



# Toxicity, Antioxidant and Antibacterial Test of Ethyl Acetate Extract of Jamblang Leaves (*Syzygium cumini* L.)

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**Abstract:** Jamblang (*Syzygium cumini* L.) is a plant from the Myrtaceae family that is used as a traditional medicine, especially its leaves. This study aims to determine the toxicity, antioxidant, and antibacterial activities of the ethyl acetate extract of jamblang leaves and to identify its secondary metabolite compounds. The toxicity test using the Brine Shrimp Lethality Test (BSLT) method showed that the ethyl acetate extract of jamblang leaves had an LC<sub>50</sub> value of 174.602 ppm, which falls into the toxic category. The antioxidant test using the DPPH method showed an IC<sub>50</sub> value of 4.268 µg/mL, which is classified as strong, although still weaker than vitamin C (IC<sub>50</sub> = 2.437 µg/mL). The antibacterial test against *Streptococcus mutans* using the disc diffusion method produced inhibition zones of 2.58 mm (20%), 6.61 mm (30%), and 9.08 mm (40%), with a Minimum Inhibitory Concentration (MIC) value of 25%. Identification of secondary metabolites using GC-MS revealed 38 compounds belonging to terpenoid, ester and aromatic Hydrocarbons groups. These results indicate that the ethyl acetate extract of jamblang leaves possesses toxicity, antioxidant, and antibacterial properties, thus having the potential to be developed as a phytopharmaceutical.

**Keywords:** Antibacterial; Antioxidant; Jamblang; *Streptococcus mutans*; Toxicity

## Introduction

Jamblang (*Syzygium cumini* L.) is a plant from the Myrtaceae family. Jamblang plants are found in countries with tropical and subtropical climates (Rosannah et al., 2015). Jamblang leaves (*Syzygium cumini* L.) contain active compounds such as flavonoids, tannins, and polyphenols that can have potential as antioxidants (Hidayah et al., 2023). Some studies report that jamblang leaves and seeds have antioxidant properties (Abriyani et al., 2023). Antioxidants are compounds that are able to reduce or fight free radicals. One of the mechanisms of antioxidant compounds is by donating hydrogen atoms or protons to free radical compounds (Aryanti et al., 2021).

Antioxidant testing on jamblang leaves was carried out using the DPPH method. The DPPH method is a method used to measure antioxidants using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The principle of the DPPH method is to reduce the intensity of the color caused by the reduced amount of DPPH that reacts with the sample. Antioxidants will inhibit radicals

by providing hydrogen atoms so that DPPH will be reduced (Pambudi et al., 2023).

Toxicity testing can be done using the Brine Shrimp Lethality Test (BSLT) method. BSLT is one of the earliest widely used to test the toxicity/screening of new anticancer compounds derived from plants using the *Artemia salina* Leach larval test animal. BSLT testing is done by observing the mortality rate that occurs after the administration of the extract to *Artemia salina* Leach, the results obtained are then calculated as the LC<sub>50</sub> (Lethal Concentration) value of the extract which shows the concentration that causes the death of *Artemia salina* Leach by 50% (Chusniasih et al., 2020).

Antibacterial activity of ethanol extract of jamblang leaves can inhibit the growth of *Streptococcus mutans* bacteria that can cause dental caries. The role of microorganisms is so important for the process of dental caries, the occurrence of the caries process is characterized by the level of activity of microorganisms in the oral cavity (Primasari et al., 2018). *Streptococcus mutans* is the main cause of dental caries, this bacterium has the enzyme Glucosyltransferase (GTF) which can catalyze sucrose into glucan which is adhesive and can

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increase bacterial attachment (Setiani et al., 2020). Several factors that cause caries consist of microorganisms that cause plaque, *Streptococcus mutans* and *Lactobacillus*, the substrate factor helps the growth of microorganisms on the enamel surface, the time factor causes changes and damage to the enamel surface (Efrida et al., 2016).

Research on the specific effectiveness of ethyl acetate extract of jamblang leaves from Aceh Besar against *Streptococcus mutans* is still limited.

## Method

2 kg of jamblang leaves as a sample was taken from Kajhu, Aceh Besar. The leaves were cleaned and dried at room temperature for 7 days. The dried jamblang leaves were then pulverized with a blender and filtered using a sieve to produce a fine dry powder (Wulandari et al., 2023). The initial stage of extraction by putting 300 grams of jamblang leaf powder into a beaker glass and mixing it with ethyl acetate solvent as much as 3 liters (1:10 ratio) was allowed to stand for 24 hours. The result of the soaking was filtered to distinguish the filtrate and the residue. Soaking is done twice until the filtrate is close to clear. Then, the filtrate resulting from maceration was combined with the solvent, concentrated with a Rotary Vacuum Evaporator, and dried in a drying cabinet at 40°C to obtain a thick extract (Irawan et al., 2020).

### Hatching of *Artemia salina* Leach Eggs

*Artemia salina* Leach weighing between 50 and 150 mg were soaked in distilled water for approximately one hour. Next, the eggs were filtered and put into a plastic container with 500 mL of sterile seawater (salinity 30 ppt, pH 7-8, and temperature 28-29°C), and equipped with aeration and 40 Watt lighting for 24 hours (Lestari et al., 2019). *Artemia salina* Leach eggs were left for 36 to 48 hours to hatch into nauplii. For testing, *Artemia salina* Leach larvae that are active and 48 hours old will be selected (Leboe et al., 2018).

### Preparation of Test Solution

2 grams of dry extract from jamblang leaves was put into a 1 L Erlenmeyer flask, then dissolved with several mL of sterile sea water. If the extract did not dissolve then DMSO was added a few drops, but not more than 0.5 mL. after the extract was homogenized, then sterile seawater was added to 1 L which was used as the mother solution with a concentration of 2000 ppm. Furthermore, several concentrations were made, namely 100, 250, 500 and 1000 ppm (Virtalia et al., 2016).

### Toxicity Test with Brine Shrimp Lethality Test (BSLT) Method.

Extracts that have been prepared with concentrations of 100, 250, 500 and 1000 ppm are then put into a vial bottle as much as 5 mL, then 10 larvae of

*Artemia salina* Leach are inserted each (Sangi et al., 2012). The test vial bottle was placed in a place that received lamp lighting (40 Watts) for 24 hours, with a temperature of 28°C-29°C. Each concentration was repeated 3 times. Mortality observations were made every 6 hours for 24 hours, characterized by larvae that had no movement and tended to be at the bottom of the vial bottle.

### Antioxidant Test of Ethyl Acetate Extract of Jamblang Leaf with DPPH Free Radical Immersion Method

25 mg of extract was dissolved in 25 mL of methanol to obtain a mother solution with a concentration of 1000 µg/mL. Then dilution was done using methanol PA and made a concentration variation of 2.5 µg/mL, 5 µg/mL, 7.5 µg/mL, and 10 µg/mL. each concentration was taken 2 mL and added DPPH as much as 1 mL with a concentration of 100 µg/mL. The sample was homogenized and then incubated for 30 minutes. The same procedure was applied to the vitamin C sample as a positive control; 1 mL of DPPH solution (100 µg/mL) was added to 2 mL of vitamin C, prepared in five concentration variants, including 2.5 µg/mL, 5 µg/mL, 7.5 µg/mL, and 10 µg/mL. Furthermore, each solution was measured for absorbance using a Uv-Vis spectrophotometer at a wavelength of 517 nm, and the treatment was repeated 3 times. The inhibition presentation value represented by the IC50 value was calculated using the following formula (Fadilla et al., 2025).

$$\% \text{ Inhibition} = \frac{\text{Abs Blanko} - \text{Abs Sample}}{\text{Abs Sample}} \times 100\% \quad (1)$$

### Antibacterial Activity Test

Antibacterial activity test was conducted using the disc diffusion method on Mueller Hinton Agar (MHA) media (Nugrahani et al., 2016). The thick extract of jamblang leaves from ethyl acetate solvent was diluted using *Dimethyl Sulfoxide* (DMSO) into concentrations of 20%, 30% and 40% as much as 10 mL (Mahmiah et al., 2020). The bacteria tested were *Streptococcus mutans*. The positive control uses chloramphenicol while the negative control uses DMSO. Scratching of bacteria on growth media is done using a sterile cotton swab (Restyana et al., 2019). A total of 20 µl of jamblang leaf extract was dripped on a 6 mm diameter disc paper and then incubated at 37°C for 24 hours (Rahman et al., 2017). The inhibition zone/clear zone of each concentration of extract and the resulting bacteria was measured using a caliper. The number of replicates for each concentration of extract and bacteria.

### Minimum Inhibitor Concentration (MIC) Test

MIC method testing uses the turbidimetric method, which has been used previously (Soelama et al., 2015) and then tested by spectrophotometry, which is considered more accurate. Using a sterile ose needle, *Streptococcus mutans* bacterial preparations stored on

agar media were taken, then streaked on a slanted agar medium. Bacteria scratched on agar media were incubated for one day at 37°C for 1 x 24 hours. The incubated bacteria were taken using a sterile ose needle and inserted into NB media until the turbidity was in accordance with McFarland standard 1. Next, a total of 11 sterile test tubes were prepared. The test tubes were labeled 1-9. Tube 10 was labeled K(+), which is the positive control, containing media with bacterial suspension equivalent to McFarland 1 turbidity standard. Tube 11 was labeled K(-), which is the negative control, meaning that it contained media and jamblang leaf extract. Tubes 2-9 were filled with 2 ml of NB media. Tube 1 contains the pure/highest concentration extract (100%) before being serially diluted into the next tube. Tube 1 contains the pure/highest concentration extract (100%) before being serially diluted into the next tube until all extract concentrations were obtained with a ratio of 1:2 (v/v). then all tubes were put into an incubator and incubated for 1 x 24 hours with three repetitions at 37°C. Turbidity observation was done by the turbidimetric method. Suppose the turbidity of the tube is still equal to or more turbid than the K(+) tube

containing McFarland 1 bacterial suspension. In that case, it means that the bacteria can still grow vigorously. Still, when the solution in the tube looks clearer than the K(+) tube, it means that bacterial growth is starting to be inhibited, this is what shows the Minimum Inhibitory Concentration (MIC). After 24 hours of incubation, turbidimetric observation was then tested by the spectrophotometric method at a wavelength of 600 nm.

## Result and Discussion

### *Toxicity Test with Brine Shrimp Lethality Test (BSLT) Method*

Based on the results of toxicity testing of ethyl acetate extract of jamblang leaves using the Brine Shrimp Lethality Test (BSLT) method at a concentration of 1000 ppm the average larval mortality after 24 hours reached 90%, while at concentrations of 500 ppm and 250 ppm the larval mortality rate was 73.33% and 53.33%, respectively. The lowest concentration of 100 ppm caused 40% larval mortality with an LC<sub>50</sub> value at 24 hours of 174.602 ppm which is included in the toxic category (Table 1).

**Table 1.** Mortality and LC<sub>50</sub> of Ethyl Acetate Extract of Jamblang Leaf

Sample	Concentration (ppm)	% Mortality in 24 Hours	LC <sub>50</sub> (ppm)	Category
Ethyl Acetate	100	40	174.602	Toxic
	250	53.33		
	500	73.33		
	1000	90		

Research conducted by Safriani et al. (2023) tested the toxicity of jamblang leaves extracted with 96% ethanol through maceration and soxhletation methods. The test results showed that the meserated extract had an LC<sub>50</sub> value of 152.2942 µg/mL, while the soxhletation extract showed a value of 60.3905 µg/mL, which is a very toxic value and has potential as an anticancer. Rachmawati et al. (2021), reported the toxicity of ethanol extract of jamblang leaves resulting in an LC<sub>50</sub> value of 485.50 ppm and Muhammad et al. (2020), tested compounds isolated from the dichloromethane extract of juwet (*Syzygium cumini* L.) stem bark, and showed that the LC<sub>50</sub> value of 119.183 µg/ml was in the toxic category. Potu et al. (2021), the greater the LC<sub>50</sub> value, the lower the toxicity level of a compound and vice versa, the smaller the LC<sub>50</sub> value, the higher the toxicity. Thus the concentration of the extract also affects the mortality rate of the test larvae. This statement is supported (Makalalag et al., 2019), which states that the higher the concentration of the extract, the greater the percentage of larval mortality.

The use of ethyl acetate solvent was chosen because it has a low level of polarity, so it is expected to be able to extract compounds that also have low polarity (Vitalia et al., 2016). An extract is categorized as active and toxic if it causes the death of up to 50% of test animals at a concentration of less than 1000 ppm. The death of

*Artemia salina* Leach larvae is caused by toxic secondary metabolite compounds. These toxic compounds can enter through the mouth of shrimp larvae, be absorbed into the digestive tract, and disrupt metabolic processes, which ultimately cause death.

### *Antioxidant Test of Ethyl Acetate Extract of Jamblang Leaf with DPPH Free Radical Inhibition Method*

The results of antioxidant activity test of ethyl acetate extract of jamblang leaves showed that the higher the concentration of the extract, the greater the percentage of inhibition produced. At a concentration of 2.5 µg/mL the inhibition value was 33.15%, increased to 52.89% at a concentration of 5 µg/mL and continued to increase until it reached 96.89% at a concentration of 10 µg/mL. Based on the IC<sub>50</sub> value obtained, which amounted to 4.268 µg/mL, the extract showed very strong antioxidant activity (Table 2). Ismail et al. (2024) stated that an extract is categorized as having strong antioxidant activity if the IC<sub>50</sub> value is <10 µg/mL.

Antioxidant testing reported by Widyastuti et al. (2021) showed that the IC<sub>50</sub> values of ethanol, ethyl acetate and n-hexane jamblang fruit extracts were 4441.6 ppm, 1754.2 ppm and 16964.6 ppm, respectively. Meanwhile, Mauldyda et al. (2023) reported that jamblang leaf tea has antioxidant activity with an IC<sub>50</sub> value of 5.84 ppm which is included in the very strong

category. Junairiah et al. (2023) also conducted antioxidant testing using n-hexane, ethyl acetate and methanol extracts, which each produced IC<sub>50</sub> values of 23.79 ppm, 171.53 ppm and 64.44 ppm. Meanwhile,

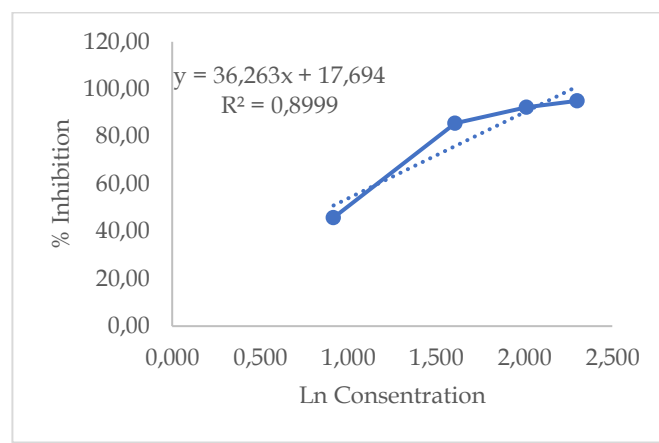
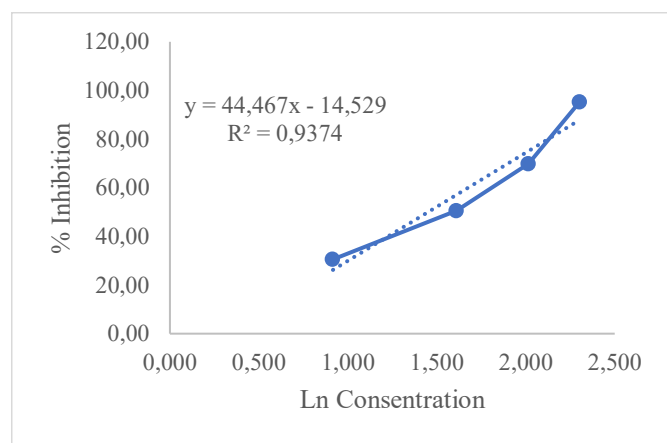
according to Ismail et al. (2024) the ethyl acetate fraction of jamblang stem has antioxidant activity with an IC<sub>50</sub> value of 496.985 µg/mL and is categorized as weak antioxidant activity.

**Table 2.** Antioxidants of Ethyl Acetate Extract of Jamblang Leaf

Sample	Concentration (µg/mL)	Abs Sample	%Inhibition	IC <sub>50</sub> (µg/mL)
Ethyl Acetate	2.5	0.374 ± 0.043	33.15	4.268
	5	0.266 ± 0.055	52.89	
	7.5	0.163 ± 0.081	71.79	
	10	0.125 ± 0.056	96.89	
Vitamin C	2.5	0.240 ± 0.009	45.78	2.437
	5	0.063 ± 0.004	86.67	
	7.5	0.034 ± 0.003	92.38	
	10	0.022 ± 0.005	95.10	

As a comparison, vitamin C was used as a positive control and showed higher antioxidant activity. At a concentration of 2.5 µg/mL, vitamin C produced an inhibition value of 45.78% µg/mL and continued to increase, as the concentration increased, reaching 95.10% at a concentration of 10 µg/mL. The IC<sub>50</sub> value of vitamin C was 2.437 µg/mL lower than that of jamblang leaf

extract (Table 2). The results of the antioxidant activity test of jamblang leaves and vitamin C showed that vitamin C has higher effectiveness in counteracting free radicals compared to jamblang leaves. Sari (2017) states that the lower the IC<sub>50</sub> value, the higher the antioxidant activity.



**Figure 1.** (a) Linear regression graph of inhibition of ethyl acetate extract of jamblang leaves; (b) Linear regression graph of vitamin C inhibition

According to Purwanto et al. (2017) antioxidant activity can be categorized into five levels, namely very strong, strong, moderate, weak and very weak. Antioxidants are said to be very strong if they have an IC<sub>50</sub> value of less than 50 ppm, strong antioxidants if the IC<sub>50</sub> value is in the range of 50-100 ppm, moderate antioxidants if the IC<sub>50</sub> value ranges from 100-150 ppm, weak antioxidants if the IC<sub>50</sub> value is 150-200 ppm and categorized as very weak if the IC<sub>50</sub> value is more than 200 ppm.

The IC<sub>50</sub> value was determined using a linear regression equation from the relationship curve of sample concentration (µg/mL) as the X-axis and percent inhibition as the Y-axis, with the regression equation  $y=ax+b$  (Figure 4.2). This shows that the IC<sub>50</sub> value is the concentration required to inhibit 50% of free radicals.

*Antibacterial Activity Test*

Based on the results of antibacterial testing of ethyl acetate extract of jamblang leaves, different inhibition zones were obtained at each concentration. The lowest concentration, 20%, obtained an average of  $2.58 \pm 0.39$ , included in the weak category, while 30% and 40% concentrations obtained an average value of  $6.61 \pm 0.76$  and  $9.08 \pm 0.77$  and included in the moderate category. The positive control using chloramphenicol obtained a strong clear zone with a value of  $32.85 \pm 0.00$ . The difference in concentration resulted in different levels of bacterial inhibition. Figure 4.3 shows the inhibition zone formed on the media overgrown by *Streptococcus mutans* bacteria because around the disk has spread secondary metabolic compounds from the jamblang leaf extract.

Ariyani et al. (2018) the criteria for the strength of antibacterial power with an inhibition zone diameter of

5 mm or less is categorized as weak, an inhibition zone of 5-10 mm is categorized as moderate, an inhibition zone of 10-20 mm is categorized as strong and an inhibition zone of 20 mm or more is said to be very strong.

**Table 3.** Diameter of Zone of Inhibition of Ethyl Acetate Extract of Jamblang Leaf.

Concentrations	Average (mm)	Category
K+	32.85 ± 0.00	Strong
K-	0.00 ± 0.00	No Activity
20%	258 ± 0.39	weak
30%	6.61 ± 0.76	Medium
40%	9.08 ± 0.77	Medium

*Minimum Inhibitory Concentration (MIC) Test*

MIC testing is done by observing the test tube of each concentration treatment marked turbid and whether or not the tube. Clear tubes indicate that the treatment concentration is the Minimum Inhibitory Concentration (MIC). The results of MIC testing of ethyl acetate extract of jamblang leaves showed that the treatments 12.50%, 6.25%, 3.13%, P 1.56%, 0.78% and 0.39 still looked cloudy, while 100%, 50% and 25% were clear. The lowest concentration that is not covered by bacteria (clear) is determined as the MIC value, so the MIC value is found at a concentration of 25% (Figure 2).



**Figure 2.** Turbidity (MIC) (a) P 100%; (b) P 50%; (c) P 25%; (d) P 12.50%; (e) P 6.25%; (f) P 3.13%; (g) P 1.56%; (h) P 0.78%; (i) P 0.39%

**Table 4.** Minimum Inhibitor Concentration (MIC) Test of Ethyl Acetate Extract of Jamblang Leaf

Concentrations	Average Value Absorbance (Abs)
100%	0.168 ± 0.042
50%	0.183 ± 0.051
25%	0.172 ± 0.083
12,50%	0.265 ± 0.053
6,25%	0.324 ± 0.008
3,13%	0.420 ± 0.097
1,56%	0.434 ± 0.066
0,78%	0.508 ± 0.097
0,39%	0.540 ± 0.033
K+	0.381 ± 0.000
K-	0.278 ± 0.000

Based on observations of absorbance values at concentrations of 100%, 50%, and 25%, it has a smaller numerical value compared to the positive control containing a suspension with McFarland 1 turbidity standard, namely with absorbance values of 0.168, 0.183, 0.192, respectively (Table 4). This shows that in tubes a, b and c there has been an increase in the growth of

*Streptococcus mutans*. Antibacterial activity can be seen from the decrease in the number of colonies at each test concentration. The greater the concentration of jamblang leaf extract, the fewer the number of colonies that grow. The results obtained are in accordance with research Chismirina et al. (2014) that the higher the concentration used, the greater the ability of microbial inhibition.

*Gas Chromatography and Mass Spectrometry (GC-MS) Identification*

38 secondary metabolite compounds were identified in the ethyl acetate extract of jamblang leaves and belonged to the group of terpenoid, ester and aromatic hydrocarbons compounds. The compound composition with the highest percentage is Propanoic acid, ethyl ester as much as 20% and Acetic acid, butyl ester as much as 16.8% (Table 5). Both compounds are included in the type of ester compounds. Poyil et al. (Poyil et al., 2025) stated that the compound Acetic acid, butyl ester has potential antibacterial activity against *Staphylococcus aureus*. Other compounds with antibacterial potential include Pentanoic acid, 4-methyl (Raja et al., 2023), Ethylbenzene (Wang et al., 2022), Benzene, 1-ethyl-3-methyl- (Reza et al., 2024), γ-Terpinene (Bhardwaj et al., 2022), 1-Dodecene (Abdelwahab et al., 2017) and Selina-3,7(11)-diene (Radi et al., 2024). Some compounds identified as antioxidants include Formic acid, butyl ester (Hemenway et al., 2012), o-Xylene (Iltaf et al., 2025), n- Butyl ether (Jiang et al., 2013), Benzene, 1,3-dimethyl- (Rahman et al., 2017), ζ-Terpinene (Bhardwak et al., 2022) and 1-Dodecene (Abdelwahab et al., 2017).

Junairiah et al. (2023) the ethyl acetate extract of *Syzygium cumini* L. leaves only contained flavonoid compounds identified as many as 57 bioactive compounds identified. Flavonoid compounds come from polyphenols which are a group of phenol compounds (Sayuti et al., 2015). The terpenoid compounds are dehydrogenated and oxidized derivatives of terpene compounds. Terpenes are a group of hydrocarbons, mainly produced by plants and some animals such as insects. The derivatives of terpenoid compounds are triterpenoids (Nola et al., 2021) Compounds of the triterpenoid group show significant pharmacological activities, such as antiviral, antibacterial, anti-inflammatory which inhibit cholesterol synthesis and as anticancer (Balafif et al., 2013).

The mechanism of action of terpenoid compounds as antibacterial substances involves membrane damage by lipophilic compounds. Terpenoids can react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymeric bonds and damaging porin, reducing the permeability of the bacterial cell wall so that bacterial cells lack nutrients, inhibited bacterial growth or death (Retnowati et al., 2011).

**Table 5.** GC-MS Analysis of Ethyl Acetate Extract of Jamblang Leaf (*Syzygium cumini* L.)

Retention Time	Compounds	Area %	Class of Compounds
3.458	Butane, 2-ethoxy	0.250	Ester
3.498	Pentanoic acid, 4-methyl	0.815	Ester
3.720	Propanoic acid, ethyl ester	20.074	Ester
3.994	Formic acid, butyl ester	0.601	Ester
4.275	2-Pentanol, 2-methyl-	0.892	Terpenoid
4.765	sec-Butyl acetate	1.744	Ester
4.917	Toluena	3.542	Aromatic Hydrocarbons
5.214	Isobutyl acetate	0.874	Ester
5.833	2-Penten-1-ol, 2-methyl-, (Z)	0.501	Terpenoid
5.979	Butanoic acid, ethyl ester	0.336	Ester
6.422	Acetic acid, butyl ester	16.837	Ester
8.068	Ethylbenzene	2.785	Aromatic Hydrocarbons
8.441	o-Xylene	1.903	Aromatic Hydrocarbons
9.142	n-Butyl ether	0.272	Terpenoid
9.375	Benzene, 1,3-dimethyl-	0.914	Terpenoid
11.260	alpha-Pinene	4.567	Terpenoid
12.550	Benzene, 1-ethyl-3-methyl-	0.307	Terpenoid
12.953	Mesitylene	0.244	Aromatic Hydrocarbons
13.338	alpha-Pinene	7.154	Terpenoid
14.067	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-	1.396	Terpenoid
14.131	Mesitylene	0.950	Terpenoid
15.421	Benzene, 1,2,3-trimethyl-	0.387	Terpenoid
15.701	o-Cymene	0.239	Terpenoid
15.940	Cyclohexene, 1-methyl-5-(1-methylethenyl)-,	1.811	Terpenoid
16.378	3-Carene	2.018	Terpenoid
16.886	α-Ocimene	0.422	Terpenoid
17.406	γ-Terpinene	0.223	Terpenoid
19.565	(+)-3-Carene	0.642	Terpenoid
24.058	1-Dodecene	0.830	Terpenoid
35.088	Cyclotetradecane	0.400	Terpenoid
36.769	Longifolene-(V4)	6.595	Terpenoid
36.956	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-	2.615	Terpenoid
38.304	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-	3.662	Terpenoid
39.220	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-	0.217	Terpenoid
39.921	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-	0.548	Terpenoid
40.364	á-Longipinene	1.378	Terpenoid
55.159	á-Guaiene	0.260	Terpenoid
55.316	Selina-3,7(11)-diene	0.381	Terpenoid

Terpenoid compounds also act as stomach poison that disrupts the digestive function of *Artemia salina* Leach larvae. This compound damages taste receptors in larvae, causing food recognition disorders resulting in death from starvation (Puspa et al., 2017). Terpenoids or steroids work as antioxidants with the primary antioxidant mechanism of action, which is able to reduce the formation of new free radicals by breaking chain reactions and converting them into more stable products, such as superoxide (Rahmawati et al., 2025).

Ester compounds are organic compounds formed from the reaction between carboxylic acids and alcohols through a process called esterification. Ester compounds have their own toxic activity or modify the toxicity of the original compound. This metabolic process plays an important role in removing ester compounds, thus affecting the level of toxicity (Sari et al., 2018). Ester compounds such as 1,2-Benzendicarboxylic acid, bis(2-ethylhexyl) ester found in ethyl acetate extracts showed toxicity to test organisms such as *Artemia salina*. This

compound belongs to a group of esters that may contribute to the toxic effects of the extract (Agustini et al., 2017).

Ester compounds also have an antibacterial role by damaging bacterial cell membranes and walls and inhibiting the growth of pathogenic bacteria (Karunia et al., 2017). The mechanism of esters towards antioxidants is by increasing the solubility and stability of native antioxidant compounds in lipid media, maintaining free radical capture ability, and facilitating wider applications in oil or fat-based systems. Enzymatic synthesis also plays an important role in producing effective and stable antioxidant esters (Wijayati et al., 2016).

## Conclusion

Ethyl acetate extract of jamblang leaves has toxicity with an LC<sub>50</sub> value of 174.602 ppm which is included in the toxic category. Ethyl acetate extract of jamblang

leaves has strong antioxidant with IC<sub>50</sub> value of 4.268 µg/mL and weaker than vitamin C with IC<sub>50</sub> value of 2.437 µg/mL which acts as a positive control. Antibacterial testing using concentrations of 20%, 30% and 40% obtained inhibition zone diameters of 2.58 (weak), 6.61 (medium) and 9.08 (medium), respectively. Ethyl acetate extract of jamblang leaves effectively inhibits the growth of *Streptococcus mutans* bacteria characterized by Minimum Inhibitory Concentration (MIC) of 25%. Secondary metabolite compounds identified in the ethyl acetate extract of jamblang leaves were 38 compounds and belonged to the terpenoid, ester and aromatic hydrocarbons compound groups.

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Conceptualization: N.N.R. & I.P.W.A.J; Methodology and conductor of experiment G.N.S, I.P.W.A.J & I.W.B.S; data analyzer and data visualization N.P.Y.N & I.P.T.I & I.P.W.A.J.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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