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# Antibacterial Activity of Ethanol Extract of Dragon's Blood (Daemonorops didymophylla) Against Salmonella typhi and Streptococcus mutants

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Abstract. Dragon's blood essentially is a red-colored resin secreted by the fruits of rattan species. Dragon's blood originated from Indonesia becomes widespread in the international market is indigenous from Daemonorops sp. Dragon's blood has been popularly used as traditional medicines. The benefit of dragon's blood as medicine certainly cannot be separated from the content of secondary metabolites. To confirm such dragon's blood efficacy, an assessment was already conducted regarding the phytochemical screening and activity dragon's blood produced by the rattan species, i.e. Daemonorops didymophylla. Ethanol extract of dragon's blood contains secondary metabolites which are effective as an antibacterial against Salmonella typhi and Streptococcus mutants. This study aims to determine what are the classes of compounds contained in the ethanol extract of dragon's blood Daemonorops didymophylla which is effective as an antibacterial against the bacteria Salmonella typhi and Streptococcus mutants. Results revealed that the separation of the active compound ethanol extract of dragon's blood Daemonorops didymophylla through liquid vacuum chromatography has 4 combined fractions. The class of compounds found in the ethanol extract of dragon's blood Daemonorops didymophylla are flavonoids, triterpenoids and alkaloids. Antibacterial activity of extract ethanol of Daemonorops didymophylla dragon's blood against Salmonella typhi and Streptococcus mutans showed that the higher the concentration, the higher the inhibitory power produced. For Salmonella typhi in the 1000 ppm extract obtained an inhibition zone of 13.63 mm with a strong category, while Streptococcus mutans in the 1000 ppm extract obtained a inhibition zone of 11.86 mm with a strong category. Fraction 1 is the fraction that has the largest inhibition zone than the other three fractions with a large inhibition zone of 14.6 mm testing in Salmonella typhi and 12.1 mm testing in Streptococcus mutants.

## Introduction

Non-timber forest products (NTFPs) are the products of Dragon's blood is a resin produced by dragon's blood rattan (*Daemonorops* sp) which is endemic in Southeast Asia. The resin attaches and covers the outer part of the both vegetal and animal forest and their derivative products and cultivation except wood originating from the forest. Based on the Minister of Forestry Regulation No. P.35/Menlhk-

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Honey Bees, Silk and Agarwood. In addition to the 5 national flagship NTFP commodities, regions can develop NTFP commodities that are seeded based on the potential of NTFPs and regional capacity, one of Jambi Province's leading NTFP commodities is Jernang (Permenhut, 2007).

Dragon's blood is a resin produced by dragon's blood rattan (*Daemonorops* sp) which is endemic in Southeast



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Asia. The resin attaches and covers the outer part of the rattan fruit, and the extraction process is needed to get it (Toriq 2013). Types of rattan capable of producing Dragon's blood are *Daemonorops draco, Daemonorops micracantha, Daemonorops Didymophylla*, and *Daemonorops mattanensis* (Soemarna., 2009). Rattan plants are clear in 3 countries, namely: Indonesian, Malaysian and Indian. Dragon's blood rattan in Indonesia is the largest with distribution in Sumatra, Kalimantan, and Java. In Sumatra, dragon's blood can be found in the provinces of Nanggroe Aceh Darusalam, Riau, Jambi, and South Sumatra.

The benefit of dragon's blood as medicine certainly cannot be separated from the content of secondary metabolites. Based on phytochemical screening results, crystal resin contains flavonoids, triterpenoids and tannins. The group of triterpenoid compounds consists of more than 4,000 compounds, useful for anti-inflammatory, hepatoprotective, analgesic, antimicrobial, and others (Dzubak et al., 2006). While the groups of tannin compounds are useful, among others, to reduce high blood pressure, anticarcinogens, antimutasigen, antimicrobials and others (Chung et al., 1998). Thus, it has the potential to be an antibacterial, antifungal, anti-inflammatory, antiallergic. anticancer, antioxidant, analgesic, hepatoprotective, anticarcinogen, reduce high blood pressure and antigen-oxygen. (Waluyo and Pasaribu., 2013).

Daemonorops didymophylla is one of the species that is quite abundant in Jambi Province, especially Sarolangun Regency. Testing of n-hexane, ethyl acetate and methanol Daemonorps sp extract has the potential to be antibacterial to Basilus subtillis and Staphylococus aureus Waluyo and Pasaribu (2013). In addition Sarman (2014) has isolated Daemonorops Draco with a comparison of several solvents and found that the ethanol solvent is the best solvent. Special research on D. didymophylla has not been done much, but it is possible that this species is less well known than D. draco. Because of the lack of research on Daemonorops didymophylla, the author took the title "Antibacterial Activity of Ethanol Extract of Dragon's blood (Daemonorops didymophylla) Against Salmonella typhi and Streptococcus mutants".

## Experimental

#### Materials and Methods

#### Sample Preparation.

The sample to be examined is the dragon's blood *Daemonorops didymophylla*. Daemonorops didymophylla dragon's blood obtained from Sepintun, Pauh, Sarolangun District, Jambi Province. *Daemonorops didymophylla* first

need to be cleaned to remove dust and dirt. 260 grams of cleaned ratta were macerated with ethanol as much as 1.5 liters for 3x24 hours.

#### Sample Extraction.

The sample first extracted by ethanol, then following by filtered. The extract obtained was thick and concentrated, so that it was weighed.

#### **Phytochemical Screening.**

Phytochemical screening includes alkaloid test, flavonoid test, saponin test, tannin test, and steroid and triterpenoid test.

#### **Alkaloid Test**

A total of 1 mL of sample was mixed with a few drops of sulfuric acid 2N, then tested with three alkaloid reagents namely Dragendorff reagent, Meyer reagent and Wagner reagent. The test results were positive if red to orange deposits were formed in Dragendorff reagents, with Meyer reagents formed yellowish white deposits and with Wagner reagents formed brown deposits (Harbone., 1987).

#### **Flavonoid Test**

The sample was added a few drops of concentrated HCl and then added Mg powder. The positive results of reacting HCl and Mg powder when the froth is formed and the color change of the solution becomes orange (Harbone., 1987).

#### **Saponin Test**

Saponins can go through foam tests in hot water. Stable foam that can last long and does not disappear with the addition of 1 drop of HCl 2N shows the presence of saponins (Harbone., 1987).

#### **Tanin Test**

The sample is mixed with FeCl<sub>3</sub> then the mixture is homogenized. If the formation of greenish black color in the mixture is positive compared to tannin (Harbone., 1987).

#### **Steroid and Triterpenoid Test**

The sample was mixed with anhydrous acetic acid and concentrated sulfuric acid (Liebermann-Burchad reagent). If blue or green is formed it indicates steroids. If it is struck by purple or orange, it indicates the existence of a triterpenoid (Harbone., 987).

#### Separation and Purification of Compounds

#### Thin Layer Chromatography (TLC)

Prepared TLC plate measuring 1x5 cm with a lower limit of 1 cm and an upper limit of 0.5 cm so that the eluent distance is 3.5 cm. Eluents are then made by comparing organic solvents with multilevel polarity. The extract is

bottled at the lower limit of the plate with a capillary tube, then eluted with the mobile/eluent phase. After the movement of the developer solution reaches the upper limit, the elution process is stopped. Furthermore, the stain shape is observed directly and under the UV lamp 254 nm. TLC was carried out to find the best solvent in the separation of compounds, so that it can be continued on column chromatography. After column chromatography, all fractions were carried out TLC tests to see the stain components. Fractions that have the same spot spot are combined and reanalyzed with TLC.

#### **Column Chromatography**

Liquid vacuum column chromatography (KVC) was performed using the stationary phase of silica gel with a comparison of samples: silica gel. The sample extract was impregnated using silica gel, then added to the column that contained the stationary phase. While the mobile phase used is in accordance with the best separation in TLC. The obtained fraction is accommodated in vial bottles, eluate which is accommodated based on each obtained ribbon and then evaporated. The results of column chromatography were carried out by KLT again. Eluate which has an identical stain pattern combined based on the Rf value on the chromatogram.

#### **Testing of Antibacterial Activity**

#### **Sterilization of Tools and Materials**

All tools and materials to be used are washed thoroughly and continued drying. Then sterilization was carried out with alcohol 70% and using autoclave for 15 minutes at a temperature of 121°C. Sterilization of the equipment was also carried out by using the oven for 15 minutes at a temperature of 210°C, by wrapping all the equipment using paper.

#### Antibacterial Testing Preparation.

Making a paper disc. Prepared whatman filter paper and made a paper disc using a paper hole. Then sterilized in an autoclave at 121°C for 15 minutes.

#### **Making test solutions**

The test solution used was ethanol extract with a concentration of 10; 50; 100; 500; 1000  $\mu$ g/mL (ppm) and the fraction obtained is taken 1 gram dissolved in ethanol solvent (Yanti and Mitika 2017).

#### Making positive and blank control solutions

The positive control used was chloramphenicol 0.3% w/v for antibacterial testing in ethanol and ethanol blanks (Yanti and Mitika 2017).

#### Preparation of media for growing bacteria

The growing media used are solid media using Nutrient Agar (NA) for testing antibacterial and potato dextrose agar (PDA)

until homogeneous, heated with hotplate and stirred with a magnetic stirrer. Put in 500 mL erlenmeyer and sterilized by autoclaving at 121°C for 15 minutes. Mixed 4 g of PDA in 250 mL of distilled water until homogeneous, heated with hotplate and stirred with a magnetic stirrer. Put in 250 mL erlenmeyer and sterilized by autoclaving at 121°C for 15 minutes. The prepared media is transferred to a petri dish and then left to order.

#### **Inoculation of Bacteria**

Bacteria *Salmonella typhi* and *Streptococcus mutants*, inoculated from pure culture to petri dishes. The cultures of S. mutans, S. were taken using ose needles and then scratched onto prepared media, then incubated for 24 hours.

#### **Test Antibacterial Activity**

Paper discs are dipped in the test solution, positive controls and blanks are then affixed to the agar media. After incubation for 24 hours, the diameter of the inhibitory zone was measured, namely the area that was not attacked by bacteria using the calipers. As well as being compared with positive chloramene protocol control for antibacterial. Testing of this activity is carried out with three replications Waluyo and Pasaribu (2013).

#### **Data Analysis**

#### Determination of phytochemical analysis

For phytochemical analysis with the emergence of changes in color, sediment or formation of layers and temperature, which indicates the reaction between the reagent and the sample.

#### Determination of analysis of antibacterial activity

If a clear zone is formed from the extract tested, it can be stated that the extract has the potential to be antibacterial, then the antibacterial categories are measured by measuring the clear zone formed by the calipers.

#### **Results And Discussion**

#### **Extraction and Separation of Compounds**

The choice of maceration method in extraction is due to an easy and simple process. This principle is based on immersion of samples with certain solvents at room temperature and protected from light, besides that in this study single maceration was used to obtain a high percent yield (Tasmin et al., 2014). Ethanol solvents are used because based on previous research (Sarman 2014) conducted extraction of dragon's blood (*Daemonorops draco*) with various solvents namely ethanol, acetone and ethyl acetate and the results showed that ethanol solvents could extract compounds contained in dragon's blood better than other solvents, besides also according to

Waluyo and Pasaribu (2013) the compounds contained in dragon's blood are semipolar to polar, so by using ethanol which is polar, it can attract polar compounds as well. Ethanol as a solvent can damage cell membranes on the dragon's blood, ethanol will enter the cell cavity through the clear cell wall to dissolve the active substance so that there are differences in a concentration inside and outside the cell causing diffusion of active substances in cells towards the outside of cells (Atun 2014).



Figure 1. (a) Results maceration of Dragon's blood; (b) Concentration of Extract with Vaccum Rotary Evaporator

Dragon's blood which has been macerated then concentrated with vacuum rotary evaporator, this is done so that the dried dragon's blood extract can be calculated to be mass. Vaccum Rotary Evaporator is a device that functions to separate a solution from its solvent so that the extract is produced with certain chemical content as desired. The liquid you want to evaporate is usually placed in a flask which is then heated with the help of a bath,and rotated. The liquid-vapor produced is cooled by a cooler (condenser) and accommodated in a place (receiver flask). The speed of this tool in evaporation is very fast, especially if aided by a vacuum. Another advantage of this tool is the recovery of evaporated solvents (Nugroho et al., 1999). In this study, the Rotary Evaporator Vaccuum was set at 50°C because the boiling point of ethanol (solvent) ranged from 78.73°C. The results of Vacum rotary evaporator obtained extracts of 21.136 grams with a yield of 8.12%.

The extract obtained was then phytochemically screened to determine the secondary metabolites contained in the ethanol extract of the dragon's blood. The phytochemical screening carried out in the form of flavonoids, tannins, steroids/ triterpenoids, dragendorf, mayers and saponins. The results of the phytochemical screening are as follows:

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No.	Chemical Compounds	Result	Information
1	Flavonoid	+	Bubbly and orange
2	Alkaloid (Dragendorf)	+	Reddish sediment.
3	Tanin	-	Turbidity
4	Triterpenoid	+	Orange Color
5	Alkaloid (Mayer)	-	Red Color
6	Saponin	-	There is no foam

Tabel 1. Phytochemical Screening of Ethanol Extract of dragon's blood Daemonorops didymophylla.

From Table 1 it can be seen that the ethanol extract of dragon's blood *Daemonorops didymophylla* contains secondary metabolites in the form of flavonoids, alkaloids and triterpenoids. Secondary metabolites found in dragon's blood *Daemonorops didymophylla* resin have similarities with crystal resin from *Daemonorops longipes*, *Daemonorops draco* and *Daemonorops melanochets* which have been carried out by phytochemical screening (waluyo and Pasaribu 2013) using ethyl acetate, n-hexane and

#### methanol.

The extract was obtained then carried out thin layer chromatography (TLC) to find the best eluent (mobile phase) for separation using column chromatography. The principle of layer chromatography is the difference in physical and chemical properties of compounds, namely the tendency of molecules to dissolve in liquids (solubility), the tendency of molecules to evaporate and

the tendency of molecules to attach to the surface (adsorption, absorption) (Hendayana., 2006). In this study used a stationary phase in the form of silica plate with a size of 5x10 cm with a distance of 3.5 cm and a mobile phase variation in the form of n-hexane: ethyl acetate with a ratio of 10: 0, 9: 1, 8: 2, 7: 3, 6: 4, 5: 5, 4: 6, 3: 7, 2: 8, 1: 9, 0:10 and ethyl aetate: methanol with a ratio of 9: 1, 8: 2, 7: 3, 6: 4, 5: 5, 4: 6, 3: 7, 2: 8, 1: 9, 0:10 and ethyl aetate: methanol with a ratio of 9: 1, 8: 2, 7: 3, 6: 4, 5: 5, 4: 6, 3: 7, 2: 8, 1: 9, 0:10. From the results of the TLC, the best separation was obtained from the variation of the mobile phase (eluent) n-hexane: ethyl acetate with a ratio of 7: 3, based on the results of this TLC that would be a reference in the separation of compounds using column chromatography.



Figure 2. TLC result for eluen KVC

Ethanol extract of dragon's blood *Daemonorops* didymophylla was carried out by liquid vacuum chromatography (KVC) using n-hexane: ethyl aetate eluent with a ratio of 7: 3 based on the results of the previous KLT. KVC aims to separate the compounds in the extract. The sample migrates to the stationary phase and the mobile phase quickly because it is in a vacuum (Oktaviani et al., 2015). The stationary phase used in liquid vacuum chromatography is silica gel G60 size  $\pm$  200-400 mesh as much as 65 grams and the column used is 5 cm in diameter. dragon's blood extract used for 5 grams of KVC which is impregnated with silica as much as 20 grams, the purpose of impregnation is to distribute the sample evenly so that there is no accumulation or clumping and so that the sample easily passes through silica during elution.

Column packing is done by means of silica inserted into the KVC column with the help of vacuum, pressed-pressed until smooth, then saturated with n-hexane as much as 10 times until there is no cracking of silica and evenly distributed by eluent. The most basic composition of CVC is silica, filter paper, samples, and filter paper, the use of filter paper aims to avoid the spread of extract into the eluent. Liquid vacuum chromatography in this study began in the best solvent from KLT, namely n-hexane: ethyl acetate with a ratio of 7: 3, the solvent used was a 100 ml sper for each increase in the gradient of polarity. In this study the n-hexane: ethyl acetate (7: 3) solvent was accommodated in the first 17 vials, because there was no band that went down then n-hexane: ethyl acetate 6; 4, 5: 5, 4: 6, 3: 7, 2: 8, 1: 9, and 0:10 to continue on ethyl: methanol 9: 1, 8; 2, 7: 3, 6; 4, 5: 5, 4: 6, 3: 7, 2: 8, 1: 9 and 0:10.



Figure 3. Chromatography Vacuum liquid

The results of liquid vacuum chromatography obtained 34 vial reservoirs. The vials are left for about 21 days so that the solvent can evaporate. After the solvent evaporates, TLC is carried out with n-hexane: ethyl solvent in each vial to see the stain component formed, the same spot spot component is put together.

Table 2. Mix fraction					
Fraction	Vial Number				
F.1	2-10				
F.2	11-24				
F.3	25-28				
F.4	29-34				

From Table 2 it can be seen that 34 of the results of the VCC are vial. After the merger is done, 4 combined fractions are obtained. The 4 combined fractions obtained will be tested for agents antibacterial.



Figure 4. Mix Fraction

The combined fraction obtained was done by phytochemical screening to see which chemical compounds were contained in each of these vials. The results of the combined fraction phytochemical screening are as follows Table 3.

From Table 3 it can be seen that the four positive fractions contain flavonoid metabolites which are thought to be dracorodine compounds, because the extract obtained is red. According to (Toriq., 2013) Drakorodim is an anthocyanin-derived flavonoid compound that gives natural red color to the dragon's blood.

Sample Coodes	Flavonoid	Steroid	Triterpenoid	Tanin	Mayer	Dragendorf	Saponin
F.1	+	+	-	-	-	-	-
F.2	+	-	+	-	-	+	-
F.3	+	-	+	-	-	-	-
F.4	+	+	-	-	-	-	-

#### Antibacterial testing

Antibacterial testing was carried out on ethanol extract of dragon's blood *Daemonorops didymophylla* with variations in concentrations of 1000 ppm, 500 ppm, 100 ppm, 50 ppm and 10 ppm. This variation in concentration is made so that the optimal concentration of extract is known as antibacterial. In addition, 4 combined fractions were also tested by dissolving 1 gram of each extract with ethanol solvent.

The bacteria used in this test were *Salmonella typhi* and *Streptococcus mutants*, which were obtained from the Microbiology Laboratory at the University of North Sumatra. These bacteria are used because they are agents in infecting the digestive tract, especially the mouth, which is the first step in making oral medicine products.

The method used in antibacterial testing in this study is the disc diffusion method, using paper discs where in this technique agar media that has been inoculated with bacteria is then inserted into the paper disc containing test compounds. The results of antibacterial testing with this disc diffusion method will form a clear zone around the paper disk and measured using a caliper. The choice of disc diffusion method is because it is a simple method and the results obtained are quite accurate.

Before carrying out antibacterial testing all the equipment used must be cleaned first, that is by washing clean all the tools and spraying with alcohol 70%, alcohol

is used because alcohol is a disinfectant that can kill other bacteria that can cause contaminants. After all the tools have been cleaned then autoclave at 121°C for 15 minutes, the function of this autoclave is to sterilize all equipment in antibacterial testing.

The bacterial growth media used in this study is nutrient agar (NA). Nutrient agar is one of the media commonly used for antibacterial testing because it contains beef extract, peptone and agar which are nutrients for bacterial growth. Nutrients are made by dissolving 13 grams of nutrein so that in 500 ml of distilled water above the hotplate and stirring until homogeneous. Nutrein storage containers must be sterilized beforehand. After complete dissolution, nutrein is sterilized in an autoclave at 121 °C for 15 minutes after nutrient sterilization is carried out to be used.

Pure cultures of *Salmonella typhi* and *Streptococcus mutants* were first fertilized on petri dishes to multiply test baketri stock. Pure culture of *Salmonella typhi* and *Streptococcus mutants* which are in slanted agar are transferred aseptically using a needle into the petri dish containing nutrient agar. After being streaked evenly on a petri dish, then incubated at 37 °C for 24 hours, set to 37 °C because the bacteria *Salmonella typhi* and *Streptococcus mutants* are mesophilic bacteria which can grow optimally at a temperature of 30 °C - 40 °C.

In antibacterial testing, all tools autoclaved were resterilized with a UV lamp in Laminar Air Flow for 30

minutes. The antibacterial testing process is carried out in laminar air flow, where the working principle of the laminar air flow is that the blower blows sterile air continuously through the inoculation chamber so that the room is free from dust and spores that might become contaminants. Furthermore, receive to be poured on a petri dish and wait until it hardens and is ready to be done the scraping.

As a positive control in this study we use chloramphenicol 0.3% which is a broad spectrum antibiotic that can inhibit the growth of gram-positive and

gram-negative bacteria, this is based on the use of *Salmonella typhi* gram-negative test bacteria and grampositive *Streptococcus mutants*. For the blanks used in this study, ethanol, which is a solvent from the extract of the dragon's blood *Daemonorops didymophylla*.

To determine the antibacterial activity of *Daemonorops draco* and 4 combined fractions of *jernang resin* ethanol extract, the inhibitory zone values produced against *Salmonella typhi* and *Streptococcus mutants* were used. Determination of the level of antibacterial activity refers to Davis and Stout (1971). The results obtained are as follows:

	Diameter Zone inhibitory (mm)				
Concentration (ppm)	1	2 3		Average(mm) ± SD	Explanation
1000	11.8	14.1	15	13.63±1.65	Strength
500	11.35	13.3	12	12.21±0.99	Strength
100	10.2	9.8	9.7	9.9±0.26	Average
50	9	8.8	9	8.93±0.11	Average
10	0	0	0	0±0	None
F.1	14	15	14.8	14.6±0.52	Strength
F.2	2.8	5	3	3.6±1.21	Weak
F.3	6	5.8	7	6.26±0.64	Average
F.4	12	11	10.2	11.06±0.90	Strength
Positive control (chloramphenicol 0.3%)	16.1	23	24	21.03±4.30	Strength
Blank (ethanol)	0	0	0	0±0	None

From Table 4 above it can be seen that the ethanol extract of dragon's blood *Daemonorops didymophylla* has the potential as an antibacterial against *Salmonella typhi* where the higher the concentration of the extract the higher the inhibition zone produced. In fraction 1 it is known to have the highest activity than other fractions as antibacterial against bacteria *Salmonella typhi*. In the antibacterial testing of *Streptococcus mutants* the following results were obtained:



Figure 5. Antibacterial Testing of Salmonella typhi

Concentration (ppm)	Diameter Zone inhibitory (mm)			Average (mm) ± SD	Explanatior
	1	2	3		
1000	10	12.5	13.1	11.86±1.64	Average
500	9.4	10.1	11	10.1±0.80	Strength
100	7.9	9	8.2	8.3±0.56	Strength
50	6.45	5.7	7	6.38±0.65	Strength
10	0	0	0	0±0	None
F.1	12	12	12.3	12.1±0.17	Average
F.2	6	6	5.2	5.73±0.46	Strength
F.3	11.2	10	9.7	10.3±0.79	Strength
F.4	7	6	6.3	6.43±0.51	Strength
Positive control (chloramphenicol 0.3%)	26.1	27	24	25.7±1.53	average
Blank (ethanol)	0	0	0	0±0	None

From Table 5 above it can be seen that the ethanol extract of dragon's blood Daemonorops didymophylla has the potential as an antibacterial against Streptococcus *mutants* where the higher the concentration of the extract the higher the inhibition zone produced. In fraction 1 it is known to have the highest activity compared to other fractions as against antibacterial the bacterium Streptococcus mutants. Research on dragon's blood against bacteria Streptococcus mutants has never been done before. dragon's blood belongs to the family Arecaceae, one family with areca nut (Areca catechu), antibacterial testing of Streptococcus mutants areca nut ethanol extract has been done previously Ningsih (2018) with areca nut ethanol extract concentrations of 2.5%, 3% and 3.5% has antibacterial activity of Streptococcus mutants with an average inhibition zone of 14.96 mm, 15.49 mm and 17.05 mm.



Figure 6. Antibacterial testing against Streptococcus mutants

Based on research was conducted (Purwanti 2017) using Daemonorops draco dragon's blood on S. aureus and E. coli bacteria, the antibacterial activity of the dragon's blood was due to the presence of dracorodine and dracorubin compounds, which are flavonoid groups. The mechanism of action of flavonoids as an antibacterial is to form complex compounds with extracellular proteins and dissolve so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds (Bobbarala., 2012). According to Cushnie (2005), in addition to playing a role in the inhibition of DNA-RNA synthesis by intercalation or hydrogen bonds with the buildup of nucleic acid bases, flavonoids also play a role in inhibiting energy metabolism. These compounds will interfere with energy metabolism in a manner similar to inhibiting the respiratory system, because sufficient energy is needed for the active absorption of various metabolites and for the biosynthesis of macromolecules. Triterpenoid compounds are also known to be active against bacteria, but the triterpenoid antibacterial mechanism is still not really known. Antibacterial triterpenoid activity is thought to involve membrane breakdown by lipophilic components Bobbarala (2012) and Cowan (1999). In addition, according to Leon et al., (2010), phenolic and triterpenoid compounds have the main target, namely the cytoplasmic membrane which refers to its hydrophobic nature.

Based on the research that has been done, it can be concluded that *Daemonorps didymophylla* dragon's blood extracted with ethanol contains secondary metabolites based on phytochemical screening with flavonoids, alkaloids and triterpenoids which can inhibit the growth of

bacteria *Salmonella typhi* and *Streptococcus mutants* which can infect the digestive tract.

# Conclusion

In this study, the separation of the active compound ethanol extract of dragon's blood Daemonorops didymophylla, where the separation of compounds through liquid vacuum chromatography has 4 combined fractions. The class of compounds found in the ethanol extract of dragon's blood Daemonorops didymophylla are flavonoids, triterpenoids and alkaloids. Antibacterial activity of Daemonorops didymophylla dragon's blood ethanol extract against Salmonella typhi and Streptococcus mutants showed that the higher the concentration the higher the inhibitory power produced, for Salmonella typhi in the 1000 ppm extract obtained a inhibition zone of 13.63 mm with a strong category while Streptococcus *mutants* in the 1000 ppm extract obtained a inhibition zone of 11.86 mm with a strong category and fraction 1 is the fraction that has the largest inhibition zone than the other three fractions with a large inhibition zone of 14.6 mm in testing Salmonella typhi and 12.1 mm in testing Streptococcus mutants.

# References

- Atun S. (2014)., Isolation Methods and Structure Identification of Natural Organic Compounds. *Brobudur Cultural Heritage Conservation Journal*; 8(2), 53-61.
- Bobbarala V., (2012)., Antimicrobial Agents. Intech: Croatia chapter 11; pp. 239–254.
- Brooks GF, Butel JS, Morse SA., (2007). Medical Microbiology Jawetz, Melnick & Adelberg Ed -23. Jakarta: Medical Book Publisher EGC; pp. ISBN-13: 978-0 -07-128735-7.
- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: a review. *Critical reviews in food science and nutrition*, *38*(6), 421-464.
- Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.
- Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, *26*(5), 343-356.
- Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay: I. Factors influencing variability and error. *Applied microbiology*, 22(4), 659-665.
- Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Kvasnica, M., Biedermann, D., ... & Sarek, J. (2006). Pharmacological activities of natural triterpenoids and their therapeutic implications. *Natural product reports*, *23*(3), 394-411.

- Gupta, D., Bleakley, B., & Gupta, R. K. (2008). Dragon's blood: botany, chemistry and therapeutic uses. *Journal of ethnopharmacology*, *115*(3), 361-380.
- Harborne, J. B. (1987). *Phytochemical methods: A guide to the modern way of analyzing plants*. Bandung: ITB.
- Hendayana S., (2006)., *Chemical Separation Chromatography and Modern Electrophoresis Methods.* PT. Rosdakarya Youth: Bandung 2006.
- De León, L., López, M. R., & Moujir, L. (2010). Antibacterial properties of zeylasterone, a triterpenoid isolated from Maytenus blepharodes, against Staphylococcus aureus. *Microbiological Research*, *165*(8), 617-626.
- Ningsih, W. (2018). Formulation and Test of Antibacterial Effectiveness of Edible Film Extract of Areca Seed (*Areca catechu* Linn). *Journal of Pharmacy and Clinical Pharmacy*, 15(2), 71-76.
- Nugroho BW, Dadang, Prijono D., (1999)., *Development* and Utilization of Natural Insecticides. Strata 1 (Thesis). Bogor (ID): Center for Integrated Pest Control Studies 1999.
- Oktaviani, E. P. (2014). Quality and antioxidant activity of probiotic drink with a variation of red dragon fruit extract (*Hyloreceus polyrhizus*). *Journal of Technobiology*, 1-15.
- Permenhut., 2007., Minister of Forestry Regulation No.: P.35/Menhut-II/2007 concerning Jakarta's Non-Timber Forest Products.
- Purwanti, S., (2017)., Antioxidant, Antibacterial, and Antibiofilm Activity of Jernang (*Daemonorops draco*) resin (Doctoral dissertation, Bogor Agricultural University (IPB)).
- Sarman SA., (2014)., Drakorodin Isolation from Daemonorops draco Resin. Strata 1 (Thesis): ITB Bandung 2014.
- Soemarna Y., (2009)., Ecology and germination and nursery techniques of jernang pulut (*Daemonorops draco* (Willd.) Blume) rattan. *Journal of Forest Products Research.*, 6(1), 3-39.
- Tasmin, N., & Kusuma, I. W. (2015). Isolation, Identification and Toxicity Test of Flavonoid Compounds from Chloroform Fraction from Terap Leaves (*Artocarpus Odoratissimus Blanco*). *Mulawarman Chemical Journal*, 12(1).
- Toriq U., (2013)., Chemical Compounds to Identify Jernang for Parameter Update of Indonesian National Standards. Strata 1 (Thesis); Bogor (ID): Bogor Agricultural University.
- Waluyo, T. K., & Pasaribu, G. (2015). Antifungal, antibacterial and wound healing activity of Jernang resin extract. *Journal of Forest Products Research*, 33(4), 377-385.
- Waluyo, T. K., & Pasaribu, G. (2013). Antioxidant and anticoagulation activity of jernang resin. *Journal of Forest Products Research*, 31(4), 306-315.
- Yanti YN, Mitika S., (2017)., Antibacterial Effectiveness Test of Ethanol Extract of Sambiloto Leaves (*Andrographis paniculata* Nees) Against

Staphylococus aureus. Scientific Journal of Ibn Sina; 2(1), 158-168.