes*. Also, incubation time is an important factor, as in the study of Sivasankar C, *et al.*, 2016 incubation time of 48 hour is sufficient time to grow biofilms on *P. acnes*. The results of biofilm formation showed the addition of 5% glucose (Figure 6).



Figure 6. The addition results of 5% glucose to the media formed a biofilm in liquid culture (right) and without addition glucose (left)

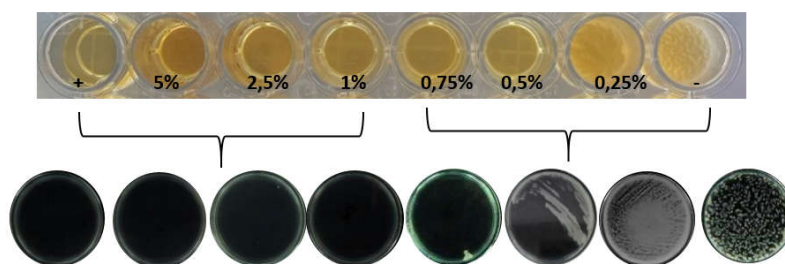


Figure 7. Test results of 0.25-5% antibiofilm concentration

After obtaining the optimal culture conditions for the antibiofilm test, the test was continued with a biofilm formation inhibition test. The results of the biofilm test are shown in Figure 7, the methanol fraction in the concentration range of 0.5-5% did not show any biofilm formation. To determine the bactericidal and bacteriostatic, it is necessary to confirmation test, the liquid culture that is inoculated on nutrient agar media. Confirmation results at a concentration of 0.5-0.75% bacterial culture can still form colonies, while at a concentration of 1-5% it does not show any colony growth. In conclusion, the methanol fraction with concentration of 1-5% it has bactericidal activity, while at a concentration of 0.5-0.75% has bacteriostatic activity. The MIC value was at 0.5%, so the antibiofilm assay was tested below that concentration.

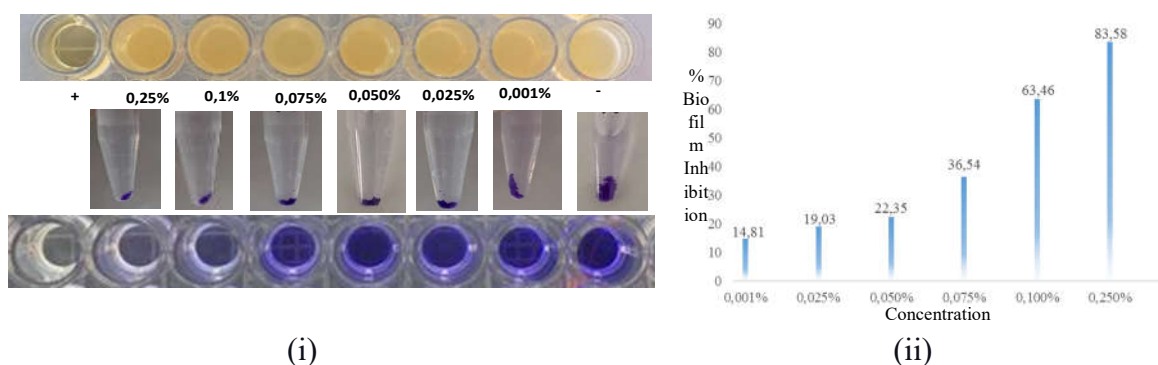


Figure 8. (i) Antibiofilm test results with concentrations of 0.25-0.001%, (ii) Inhibitory value of biofilm formation

After MIC data was obtained activity of the methanol fraction, further dilution in the concentration range of 0.001-0.25%. In this concentration range, *P. acnes* can form biofilms with various thicknesses. The results of the biofilm inhibition test (Figure 8(i)) showed that the methanol fraction was able to inhibit the formation of the largest biofilm at a concentration of 0.25%. However, the the minimum inhibitory concentration of biofilm (MICoB) was at 0.5%. At that concentration, the methanol fraction could inhibit the biofilm without killing *P. acnes* bacteria.

### Methanol Active Fraction GC-MS Analysis

The results of the GC-MS analysis (Figure 9) showed that 18 (Table 7) active methanol fractions were identified. The compound aR-turmerone is the dominant compound found in the methanol fraction of *C. longa L.* The compound aR-turmerone is a sesquiterpene group

which is the main component of *C. longa* L. essential oil and is known to have anti-bacterial activity (Negi, P. S *et al.* , 1999).

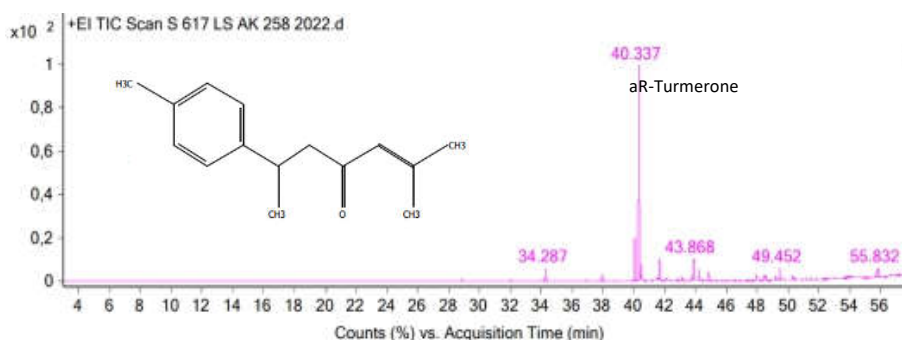
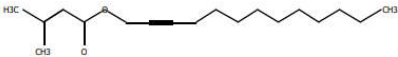
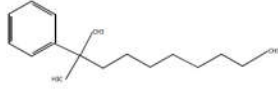
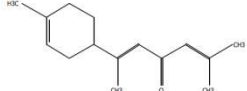
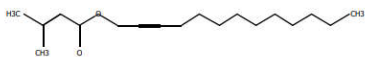
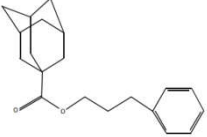
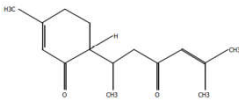
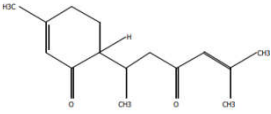
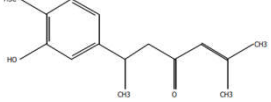
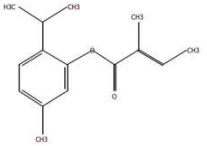
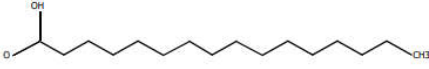
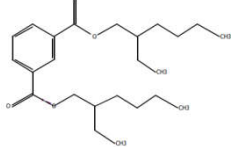


Figure 9. Results of GC-MS analysis showing the presence of phytochemical compounds in the methanol fraction extract of *C. longa* L.

Table 7. Phytochemical components of the active fraction of methanol

Peak	Area %	RT	Mr	Lib Score	Compound Name	Formula	Structure
1	516	34287	206,2	89,43	Phenol, bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	
2	330	37941	134,1	75,4	1,3,8-p-Menthatriene	$C_{10}H_{14}$	
3	1756	40031	218,2	88,3	(Z)-.gamma.-Atlantone	$C_{15}H_{22}O$	
4	10000	40337	216,2	92,67	aR-Turmerone	$C_{15}H_{20}O$	
5	431	40473	218,2	89	Tumerone	$C_{15}H_{22}O$	
6	832	41649	176,1	74,93	Benzenebutanal, .gamma.,4-dimethyl-	$C_{12}H_{16}O$	
7	130	43137	220,2	89,51	6R,7R)-Bisabolone	$C_{15}H_{24}O$	

Peak	Area %	RT	Mr	Lib Score	Compound Name	Formula	Structure
8	112	43757	278,2	69,86	3-Methyl-2-butenic acid, tridec-2-ynyl ester	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	
9	878	43868	232,2	69,04	Benzene, (1,1-dimethylnonyl)	C <sub>17</sub> H <sub>28</sub>	
10	358	44228	218,2	86,49	(E)-Atlantone	C <sub>15</sub> H <sub>22</sub> O	
11	328	44822	278,2	70,46	3-Methyl-2-butenic acid, tridec-2-ynyl ester	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	
12	162	47918	298,2	69,66	1-Adamantanecarboxylic acid, 3-phenylpropyl ester	C <sub>20</sub> H <sub>26</sub> O <sub>2</sub>	
13	219	48449	234,2	87,96	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	
14	115	48557	234,2	84,74	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	
15	182	49210	232,1	71,86	6-(3-Hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one	C <sub>15</sub> H <sub>20</sub> O <sub>2</sub>	
16	425	49452	232,1	75,22	Thymyl tiglate	C <sub>15</sub> H <sub>20</sub> O <sub>2</sub>	
17	186	50280	256,2	85,86	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	
18	803	55832	390,3	82,46	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	

## CONCLUSION AND RECOMMENDATION

The methanol fraction of *C. longa* L. is an active fraction that can inhibit the formation of biofilms. The largest inhibition percentage was at a concentration of 0.25% and the minimum inhibitory concentration of biofilm (MICoB) was found at a concentration of 0.5%. The MICoB value is the lowest concentration where the fraction can inhibit the formation of

biofilms but can still grow bacteria. The main compound identified in the active methanol fraction was aR-turmerone.

Based on the results *Curcuma longa* L. rhizome as a drug candidate in the treatment of *Propionibacterium acnes* resistance, it has been proven that the methanol fraction has antibiofilm activity. One of the main components detected from the methanol fraction is aR-turmerone compound. aR-turmerone is the main component in the essential oil of *Curcuma longa* L., so that further research can recommend testing the essential oil or the pure active substance of aR-turmerone as an antibiofilm tested in vitro and in vivo. *Curcuma longa* L. is quite potential as a drug candidate, in the treatment of *P.acnes* resistance, so this research needs to be tested for the safety.

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