

# Antibacterial Activity and Active Fraction of *Zingiber officinale* Roscoe, *Zingiber montanum* (J.Koenig) Link ex A., and *Zingiber zerumbet* (L.) Roscoe ex Sm. Against *Propionibacterium acnes*

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## ABSTRACT

**Background:** *Propionibacterium acnes* is a commensal bacteria that play a role in developing acne vulgaris on the skin. Antibacterial activity assay in the last decades have shown that *Propionibacterium acnes* is resistant to several antibiotics. Potential zingiber genera such as *Z. officinale*, *Z. montanum*, and *Z. zerumbet* have potential as antibacterial. **Objective:** This study aims to compare the antibacterial activity of *Z. officinale*, *Z. montanum* and *Z. zerumbet* against *Propionibacterium acnes*, the active fraction, and the components of the active fraction of the plant. **Materials and Methods:** Antibacterial activity test using agar diffusion method, extracts with high antibacterial activity were partitioned with water: n-hexane, n-hexane: methanol, water: ethyl acetate. The fraction was tested for antibacterial activity against *P. acnes*. The active fraction obtained was identified for its phytochemical content using TLC and GC-MS methods. **Results:** The results of the antibacterial activity test of *Z. officinale* extract had a larger inhibition zone. The test results of the active fraction *Z. officinale* had a higher activity than the extract. The results of screening using the GC-MS method obtained that the main components identified from *Z. officinale* were volatile oil components ( $\alpha$ -curcumene,  $\alpha$ -zingiberene and zingerone) and oleoresin (6-shogaol). **Conclusion:** Extracts of *Z. officinale*, *Z. montanum* and *Z. zerumbet* had antibacterial activity against *P. acnes*. The highest antibacterial activity in *Z. officinale* both extract and ethyl acetate fraction which is known to contain terpenoids and oleoresin compounds (6-shogaol).

**Key words:** Antibacterial, *Zingiber officinale*, *Zingiber montanum*, *Zingiber zerumbet*, *Propionibacterium acnes*.

## INTRODUCTION

*Propionibacterium acnes* is a commensal bacteria found in the sebaceous glands.<sup>1</sup> Apart from being a normal flora on the skin, these bacteria can act as opportunistic pathogenic bacteria in acne vulgaris. Colonization of pilosebaceous follicles by *P. acnes* is one of the main factors that cause acne by taking part in the skin inflammatory response.<sup>2</sup> In addition to manipulating the host immune response, *P. acnes* can form biofilms and make associations with other bacteria<sup>3</sup>. Resistance of *P. acnes* to antibiotic therapy has gradually emerged over the years as a global problem, with high rates of resistance reported for erythromycin (macrolide) and clindamycin (lincosamide) between 21%-70%, for tetracyclines between 4%-70.30%. The most common mechanism of antibiotic resistance in *P. acnes* is chromosomal point mutations, particularly in the 23S rRNA gene for macrolide resistance and the 16S rRNA gene for tetracycline resistance.<sup>2</sup>

The genus *Zingiber* has been widely used in the world of health as alternative medicine. Several groups of compounds in the *Zingiber* genus are known to have an antibacterial activity such as oleoresins, terpenoids, and flavonoids.<sup>4,5</sup> Several plants of the genus *Zingiber* are widely distributed in Indonesia, three of which are: *Zingiber officinale*, *Zingiber montanum* and *Zingiber zerumbet*. The three species have advantages in terms of the main content they have. *Zingiber officinale* L. contains oleoresins such as gingerol and shogaol,<sup>6</sup> *Zingiber*

*montanum* contains the main content of Terpinen-4-ol, sabinene, kassumunin and flavonoids.<sup>7,8</sup> While *Zingiber zerumbet* has the main component in the form of sesquiterpene derivative compounds such as zerumbone and humuladien.<sup>9</sup> Seeing the potential of the three plants, it is necessary to research the antibacterial potential of the three species of the genus *Zingiber* against *Propionibacterium acnes* and screening for active compounds.

## MATERIALS AND METHODS

### Materials

The plant material used was taken from Ciamis Regency, West Java Province, Indonesia. Crops were harvested on March 24, 2021 and then determined. Voucher specimens are stored at the Bandungense Herbarium, Bandung Institute of Technology, with registration numbers: *Zingiber officinale* Roscoe namely FIPIA-DEP30, *Zingiber montanum* (J.Koenig) Link ex A., namely FIPIA-DEP29 and *Zingiber zerumbet* (L.) Roscoe ex Sm., namely FIPIA-DEP27. The bacteria used were *Propionibacterium acne* ATCC 11827, the culture medium was Nutrien Agar (Oxoid), and dimethyl sulfoxide /DMSO 99.9% (Merck) as extract solvent. The materials used in the extraction process are ethanol 70%, the materials used for fractionation are methanol, ethylacetate 99.8% (Satyam), n-hexane 99.9% (Alfa), and aquadestylates (Brataco). Materials used for TLC: Silica Gel 60 F254 (Merck) was used in stationary phase with methanol (Merck) as mobile phase, and

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Chloroform (Fisher Scientific). The tools used for GC-MS analysis are: GC Aginelt series 7890A MS detector agilent 5977B GC/MSD brand, the column used is DB-5MS (5%-phenyl)-methylpolysiloxane) column with a length of 30 m with a diameter of 0.25mm.

### Extraction and quality parameter of extract

A total of 1000 grams of simplicia powder *Z. officinale*, *Z. montanum*, and *Z. zerumbet* were extracted using the maceration method with 70% ethanol solvent, the amount of solvent used was 1:10 (g: mL) of the total simplicia. The duration of maceration is once 24 hours with two replacement solvents with the same amount and type of solvent.<sup>10</sup> The extract was concentrated using a rotary evaporator at a temperature of 50°C with a pressure of -0.8 BAR. The extract was then screened for phytochemicals including: alkaloids, polyphenols, tannins, flavonoids, saponins, troterpenoids and steroids. The phytochemical screening procedure was adopted from the Farnsworth method.<sup>11</sup> The characteristic test of the extract included: water content, essential oil content, residual solvent content, total ash content, acid insoluble ash content, drying shrinkage, and total curcuminoid content for *Zingiber montanum*, the test procedure and extract quality parameters refer to the Indonesian Herbal Pharmacopoeia 2nd Edition,<sup>12</sup> except that the measurement of the residual ethanol content refers to the Indonesian Pharmacopoeia 3rd Edition.<sup>13</sup>

### Total flavonoid assay

A sample of 200 mg was put into an erlenmeyer, added 25 mL of ethanol P, stirred for one hour with a magnetic stirrer. Filter into a 25 mL volumetric flask, rinse the filter paper with ethanol P and add up to the mark. Preparation of the comparison solution by weighing 10 mg of quercetin using a micro-analytical balance, put it into a 25 mL volumetric flask, dissolve and add ethanol P, until the mark. Make a series of dilutions of solutions of 100, 75, 60, 50, 25, and 10 ppm. Pipette separately 0.5 mL of the test solution and each series of reference solutions into a suitable container, add each 1.5 mL of ethanol P, 0.1 mL of 10% AlCl<sub>3</sub> P, 0.1 mL of 1 M sodium acetate and 2.8 water. Shake and let sit for 30 minutes at room temperature. Measure the absorption at max. Create a calibration curve and calculate the percentage of flavonoids.<sup>12</sup>

### Fractionation

Fractionation using liquid-liquid phase extraction. The solvent is selected based on the polarity index gradient taking into account the miscibility of the solvent phase. The solvents used are water: n-hexane (1:1), n-hexane: methanol (1:1), water: ethyl acetate (1:1). The flow of fractionation can be seen in Figure 1.<sup>14</sup>

### Antibacterial assay

Antibacterial activity was tested by disc diffusion method. The media used is Nutrient Agar. The extract solvent was DMSO 10% and the number of samples tested was 20 µL which was dripped into a blank disk (Ø 6mm). Incubation at 37°C for 24 hours under anaerobic conditions.<sup>15</sup> Extracts of *Z. officinale*, *Z. montanum* and *Z. zerumbet* were prepared in three concentrations of 5%, 10%, 15% and DMSO 10% as blanks. Extracts that have a high inhibition zone against *P. acnes* were continued with fractionation and identification of active compounds.

### Screening of active fraction phytochemical components using TLC and GC-MS

The first step of screening active fraction components using the TLC method with the stationary phase is silica gel 60 F254. The mobile phase used was methanol and chloroform (5:95) for the identified curcumin and quercetin of active fraction. Component screening for volatile compounds using GC-MS with the following conditions: run time 49.5 minutes, heater 200 °C, pressure 7.6522 psi, total flow 54 mL/min.

### Data analysis

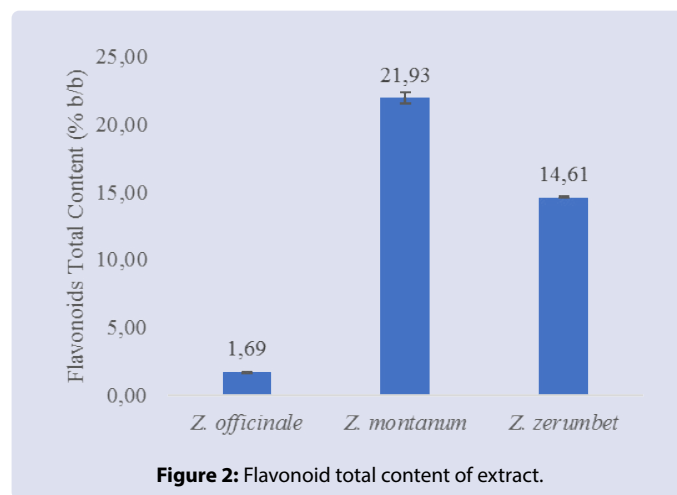
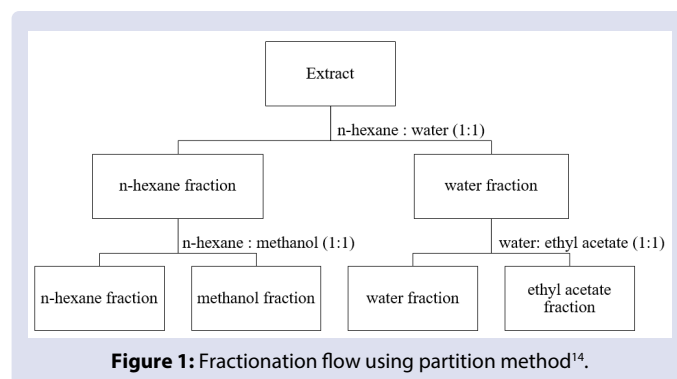
The data were analyzed using the ANOVA (Analysis of Variance) method to see the difference between the test groups with a 95% confidence level. Prior to the ANOVA test, homogeneity and normality tests were carried out on the data. The results of the ANOVA test were followed by LSD (Least Significant Difference) to see the differences between the groups.

## RESULTS

The results of the extraction from each of 1000 grams of simplicia powder obtained 125.8 grams of *Z. officinale* extract (yield 12.58%), 135 grams of *Z. montanum* extract (13.50% yield) and 99.59 grams of *Z. zerumbet* extract (9.96% yield) (Table 1). The amount of yield of *Z. montanum* did not meet the requirements of the Indonesian Herbal Pharmacopoeia 2nd Edition.

The results of phytochemical screening showed that the extracts of *Z. officinale*, *Z. montanum* and *Z. zerumbet* contained alkaloids, polyphenols, flavonoids, and triterpenoids. The differentiator of the phytochemical content is that *Z. montanum* contains steroids and *Z. zerumbet* contains saponins (Table 2). The test results of extract parameters can be seen in Table 3. Determination of extract parameters is very important, several parameters can affect the test activity such as water content, ethanol residue, essential oil content, total ash content, acid-insoluble ash content and total curcuminoid content. Extract parameter requirements can be seen in Table 4.

The results for total flavonoid levels are shown in Figure 2. The highest total flavonoid content was in the *Z. montanum* extract and the lowest was in the *Z. officinale* extract. Measurement of flavonoid levels using UV-Vis spectrophotometer method with reagents AlCl<sub>3</sub>(10%) and 1 M Na-Acetate.<sup>12</sup> Measurements were made at λ 455 nm, the wavelength is the maximum absorption for the reaction of flavonoids with AlCl<sub>3</sub> (Figure 3).



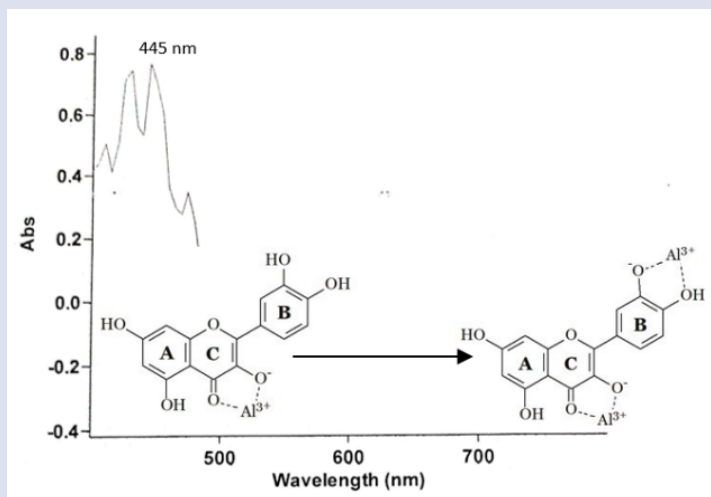


Figure 3:  $\lambda_{max}$  of the complex reaction of flavonoids and aluminium ion<sup>17</sup>.

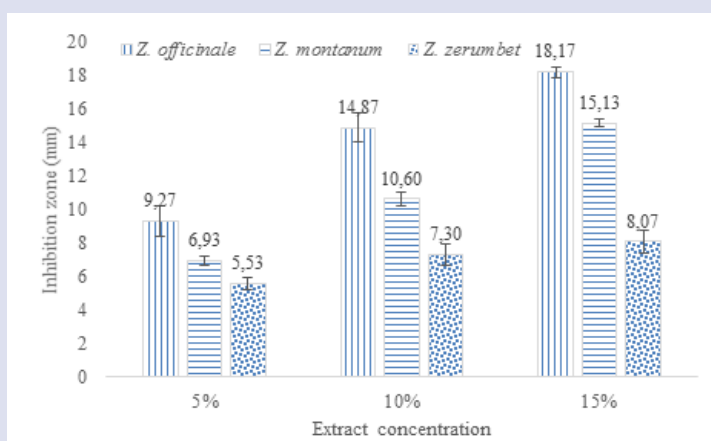


Figure 4: Antibacterial activity of *Z. officinale*, *Z. montanum* and *Z. zerumbet* extract.

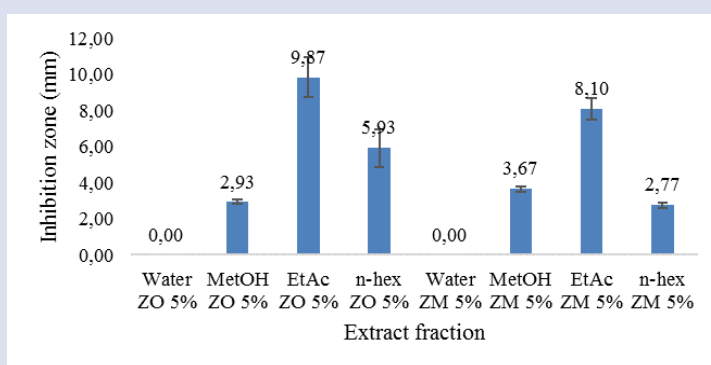


Figure 5: Antibacterial activity of *Z. officinale*(ZO), *Z. montanum*(ZM) fraction.

Table 1: The Result of extract through the maceration method.

Extract	Simplicia Weight (gram)	Extract weight (gram)	Yield (% b/b)	Requirements(12) (%)
<i>Z. officinale</i>	1000	125.8	12.58	5.9
<i>Z. montanum</i>	1000	135	13.50	15
<i>Z. zerumbet</i>	1000	99.58	9.96	5.5

**Table 2: Phytochemical screening results.**

No	Compound Group	Z. officinale	Z. montanum	Z. zerumbet
1	Alkaloids	+	+	+
2	Polyphenol	+	+	+
3	Tannin	-	-	-
4	Flavonoids	+	+	+
5	Saponins	-	-	+
6	Steroids	-	+	-
7	Triterpenoids	+	+	+

Note:

+: positive towards compounds.

-: negative towards compounds.

**Table 3: Extract parameter test results.**

No.	Kind of Assay	Z. officinale (%)	Z. montanum (%)	Z. zerumbet (%)
1	Total ash content (w/w)	3.98±0.35	3.57±0.19	1.61±0.08
2	Acid-insoluble ash content (w/w)	1.16±0.01	1.09±0.03	0.57±0.01*
3	Essential oil content (v/w)	1.60±0.69*	3.73±0.61	1.33±0.61*
4	Residual ethanol solvent (w/w)	0.44±0.04	0.84±0.02	0.06±0.05
5	Water content (v/w)	9.76±0.34	9.11±0.59	7.32±0.25
6	Curcuminoid Total (w/w/)	-	1.65±0.10*	-

Note: \* = Does not fulfill the requirements.

**Table 4: Extract parameter test requirements.**

No.	Kind of Assay	Z. officinale (%)	Z. montanum (%)	Z. zerumbet (%)
1	Total ash content (w/w) <sup>12</sup>	< 5.00	< 7.60	< 0.70
2	Acid insoluble ash content (w/w) <sup>12</sup>	< 3.50	< 1.90	< 0.40
3	Essential oil content (v/w) <sup>12</sup>	≥ 2.40	≥ 1.20	≥ 2.30
4	Residual ethanol solvent (w/w) <sup>16</sup>	< 5.00	< 5.00	< 5.00
5	Water content (v/w) <sup>12</sup>	< 10.00	< 10.00	< 10.00
6	Curcuminoid Total (w/w/) <sup>12</sup>	-	≥ 1.65	-

**Table 5: Phytochemical components identified in the Z. officinale ethyl acetate fraction by GC-MS.**

No.	Name of the Compound	Retention Time	Molecular weight (g/mol)	Peak Area (%)	Formula
1	Decanal	15.40	156.27	2.17	C <sub>10</sub> H <sub>20</sub> O
2	Trifluoroacetyl-lavandulol	20.06	250.26	0.79	C <sub>12</sub> H <sub>17</sub> F <sub>3</sub>
3	α-Curcumene	22.73	202.34	1.8	C <sub>15</sub> H <sub>22</sub>
4	α-Zingiberene	23.07	204.35	2.76	C <sub>15</sub> H <sub>24</sub>
5	trans-β-Farnesene	23.78	204.35	1.21	C <sub>15</sub> H <sub>24</sub>
6	Frambinone	24.44	164.20	1.27	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
7	4-(1-Hydroxyallyl)-2-methoxyphenol	24.91	180.20	0.86	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>
8	Zingerone	26.56	194.23	32.07	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>
9	(3S,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-1-ene	26.93	168.23	0.82	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>
10	Nonane, 1,9-dibromo	27.57	286.05	0.6	C <sub>9</sub> H <sub>18</sub> Br <sub>2</sub>
11	1,3-Benzenediol, 4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]	38.10	276.13	0.91	C <sub>9</sub> H <sub>6</sub> F <sub>6</sub> O <sub>3</sub>
12	(6)-Shogaol	39.01	276.37	21.98	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>
13	Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	40.47	250.38	0.81	C <sub>16</sub> H <sub>26</sub> O <sub>2</sub>
14	β-Dihydroagarofuran	41.23	222.37	8.5	C <sub>15</sub> H <sub>26</sub> O
15	Phthalic acid, di(2-propylpentyl) ester	41.46	390.56	95.29	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>

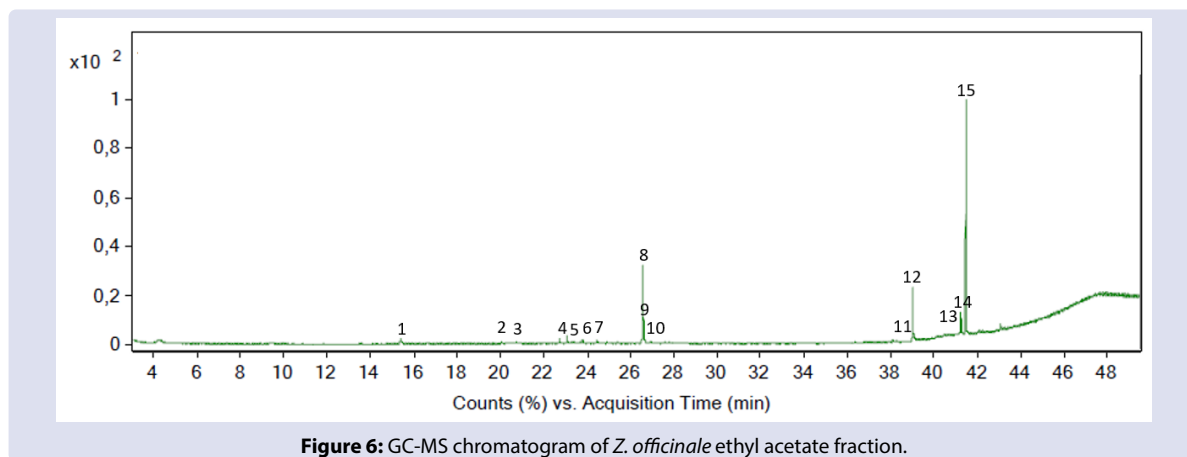
**Table 6: Phytochemical components identified in the *Z. montanum* ethyl acetate fraction by GC-MS.**

No.	Name of the compound	Retention Time	Molecular weight (g/mol)	Peak Area (%)	Formula
1	Terpinen-4-ol	14.66	154.25	3.44	C <sub>10</sub> H <sub>18</sub> O
2	Isovanillin	20.61	152.15	1.36	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>
3	Menthane, 1,2,4-trihydroxy	23.02	188.26	1.73	C <sub>10</sub> H <sub>20</sub> O <sub>3</sub>
4	Methylisoeugenol	25.37	178.23	1.79	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>
5	Benzene, 4-(1E)-1,3-butadien-1-yl-1,2-dimethoxy	26.27	190.24	38.04	C <sub>12</sub> H <sub>14</sub> O <sub>2</sub>
6	(Z)-1-(Buta-1,3-dien-1-yl)-2,4,5-trimethoxybenzene	30.22	220.26	6.74	C <sub>13</sub> H <sub>16</sub> O <sub>3</sub>
7	(E)-4-(3,4-Dimethoxyphenyl)but-3-en-1-ol	31.42	208.25	99.68	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
8	1-(3,4-Dimethoxyphenyl)-2-(N-hydroxyamino)propan-1-ol	32.24	177.24	1.32	C <sub>11</sub> H <sub>15</sub> NO
9	(E)-4-(3,4-Dimethoxyphenyl)but-3-en-1-yl acetate	34.08	250.29	22.82	C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>
10	Hexanedioic acid, dioctyl ester	40.07	370.57	1.7	C <sub>22</sub> H <sub>42</sub> O <sub>4</sub>
11	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	41.45	390.56	11.07	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>
12	trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3',4'-dimethoxystyryl]cyclohex-1-ene	45.46	380.48	17.56	C <sub>24</sub> H <sub>28</sub> O <sub>4</sub>

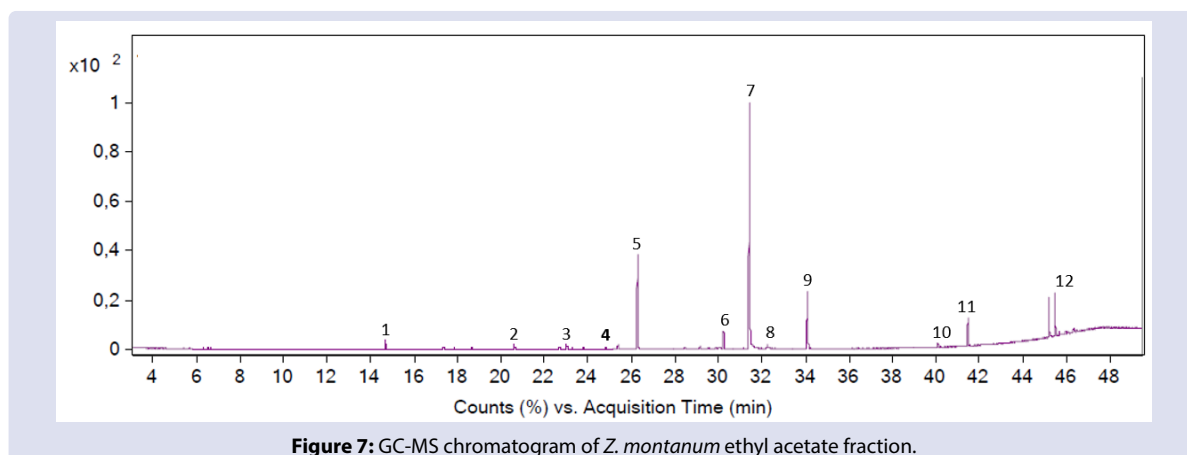
**Table 7: TLC results of ethyl acetate fraction.**

No. Rf	<i>Z. officinale</i>	<i>Z. montanum</i>	Quercetin	Curcumin
1	0.14	0.14	0.91	0.57*
2	0.29	0.27	-	-
3	0.43	0.30	-	-
4	0.55	0.53	-	-
5	0.71	0.57*	-	-
6	0.83	0.73	-	-
7	-	0.94	-	-

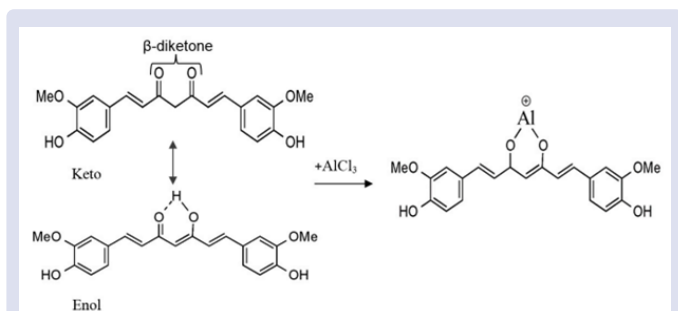
Note : \*Rf is equal to standard.



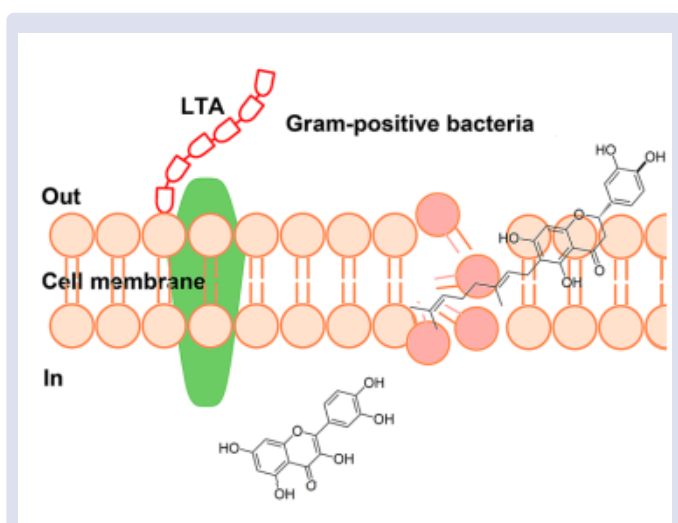
**Figure 6: GC-MS chromatogram of *Z. officinale* ethyl acetate fraction.**



**Figure 7: GC-MS chromatogram of *Z. montanum* ethyl acetate fraction.**



**Figure 8:** Transformation dike to enol group fungus ion and complex with aluminium ion<sup>20</sup>.



**Figure 9:** Diagrammatic mechanisms of plant favonoids to cell membrane bacteria<sup>22</sup>.

The antibacterial activity of the three extracts can be seen in Figure 4. The results of the analyses of variant the antibacterial activity of the three species were significantly different (Sig. < 0.05). The highest activity was in *Z. officinale* extract, followed by *Z. montanum* and the smallest was *Z. zerumbet*. Based on the antibacterial activity test, the extracts of *Z. officinale* and *Z. montanum* extracts were continued for fractionation. The results of the fractionation and antibacterial activity test showed two fractions had a high inhibition zone, the active fractions are ethyl acetate *Z. officinale* and ethyl acetate *Z. montanum* (Figure 5). Comparison of the antibacterial test results of the extract with the ethyl acetate fraction experienced a significant increase in the inhibition zone for *Z. montanum* (Sig. < 0.05) while *Z. officinale* an increase in the inhibition zone but not significant (Sig. > 0.05). The increase in antibacterial activity was due to the phytochemical compounds being less, so that in the fraction which allowed the interaction between the antagonist active substances to be reduced.

The active ethyl acetate fractions of *Z. officinale* and *Z. montanum* were screened using GC-MS and obtained 15 components of chemical compounds from the ethyl acetate fraction *Z. officinale* (Table 5) and 12 components from the ethyl acetate fraction *Z. montanum* (Table 6). In addition, curcumin is a compound found in the ethyl acetate fraction through thin layer chromatography (TLC) identification, curcumin was identified in the 5th spot with an R<sub>f</sub> value of 0.57 (Table 7).

## DISCUSSION

Extraction using 70% ethanol yield is shown in Table 1. The yield was highly dependent on the water content and ethanol residue. The

water content and ethanol residue in the extracts of *Z. officinale*, and *Z. montanum* had high levels compared to that of *Z. zerumbet* but still met the requirements. In addition, the yield value of the extract is influenced by the polarity of the substance that can be attracted by 70% ethanol solvent. Conformity of characteristics of solute-solvent interactions according to the classical “like dissolves like” rule.<sup>18</sup> Ethanol 70% has a polarity index of 8.2, ethanol includes a semi-polar solvent in which polyphenolic compounds, flavonoids and some secondary metabolites as shown in Table II can be dissolved at this polarity index.<sup>19</sup>

The extract parameter of essential oils *Z. officinale* does not meet the requirements, and *Z. zerumbet* from the parameters of acid-insoluble ash content total content and essential oils. Meanwhile, *Z. montanum* from the parameter of total curcuminoid content. The minimum number of requirements based on the Indonesian Herbal Pharmacopoeia 2nd Edition standards for *Z. officinale* essential oil is 2.40% for *Z. officinale* and 2.30% for *Z. zerumbet*. The essential oil content factor can be influenced by many factors, some of which are the process of making simplicia, the type of extract solvent and the concentration process of the extract. The extract concentration process uses a temperature of 50°C. The components of the essential oil may contain many compounds that can evaporate under these conditions.

Testing the levels of flavonoids with UV-Vis spectrophotometric method, the principle of the reaction of flavonoids with the addition of AlCl<sub>3</sub> will form a stable acid complex with a C-4 ketone group, as well as on the C3 or C-5 hydroxyl group of flavones and flavonoids as in Figure 3. The AlCl<sub>3</sub> also forms a stable acid complex with an orthodihydroxyl group on ring A or B<sup>17</sup> (Figure 3). The high levels of flavonoids in *Z. montanum* extract, possibly due to the curcumin compound that binds AlCl<sub>3</sub> through the diketone group which also provides absorption at a wavelength of 445 nm (Figure 8).<sup>20</sup>

The antibacterial activity of ginger has the greatest inhibition zone compared to the other two species (*Z. montanum* and *Z. zerumbet*) this is due to the presence of phytochemicals in the form of flavonoid compounds (the results of phytochemical screening). The antibacterial action mechanism of flavonoids is highly dependent on the flavonoid class structure contained in ginger. Based on research by Zubair MS, et al<sup>21</sup> the flavonoid contained in *Z. officinale* is 5-hydro-7,8,20-trimethoxyflavanone. The flavonoid group of the flavonone class has an anti-bacterial mechanism of action by inhibiting the cytoplasmic membrane function on membrane fluidity was studied using liposomal model membranes (Figure 9).<sup>22</sup>

The ethyl acetate fraction of *Z. officinale* has antibacterial activity identified by GC-MS including phenol compounds: frambinone, 4-(1-Hydroxyallyl)-2-methoxyphenol; zingerone; 1,3-benzenediol; and 4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]. The phenol-derived compound that has antibacterial activity by damaging bacterial cell membranes.<sup>23</sup> The sesquiterpenes are: α-curcumene; α-zingiberene; trans-β-farnesene; and dihydro-beta-agarofuran. The detected oleoresin compound was 6-shogaol, both sesquiterpene and oleoresin compounds having phenol groups have the same antibacterial mechanism of action as monoterpenes. The compound, 6-Shogaol is the dominant compound in ginger resulting from dehydration of 6-gingerol, the conversion process can be triggered by heating.<sup>24</sup>

The ethyl acetate fraction also detected an unsaturated aliphatic aldehyde compound is decanal. Based on Soliman, S.S., et al<sup>25</sup> decanal is an antibacterial agent. Decanal compounds have a mechanism of action due to aldehyde groups which are very likely to cause the function of cell membrane proteins, thereby increasing their permeability.<sup>26,27</sup> The ester compounds such as Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester; and phthalic acid, di(2-propylpentyl) ester is not yet known with certainty how the effect as an antibacterial and the mechanism of action. The zingerone<sup>28</sup> and 6-shogaol<sup>29</sup> compounds in ginger are

known to have the activity of inhibiting the formation of biofilms on bacteria. Biofilm formation in *P. acnes* is one of the mechanisms of colonization and antimicrobial resistance.<sup>30,31</sup>

The second extract that had high antibacterial activity against *P. acnes* was *Z. montanum*. Compounds related to antibacterial activity in the extract are flavonoids (the result of phytochemical screening). The active fraction of *Z. montanum*, are ethyl acetate fraction, showed that the results of the GC-MS screening contained monoterpenic compounds: Terpinen-4-ol, phenol compounds: isovanillin; benzene, 4-(1E)-1,3-butadiene-1-yl-1,2-dimethoxy; 1-(3,4-dimethoxyphenyl)-2-(n-hydroxyamino)propan-1-ol; Cyclic polyalcoholic compounds: menthane, 1,2,4-trihydroxy, phenol esters: methylisoeugenol; (E)-4-(3,4-dimethoxyphenyl)but-3-en-1-ol; (E)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl acetate. Others : (Z)-1-(Blind-1,3-dien-1-yl)-2,4,5-trimethoxybenzene; 1-(3,4-dimethoxyphenyl)-2-(n-hydroxyamino)propan-1-ol; hexanedioic acid, dioctyl ester; 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester; trans-3-(3,4-Dimethoxyphenyl)-4-[(E)-3',4'-dimethoxystyryl]cyclohex-1-ene. Same as in *Z. officinale* phenolic compounds, monoterpenes and sesquiterpenes have mechanisms against bacterial cell membranes.<sup>32</sup>

Terpinen-4-ol is the main component of *Z. montanum* rhizomes. This compound has antibacterial activity and can also inhibit the formation of biofilms from bacteria.<sup>33,34</sup> The results of thin layer chromatography in the ethyl acetate fraction contain a non-volatile compound is curcumin. The mechanism of curcumin is blocks bacterial growth, that inhibit bacterial virulence factors, inhibit bacterial biofilm formation and prevent bacterial adhesion to host receptors through the bacterial quorum sensing regulation system.<sup>35</sup>

## CONCLUSION

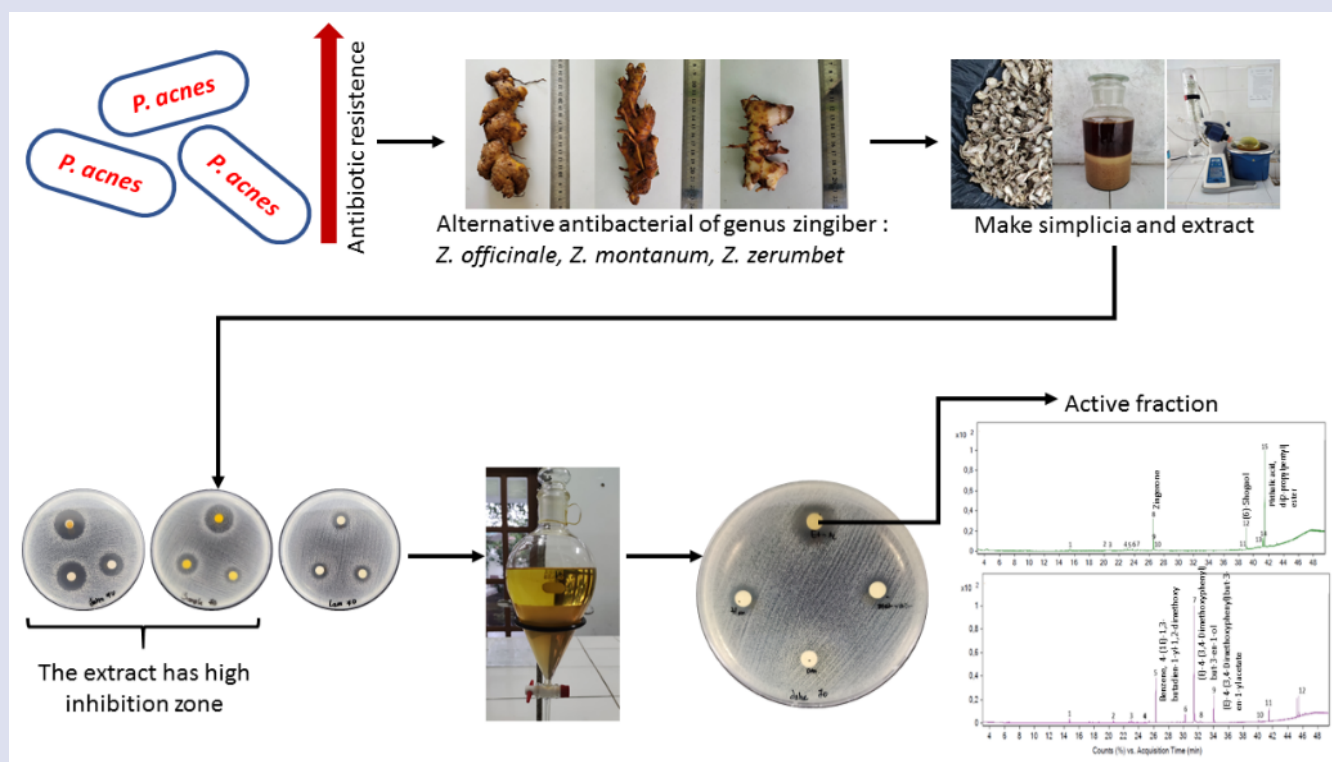
The 70% ethanol extract of *Z. officinale*, *Z. montanum* and *Z. zerumbet* had antibacterial activity against *P. acnes*. The highest activity was on *Z. officinale* both extract and active fraction. The active fraction ethyl acetate is the fraction with the highest inhibition zone, containing compounds consisting of 15 components including monoterpenoids-sesquiterpenes and oleoresin (6-shogaol) groups.

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## GRAPHICAL ABSTRACT





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