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# ANTIBACTERIA ACTIVITY ETHANOL EXTRACT OF *ACHRAS ZAPOTA* L. FRUIT AND *LAGENARIA SICERARIA* (MOLINA) STANDL. AGAINST *SALMONELLA TYPHI*

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Detail Artikel

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Kata Kunci

*Synergistic*
*Salmonella typhi*
*Achras zapota L.*
*Lagenaria siceraria (Molina) Standl.*
*FICI*

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***ABSTRACT***

*The Synergistic of antibacterial is an interaction of two antibacterial agents that produces a greater inhibitory effect on bacteria, compared to the individual antibacterial effects of the two agents. The purpose of this study was to determine the synergistic effect of anti-bacterial extract of sapodilla fruit (Achras zapota L.) and water gourd (Lagenaria siceraria (Molina) Standl.) extract against Salmonella typhi bacteria. Extraction of sapodilla fruit and water gourd fruit was carried out by maceration method using 96% ethanol (1:10). Determination of the value of the minimum inhibitory concentration (MIC) of anti-bacterial sapodilla manila fruit and water gourd fruit was carried out by the checkerboard assay method using a 96 wells microplate by calculating the Fractional Inhibition Concentration Index (FICI) value. The results showed that the single MIC value of sapodilla manila fruit and water gourd fruit respectively are 50 mg/mL and 100 mg/mL. The interaction between the two*



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*is expressed as a Fractional Inhibitory Concentration Index (FICI) with a value of 1.5. The FICI values indicate different antibacterial effects of the combination of sapodilla fruit and water gourd fruit against Salmonella typhi.*

## INTRODUCTION

*Salmonella typhi* bacteria are gram-negative bacteria that cause typhoid fever or typhus. Typhoid fever is transmitted via fecal-oral route which enters the human body through contaminated food and drink . This disease is still often found in tropical and subtropical areas, especially in areas with inadequate quality water sources with low hygienic and sanitation standard (Kasim, 2020). As a tropical country that is rich in a diversity of natural biological resources, Indonesia has natural ingredients that have the potential to be used as medicine and can be used for alternative treatments.

Plants that are popularly used by the community as treatment for typhoid fever sufferers are manila sapodilla (*Achras zapota* L.) and water pumpkin (*Lagenaria siceraria* (Molina) Standl). The manila sapodilla fruit is empirically used by Indonesian people as a treatment for typhoid fever in the form of juice for drinking. Unripe Manila sapodilla fruit is very hard and has an astringent or chewy taste due to the tannin content (Akwa & Nguimbous, 2021).

Manila sapodilla fruit contains chemical compounds such as alkaloids, flavonoids, tannins (Trisnawati, 2019) and terpenoids (Punia Bangar dkk., 2022) which can act as antibacterials against Salmonella typhi. J & M.S,( 2019) stated that " the 70% acetone extract for sapodilla bar and juice exhibited stronger antibacterial activity against gram-positive bacteria. Despite some antioxidants being lost in processed food, these still retained important sources of bioactive compounds. Antimicrobial activity done showed different selectivity for sapodilla juice and bar". Based on research conducted(Salnus & Artati, 2019), young manila sapodilla fruit extract inhibits Salmonella typhi with a large inhibition zone of 18 mm at a concentration of 55%.

Water gourd fruit contains chemical compounds including saponins, phenols, flavonoids, curcumin and alkaloids(Shantia dkk., 2021). Water gourd fruit is used empirically for the treatment of typhoid fever. Based on research conducted (Srikacha & Ratananikom, 2020), the ethanol extract of *Piper betle* Linn showed the highest antibacterial activity against Gram-positive and the negative bacteria. MIC and MBC of the ethanol extract of *Piper betle* Linn against *Salmonella typhimurium* were the same (1 562.50 mg/L); while it showed the highest MIC and MBC against Pseudomonas aeruginosa of 6 250 mg/L and 12 500 mg/L,

respectively. *Salmonella typhimurium* is the most susceptible bacteria while *Pseudomonas aeruginosa* is the most resistant bacteria towards the ethanol extract of *Piper betle* Linn. *Piper betle* possesses compounds with potential antibacterial activity and might be useful as an alternative to control infectious diseases.

The interaction of two antibacterial agents produces a greater inhibitory effect against bacteria, compared to the individual antibacterial effects of the two agents. So, research was carried out to test the antibacterial synergy of manila sapodilla fruit extract and

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pumpkin fruit extract in inhibiting the growth of *Salmonella typhi* using the checkerboard assay method.

## CHEMICALS AND METHODS

### Chemicals

The tools and materials used in the research are as follows. The tools used in this research were, autoclave (Gea®), porcelain cup, Eppendorf, Erlenmeyer (Iwaki®), beaker (Iwaki®), measuring cup (Iwaki®), incubator (Memmert®), tube needle, micropipette (Dragon Lab®), oven (Thermo®), Rotary evaporator (IKA®), syringe, test tube (Pyrex®), analytical balance (Mettler Toledo®), and wells microplate 96 (Biologix®). The ingredients used in this research were Aquadest, DMSO 10%, manila sapodilla fruit (Achras zapota L.), water gourd fruit (Lagenaria siceraria (Molina) Standl), 96% ethanol, Mc solution. Farland 0.5, NaCl 0.9%, Nutrient Agar (NA), Nutrient Broth (NB) and 2,3,5- Triphenyltetrazolium chloride. The test bacteria used in this research was Salmonella typhi obtained from the *Salmonella typhi* collection of the Microbiology Laboratory, Faculty of Health Sciences, Almarisah Madani University.

### Methods

### Sample Preparation

The sample used was young sapodilla fruit (*Achras zapota* L.), obtained from Jl. Ir. Sutami, Sudiang, District. Biringkanaya, Makassar City, South Sulawesi and young water pumpkin (*Lagenaria siceraria* (Molina) Standl) were obtained from Daya market, Biringkanaya District, Makassar City, South Sulawesi.

The young manila sapodilla fruit (*Achras zapota* L.) obtained were washed with running water, then wet sorted and chopped to facilitate the drying process, after that they were weighed, then dried using an oven at a temperature of 50°C. Next, it is sorted to dry to separate the dirt that was involved during drying. The dried simplicia is ground and then extracted using the maceration method. The young water pumpkin (*Lagenaria siceraria* (Molina) Standl.) fruit obtained was washed with running water, then sorted wet and chopped to facilitate the drying process, after that it was weighed, then dried using an oven at a temperature of 50°C. Next, it is sorted to dry to separate the dirt that was involved during drying. The dried simplicia is ground and then extracted using the maceration method(Utami, Mubarak, dkk., 2023).

### Sample Extraction

### Preparation of Mandarin Sapodilla Fruit Extract *(Achras zapota L.)*

Simplisia sapodilla fruit is extracted using the maceration method. 400 grams of simplicia powder was soaked in a maceration container using 4000 mL of 96% ethanol solvent with a powder: solvent ratio of 1:10 (w/v). The simplicia is first wetted with some 96% ethanol solvent until it is completely wet, after that it is filled up to 4000 mL. Next, leave the macerate for 3 x 24 hours and stir occasionally. The macerate is filtered using filter paper. The filtrate is collected and then the maceration residue is remaciated once. Then, the

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macerate is filtered, then the filtrate resulting from maceration and remaceration is evaporated using a Rotary Evaporator at a temperature of 50°C until a thick extract is obtained and the % yield is calculated(Utami, 2020).

**Preparation of Pumpkin Fruit Extract** (*Lagenaria siceraria* (Molina) Standl.)

Water gourd fruit simplicia is extracted using the maceration method. 260 grams of simplicia powder was soaked in a maceration container using 2600 mL of 96% ethanol solvent with a powder: solvent ratio of 1:10 (w/v). The simplicia is first wetted with some 96% ethanol solvent until it is completely wet, after that it is filled up to 2600 mL. Next, leave the macerate for 3 x 24 hours and stir occasionally. The macerate is filtered using filter paper. The filtrate is collected and then the maceration residue is remaciated once. Then, the macerate is filtered, then the filtrate resulting from maceration and remaceration is evaporated using a Rotary Evaporator at a temperature of 50°C until a thick extract is obtained and the % yield is calculated(Utami, 2021).

$$\% \text{ yield} = \frac{Extract\ Weight}{Sample\ Weight} \times 100\%$$

**Identification of Compound Classes**

**Alkaloids**

The extract was mixed with 1 mL of chloroform and 1 mL of ammonia put into a test tube, then heated over a water bath, shaken and filtered. The filtrate obtained is divided into three equal parts, then put into a 2 N sulfuric acid test tube, shake and let stand for several minutes until separated. The top of each filtrate was taken and tested with Mayer, Wagner, and Dragendroff reagents. The formation of orange, brown and white precipitates in each test result indicates the presence of alkaloids(Utami, Yulianty, dkk., 2023).

**Flavonoids**

Weigh 0.5 gram of the extract, put it in a test tube then add 70% ethanol and 5-6 drops of concentrated HCl. If a red color forms, it indicates a flavonoid compound and if an orange color forms, a flavonoid compound is formed(Imrawati dkk., 2023).

**Saponin**

Weighed 0.5 gram of the extract then put it in a test tube then added 10 ml of hot water, shaken vigorously for 10 seconds. If froth or foam forms for approximately 10 minutes as high as 1 cm to 10 cm, and when 1 drop of 2 N hydrochloric acid is added and the foam does not disappear, it means that it is positive for containing saponin(Utami & Jariah, 2023).

**Tannin**

The extract is weighed at 0.5 grams, put into a test tube then dissolved in 10 ml of warm water, added with 1% $FeCl_3$ 1-2 drops. If dark blue or blackish green is formed, the extract indicates a tannin group compound (Utami & Jariah, 2023).

**Steroid/Triterpenoid**

The extract was mixed with 3 mL of chloroform and added with 2 mL of concentrated sulfuric acid and 2 mL of anhydrous acetic acid. The color change from purple

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to blue or green indicates the presence of steroids or the formation of a brownish red color at the interface indicates the presence of triterpenoids(Imrawati dkk., 2023).

**Phenolic**

A number of extracts were mixed with 3 mL of 96% ethanol into a test tube, then heated over a water bath, then filtered. The filtrate obtained was added with a few drops (2-3 drops) of 1% FeCl3 and the formation of green, red, yellow, orange, blue and black colors indicated the presence of phenolics(Ahmad dkk., 2015).

**Preparation for Determining Minimum Inhibitory Concentration (MIC)**

**Tool Sterilization**

The tools used are sterilized first. Glass utensils are washed, dried and then wrapped in paper. For glassware that has a scale, it is sterilized using an autoclave at a temperature of 121ºC for 15 minutes, glassware that does not have a scale is sterilized using an oven at a temperature of 180ºC for 1 hour, and round glassware is sterilized by lighting it using a Bunsen flame(Utami, Ismail, dkk., 2023).

**Media Creation**

**Nutrient Agar (NA) Media**

A total of 20 grams of NA media was weighed and dissolved in 250 mL of distilled water. Then stirred and heated using a microwave until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes(Utami, Ismail, dkk., 2023).

**Media Nutrient Broth (NB)**

A total of 13 grams of NB media was weighed and dissolved in 250 mL of distilled water. Then stirred and heated using a microwave until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes(Utami dkk., 2024).

**Rejuvenation of Test Bacteria**

The Salmonella typhi test bacteria were taken using a sterile loop needle and then inoculated on NA slant media. Incubated in an incubator at 37°C for 1× 24 hours(Utami dkk., 2024).

**Preparation of Test Bacterial Suspension**

One test culture of Salmonella typhi bacteria that has been rejuvenated in NA medium is suspended in a sterile test tube containing 3 mL of 0.9% NaCl, then the turbidity is seen by comparing the standard turbidity of 0.5 Mc. Farland (equivalent to 1.5 × 108 CFU/mL)(Utami dkk., 2024).

**Determination of Minimum Inhibitory Concentration (MIC) of Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Gourd Fruit Extract (*Lagenaria siceraria* (Molina) Standl)**

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Determination of the MIC of manila sapodilla fruit extract and water gourd fruit extract was carried out by microdilution in 96 wells microplate wells. An extract concentration of 100 mg/mL was made; 50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL. A concentration of 50 mg/mL was made by taking 0.5 mL of the extract stock solution with a concentration of 100 mg/mL, placing it in an Eppendorf tube, then adding 0.5 mL DMSO. Then, 0.5 mL of a concentration of 50 mg/mL was put into a new Eppendorf tube and 0.5 mL of DMSO was added to make a concentration of 25 mg/mL. The next concentration is made in this way.

Then, 20 µL of each concentration was put into the microplate wells and diluted with 175 µL NB medium and 5 µL *Salmonella typhi* bacterial suspension in each well (equivalent to 0.5 Mc Farland 1.5 × 106 CFU/mL), then homogenized. Thus, the volume in each well becomes 200 µL and the extract concentration becomes 10 mg/mL; 5 mg/mL; 2.5 mg/mL; 1.25 mg/mL; 0.625 mg/mL. The other wells are filled with negative control testing (test bacteria plus medium) and medium control. Then, the microplate was incubated at 37˚C for 1 x 24 hours. After that, observations were made by dripping each well with 5 µL of 2,3,5-Triphenyltetrazolium chloride, then incubating for 30 minutes. Then, observe visually by looking for changes in the color of the solution in each well, namely a change in color to red. In the wells that did not change color to red, it showed that there was no bacterial growth and at the lowest concentration it showed the MIC value of manila sapodilla fruit extract (*Achras zapota* L.) and water pumpkin fruit extract (*Lagenaria siceraria* (Molina) Standl) (Kowalska-Krochmal & Dudek-Wicher, 2021).

**Determination of Minimum Inhibitory Concentration (MIC) of Sapodilla Fruit Extract (*Achras zapota* L.) Combination with Water Pumpkin Extract (*Lagenaria siceraria* (Molina) Standl.)**

Determination of the Minimum Inhibitory Concentration (MIC) of a combination of manila sapodilla fruit extract (*Achras zapota* L.) and water gourd fruit extract (*Lagenaria siceraria* (Molina) Standl) using the microdilution method. From each initial solution, manila sapodilla fruit extract (*Achras zapota* L.) and water gourd extract (*Lagenaria siceraria* (Molina) Standl) (100 mg/mL; 50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6 .25 mg/mL), 20 µL was taken from each extract dilution and placed in each microplate well. Then 155 µL of medium was added, and 5 µL of Salmonella typhi bacterial suspension in each well (equivalent to 0.5 Mc Farland 1.5 × 106 CFU/mL), then homogenized, so that the volume of solution in each well was 200 µL. The concentration of manila sapodilla fruit extract and pumpkin fruit extract is 10 mg/mL; 5 mg/mL; 2.5 mg/mL; 1.25 mg/mL; 0.625 mg/mL. The other wells are filled with negative control testing (test bacteria plus medium) and medium control. Then, the microplate was incubated at 37˚C for 1 x 24 hours.

After that, observations were made by dripping each well with 5 µL of 2,3,5-Triphenyltetrazolium chloride, then incubating for 30 minutes. Then, observe visually by looking for changes in the color of the solution in each well, namely a change in color to red. In wells that do not change color to red, it shows that there is no bacterial growth and at the lowest concentration it shows the MIC value of a combination of manila sapodilla fruit extract (*Achras zapota* L.) and water pumpkin fruit extract (*Lagenaria siceraria* (Molina) Standl) (Kowalska-Krochmal & Dudek-Wicher, 2021).

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**Determination of Fractional Inhibitory Concentration Index (FICI) of Sapodilla Fruit Extract (*Achras zapota* L.) Combination with Water Pumpkin Extract (*Lagenaria siceraria (Molina) Standl.):***

$$FIC_1 = \frac{MIC\ Manila\ Sapodilla\ Fruit\ Extract\ in\ Combination}{MIC\ Manila\ Sapodilla\ Fruit\ extract}$$

$$FIC_2 = \frac{MIC\ Water\ Pumpkin\ Extract\ in\ Combination}{MIC\ Water\ Pumpkin\ Extract}$$

$$FICI = FIC_1 + FIC_2$$

In vitro interactions between antimicrobial agents are determined by calculating the formula, so the effects are described as follows (Abdulabbas dkk., 2022) :

1. Synergistic: FICI value ≤ 0.5
2. Additive effect: FICI value >0.5 or ≤ 1
3. Different effects: FICI value >1 or ≤ 4
4. Antagonist: FICI value > 4

**Data analysis**

Data analysis was carried out based on the results of the calculation of the Fractional Inhibitory Concentration Index (FICI). Then, a discussion is carried out on the observational data obtained and conclusions are drawn.

## RESULT AND DISCUSSION

The stages of this research start from processing the samples until they become simplified, then continue with extracting the samples using the maceration method. The following are the results of the percent yield of manila sapodilla fruit extract (*Achras zapota* L.) and water gourd fruit extract (*Lagenaria siceraria* (Molina) Standl.).

In this study, the plant samples used were manila sapodilla fruit (*Achras zapota* L.) and water gourd fruit (*Lagenaria siceraria* (Molina) Standl.) with the aim of determining the synergistic antibacterial effect of the two extracts on *Salmonella typhi* bacteria. The extraction method used for this research is the maceration method using 96% ethanol filter fluid at a sample to filter ratio of 1:10. The choice of extraction method is used to avoid damage to thermolabile compounds (Punia Bangar dkk., 2022). 96% ethanol was chosen because of its good absorbance and high filtering ability so that it can filter non-polar, semi-polar and polar compounds. 96% ethanol solvent penetrates more easily into the sample cell walls than low concentration ethanol solvent, resulting in a concentrated extract(Wendersteyt dkk., 2021).

The extraction results obtained are then weighed and the percent yield of the extract is calculated. The percentage yield obtained has met the requirements according to the Indonesian Herbal Pharmacopoeia, namely a yield of not less than 7.2%. According (Shantia dkk., 2021) the high number of active compounds contained in a sample is indicated by the high amount of yield produced (table 1).

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**Table 1**. Calculation Results of Yield of Manila Sapodilla Fruit Extract *(Achras zapota* L.) and Water Pumpkin Extract (*Lagenaria siceraria* (Molina) Standl.)

| Simplicity | Simplicity Weight (g) | Extract Weight (g) | Extract Yield (%) |
|---|---|---|---|
| Manila Sapodilla Fruit Extract (*Achras zapota* L.) | 400 | 159,90 | 39,9 |
| Water Pumpkin Extract (*Lagenaria siceraria* (Molina) Standl.) | 260 | 57,09 | 21,95 |

The next stage is a qualitative phytochemical screening, where the test is carried out using a test tube with the addition of chemical reagents according to the compound class tested. This phytochemical screening was carried out to identify the types of chemical compounds contained in manila sapodilla fruit extract and water gourd fruit extract. The results of the phytochemical screening test can be seen in table 2.

**Table 2.** Results of Phytochemical Screening Tests for Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Pumpkin Extract (*Lagenaria siceraria* (Molina) Standl.)

| No. | Phytochemical Screening Tests | | Test Result | |
|---|---|---|---|---|
| | | | **Manila Sapodilla Fruit Extract (*Achras zapota* L.)** | **Water Pumpkin Extract (*Lagenaria siceraria* (Molina) Standl.)** |
| 1. | Alkaloids | Mayer | - | - |
| | | Wagner | - | - |
| | | Dragendroff | - | - |
| 2. | Phenolic | | + | + |
| 3. | Flavonoids | | - | + |
| 4. | Saponin | | + | + |
| 5. | Steroid & triterpenoid | | + | + |
| 6. | Tannin | | + | + |

Based on the results of the phytochemical screening test, manila sapodilla fruit extract (*Achras zapota* L.) was positive for containing phenolic compounds, saponins, tannins

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and triterpenoids. The results of the same research conducted by(Akwa & Nguimbous, 2021) showed that the ethanol extract of manila sapodilla fruit (*Achras zapota* L.) contains triterpenoid, alkaloid and phenolic compounds. As for Table 3, the results of the phytochemical screening test for water gourd extract (*Lagenaria siceraria* (Molina) Standl.) are positive for containing phenolics, flavonoids, saponins, tannins and triterpenoids. The same results in research conducted by[8] showed that water pumpkin fruit extract (*Lagenaria siceraria* (Molina) Standl.) contains saponins, phenols, flavonoids, curcumin and alkaloids.
Tests for determining the MIC (Minimum Inhibitory Concentration) value of single manila sapodilla fruit extract and water gourd fruit extract using the checkerboard microdilution method using a 96 well microplate containing 200 µL in each well, and dilution at a concentration that is a multiple of twice the initial concentration in each solution. stock of manila sapodilla fruit extract and water gourd fruit, namely, 100 mg/mL; 50 mg/mL; 25 mg/mL; 12.5 mg/mL and 6.25 mg/mL. The results of determining single and combined MIC values can be seen in figure 1 and figure 2.


**Figure 1**. Results of Determination of Single and Combination MIC Values of Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Pumpkin Extract (*Lagenaria siceraria* (Molina) Standl.) Using the Checkerboard Assay Method

Information :
- = Clear (no bacterial growth)
+ = Red (there is bacterial growth)
A1 = Manila sapodilla fruit extract with a concentration of 100 mg/mL
A2 = Manila sapodilla fruit extract with a concentration of 50 mg/mL
A3 = Manila sapodilla fruit extract with a concentration of 25 mg/mL
A4 = Manila sapodilla fruit extract with a concentration of 12.5 mg/mL
A5 = Mandarin sapodilla fruit extract with a concentration of 6.25 mg/mL
B1 = Pumpkin fruit extract with a concentration of 100 mg/mL
C1 = Medium control
C2 = Control medium + DMSO
C3 = Medium control + *Salmonella typhi* suspension
C4 = Medium control + DMSO + *Salmonella typhi* suspension

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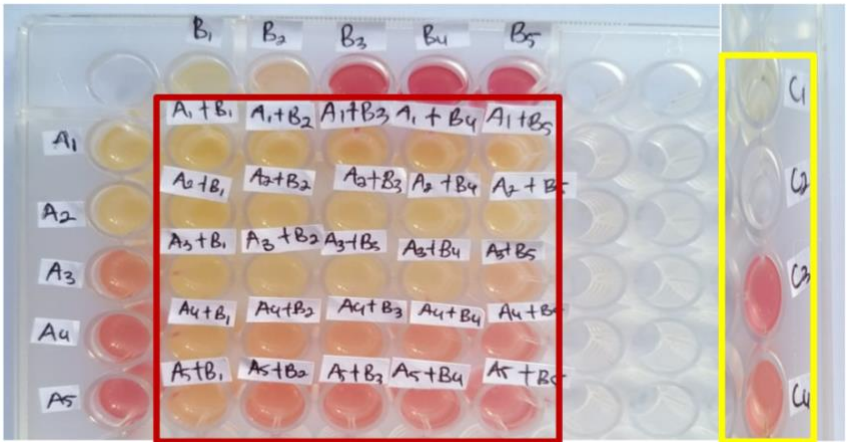

**Figure 2.** Results of Determination of Single and Combination MIC Values of Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Gourd Fruit Extract (*Lagenaria siceraria* (Molina) Standl.) Using the Checkerboard Assay and Control Method.

Information :
- = Clear (no bacterial growth)
+ = Red (there is bacterial growth)
B1+A1 = 20 µL Concentration of pumpkin fruit extract 10 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 10 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B1+A2 = 20 µL Concentration of pumpkin fruit extract 10 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B1+A3 = 20 µL Concentration of pumpkin fruit extract 10 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 2.5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B1+A4 = 20 µL Concentration of pumpkin fruit extract 10 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 1.25 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B1+A5 = 20 µL Concentration of pumpkin fruit extract 10 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 0.625 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B2+A1 = 20 µL Concentration of pumpkin fruit extract 5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 10 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B2+A2 = 20 µL Concentration of pumpkin fruit extract 5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B2+A3 = 20 µL Concentration of pumpkin fruit extract 5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 2.5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B2+A4 = 20 µL Concentration of pumpkin fruit extract 5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 1.25 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B2+A5 = 20 µL Concentration of pumpkin fruit extract 5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 0.625 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B3+A1 = 20 µL Concentration of pumpkin fruit extract 2.5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 10 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B3+A2 = 20 µL Concentration of pumpkin fruit extract 2.5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B3+A3 = 20 µL Concentration of pumpkin fruit extract 2.5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 2.5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B3+A4 = 20 µL Concentration of pumpkin fruit extract 2.5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 1.25 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B3+A5 = 20 µL Concentration of pumpkin fruit extract 2.5 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 0.625 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B4+A1 = 20 µL Concentration of pumpkin fruit extract 1.25 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 10 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B4+A2 = 20 µL Concentration of pumpkin fruit extract 1.25 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media

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B4+A3 = 20 µL Concentration of pumpkin fruit extract 1.25 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 2.5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B4+A4 = 20 µL Concentration of pumpkin fruit extract 1.25 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 1.25 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B4+A5 = 20 µL Concentration of pumpkin fruit extract 1.25 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 0.625 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B5+A1 = 20 µL Concentration of pumpkin fruit extract 0.625 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 10 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B5+A2 = 20 µL Concentration of pumpkin fruit extract 0.625 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B5+A3 = 20 µL Concentration of pumpkin fruit extract 0.625 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 2.5 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B5+A4 = 20 µL Concentration of pumpkin fruit extract 0.625 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 1.25 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
B5+A5 = 20 µL Concentration of pumpkin fruit extract 0.625 mg/mL + 20 µL Concentration of manila sapodilla fruit extract 0.625 mg/mL + 5 µL Salmonella typhi suspension + 155 µL NB media
C1 = 200 µL Medium NB
C2 = 180 µL Medium NB + 20 µL DMSO
C3 = 195 µL Medium NB + 5 µL Salmonella typhi
C4 = 175 µL Medium NB + 20 µL DMSO + 5µL Salmonella typhi

Determination of the MIC value can be observed visually by looking at the red color change in the wells that grow bacteria after adding the TTC reagent (2,3,5-Triphenyltetrazolium chloride). In wells that do not experience a change in color to red and at the lowest concentration it shows the MIC value. The red color in the solution in the well is the reduction of the TTC reagent as an electron acceptor by the enzyme dehydrogenase/Dehydrogenase Activity (DHA) produced by living bacteria. Reduction by the dehydrogenase enzyme will form a red compound, namely red triphenyl formazan (TPF) (Tanaka dkk., 2021)

Based on Figure 1, the results obtained show that the single MIC value of manila sapodilla fruit against Salmonella typhi is 50 mg/mL and the single MIC value of water gourd fruit against Salmonella typhi is 100 mg/mL. Then, in the results of the combination MIC test, the combined MIC value was obtained in wells A2+B1, where sapodilla fruit extract with a concentration of 50 mg/mL was combined with pumpkin fruit extract with a concentration of 100 mg/mL.

The Minimum Inhibitory Concentration (MIC) values for single and combined manila sapodilla fruit extract (*Achras zapota* L.) and water gourd fruit extract (*Lagenaria siceraria* (Molina) Standl.) (table 4).

Based on the research results in table 4, it shows that there is no change in the MIC value of manila sapodilla fruit extract after combining it with 100 mg/mL water pumpkin extract from the single MIC value of manila sapodilla fruit. Meanwhile, the MIC in a single pumpkin fruit extract was 100 mg/mL to 50 mg/mL after combining it with 50 mg/mL sapodilla fruit extract. The results of the combination of manila sapodilla fruit extract with water gourd extract obtained the FIC value (appendix 10) of manila sapodilla fruit extract is 1 and the results obtained from the combination of water gourd extract and manila sapodilla fruit extract obtained the FIC value (appendix 10) of water gourd fruit is 0.5. From the results of synergy testing using the Checkerboard microdilution test method, a FICI (Fraction Index Combination Inhibitor) of 1.5 was obtained, which means that each sample had a different effect in inhibiting the growth of *Salmonella typhi* bacteria.

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**Table 4**. Single and Combination Minimum Inhibitory Concentration (MIC) Values of Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Gourd Fruit Extract (*Lagenaria siceraria* (Molina) Standl.)

| Extract | MIC (mg/mL) |
|---|---|
| Manila Sapodilla Fruit Extract (*Achras zapota* L.) | 50 |
| Water Gourd Fruit Extract (*Lagenaria siceraria* (Molina) Standl.) | 100 |
| Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Gourd Fruit Extract (*Lagenaria siceraria* (Molina) Standl.) 100 mg/mL | 50 |
| Manila Sapodilla Fruit Extract (*Achras zapota* L.) and Water Gourd Fruit Extract (*Lagenaria siceraria* (Molina) Standl.) 50 mg/mL | 50 |

Based on research conducted by(Stefanović dkk., 2012), a different effect is a FICI value that is >1 but ≤4. Different effect is a situation where the effect produced by the combination of the two samples is no different from the single preparation or only one of the samples experiences increased effectiveness(Putranti dkk., 2021). In this case, the one that experienced increased effectiveness after being combined was pumpkin fruit extract. Ethanol extract of manila sapodilla fruit and pumpkin fruit extract have antibacterial effects against Salmonella typhi. Inhibition of bacterial growth is thought to come from secondary metabolite compounds contained in the extract, namely phenols, triterpenoids, flavonoids and saponins.

Phenol is a polar compound and acts as an antibacterial. The mechanism of action of phenolic compounds in killing bacteria is by denaturing bacterial cell proteins. As a result of denaturation of bacterial cell proteins, all bacterial cell metabolic activities stop because all bacterial cell metabolic activities are catalyzed by enzymes which are proteins (Tanaka dkk., 2021). In high concentrations, the phenol content penetrates and disrupts bacterial cell walls and precipitates proteins in bacterial cells. In lower concentrations, phenol inactivates important enzyme systems in bacterial cell(Kundu dkk., 2022) . The mechanism of action of triterpenoids is to react with porins (transmembrane proteins) on the outer membrane of bacterial cell walls, forming strong polymer bonds resulting in damage to the porins. Damage to porins can cause a lack of permeability of bacterial cell walls which will result in bacterial cells lacking nutrition, so that bacterial growth is hampered and they die (Maher & Hassan, 2023). The mechanism of flavonoid compounds against bacteria is carried out by damaging

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the cell walls of bacteria which consist of lipids and amino acids which react with the alcohol groups in flavonoid compounds(Yuan dkk., 2021) . According to the statement by (Luo dkk., 2025) that the mechanism of flavonoids as antibacterials is by inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism.

The next secondary metabolite compound is saponin, the mechanism of saponin as an antibacterial is by causing leakage of proteins and enzymes from inside the cell which results in cell death, in other words it is bactericidal (Dong dkk., 2020).

## CONCLUSION

Based on the results of the research carried out, it can be concluded that there is a different antibacterial effect of the combination of manila sapodilla fruit extract and pumpkin fruit extract against Salmonella typhi with a Fractional Inhibitory Concentration Index (FICI) of 1.5.

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