**Indonesian Journal of Global Health Research**
Volume 3 Number 1, February 2021, pp. 29 - 42
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

# THE EFFECT OF FRACTION AND ACTIVE COMPOUNDS OF MOMORDICA BALSAMINA L. ON BACTERIA SALMONELLA TYPHI CAUSING SALMONELLOSIS

**Aryanti*, Rinda Lamdayani**
STIKES Abdurahman Palembang, Jl. Suka Jaya No.7, Suka Bangun, Kec. Sukarami, Kota Palembang, Sumatera Selatan, Indonesia 30151
*aryantianti89@gmail.com

**ABSTRACT**

*Salmonella typhi* is one of the bacteria that causes typhus. The handling of typhus by bacteria generally requires the provision of antibacterial substances, namely antibiotics. Excessive and irrational use of antibiotics causes bacteria to be resistant so that over time the benefits of using antibiotics will decrease. Pare leaves are an alternative treatment for various diseases, one of which is antibacterial. The purpose of this study was to determine the ability of bitter melon leaf fractions and active compounds against Salmonella typhi bacteria with various concentrations. This study was a laboratory experimental study through in vitro. The subjects in this research were Salmonella typhi bacteria. The results of this study indicated that the concentration of ethyl acetate fraction is a strong fraction between n-hexane and water methanol fractions in inhibiting Salmonella typhi bacteria. The determination of the active compound group from the purification of the ethyl acetate fraction of forest bitter melon leaves obtained flavonoid active compounds with an Rf value of 0.1 in the eluent n-hexane: ethyl acetate (7:3). The minimum inhibitory concentration (MIC) of ethyl acetate fraction was 125 µg/ml, while the MIC of flavonoid compounds was 62.5 µg/ml against Salmonella typhi bacteria. From the results of the equivalence test of the ethyl acetate fraction with ampicillin against Salmonella typhi bacteria, it showed that the concentration of the active fraction of ethyl acetate 1 µg/ml was equivalent to 0.007 µg/ml ampicillin, while the equivalence of flavonoid compounds was obtained 1 µg/ml concentration of active compounds equivalent to 0.011 µg/ml ampicillin.

**Keywords**: active compounds; fractions; momordica balsamina L.; salmonella typhi bacteria

| First Received | Revised | Accepted |
|---|---|---|
| 12 December 2020 | 17 December 2020 | 28 December 2020 |
| **Final Proof Received** | | **Published** |
| 05 January 2021 | | 01 February 2021 |

**How to cite (in APA style)**
Aryanti, A., & Lamdayani, R. (2021). The Effect of Fraction and Active Compounds of Momordica Balsamina L. on Bacteria Salmonella Typhi Causing Salmonellosis. *Indonesian Journal of Global Health Research*, *3*(1), 29-42. https://doi.org/10.37287/ijghr.v3i1.291

## INTRODUCTION

Indonesia is a country that has a tropical climate. Infectious diseases are generally and widely spread in tropical areas such as in Indonesia. The causes of many infectious diseases are caused by bacteria, viruses and other parasites. In general, infection is a condition in which a person's body is infected by pathogenic microorganisms that can cause infection. Based on the survey, the number of infectious patients who died was 28.1%, this number was greater than due to vascular disease which was at 18.9% and respiratory diseases at 15.7% (Ministry of Health, 2008).

Infection is a disease that is generally caused by microorganisms such as bacteria and viruses. Salmonella typhi is one of the bacteria that causes typhus. This bacterial infection occurs from eating food contaminated with dirt and feces which contain *Salmonella typhi* bacteria of the carrier organism (host). After entering the digestive tract, these bacteria will invade the

intestinal wall causing damage and inflammation (Jawetz et al, 2001, Jawetz et al., 2001 in Komala 2012).

The main treatment for infections caused by bacteria is antibiotics. Antibiotics are natural or synthetic compounds that have the ability to inhibit, suppress or stop biochemical processes in an organism's body which will later inhibit the growth process of that organism (Utami, 2012). Antibiotics are devoted to treat bacterial infections because antibiotics will work by destroying the biochemical system and a means of selecting bacteria that have genetically changed shape and properties.

Nowadays, the use of natural ingredients is preferred by the public. One of the plants that has antibacterial potential is bitter melon (Momordica balsamina L). Pare leaf (Momordica charantia L.) is a plant that contains medicinal compounds such as tannins, alkaloids, saponins, flavonoids, and triterpenoids which are considered antibacterial and momordic acid (Nurdina, 2012). The results of research conducted by Oktaviani (2016) found that bitter melon leaves (Momordica balsamina L) have antibacterial activity against Escherichia coli and Staphylococcus aureus. In this study, active compounds were also found, namely tannins and alkaloids which are considered to function as antibacterial.

The people of Bangun Jaya, Meranjat, Ogan Ilir used forest pare leaf plant (Momordica balsamina L.) as a medicine to treat typhus. The efficacy of bitter gourd leaves (Momordica balsamina L.) in overcoming typhoid has never been conducted, but for the bitter melon and bitter melon leaves with the same genus and family, namely bitter melon (Momordica charantia L), many studies have been conducted. This research was a laboratory experimental study through in vitro to determine the effect of the active fraction and active compounds of bitter melon leaves (Momordica balsamina L.) on Salmonella typhi bacteria. The determination of the minimum inhibitory concentration (MIC) of the active fraction and active compounds used a completely randomized design

## METHOD

This research was conducted in vitro where the tools and materials we used were as follows;

### Research Tools

The tools used in this research were autoclave, spectrophotometer, stirring rod, blender, maserator, glass beaker, flacon bottle, vial bottle, bunsen lamp, petri dish, hair dryer, incubator, ose needle, syringe, camera, disc paper, filter paper, label paper separating flask, refrigerator, column chromatography, oven, capillary tube, bath, Thin Layer Chromatography (TLC) plate, test tube rack, rotary evaporator, regression tube, analytical scales, roll wipes and vortex, as well as GF254 silica gel plate.

### Research Materials

The materials used in this study were distilled water, 70% alcohol, pare leaf simplicia (Momordica balsamina L.), N-hexane solution, ethyl acetate solution, methanol solution, nutrient agar (NA), nutrient broth (NB), DMSO solution, silica gel powder G-60 (0.063-0,200), Salmonella typhi bacteria cultures, and Ampicilin.

### Research process

The research process was carried out in accordance with the objectives of the study including extracting simplicia powder to obtain dry extracts. After that, fractionation was done by using the FCC (Liquid-Liquid Fractionation) method which was carried out continuously with n-

hexane, ethyl acetate and methanol (polar solvent) solvents to obtain a viscous fraction to dry fraction. Rejuvenation of Salmonella typhi bacteria culture was performed by aseptic method. One ose was taken and then inoculated into NB media aseptically by placing a loop needle containing the culture. Antibacterial activity test of fractions resulting from N-hexane, ethyl acetate and methanol fractions. This was done to find out which fraction the active compound was in. The bacterial activity test was carried out by the agar diffusion method for Salmonella typhi. The KHM value was determined by the agar diffusion method using disc paper with a diameter of 5 mm. The working procedure for MIC determination used the active fraction with different concentrations.

The bioautography test was carried out to determine the Rf value of the antibacterial active compound using thin layer chromatography. This was done to determine what compounds were present from the active fraction. Isolation was done on the active compound. The results of the bioautographic test were known from the spots showing the characteristics of the active compound. It was then continued by making the value of the minimum inhibitory concentration (MIC) of the active compound. The active fraction from the results of the antibacterial activity test, the fraction was weighed using an analytical balance as much as 0.02 ml and dissolved in accordance with the active fraction carried out with different concentrations of 4 times. The equivalence test of the active fraction and active compound with the antibiotic Ampicillin was carried out by entering the resistance diameter data into the standard Ampicillin curve. To determine the diameter of the resistance Ampicillin, Ampicillin solutions was made with different concentrations.

## RESULTS

### The Extraction of Pare Leaf (Momordica balsamina L.)

Pare leaves that have been cleaned weighing 1 kg were dried, obtaining dry simplicia then blended until smooth so that powder simplicia was obtaine. Then, powder simplicia was extracted by maceration with methanol solvent and produced as much as 50 g of extract which was 25% of the total simplicia. The extract percentage obtained can be considered to have met the quality standard requirements of the drug. BPOM (2005) stated that the percentage of thick extract from a plant with a percentage of ≥ 11% can be used as raw material for the manufacture of medicines and products. The amount of extract depends on the number and type of components or substances dissolved in it. Apart from the amount, the quality of the extract is also related to the chemical compounds contained because the biological response caused by the extract is caused by chemical compounds. According to the Indonesian Ministry of Health (2000), methanol solvent used in the maceration process can dissolve organic compounds contained in simplicia. Methanol solvent is capable of dissolving almost all components both polar, semi-polar and non-polar (Harborne, 1987).

### Pare Leaf Extract Fractionation (Momordica balsamina L.)

The extract from the simplicia of bitter gourd leaves (Momordica balsamina L.) obtained methanol extract, then the extract was fractionated using the Liquid-Liquid Fractionation (FCC) method using n-hexane, ethyl acetate, and water methanol, each 1 L gradually. Then, each liquid fraction obtained was evaporated by a rotary evaporator, so that the viscous fraction was obtained. From the fractionation process, the results are shown in Table 1.

Table 1.
Fractionation of bitter melon leaf extract (Momordica balsamina L.)

| Solvent | Weight Fraction (g) | Percentage (%) |
|---|---|---|
| N-hexane | 23.7 | 47 |
| Ethyl acetate | 16.3 | 33 |
| Methanol water | 10.00 | 20 |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*

In Table 1, it can be seen that the fractionation of bitter melon leaf extract (Momordica balsamina L.) from the extract weighing 50 grams was obtained by the n-hexane fraction weighing 23.7 g (47%), ethyl acetate fraction weighing 16.3 g (33%), and a methanol fraction weighing 10.0 g (20%) in paste or thick form.

**Test Activity Antibacterial Leaf Pare *(Momordica balsamine* L.)**
The results of the antibacterial activity test for each fraction can be seen in Table 2.

Table 2.
Antibacterial activity test results of the fraction of n-hexane, ethyl acetate, water methanol of bitter melon leaves (Momordica balsamina L.) at a concentration of 4% against Salmonella typhi bacteria

| Faction Type | Mean ± Standard Deviation Inhibition diameter (mm) |
|---|---|
| N-hexane | 0.00 ± 0.00 |
| Ethyl acetate | 15.90 ± 0.31 |
| Methanol water | 5.21 ± 0.02 |
| Control | 0.00 ± 0.00 |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*

*Source: Research results in the Biology lab of Sriwijaya University in 2016*
Picture 1: The results of the antibacterial activity test of n-hexane, ethyl acetate, and water methanol fractions at a concentration of 4% against Salmonella typhi bacteria.

**Determination of Minimum Inhibitory Concentration (MIC) of Pare Leaf Fraction (Momordica balsamina L.)**
From the analysis of variance or ANOVA, it was found that the p value was less than 0.05, which means that the treatment concentration of the active fraction of ethyl acetate had an effect on the inhibitory diameter. While the results of the Post Hoc Duncan follow-up test can be seen in table 3.

Table 3.
Mean Inhibitory Diameter (mm) ethyl acetate fraction against Salmonella typhi bacteria at various concentrations

| Fraction Concentration | N | Mean ± Standard deviation of diameter ethyl acetate inhibitor | Notation |
|---|---|---|---|
| 4000 µg / ml | 4 | 11.57 ± 0.02 | a |
| 2000 µg / ml | 4 | 11.15 ± 0.29 | a |
| 1000 µg / ml | 4 | 10.35 ± 0.60 | b |
| 500 µg / ml | 4 | 9.65 ± 0.17 | c |
| 250 µg / ml | 4 | 8.60 ± 0.43 | d |
| 125 µg / ml | 4 | 5.75 ± 0.30 | e |
| 62.5µg / ml | 4 | 0.00 ± 0.00 | f |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*
Note: numbers written in different lowercase letters show significantly different results for further tests ($p \leq 0.05$)


Figure 2. KHM determination ethyl acetate fraction against Salmonella typhi bacteria

Information: 1: Concentration 4000 µg/ml, 2: Concentration 2000 µg/ml, 3: Concentration 1000 µg/ml, 4: Concentration 500 µg/ml 5: Concentration 250 µg/ml, 6: Concentration 125 µg/ml, 7: Concentration 62.5 µg/ml

Based on Table 3 and Figure 2, it can be seen that the ethyl acetate fraction of the bitter melon leaf extract shows the diameter of the inhibition zone formed around the disc paper which was an indication of the strength of Salmonella typhi activity. Based on this value, it was known that the greater the percentage of concentration, the greater the diameter of the formed inhibition zone. The largest diameter of the inhibition zone was at a concentration of 4000 µg/ml, namely 11.57 mm. Then,the diameter of the inhibition decreased with decreasing the concentration of the fraction. The smallest inhibition zone diameter was found at a concentration of 125 µg/ml with an inhibition diameter of 5.75 mm and a concentration of 62.5 µg/ml with no inhibition zone.

**Bioautographic Test Results and Determination of Active Compounds in Pare Leaves (Momordica balsamina L.)**
The results of bioautographic tests and the determination of the active compound group of forest bitter melon leaves (Momordica balsamina L.) can be seen in Table 4.

Table 4.
Bioautographic test results and determination of the active compound group of forest bitter melon leaves (Momordica balsamina L.)

| Faction Type | Eluent (solvent) | Rf | Color | Active Compounds |
|---|---|---|---|---|
| Ethyl acetate | n-hexane: ethyl acetate (7: 3) | 0.1 | Yellow | Flavonoids |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*

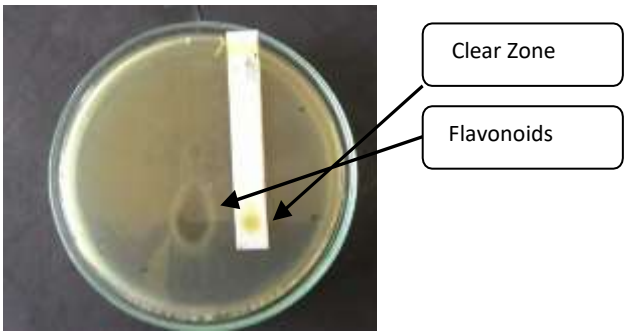


Figure 3. Bioautographic test results

**Determination of Minimum Inhibitory Concentration (MIC) of Active Compound of Pare Leaves (Momordica balsamina L.)**

From the analysis of variance or ANOVA, it was found that the p-value was less than 0.05, which means that the concentration of the active compound treatment had an effect on the inhibitory diameter. Meanwhile, the results of the Post Hoc Duncan follow-up test can be seen in Table 5.

Table 5.
Results of Determination of Minimum Inhibitory Concentration Value (MIC) of Active Compounds against Salmonella typhi

| Fraction Concentration | N | Mean ± Standard deviation of ethyl inhibitor diameter | Notation |
|---|---|---|---|
| 2000 µg/ml | 4 | 2.10 ± 0.26 | a |
| 1000 µg/ml | 4 | 1.05 ± 0.21 | b |
| 500 µg/ml | 4 | 0.70 ± 0.04 | b |
| 250 µg/ml | 4 | 9.90 ± 0.14 | c |
| 125 µg/ml | 4 | 8.50 ± 0.26 | d |
| 62.5 µg/ml | 4 | 5.50 ± 0.43 | e |
| 31.25 µg/ml | 4 | 0.00 ± 0.00 | f |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*

Note: numbers written in different lowercase letters show significantly different results for further tests ($p \leq 0.05$).

Figure 4. Results of Minimum Inhibitory Concentration Test for Active Compound of Pare Leaves (Momordica balsamina L.) against Salmonella typhi

Information: (1). 2000 µg/ml, (2). 1000 µg/ml, (3). 500 µg/ml, (4). 250 µg/ml, (5). 125 µg/ml, (6). 62.5 µg/ml, (7) Concentration 31.2 µg/ml

**Equivalence Test of Active Fractions and Active Compounds with Ampicillin**

The results of the analysis of the average diameter of ampicillin inhibition against Salmonella typhi bacteria at concentrations of 1000 µg/ml, 500 µg/ml, 100 µg/ml, 50µg/ml, 10 µg/ml, and 1 µg/ml:

Figure 5. Ampicili antibiotic activity test against the growth of Salmonella typhi bacteria with various concentrations

Table 6.
Results of Ampicillin antibiotic testing against the growth of Salmonella typhi bacteria

| Concentration (µg / ml) | Concentration (µg / disc) | Log | Mean ± Standard deviation of ethyl acetate inhibitory diameter |
|---|---|---|---|
| 1000 µg/ml | 10 | 1 | 13.60 ± 0.12 |
| 500 µg/ml | 5 | 0.699 | 12.45 ± 0.54 |
| 100 µg/ml | 1 | 0 | 11.70 ± 0.24 |
| 50 µg/ml | 0.5 | -0.301 | 10.75 ± 0.22 |
| 10 µg/ml | 0.1 | -1 | 7.70 ± 0.63 |
| 1 µg/ml | 0.01 | -2 | 6.05 ± 0.73 |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*
Note: numbers written in different lowercase letters show significantly different results for further tests ($p \leq 0.05$).

The test of the equivalence of fractions and active compounds with ampicillin antibiotics was done by entering the inhibitory diameter data into the regression analysis. The results of the analysis will be obtained a regression line equation. The value of the inhibitory diameter of the fraction and active compound at MIC, the regression value of ampicillin, the regression

constant value of ampicillin were included in the regression line equation to obtain an equivalence value.

The equivalence of the ethyl acetate fraction of forest pare leaves with ampicillin was obtained by entering the inhibition diameter in the regression equation, namely:

$Y = a + bX$

$Y = 11.06 + 2.582X$

Table 7.
Equivalence Test Results of the ethyl acetate fraction of Pare Leaves (Momordica balsamina L) with Ampicillin against bacteria *Salmonella typhi*

| Concentration of ethyl acetate fraction | Ampicillin concentration |
|---|---|
| 125 µg/ml | 0.87 µg/ml |
| 1 µg/ml | 0.007 µg/ml |
| 142 µg/ml | 1 µg/ml |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*

Based on table 7 it can be seen that the 125 µg/ml concentration of the active fraction is equivalent to 0.87 µg/ml of the concentration of ampicillin. This shows that the required ampicillin concentration is smaller than the active fraction concentration in inhibiting Salmonella typhi bacteria.

The equivalence of the active compound of forest bitter melon leaves with ampicillin was obtained by entering the inhibitory diameter in the regression equation, namely:

$Y = a + bX$

$Y = 11.06 + 2.582X$

Table 8.
Equivalence Test Results of the active compound of Pare Leaves (Momordica balsamina L) with Ampicillin against Salmonella typhi bacteria

| Concentration of Compounds Active | Ampicillin concentration |
|---|---|
| 62.5 µg/ml | 0.70 µg/ml |
| 1 µg/ml | 0.11 /ml |
| 91 g/ml | 1 µg/ml |

*Source: Research results in the Biology lab of Sriwijaya University in 2016*

## DISCUSSION

### Fractionation of Pare Leaf Extract (Momordica balsamina L.)

Based on the Table 1, it can be seen that the fractionation of bitter melon leaf extract (Momordica balsamina L.) from the extract weighing 50 grams was obtained by the n-hexane fraction weighing 23.7 g (47%), ethyl acetate fraction weighing 16.3 g (33%), and a methanol fraction weighing 10.0 g (20%) in paste or thick form. In table 1, it can be seen that the n-hexane fraction had a greater weight than ethyl acetate and methanol fraction of water. The solvents used in the fractionation have the ability to attract the compounds contained in the simplicia differently so that each extraction product contains compounds from different groups. The difference in weight fraction obtained from each solvent used did not affect the antibacterial activity. These solvents have the ability to separate compounds in the extract based on polarity.

**Test Activity Antibacterial Leaf Pare *(Momordica balsamine* L.)**
DMSO was used as a solvent because DMSO is a polar aprotic solvent, colorless and can dissolve polar and non-polar compounds which have a wide range of organic solvents such as water and have no biological activity. From Table 2 and Figure 1, it can be seen that the n-hexane fraction had no inhibitory diameter, the ethyl acetate fraction had an inhibition diameter of 15.90 mm, and the methanol fraction of water had an inhibition diameter of 5.21 mm. This shows that from this fraction, the ethyl acetate fraction had the strongest inhibitory diameter. This is in accordance with research on the antibacterial activity test and the identification of the active compound class of the chayote (sechium edule) (Swartz, 2009) where ethyl acetate extract had the highest antibacterial activity compared to hexane extract, chloroform extract, butanol extract, and water extract against P. aeruginosa and E. coli.

From the results of the antibacterial activity test, it was found that the fraction of bitter melon leaves (Momordica balsamina L.) had antibacterial activity against Salmonella typhi by forming an inhibition zone around the disc paper of 15.9 mm with a strong antibacterial power category. The results of this study are in accordance with the research of Ambarwati (2007), where the strength of antibacterial power is divided into 4, namely the inhibition zone > 20 mm means very strong, 10-20 mm inhibition zone means strong, 5-10 mm inhibition zone means medium and the inhibition zone < 5 mm or less is weak.

The active fraction contains active antibacterial compounds. This active compound will attack the components of bacterial cells which have a large number of nucleic acid proteins, enzymes, semipermeable membranes and cell walls. If the active compound component of the bitter melon leaf fraction (Momordica balsamina L.) attacks one of the components of the bacterial cell, it will cause damage to the bacterial cell, causing inhibition of bacterial growth. This assumption is made based on the statement of Hugo & Russel (1977)(Yusran, 2009) which explained that damage to bacterial cell components can be caused by the reaction of antibacterial active compounds with parts of bacterial cells.

**Determination of Minimum Inhibitory Concentration (MIC) of Pare Leaf Fraction (Momordica balsamina L.)**
In this study, the determination of the minimum inhibitory concentration was based on a concentration derivative starting from 4000 µg/ml, 2000 µg/ml, 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and a concentration of 62.5 µg/ml with 4 repetitions. The aim was to determine the smallest amount of antibacterial active substances needed to inhibit the growth of the tested organisms. MIC is stated as the lowest concentration of antibacterial substance ethyl acetate fraction from forest bitter melon (Momordica balsamina L.) which can inhibit bacterial growth.

Based on the oneway Anova statistical test, it was found that p value = 0.000 with a value of $\alpha = 0.05$ ($p < \alpha$), which means that there was a difference that affects the average inhibition diameter of each concentration, because the variance of the data was homogeneous, then the analysis was continued using the Post Hoc Duncan test to see the magnitude of the effect of each concentration on the inhibitory diameter of the ethyl acetate fraction.Based on Duncan's Post Hoc test, it can be concluded that there was a significant difference between each concentration treatment as seen from the results of Duncan's Post Hoc analysis which are in different columns.

Based on Table 3 and Figure 2, it can be seen that the ethyl acetate fraction of the bitter melon leaf extract shows the diameter of the inhibition zone formed around the disc paper which is an indication of the strength of Salmonella typhi activity. Based on this value, it is known that the greater the percentage of concentration, the greater the diameter of the formed inhibition zone. The largest diameter of the inhibition zone was at a concentration of 4000 µg/ml, namely 11.57 mm, then the diameter of the inhibition decreased as the concentration of the fraction decreased. The smallest inhibition zone diameter was found at a concentration of 125 µg/ml with an inhibition diameter of 5.75 mm and a concentration of 62.5 µg/ml with no inhibition zone.

The smallest concentration of ethyl acetate fraction that still inhibits the growth of Salmonella typhi bacteria was concentration of 125 µg/ml with a diameter of 5.75 mm, so this concentration is expressed as the MIC value. The KHM value of the fraction with a concentration of 125 µg/ml was categorized as strong enough. This is consistent with Holetz's (2002) statement, which states that the conditions for antibacterial power are as follows: <100 µg/ml is very strong, 100-500 µg/ml means strong enough, 500-1000 µg/ml means weak, and> 1000 µg/ml does not exist. Based on the opinion of Greenwood (1995) in Syarifah (2006), it was stated that the activity of the fraction decreases along as the decrease in concentration, so that the diameter of the inhibition zone formed is also getting smaller.

This study is not in line with the research conducted by Mahanani, et al. (2012) on Antibacterial Power of Pare Leaf Extract (Momordica Charantia L.) in Inhibiting the Growth of Streptococcus viridians with a miminum inhibition of 6.69 mm with a concentration of 1000 µg/ml. Whereas in this study the concentration was 125 µg/ml with an inhibition diameter of 5.75 mm. This shows that the minimum inhibitory concentration in this study is better than in previous studies.

**Bioautographic Test Results and Determination of Active Compounds in Pare Leaves (Momordica balsamina L.)**

The bioautographic test stage was carried out to determine the compounds contained in the active fraction. The ethyl acetate fraction was continued to the bioautography stage using TLC plates as the stationary phase. This bioautographic test stage was carried out using the appropriate eluent so that it can be determined which group of active compounds that act as antibacterials can be determined. Eluent according to used n-hexane: ethyl acetate with a ratio of 7: 3 as the mobile phase. As much as 2% H2SO4 was used to clarify the color spots and to see the compounds that act as antibacterials, it can be seen from the TLC plate printed in a petri dish.

The bioautographic test results obtained the active compound with an Rf value of 0.1 with n-hexane: ethyl acetate (7: 3) as eluent. This can be seen by the formation of a clear area (bacterial growth inhibition) at Rf 0.1 as shown in the picture above. The spots with Rf 0.1 were sprayed with H2SO4 then heated, yellow spots appeared which indicated that the flavonoid compounds contained in the ethyl acetate fraction of forest pare leaves were active antibacterial compounds of Salmonella typhi. The value of Rf can be defined as the distance traveled by the compound from the point of origin divided by the distance traveled by the solvent from the point of origin (Stahl, 1985).

Based on the bioautography test results of bitter melon leaves, the spots that arise after being sprayed and heated over a bath show a yellow spot which is a flavonoid compound.

This compound is bioactive so that it can be used as a natural antibacterial agent. According to various literatures, flavonoids as phenol derivatives can cause damage to the cell wall structure and change the permeability mechanism of the bacterial cell wall so that they are said to have antibacterial properties (Handayani and Tendelilin, 2006). This research is in line with previous research (Rahayu, 2016) on the effect of fruit ethanol extract concentration on the growth of shigella dysenteriae bacteria in vitro which contains flavonoids which have antibacterial properties.

**Determination of Minimum Inhibitory Concentration (MIC) of Active Compound of Pare Leaves (Momordica balsamina L.)**

Based on the test results of the active compound antibacterial activity from the purification of the ethyl acetate fraction of forest bitter melon leaves (Momordica balsamina L.), it was found that the active compound was an isolate which was a flavonoid compound and continued with the determination of the minimum inhibitory concentration (MIC) value of the active compound to determine the lowest value of power. inhibit the active compound.

In this study, the determination of the minimum inhibitory concentration was based on a concentration derivative starting with a concentration of 2000 µg/ml, 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, and 31.25. µg/ml with 4 repetitions. The aim 2qw to determine the smallest amount of antibacterial active substance from the active compound of bitter melon leaves (Momordica balsamina L.) which is still able to inhibit bacterial growth.

Based on the oneway Anova statistical test, it was found that p value = 0.000 with a value of $\alpha = 0.05$ ($p < \alpha$), which means that there was a difference that affected the average inhibition diameter of each concentration, because the variance of the data was homogeneous, then the analysis was continued using the Post Hoc Duncan test to see the magnitude of the effect of each concentration on the inhibitory diameter of the active compound.

Based on Duncan's Post Hoc test, it can be concluded that there wass a significant difference between each concentration treatment as seen from the results of Duncan's Post Hoc analysis which were in different columns. Based on Table 5 and Figure 4, it can be seen that at a concentration of 2000 µg/ml, the value of the largest inhibition diameter against Salmonella typhi is 12.10 mm, while the smallest inhibition diameter value is at a concentration of 62.5 µg/ml of 5.50 mm, and a concentration of 31.25 µg/ml had no inhibition diameter. According to Pelczar and Chan (2005), there are many factors that can influence the inhibition or eradication of microorganisms by antibacterial substances, such as the concentration or intensity of antibacterial substances, types of microorganisms, the number of microorganisms, temperature, media components, Ph, and incubation time.

From table 5, the determination of the minimum inhibitory concentration value of a compound with a concentration from 2000 µg/ml to 62.5 µg/ml is active in inhibiting the growth of Salmonella typhi, so that at a concentration of 62.5 µg/ml with a diameter of 5.50 mm is expressed as the inhibitory concentration. minimum against Salmonella typhi bacteria.

The inhibition zone formed up to a concentration of 62.5 µg/ml indicates that the compounds contained are flavonoids which have potential as antibacterial. This is in accordance with the opinion stated by Holetz (2002), which explains that antibacterial

compounds with concentrations below 100 μg/ml are classified as very strong antibacterial compounds. The higher the concentration value will affect the value of antibacterial activity. This is in accordance with the statement of Pelczar and Chan (2005) which states that the higher the antibacterial concentration, the greater the resulting activity.

**Equivalence Test of Active Fractions and Active Compounds with Ampicillin**

To determine the equivalence of the fractions and compounds of bitter melon leaves with ampicillin, the equivalence test was carried out. The equivalence test was carried out by comparing the minimum inhibitory diameter of the active fraction and the active compound with the minimum inhibitory diameter of ampicillin antibacterial. The resistance diameter of the test results with Salmonella typhi antibacterial is made in the form of a linear graph. Equation of the lines so that the equality value is obtained.

Based on table 8, it can be seen from the results of the equivalence test of the ethyl acetate fraction with ampicillin against Salmonella typhi bacteria, it shows that from a concentration of 125 μg/ml equivalent to 0.87 μg/ml ampicillin and a concentration of 1 μg/ml the active fraction of bitter melon leaves is equivalent to 0.007 μg/ml with ampicillin. Meanwhile, the results of the equivalence test for the active compound of bitter melon leaves showed that from 62.5 μg/ml the active compound concentration was equivalent to 0.70 μg/ml ampicillin and from 1 μg/ml the active compound concentration was equivalent to 0.011 μg/ml ampicillin. This shows that to get the appropriate dose of ampicillin to inhibit the growth of Salmonella typhi bacteria, a larger number of fractions and compounds is needed.

This research is in line with previous research (Rahayu, 2016)), where the ampicillin antibiotic has greater inhibition than the inhibition of the ethanol extract of bitter melon (Momordica charantia L.) against Shigella dysenteriae bacteria. However, the fractions and active compounds of various concentrations have shown positive antibacterial activity in inhibiting the growth of Salmonella typhi bacteria.

## CONCLUSION

Ethyl acetate and water methanol fractions from the leaves of bitter melon (Momordica balsamina L.) have antibacterial activity against Salmonella typhi. Ethyl acetate fraction has the strongest antibacterial activity against Salmonella typhi. The class of antibacterial active compounds found in the ethyl acetate fraction of bitter melon leaves (Momordica balsamina L.) are flavonoids with an Rf value of 0.1 mm. The minimum inhibitory concentration (MIC) of the ethyl acetate fraction is 125 μg/ml with an inhibition diameter of 5.75 mm against Salmonella typhi and the MIC in the active compound is 62.5 μg/ml with an inhibition diameter of 5.50 mm against Salmonella typh. Equivalent 1 μg/ml concentration of active fraction equivalent to Ampicillin 0.007 μg/ml and 1 μg/ml of active compound equivalent to Ampicillin 0.011 μg/ml

## REFERENCES

Ambarwati. 2007. Effectiveness of Neem Seed Antibacterial Substance (Azadirachta indica) to Inhibit the Growth of Salmonella typhosa and Syaphylococcus aureus. Journal of the Muhammadiyah University of Surakarta. Vol. 8 (3)

Department of health. (2008). National Riskesdas 2007. Agency for Health Research and Development, Ministry of Health, Republic of Indonesia December 2008, 1–384.http://kesga.kemkes.go.id/images/pedoman/Riskesdas 2007 Nasional.pdf

Chambers, SR (2009). No Title 血清 及 尿液 特定 蛋白 检测 在 糖尿病 肾病 早期 诊断 中 的 意义. April.

Handayani, J., Tandelilin. 2006. The Power Influence

Antibacterial Fresh The Leaf Extract (Camelilia Sinensis) Against Streptococcus alpha. Journal of the Indonesian Dentists Association

Holetz, FB (2002). Screening of seme plats used in Brazilian Folk Medizine or the treatment of Infections Disease. Journal of Boline International. 97 (7): 1027-1031

Jawetz, EJL Melnick, EA Adelberg, GF Brooks, JS, Butel, and LNOrnston. 2008. Medical Microbiology. Edition 23. Jakarta

: EGC

Mahanani, PP, Praharani, D., Purwanto. 2012. Antibacterial Power of Pare Leaf Extract (Momordica Charantia L) In Inhibiting Growth of Streptococcus viridians. Student Research Scientific Articles. University of Jember.

Nurdina, YES (2012). Inhibition of Pare Leaf Extract (Momordica charantia) Against Lactobacillus (Antibacterial Activity of Pare Leaf (Momordica charantia) Extract Against Lactobacillus acidophilus). Scientific Articles of Student Research Results, 3–6.

Oktaviani, Y. 2016. Antibacterial Activity Test of Active Compounds from Pare Leaf Extract (Momordica balsamina L.) Against Escherichia Colidanstaphylococcus Aureus. UniversitasSriwijaya Palembang

Pelczar, M. L and ECS Chan. 2005. Basics of Microbiology (Volume 1). UI Press: Jakarta.

Rahayu, S. (2016). Effect of Concentration of Ethanol Extract of Pare (Momordica charantia L) on the Growth of Shigella dysenteriae Bacteria by In Vitro. Ibn Sina's Scientific Journal, 1 (2), 203–210.

Swartz, S. (2009). STUDY OF ANTIBACTERIAL ACTIVITIES AND IDENTIFICATION OF ANTI-ACTIVE CLASSES OF ANTIBACTERIAL COMPOUNDS OF SIAM (Sechium edule).

Syarifah. 2006. Isolation of Jmabu Bioa Leaf Bacteria Compounds (Eugenia Densilora BL) and Determination of Minimum Inhibitory Concentrations (MIC) against Staphylococcus Aureus and Escherichia coli. Sriwijaya University Postgraduate Thesis Program

Utami, P. 2003. Medicinal Plants to Overcome Diabetes Mellitus. Agro Media Pustaka. Jakarta.

Utami, P. 2012. Natural Antibiotics to Overcome Various Diseases. Agro Media: Jakarta.

Yusran, A. and K. (2009). Anti-fungal inhibition test of Cinnamomun burmanii essential oil extract on the growth of Candida albicans. Dentofacial, 8 (2), 104–110.