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ANTIBACTERIAL ACTIVITY TEST OF WATER APPLE LEAF EXTRACT Syzygium aqueum (Burm.f) Alston AGAINST Salmonella typhi BACTERIA USING ELISA READER

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ABSTRACT

The water apple plant Syzygium aqueum (Burm. f) Alston is one of the plants used as traditional medicine due to its secondary metabolites such as flavonoids, steroids, terpenoids, alkaloids, tannins, phenols, saponins which can be utilized as antibacterials, antioxidants and others. This research aims to determine the IC_{50} antibacterial activity of the water apple leaf extract against Salmonella typhi ATCC-14028 bacteria in inhibiting bacterial growth. Extraction was carried out using multi-step maceration using n-hexane, ethyl acetate, and methanol as solvents to obtain the concentrated extracts. Antibacterial activity test was conducted by using the microdilution method on Mueller Hinton Broth media by calculating optical density (OD) values at concentrations of 500 ppm, 250 ppm, 125 ppm, 62.50 ppm, 31.25 ppm, 15.63 ppm, 7.831 ppm, and 3.906 ppm. The optical density value was measured at a wavelength of 595 nm by Elisa microplate reader. Phytochemical contents were investigated and the presence of flavonoid, phenol, steroid, and terpenoid compounds in the extracts was indicated. Water apple leaf extracts have antibacterial activity in inhibiting the growth of Salmonella typhi ATCC-14028 bacteria showing an IC_{50} value of n-hexane, ethyl acetate, and methanol extracts of 155,607 ± 30,592 µg/mL, 56.492 ± 50.124 µg/mL, 107.96 ± 50.124 µg/mL *which is categorized as weak, strong, and moderate respectively.*

ABSTRAK

Tanaman jambu air *Syzygium aqueum* (Burm. f) Alston merupakan salah satu tanaman yang digunakan sebagai obat tradisional karena kandungan metabolit sekundernya seperti flavonoid, steroid, terpenoid, alkaloid, tanin, fenol serta saponin yang dapat dimanfaatkan sebagai antibakteri, antioksidan dan lain-lain. Tujuan penelitian ini adalah untuk mengetahui IC_{50} aktivitas antibakteri ekstrak daun jambu air terhadap bakteri *Salmonella typhi* ATCC-14028 dalam menghambat pertumbuhan bakteri. Ekstraksi dilakukan secara maserasi bertingkat dengan menggunakan pelarut n-heksana, etil asetat dan metanol sehingga diperoleh ekstrak pekat. Uji aktivitas antibakteri dilakukan dengan menggunakan metode mikrodilusi pada media Mueller Hinton Broth dengan menghitung nilai *optical density* (OD) pada konsentrasi 500 ppm, 250 ppm, 125 ppm, 62,50 ppm, 31,25 ppm, 15,63 ppm, 7,831 ppm, dan 3,906 ppm . Nilai *optical density* diukur pada panjang gelombang 595 nm dengan Elisa microplate Reader. Kandungan fitokimia dianalisis dan menunjukkan adanya senyawa flavonoid, fenol, steroid dan terpenoid dalam ekstrak tersebut. Ekstrak daun jambu air mempunyai aktivitas antibakteri dalam menghambat pertumbuhan bakteri *Salmonella typhi* ATCC-14028 menunjukkan nilai IC_{50} ekstrak n-heksana, etil asetat, dan metanol masing-masing sebesar 155,607 ± 30,592 µg/mL , 56.492 ± 50.124 µg/mL, 107.96 ± 50.124 µg/mL dengan kategori lemah, kuat, dan sedang.

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INTRODUCTION

Infectious diseases such as typhoid fever are caused by Enterobacteria bacteria such as *Salmonella typhi*. Conventional treatment is carried out with synthetic antibiotics such as chloramphenicol, ampicillin, and cotrimoxazole. The presence of side effects at a certain time provides new insights for the development of phytopharmaceutical products from natural sources in overcoming various forms of diseases and infections by microorganisms. The World Health Organization (WHO) has noted based on a report of 2018, each year acute infection due to *Salmonella enterica serotype typhi* can cause up to 21 million cases with a death rate ranging from 128,000 to 161,000 people (WHO, 2018). Typhoid fever, which is common in Southeast and South Asia, can result in a death rate of 10–30% if left untreated. However, with rapid and effective treatment, this figure can be reduced to 1-4% (Alba et al., 2016). Generally, treatment is carried out by involving antibiotics to overcome *Salmonella typhi* bacteria and prevent more serious complications in the therapy process. Until now, the trend of using antibiotics as a therapy for infectious diseases has been widely reported to cause quite serious antibiotic resistance. This is due to the relatively uncontrolled use of antibiotics systematically. One of the causes is the circulation of antibiotics with weak supervision by the responsible government agencies. Therefore, in encouraging strategic targets and plans to encourage a strong national health system, through increasing raw materials for medicines by utilizing plants that have the potential to be antibacterial (Minister of Health of the Republic of Indonesia, 2022).

One of the plants that can be utilized is the water apple plant (*Syzygium aqueum*). Using natural ingredients as raw materials for traditional medicine gives the advantage of minimizing the side effects it causes. Therefore, one of the plants that can be utilized is the water apple plant (*Syzygium aqueum* (Burm. f) *Alston*). The part of the plant used is the water apple leaves because it contains the chemical compounds flavonoids, polyphenols, alkaloids, tannins, and saponins (Tandi, 2017). These secondary metabolite compounds can have antibacterial activity to inhibit the growth of *Streptococcus mutans* bacteria (Annisaul et al., 2023). Research reported by (Hariyati et al., 2015; Mulqie et al., 2022) on water apple leaves with the same species has great potential as a source of antibacterial because the ethanol extract can inhibit the development of a number of clinical isolates of bacteria. Meanwhile, the ethyl acetate fraction of water apple leaves showed a bactericidal effect against *Escherichia coli* and *Staphylococcus aureus* (Choesrina et al., 2019).

The antibacterial potential of water apple leaves can also be applied in preliminary research on *Salmonella typhi* bacteria. So the selection of water apple leaves as a natural ingredient in traditional medicine needs to be initiated because its utilization is still lacking in the community. Most people think that only the fruit can be consumed. Based on the description above, this study was conducted to reduce the adverse effects associated with the use of antibiotics to treat bacterial diseases because nature has actually prepared everything the body needs to be converted naturally by the body into medicine. The information on the potential of water apple

leaves as a natural antibacterial against *Salmonella typhi* bacteria is expected to be obtained through this study. Utilization of traditional medicine has the advantage of minimizing the side effects it causes.

METHOD

Tools

The tools and equipment used include glassware (pyrex/iwaki), UV 254 nm and UV 366 nm lamps, autoclave (Hirayama HVE 50), oven (Memmert A-6), analytical balance (Ohaus), filter paper, chamber, 40 mesh sieve, incubator (Memmert IN30), laminar airflow (Otto/5A-96), hot plate (H550 Pro Dlab), rotary evaporator (Biobase RE-301), Elisa microplate reader (BK mini 200), and water bath (Memmert WNB14).

Materials

The materials in this study were water apple leaves (*Syzygium aqueum* (Burm. f) *Alston*), distilled water, n-hexane (Merck), methanol (Merck), ethyl acetate (Merck), dimethyl sulfoxide (DMSO (Merck), *Salmonella typhi* ATCC-14028 bacteria, Mueller-Hinton Agar (MHA) media, Mueller-Hinton Broth (MHB) media, Dragendorff reagent, 5% $FeCl_3$, 10% $FeCl_3$. $AlCl_3$ 1%, Lieberman-Burchard reagent and silica gel GF254 TLC plates (Merck)

Research Procedures

Sample Preparation

Water apple leaf samples were collected in Lepak Village, East Lombok Regency. The samples were cleaned with clean running water as part of the sampling procedure. The samples were cleaned, drained, and dried for approximately three days under the sunlight. Sorting was carried out to separate damaged or dirty parts of the sample when dried. The water apple leaf samples were ground into powder and sieved through a 40-mesh sieve. the final product is water apple leaf powder with a similar fineness level. The simplicia is stored in a dry state at a temperature of between 27 and 30°C wrapped in plastic (Istiqomah et al., 2021).

Extract Production

The sample process of simplicia was carried out by extraction using a multilevel maceration method. The solvents used in this process are n-hexane, ethyl acetate, and methanol due to the differences in their polarity levels. Extraction started from solvents with low to high polarity levels. 300 grams of simplicia powder of water apple leaves were put into a vessel, then the first maceration was carried out with n-hexane for 3x24 hours. The n-hexate residue was macerated with ethyl acetate and the ethyl acetate residue was macerated again with methanol. A water bath was used to evaporate the n-hexane filtrate after being concentrated using a rotary evaporator set at a temperature of 50°C. Each process will produce thick extracts of hexane, ethyl acetate, and methanol.

Phytochemical Screening

Flavonoid Test

Water apple leaf extract was spotted on a TLC plate and eluted in n-hexane: ethyl acetate eluent at a ratio of (7:3). The elution results were identified by spraying 1% $AlCl_3$ reagent. The spots formed were observed at UV 254 and UV 366 nm. A positive reaction was indicated by the formation of a brownish-yellow spot (Kinam et al., 2021).

Phenol Test

Water apple leaf extract was spotted on a TLC plate and eluted in n-hexane: ethyl acetate eluent at a ratio of (7:3). The elution results were identified by spraying

10% $FeCl_3$ reagent, if a blue-black stain was formed, it is positive for containing phenol compounds (Kinam et al., 2021).

Steroid/Terpenoid Test

Water apple leaf extract was spotted on a TLC plate and eluted in n-hexane: ethyl acetate eluent in a ratio of (7:3). The elution results were identified by spraying Lieberman-Burchard reagent, if a green-blue stain was formed, the steroid and terpenoid compounds showed a red or purple color (Aritonang, 2022).

Alkaloid Test

Water apple leaf extract was spotted on a TLC plate and eluted in n-hexane: ethyl acetate eluent at a ratio of (7:3). The elution results were identified by spraying Dragendroff, if an orange or red spot is formed in visible light, it is positive for the presence of alkaloid compounds (Kinam et al., 2021).

Antibacterial Activity Test

Tool Sterilization

The process begins with cleaning the tools and materials to be used by washing and drying. After completely drying, the tools and materials are wrapped in paper to undergo a sterilization process at a temperature of 121°C for ±15 minutes to create sterile conditions before being used in the study.

Media Preparation

Preparing MHA and MHB media by dissolving 0.3 grams of MHA in 5 milliliters of sterile distilled water and MHB in 0.1 grams in 5 milliliters of distilled water. Both solutions are heated in an Erlenmeyer flask until dissolved, then cotton is inserted and covered with aluminum foil. Before being used in the study, both were sterilized for 15 minutes at a temperature of 121°C in an autoclave (Mulqie et al., 2022).

Bacterial Rejuvenation

Salmonella typhi bacteria were rejuvenated in MHA media using an ose needle. Pure culture of *Salmonella typhi* bacteria was inoculated by taking one loop and then scratching it in a zigzag manner on the slanted media in a test tube. After scratching the medium, the bacteria were cultured for 24 hours at 37°C (Lestari et al., 2020).

Preparation of McFarland solution turbidity standard

The 0.5 McFarland concentration standard was made by preparing a solution of 9.95 mL of 1% H_2SO_4 and 0.05 mL of 1% $BaCl_2$ to be homogenized. The mixture was shaken until a cloudy solution appeared, in the microbiological testing procedure allowing for consistent adjustment of bacterial density for comparison purposes (Yusrina et al., 2022).

Preparation of Bacterial Suspension

The suspension solution was made by taking 1 loop of bacteria, then homogenized in 0.9% physiological NaCl as much as 5 mL (Zamilah et al., 2020).

Antibacterial Activity Testing

The antibacterial potential of water apple extract was carried out using an Elisa reader through the microdilution method. The stock solution of each extract was made at a concentration of 2000 ppm with 10% DMSO solvent. Multilevel dilutions were carried out to obtain final concentrations of 500; 250; 125; 62.5; 31.25, 15.63; 7.81; and 3.90 ppm. Several treatment groups were made to obtain a calculation model for the percentage of inhibition and IC_{50} values. The first treatment was to insert 100µl of

MHB media into all wells up to column 8. Then, a sample of 2000 ppm water apple leaf extract was taken as much as 100µl and inserted into the first column of rows A-D and 100µl from the first column of row A, transfer to column 2A to column 8, then take 100µl of bacterial suspension and insert it into columns 1-8 so that columns 1-8 rows A-D (100µl media + 100µl extract + 100µl suspension) as a test sample. In columns 10-11 as an extract control (100µl media + 100µl extract). Next, in column 12 rows A-D as bacterial control (100µl media + 100µl suspension + 100µl saline solution) and rows E-H contain streptomycin (100µl media + 100µl streptomycin discarded 100µl + 100µl bacterial suspension + 100µl saline solution) as antibiotic control. The microplate was incubated overnight or 24 hours at 37°C in an incubator before reading the optical density (OD) value using an elisa microplate reader at a wavelength of 595 nm. The work was carried out aseptically in Laminar Airflow (LAF).

Data Analysis

The analysis of extract yield data was carried out using the formula (1) as follows:

$$\% \text{ Yield} = \frac{\text{weight of thick extract}}{\text{weight of simple substances}} \text{ x } 100\% \quad \ldots(1)$$

Data analysis was carried out with the results of absorbance data to calculate the percentage of bacterial inhibition based on the formula (2) (Rollando, 2019) with modifications as follows:

$$\% \text{ Inhibition} = \frac{\text{"Bacterial control OD-" ("test sample OD")"-(Extract control OD)"}}{\text{Bacterial OD}} \text{ x } 100\% \quad \ldots(2)$$

Description:

Bacterial control contains media and bacterial suspension; Test sample contains media, test extract, and bacterial suspension; Extract control contains media and test extract

The calculation of the IC_{50} value is calculated using the results of % inhibition and concentration entered into the Microsoft Excel application and the results of the linear regression equation are obtained. After the study is completed and all data obtained, it is then processed to get conclusions. Data are then analyzed using SPSS (Statistical Program For Social Science) 24 to determine the normality of data.

RESULT AND DISCUSSION

Phytochemical Screening

An important step in analyzing the secondary metabolite content in water apple leaf extract is phytochemical screening using the TLC method. The method used is the spray method using a silica gel GF254 TLC plate. The identification results are shown in Table 1, which shows that the three extracts through the extraction process with multilevel maceration indicate the presence of compounds such as flavonoids, phenolics, steroids, or terpenoids.

Table 1. Results of phytochemical screening of water apple leaf extract

Compound Groups	Reagent	Methanol	Ethyl Acetate	N-Hexane
Flavonoid	$AlCl_3$	+	+	+
Steroids/Terpenoids	Lieberman-Burchard	+	+	+
Alkaloid	Dragendroff	-	-	-
Phenol	$FeCl_3$	+	+	+

Description: (+) = presence of secondary metabolite compound group
(-) = absence of secondary metabolite compound group

The appearance of yellow spots on a flavonoid phytochemical test indicated that the result is positive. The appearance of yellow color is due to the interaction between AlCl3 and the flavonoid group, resulting in a complex between the hydroxyl group adjacent to the ketone or with the hydroxyl group so that the neighboring group is what causes the yellow color to the sample that is positive for containing flavonoids. In accordance with Figure 1.

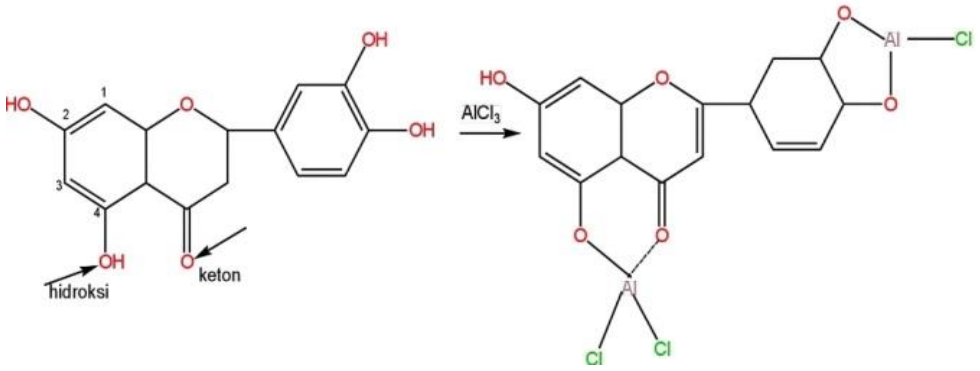


Figure 1. Flavonoid test reaction mechanism (Guntarti et al., 2017).

In the test of steroid/terpenoid compounds, the reaction mechanism in Figure 2 is as follows. This is caused by a shift in the wavelength of visible light due to the oxidation of the hydroxyl group at C4 by H_2SO_4 followed by continued oxidation of the ABCD ring in the steroid structure that forms a conjugated diene, causing a shift in the wavelength to a larger direction that can absorb visible light re-emitted by the compound that has been formed, so that a greenish or bluish spot color will appear on the TLC plate. The color gradation formed also depends on the complexity of the chemical structure of the compound analyzed with the reagent.

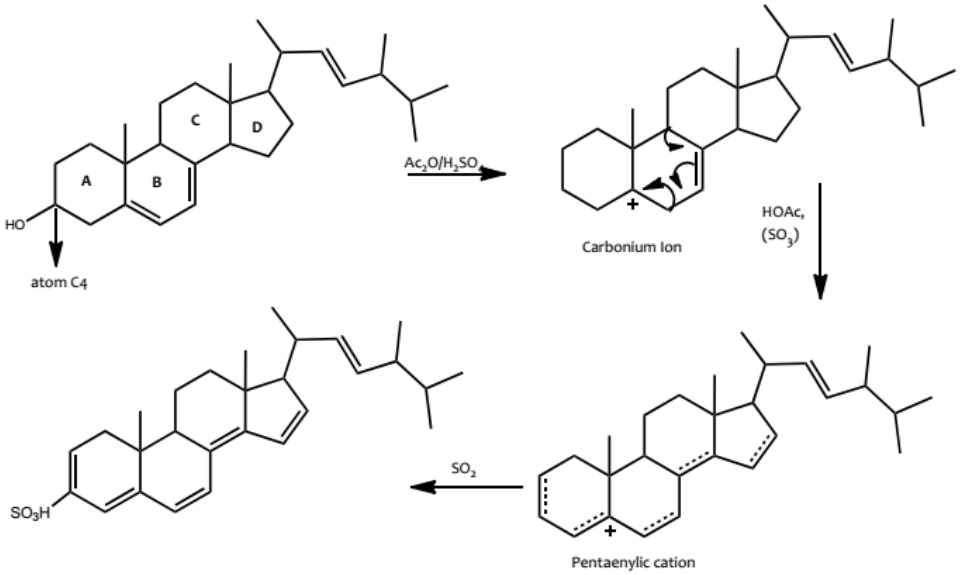


Figure 2. Reaction mechanism of steroid and terpenoid tests (Habibi et al., 2018).

Furthermore, the positive phenolic test containing phenolic compound groups, it is indicated by the formation of bluish-black stains. Phenolic compounds give positive results because $FeCl_3$, namely the Fe group, reacts with the hydroxyl group found in phenol compounds. The reaction can be seen in Figure 3.

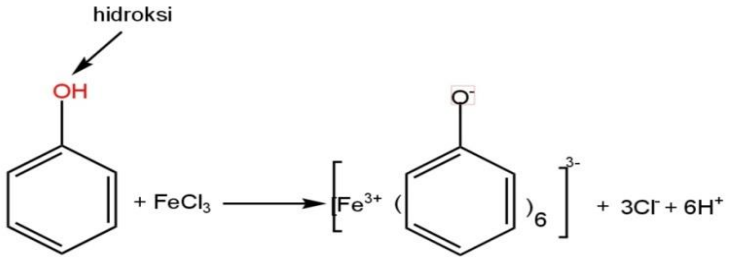


Figure 3. The chemical reaction of phenol compounds with $FeCl_3$ (Nofita, 2020)

Antibacterial Activity Test

Table 2. Results of % Inhibition of Streptomycin Extract and Control

Sample	Concentration (ppm)	% Inhibition		
		Rep I	Rep II	Rep III
N-hexane extract	500	93.638	114.314	87.276
	250	65.009	69.184	65.606
	125	55.268	52.485	49.304
	62.50	49.304	46.719	36.580
	31.25	36.381	35.984	27.634
	15.63	33.001	33.598	23.658
	7.81	26.441	19.681	18.290
	3.90	22.067	14.711	17.296
Ethyl Acetate Extract	500	107.661	117.741	106.182
	250	85.349	95.698	87.768
	125	64.247	68.548	66.532
	62.50	60.483	56.989	54.973
	31.25	57.258	45.161	46.102
	15.63	47.017	36.962	44.086
	7.81	37.500	34.139	36.155
	3.90	34.543	27.284	28.629
Methanol Extract	500	126.838	128.429	110.934
	250	85.089	79.721	66.003
	125	70.576	69.980	50.695
	62.50	66.401	52.882	42.544
	31.25	44.532	49.105	33.598
	15.63	36.580	34.393	16.501
	7.81	27.236	29.025	11.928
	3.90	20.278	18.886	4.373
Streptomycin Control	500 ppm		147.316%	

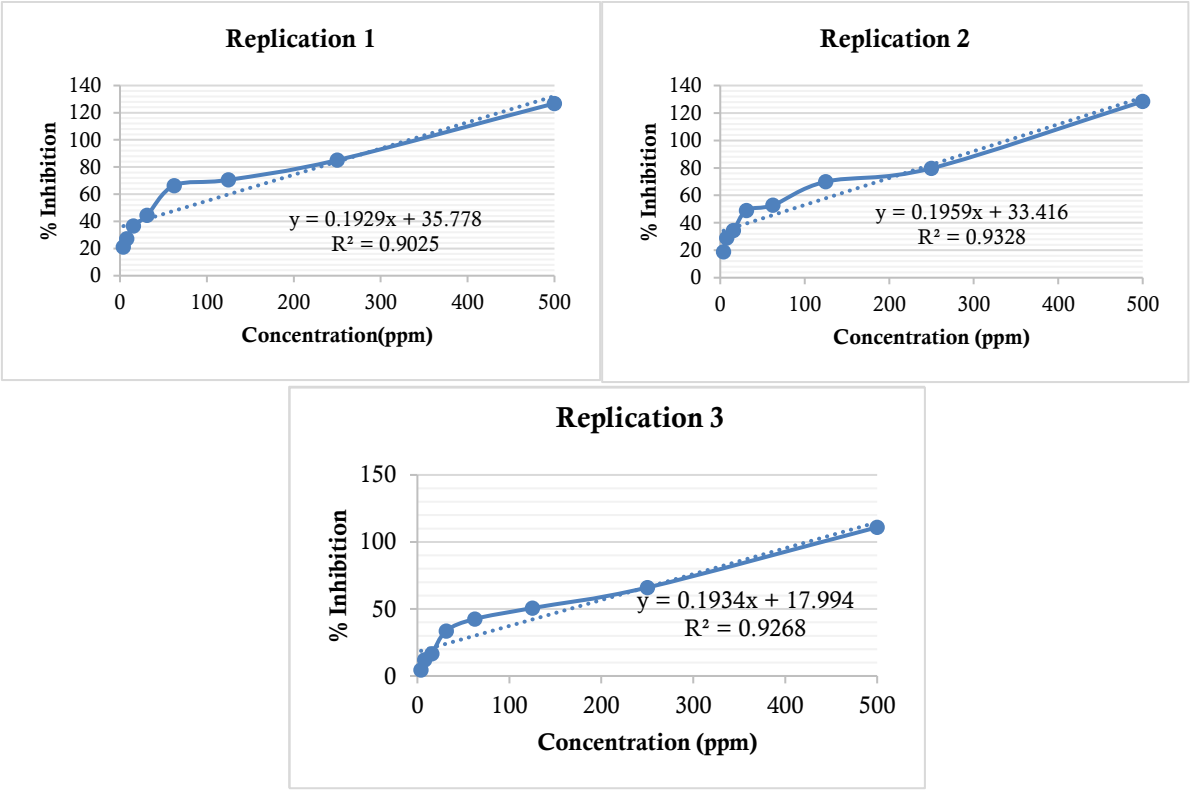
Table 2, shows that the three extracts of water apple leaves have a percentage of inhibition with the highest concentration having greater inhibition, while the smaller the concentration used, the smaller the percentage of inhibition. So the smaller the concentration, the smaller the ability as an antibacterial activity.

Table 3. IC_{50} value of water apple leaf extract

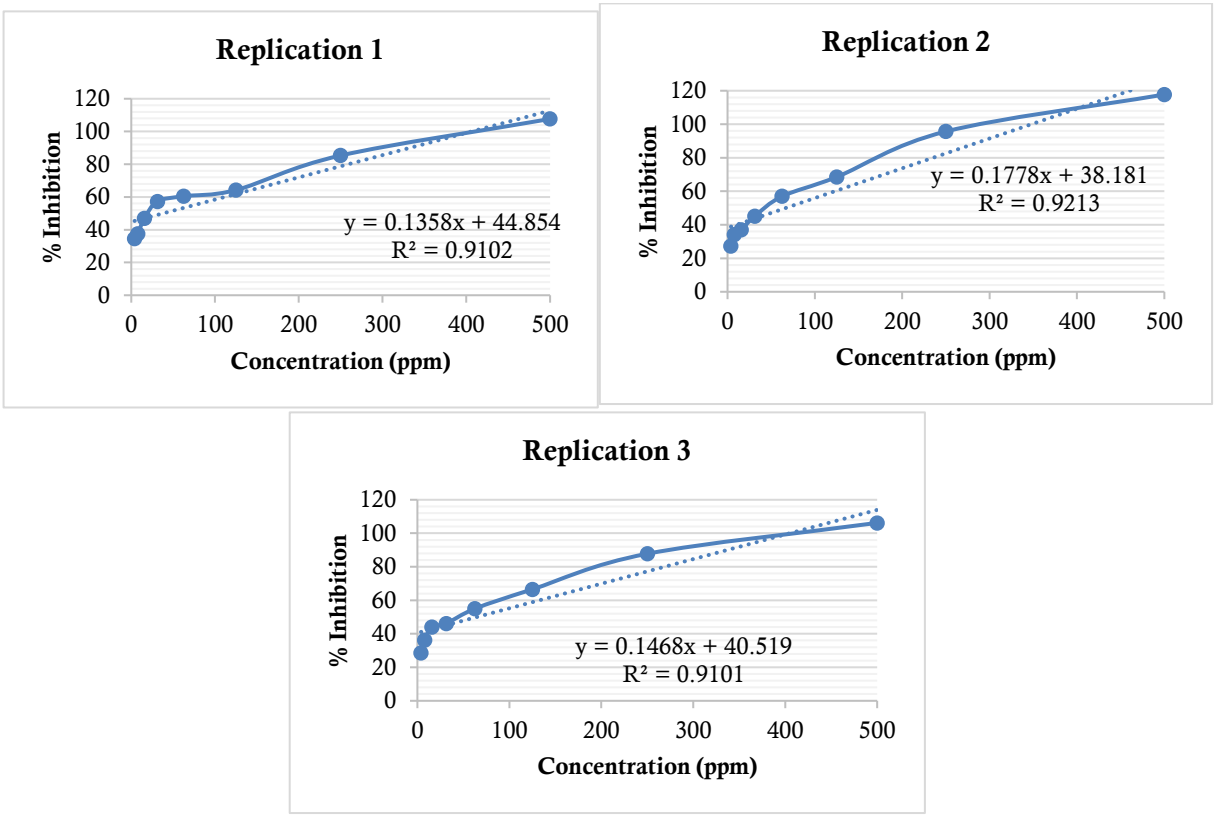
Extract Sample	IC_{50} Value µg/mL (Replicate)			Average IC_{50} µg/mL	SD	Category
	Rep 1	Rep 2	Rep 3			
N-hexane	142.4	133.752	190.569	155.607	30.592	Weak
Ethyl acetate	38.118	66.774	64.584	56.492	15.950	Strong
Methanol	73.727	84.655	165.491	107.96	50.124	Moderate

Table 3 shows the antibacterial potential of the three water apple leaf extracts against *Salmonella typhi* ATCC-14028 bacteria. Antibacterial activity is determined from the IC_{50} value. The microdilution results showed that of the three extracts in this study, the n-hexane extract had an IC_{50} value of 155,607 ± 30,592 µg / mL, the ethyl acetate extract had an IC_{50} value of 56,492 ± 15,950 µg /

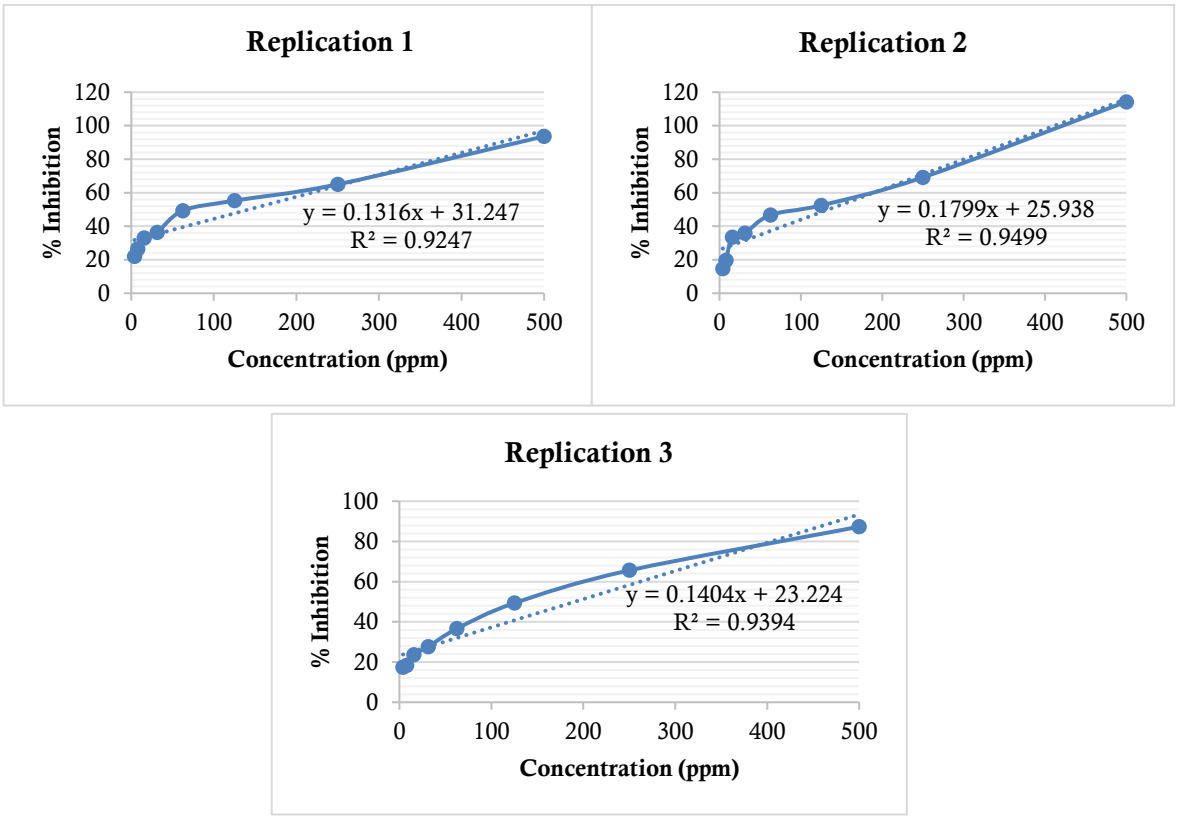
mL and methanol 107.96 ± 50,124 µg / mL, the three extracts were categorized as weak, strong and moderate according to the predetermined IC_{50} value standard. The very strong category has a value of less than 50 µg/mL, the strong category with a value of 50-100 µg/mL, then the moderate category it has a value of 100-150 µg/mL, the weak category 150-200 µg/mL and a very weak IC_{50} value ≥ 200µg/mL. There is an antibacterial activity of water apple leaf extract against *Salmonella typhi* ATCC-14028 bacteria, but the three samples have different potentials, namely in the n-hexane extract 155,607±30,592µg/mL, the ethyl acetate extract has an IC_{50} value of 56,492±15,950 µg/mL and methanol 107.96±50.124 µg/mL.



Graph 1. Linear regression of methanol fraction



Graph 2. Linear regression of ethyl acetate fraction



Graph 3. Linear regression of hexane fraction

In the linearity graph above, the average R^2 value for each fraction of methanol, ethyl acetate, and hexane is 0.92, 0.91, and 0.938 respectively which are then converted into r values with an average of 0.95, 0.95, 0.96. The r value replication 1

indicates a very good correlation between the two variables and the data can be considered, in this case, the range of 0.95 - 1.00 indicates that the level of relationship between the two variables, namely concentration and % inhibition, is a linear relationship. Based on the table and linearity graph, it can be concluded that the best activity of each fraction is the ethyl acetate fraction with an IC_{50} value of 54,692.

The results of phytochemical screening provide a picture of similar secondary metabolite content, namely flavonoids, phenols and terpenoids/steroids. Terpenoid and steroid compounds will be more fractionated in n-hexane solvents, while flavonoid and phenol compounds will be fractionated in ethyl acetate and methanol solvents. However, in this case, the semi-polar solvent (ethyl acetate) gave the best IC_{50} results because compounds containing hydroxyl groups (flavonoids and phenolics) and hydrophobic groups (alkanes, alkenes, methoxy, and benzene) were fractionated more easily with ethyl acetate so that the composition of active compounds against *Salmonella typhi* ATCC-14028 was extracted more with ethyl acetate solvent. The high antibacterial activity against these bacteria is closely related to the fractionation of phenolic compounds in the form of less polar and highly methoxy aglycones (O-CH_3) which will be fractionated into less polar solvents such as ethyl acetate (Kaczorová et al., 2021).

In addition, the cause of the difference in potential in the test samples is influenced by the polarity of the solvent, namely non-polar from n-hexane, semi-polar from ethyl acetate, and polar from methanol. Non-polar solvents such as hexane only extract a small portion of the compound components contained in water apple leaves so the potential is categorized as weak. Ethyl acetate solvent as a semi-polar solvent that can attract more compound components when compared to hexane and methanol such as flavonoids, steroids/terpenoids, and phenolics. This condition is due to the presence of non-polar groups (-CH_3) and (-CH_2-CH_3) and the ability of ethyl acetate to interact hydrogen with other compounds with the presence of its carbonyl group (C=O) so that the extraction gradient obtained is a greater composition of the extracted metabolite compounds, both polar and non-polar compounds.

Methanol solvent is a polar solvent so it can still attract the compounds contained well, but the components of the extracted compounds are generally highly polar compounds such as glycosides and tannins which generally have relatively weak antibacterial activity. In this study, ethyl acetate is the best at attracting compounds that can have antibacterial activity against salmonella typhi. In addition, the cause of the three extracts having different IC_{50} values of more than 50 µg/mL is that the adaptive nature of the natural compound in the extract is very possible for compound decomposition during the heating process (evaporation) in the water bath so that the compounds contained in the extract become damaged or change their structure and their antibacterial activity provides a significant difference. In addition, it is suspected that there is an influence on the extraction process such as the duration of extraction. The longer the extraction duration, the longer the contact between the solvent and the simple material and the more extracted compound components. The three guava leaf extracts contain chemical compounds that act as antibacterials, namely the flavonoid, phenol, and seroid/terpenoid compounds.

According to (Veronica et al., 2020), flavonoid compounds act as antibacterials through a working mechanism by inhibiting bacterial replication because they cause plasma leakage, inhibit bacterial energy metabolism, and cause bacterial cell lysis, in addition to being able to denature amino acids and enzymes in bacteria, thereby damaging the cell membrane. While the steroid and terpenoid groups inhibit the process of cell membrane formation by disrupting the synthesis and replication of membrane proteins which cause imperfect shapes in the bacterial cell membrane (Riga & Hakim, 2021). The mechanism of action of phenol as an antibacterial is by damaging the enzyme components in bacteria which have an impact on the activity of the lysozyme enzyme which results in a decrease in cell surface tension so that bacterial cell death occurs (Purwaningsih & Wulandari, 2020).

The test bacteria, *Salmonella typhi* ATCC-14028 is a gram-negative bacteria. This bacteria is a pathogen in the human body as a cause of acute typhoid fever. This disease is transmitted through contamination of food and drinks consumed by humans because the nutrient entry route is through fecal-oral (Agustina et al., 2019). The layered cell wall structure is owned by gram-negative bacteria, this structure is complex so that this type of bacteria is relatively strong against antibacterials. This complex cell wall has a lipopolysaccharide compound component that can prevent the entry of foreign substances into bacterial cells including antibiotics and other compounds. This is what causes typhoid fever to provide a fairly extra therapy process, this is also the reason why the inhibitory ability of the extract provides less potential. In this study, the three extracts had small antibacterial activity values in inhibiting *Salmonella typhi* ATCC-14028 bacteria, which was seen from the IC_{50} results, the three extracts had values of more than 50 µg / mL. The selection of streptomycin as an antibiotic control because streptomycin is one of the antibiotics that can inhibit gram-negative bacteria, gram-negative bacteria have little peptidoglycan so they require another pathway in the inhibition process, so the antibiotic used is streptomycin.

CONCLUSION

The results of phytochemical screening using the spray method on the hexane, ethyl acetate, methanol extract of *Syzygium aqueum* (Burm. f) *Alston* water apple leaves positively contained flavonoid, phenolic, steroid/terpenoid compounds. In the third sample, the *Syzygium aqueum* (Burm. f) *Alston* water apple leaf extract has antibacterial potential to be developed in inhibiting *salmonella typhi* bacteria which is indicated by the IC_{50} value in each sample, namely, n-hexane extract 155,607 ± 30,592 µg / mL, ethyl acetate extract 56,492 ± 15,950 µg / mL, and methanol extract 107.96 ± 50,124 µg / mL.

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