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Research Article

Phytochemical Screening and Antibacterial Activity Test of Ethanol Extract of Durian (*Durio Zibethinus* murr.) Soya Varieties Against Pathogen Bacteria *Escherichia Coli* in Raw Drinking Water

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ABSTRACT

Introduction: Durian (Durio zibethinus) fruit skin contains antibacterial compounds. The antibacterial content in durian skin (Durio zibethinus) such as alkaloids, flavonoids, saponins, phenols and tannins can inhibit the growth of pathogenic bacteria such as Escherichia coli, Salmonella typhosa and Staphylococcus aureus and act as a disinfection agent. This research aims to determine the secondary metabolite compounds and antibacterial activity of the ethanol extract of Soya durian peel against the pathogenic bacteria Escherichia coli ATCC 25922. **Methods:** This type of research is a laboratory experiment, including making Soya durian peel extract using the maceration method using 96% ethanol solvent. The antibacterial activity test was carried out using the liquid dilution method to determine the Minimum Inhibitory Concentrations (MIC) value and the solid dilution method to determine the Minimum Bactericidal Concentration (MBC) value. **Results:** Based on qualitative phytochemical screening, Soya durian peel ethanol extract contains secondary metabolite compounds with an average content of 4.24% alkaloids, 22.95% flavonoids, 1.74% saponins, 57.41% phenols and 2.27% tannins. Soya durian peel extract has an MIC against E. coli ATCC 25922 bacteria of 3.12%, while the MBC value of Soya durian peel extract against E. coli ATCC 25922 bacteria is 6.25%. The results of the One Way ANOVA analysis of the Minimum Bactericidal Concentration (MBC) data have a significant value of 0.00 < 0.05. The results of the Pearson correlation test (r) showed a significant number of 0.000 ($p < 0.05$), the Pearson correlation coefficient between concentration and number of bacterial colonies was (r) = 0.812. This means that the higher the concentration of Soya durian peel ethanol extract given, the less the number of *E.coli* ATCC 2592 bacterial colonies will be reduced. The results of a simple linear regression test showed that the value of Y = 245.618 – 29.016 245,618 colonies and each increase in the concentration of Soya durian peel ethanol extract by 1% will cause a decrease in the number of bacterial colonies to 29,016 colonies. **Conclusion:** Soya durian skin extract has antibacterial compounds that can kill pathogenic Escherichia coli bacteria in raw drinking water.

Keywords: Durian (Durio zibethinus) Soya variety, phytochemicals, antibacterial, Escherichia coli, raw drinking water.

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INTRODUCTION

More than 50% of drinking water treatment systems use chlorine to disinfect water, but currently chlorine is known to produce 600 by-products from disinfection which can cause various health problems.[1] So it is an urgent need to look for new, easy water disinfection materials and methods. When applied, it is cheap and can produce drinking water that is safe for consumption. The use of plants as disinfection agents in water treatment is very promising. This is because plants, apart from being used as food, are also used in the health sector, because both leaves, fruit, fruit skin, stem bark and roots contain antibacterial ingredients such as phenols, quinones, flavonols, tannins, coumarins and alkaloids.[2] Plant secondary metabolite compounds have some biological effects, such as anti-microbial and anti-oxidant properties.[3]

The use of local plants around us as disinfection materials is highly desirable. Local plants that contain antibacterial properties such as the durian plant (Durio Zibethinus Murr.), the skin and seeds of which contain antibacterial properties,[4–6].

Durian is a tropical plant native to southeast Asia, and is a fruit native to Indonesia. This plant occupies the fourth position in national fruit production with fruit production of more than seven hundred thousand tons per year, with harvest seasons that do not coincide and occur around September to February.[7] In Indonesia there are dozens of types of durian varieties spread throughout almost all regions. Indonesia is one of the countries that has the largest variety of durian varieties in the world. The center of durian diversity is Kalimantan Island, where the most types of durian varieties are found. There are around 20 species of Durio members, nine of which are edible. Research institutions have released various superior durian cultivars.[8] One type of superior durian is the Soya variety which originates from Maluku Province and is located in Eastern Indonesia.[9]

Durian is a plant that has very abundant by-products or waste. In general, what durian lovers consume is the flesh or fruit coating, which is around 20 – 35% of the whole durian fruit. Meanwhile, the remaining 60 – 75% of the fruit skin and 5 – 15% of the seeds are not utilized.[10] Several studies have revealed that

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durian skin waste contains secondary metabolite compounds such as flavonoids, alkaloids, saponins, tannins and triterpenoids which are active and able to inhibit bacterial growth.[4–6] So its use as an antibacterial agent in the water disinfection process is worthy of being tested as an alternative material to replace chlorine or chlorine.

Fruit peels usually become rubbish and are thrown away, but parts of some plants contain many antibacterial compounds that can be used. Like Durian (Durio zibethinus), the skin of the fruit contains antibacterial compounds. The results of research conducted by Arlofa,[11] found antibacterial content in durian skin such as alkaloids, saponins and triterpenoids which can inhibit the growth of pathogenic bacteria such as Escherichia coli, Salmonella typhosa and Staphylococcus aureus and as a disinfection agent. Durian skin antibacterial tests conducted by Anggraeni and Anam,[12] and Ravichandran[13] found that durian skin extract can be used as an antibacterial for human pathogens such as Escherichia coli and staphylococcus aureus. There is inhibition of the growth of Eschercia Coli and Stapilococus Aureus bacteria on durian skin according to the results of research conducted by Muawanah.[4]

These various studies provide indications that durian skin generally has antibacterial properties.[14] However, for the Soya durian, a durian native to Maluku, until now it is not known what chemical content in the skin of the fruit can inhibit the activity and even kill pathogenic bacteria. Therefore, this study aims to determine the secondary metabolite compounds and antibacterial activity of the ethanol extract of Soya variety durian peel against the pathogenic bacteria Escherichia coli in raw drinking water.

MATERIALS AND METHODS

Collection and processing of Soya durian skin

The Soya variety durian fruit used as research material was taken directly from Negeri Soya, Sirimau District, Ambon City, Maluku. The Soya durian skin used is ripe durian skin and the skin of the fruit is taken. The skin of the Soya durian fruit taken is skin that is still fresh and not moldy. Then sorted wet, namely by washing using clean running water. After that, the sample was aired indoors. Next, the sample was chopped into small pieces and then dried using an oven at 450C for approximately 48 hours. After the sample is dry, it is powdered using a blender and the sample is ready to be extracted.

Soya durian skin extraction

Samples were extracted using the maceration method. The durian rind simplicia was put into 4 maceration vessels and then the extraction process was carried out using 96% ethanol solvent. Add approximately 3000 ml of 96% ethanol to each vessel until all the simplicia powder is submerged and the solvent is 1 cm above the surface of the powder. Extraction was carried out for 3 x 24 hours. The maceration results are then filtered with filter paper. The filtrate and dregs are separated in different containers, the dregs obtained are macerated again using the same solvent 3 times, this process is carried out until the filter liquid can no longer attract the compounds contained in the sample or is saturated.[15] All the filtrates that have been obtained are collected and concentrated using a 50C rotary evaporator. The sample extract is then ethanolized by airing the extract until it becomes dry in a desiccator.[16]

Qualitative Phytochemical Screening

In the alkaloid testing stage,[17] weighed 4g of the extract, added sufficient chloroform (±1 ml), then added 10 ml of ammonia and 10 ml of chloroform. Next, filter it into a test tube and add 10 drops of 2N H2SO4 then shake for a few minutes until 2 (two) layers are formed. Transfer the top layer of 1 ml each to 3 (three) other test tubes and add Meyer (tube 1), Wanger (tube 2) and Dragendorf (tube 3) reagents. Next, look at the color change that occurs, if positive there will be a red-orange precipitate with Dragendorf, yellowish white with Meyer and brown with Wanger. In the flavonoid testing stage, 200 mg of extract was added to 5 ml of ethanol and heated for 5 minutes. Next add a few drops of concentrated HCl and 0.2 g of Mg powder. Positive result, a dark red color will occur for 3 minutes.[18]

The saponin testing stage, weigh 2 g of extract in a test tube, add distilled water until submerged and boil for 2-3 minutes. Let it cool then shake as hard as you can. Stable foam indicates positive.[19] In the phenol testing stage, 2 g of extract was extracted with 7 ml of 70% ethanol, then 1 ml was taken into a test tube and 2 drops of 5% FeCl3 solution were added. Look at the color changes that occur. If positive, a green or blue color will appear.[20] The tannin testing stage, weigh 20 mg of extract, add ethanol until submerged, then add 2-3 drops of 1% FeCl3 solution. A positive result will produce a bluish black or green color.[21] In the quinone testing stage, 1 g of extract was added with several drops of 1N NaOH. Look at the color changes that occur. If positive, the color will change to yellow.[22]

Quantitative Phytochemical Screening

Alkaloid content analysis test, weigh ± 100 mg of durian peel ethanol extract, then add 5 ml of 2N HCL, then shake, then wash the solution with 10 ml of chloroform 3 times in a separating funnel, discard the coloroform phase, neutralize the solution by adding 0 NaOH .1 N, then add 5 ml of BCG solution and 5 ml of phosphate buffer, then extract the solution with 5 ml of chloroform, stir the solution 2 times, collect the chloroform phase then evaporate with nitrogen gas, add 5 ml of chloroform. The absorbance of the extract solution was measured using a UV-Vis spectrophotometer at an absorbance wavelength of 273.00 nm. Replication was carried out three times.

The saponin content analysis test was carried out by placing 10 grams of sample into a 250 ml beaker and adding 200 ml of 20% ethanol, then evaporating in a water bath (temperature 55°C) for 4 hours. The solution is filtered and the residue is extracted again. The extract was evaporated in a water bath at 90°C until the volume became 40 ml. The concentrate was poured into a separating funnel and the water layer was taken, then 60 ml of n-butanol was added and mixed with the extract, then washed with 10 ml of 5% NaCl and then evaporated. The samples were dried in an oven until a stable weight was obtained.

To determine the total tannin test, weigh 100 mg of Soya durian peel ethanol extract, extract with 10 ml of diethyl-ether for 20 hours, then filter and the remaining diethyl-ether is evaporated, then distilled water is added to the sample up to 10 ml, and then take 1 ml sample solution and add 0.1 ml of folin-ciocalteu reagent and vortex. Next, wait for 5 minutes and add 2 ml of 20% sodium carbonate and vortex, wait 5 minutes then add distilled water to a volume of 10 ml. The absorbance of the extract solution was measured using a UV-Vis spectrophotometer at an absorbance wavelength of 649.90 nm. Replication was carried out three times.

The phenolic content analysis test was carried out by adding 1 ml of the extract solution, then adding 0.4 ml of Folin Ciocalteau reagent and leaving it for 4-8 minutes. Next, 4 ml of 7% sodium carbonate (Na2CO3) solution was added until homogeneous. Next, 10 ml of aquabidestillata was added and left for 2 hours at room temperature. The absorbance of the extract solution was measured using a UV-Vis spectrophotometer at an absorbance wavelength of 744.80 nm. Replication was carried out three times.

The analysis test for flavonoid levels was carried out by taking samples that had been prepared using a 500 µL micropipette which was poured into a test tube and 2 ml of distilled water was added. Add 150 µL of 5% NaNO2 and let sit for 6 minutes, then add 150 µL of 10% AlCl3 and let sit for 6 minutes. 2 ml of 4% NaOH was added and diluted with distilled water until the tube volume reached 15 ml and left for 5

minutes. The absorbance of the extract solution was measured using a UV-Vis spectrophotometer at an absorbance wavelength of 450.00 nm. Replication was carried out three times.

Testing of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of Soya durian fruit peel extract on Eschericia coli

Minimum inhibitory concentrations (MIC) test

In this study, the MIC test was carried out based[23,24] turbidimetrically. Prepare 11 sterile test tubes. Each test tube is labeled 1-9. Then tube 10 is labeled K(+) which is a positive control, namely a tube containing a bacterial suspension equivalent to the McFarland turbidity standard 1 and tube 11 is labeled K(-) which is a negative control, namely a tube containing BHI-B solution. Tube 1 was filled with 4 ml of 100% concentration of durian skin extract. Tubes 1-9 were filled with 2 ml of BHI-B liquid medium. Then take 2 ml of solution from tube 1, put it in tube 2. The same thing is done to tube 9, getting all the extract concentrations by dilution in multiples of 0.5.

To test turbidity, 1 ml of the suspension media from each E. coli bacteria was taken and put into test tubes for treatments 1-9. Next, all tubes were put into an anaerobic jar and incubated at 37 oC for 24 hours with 4 repetitions of incubation. After each incubation, it is observed using the turbidimetric method or turbidity observation. If the turbidity of the tube is still equal to or more turbid than the K(+) tube containing the bacterial suspension (Tube 10), it shows that the bacteria can still grow well, but when the solution in the tube looks clearer than the K(+) tube, it shows that the bacteria are growing. starting to get stuck. This is what indicates Minimum Inhibitory Concentrations (MIC).[25]

Minimum Bactericidal Concentration (MBC) Test

The MBC test is carried out according to method[26] which is a continuation of the MIC test using the plate count method. A total of 1 ml of suspension at each MIC concentration was spread evenly onto nutrient agar (NA) in a petridish in duplicate. Then, incubate at 37 oC for 24 hours. Readings of growing bacterial colonies are counted using a colony counter. The concentration that shows there is no growth of bacterial colonies is what shows the Minimum Bactericidal Concentration (MBC).

RESULTS

Durian Soya Skin Extraction

Soya durian skin extract was made by extracting Soya durian skin powder using the maceration method with 96% ethanol solvent. The purpose of filtering is to separate compounds in simplicia. The filter fluid will enter the cell cavity containing the active substance by penetrating the cell wall, so that the active substance will also dissolve in the filter fluid.Table 1 shows that from the results of extracting Soya durian skin simplicia 4 times using around 4000 grams of Soya durian skin simplicia, an average yield of 9.289 was obtained with a standard deviation (SD) value of 0.102.

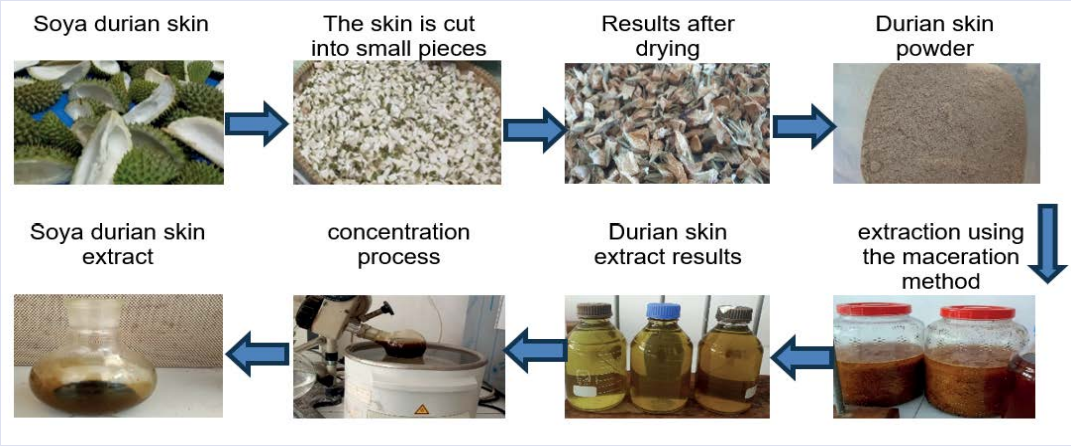

Figure 1. Process of collecting, processing and extracting Soya durian skin using 96% ethanol.

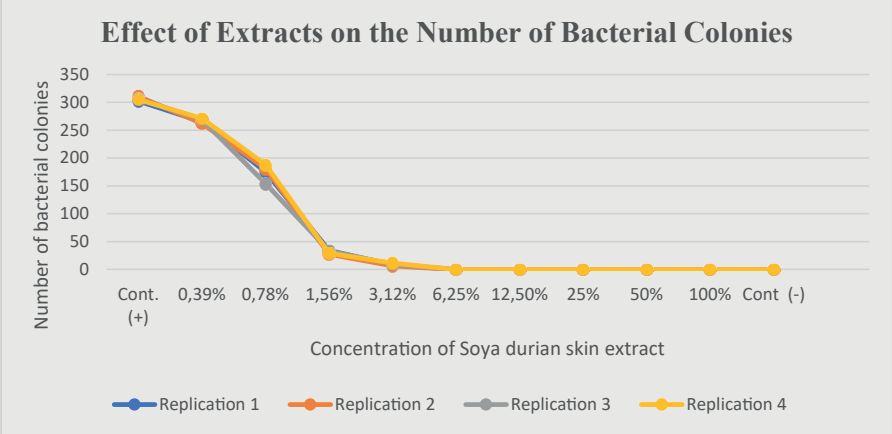

Figure 2. Effect of Several Concentrations of Durian Soya Skin Extract on Reducing the Number of Escherichia coli ATCC 25922 Bacterial Colonies.

Qualitative and quantitative test of secondary metabolite content of 96% ethanol extract of Soya durian peel

Table 2 shows that of the 6 types of secondary metabolite compounds screened in Soya durian peel extract using 96% ethanol filter fluid, there are 5 positive secondary metabolites contained in Soya durian peel, namely alkaloids, flavonoids, saponins, phenols, tannins. Meanwhile, one secondary metabolite, namely quinone, was not found in the phytochemical screening of Soya durian skin.

Table 3 shows that, of the 5 types of secondary metabolites that were positive for the test compounds in Soya durian skin in the qualitative test, the quantitative phytochemical screening showed that the highest levels of metabolite compounds were found in phenol compounds with an average compound level of 57.41%. The lowest secondary metabolite in Soya durian skin is saponin, with an average compound content of 1.74%.

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of Soya durian peel ethanol extract on Escherichia coli ATCC 25922 bacteria

Table 4 shows that from the 9 series of dilutions of Soya durian peel extract which were tested using the liquid dilution method, there were 3 extract concentrations in the test tube that appeared cloudy (+) and indicated bacterial activity, namely at a concentration of 1.56%, 0 .78%, and 0.39%. Meanwhile, 6 extract concentrations looked clear (-) and indicated that there was no bacterial activity, namely at concentrations of 3.12%, 6.25%, 12.5%, 25%, 50% and 100%. The MIC is at the lowest concentration which indicates there is no bacterial activity, namely a concentration of 3.12%. In the positive control (+) containing the bacterial colony E. coli ATCC 25922, it appears cloudy, indicating there is bacterial activity, while in the negative control tube (-) which only contains BHI-B liquid media, it appears clear, indicating there is no bacterial activity.

Table 5 shows that of the 9 solid dilution test concentrations of Soya durian skin extract, there were 4 extract concentrations that contained bacterial colonies, namely at concentrations of 3.12%, 1.56%, 0.78% and 0.39%. Meanwhile, at 5 extract concentrations there was no bacterial activity in the petri dishes, namely at concentrations of 100%, 50%, 25%, 12.5% and 6.25%. Minimum Bactericidal Concentration (MBC) is found at the lowest concentration which indicates there is no bacterial activity, namely at a concentration of 6.25%. In the positive control tube (+) there were the most bacterial colonies with an average of 306.25 colonies, while in the negative control tube (-) there were no bacterial colonies.

Figure 2 shows that a decreasing trend in the number of *E.coli* colonies has begun to appear since the concentration of Soya durian peel extract was small, namely a concentration of 0.39%, then the number of colonies decreased again at an extract concentration of 0.78%. The largest decrease in the number of E. coli colonies was at a concentration of 1.56% and a slight slope at a concentration of 3.12% and at a concentration of 6.25%, the E. coli colonies were declared 0, the number of E. coli colonies and so on at higher concentrations. The data was then analyzed using One Way ANOVA, followed by the Pearson Correlation test and Simple Linear Regression test.

Table 6 shows that the results of the One Way ANOVA analysis of the Minimum Bactericidal Concentration (MBC) data have a significant value of 0.00 ($p \leq 0.05$). This means that the data from the MBC test results shown by the decrease in the number of *E.coli* ATCC 25922 bacterial colonies in each treatment is significant, so it can be continued with the Pearson Correlation test with a confidence level of 5%.

Table 1. Calculation results of the yield of Soya durian rind extract using 96% ethanol solvent.

Replication	Empty weight of fat pumpkin (grams)	Durian skin weight (grams)	Extract Weight + pumpkin fat (grams)	Extract weight (grams)	Rendement (%)	Mean ± SD
I	108,04	1000,12	199,98	91,94	9,19	
II	108,11	1001,10	201,69	93,58	9,35	9,289 ± 0,102
III	108,16	1000,25	202,13	93,97	9,39	
IV	110,12	1000,38	202,11	91,99	9,20	

Table 2. Qualitative phytochemical screening results for secondary metabolite content of 96% ethanol extract of Soya durian peel.

Metabolit sekunder	Reactor	Observation	Result
Alkaloid	Kloroform + H_2SO_4		
	+Dragendoff reactor	Looks red-orange	+
	+Meyer reactor	Appears yellowish white	+
	+Wanger reactor	Looks brown	+
Flavonoid	CH_3COOH + HCL + bubuk Mg	Looks dark red	+
Saponin	Aquadest	White foam forms	+
Fenol	Etanol + $FeCl_3$ 5%	Looks green	+
Tanin	Etanol + $FeCl_3$ 1%	Looks bluish green	+
Kuinon	NaOH	No color change	-

Note: (+) Contains test compound (-) does not contain the test compound

Table 3. Results of quantitative phytochemical screening of secondary metabolite content of 96% ethanol extract of Soya durian peel.

Test Parameter	Test Results (% b/b)			Mean
	1	2	3	
Alkaloid	4,24	4,24	4,24	4,24
Flavonoid	22,94	22,95	22,96	22,95
Saponin	1,74	1,74	1,75	1,74
Fenol	57,25	57,49	57,49	57,41
Tanin	2,28	2,27	2,27	2,27

Table 4. Minimum Inhibitory Concentrations (MIC) test results of the liquid dilution method of Soya durian peel ethanol extract on Escherichia coli ATCC 25922 bacteria.

Extract concentration	Test results				notes
	1	2	3	4	
100%	-	-	-	-	
50%	-	-	-	-	
25%	-	-	-	-	(-): looks clear in the test tube,
12,5%	-	-	-	-	
6,25%	-	-	-	-	
3,12%	-	-	-	-	
1,56%	+	+	+	+	
0,78%	+	+	+	+	
0,39%	+	+	+	+	(+): looks cloudy in the test tube,
Conc (+)	+	+	+	+	
Conc (-)	-	-	-	-	

Table 5. Minimum Bactericidal Concentration (MBC) test results using the solid dilution method of Soya durian peel ethanol extract on Escherichia coli ATCC 25922 bacteria.

Extract concentration	MBC test result (coloni/ml)				Mean
	1	2	3	4	
Conc. (+)	302	311	307	305	306,25
0,39%	265	262	269	271	266,75
0,78%	175	181	154	188	174,50
1,56%	34	27	33	29	30,75
3,12%	8	6	9	11	8,50
6,25%	0	0	0	0	0
12,5%	0	0	0	0	0
25%	0	0	0	0	0
50%	0	0	0	0	0
100%	0	0	0	0	0
Conc.(-)	0	0	0	0	0

Table 6. One Way ANOVA Test Results Minimum Bactericidal Concentration (MBC) solid dilution method of Soya durian peel ethanol extract on Escherichia coli ATCC 25922 bacteria.

Bacteri Coloni	Sum of Squares	df	Mean Square	F	p-value
Between Groups	560568.727	10	56056.873	2364.815	.000
Within Groups	782.250	33	23.705		
Total	561350.977	43			

Table 7. Pearson Correlation Coefficient Between Durian Soya Skin Extract and the Number of Escherichia coli ATCC 52922 Bacterial Colonies.

	Bakteri coloni	
Exstract concentration	r	-812
	p	.000
	N	44

Table 8. R Square Effect of Durian Soya Skin Extract on the Number of Escherichia coli ATCC 25922 Bacterial Colonies.

Model	R	R Square	Adjusted R Square	Std. Error of the Estiat
1	,812[a]	,660	,652	67.41924

a. Predictors: (Constant), durian peel extract

Table 9. Regression Coefficient of Durian Soya Peel Extract Concentration on Reducing the Number of Escherichia coli ATCC 25922 Bacterial Colonies.

Model	Unstandardized Coefficients		Standardized Coefficients	t	p-value
	B	Std. Error	Beta		
(Constant)	245.618	21.799		11.267	.000
Ekstrak kulit durian	-29.016	3.214	-.812	-9.028	.000

Table 7 shows that the results of the Pearson Correlation test (r) show a significant number of 0.000 ($p < 0.05$) with a number of subjects (N) of 44, which means there is a relationship between increasing the concentration of Soya durian peel ethanol extract and decreasing the number of E colonies. .coli ATCC 25922. The Pearson correlation coefficient between concentration and number of bacterial colonies is (r) = 0.812. This means that the higher the concentration of Soya durian peel ethanol extract given, the less the number of *E.coli* ATCC 2592 bacterial colonies. The value of 0.812 indicates that there is a very strong relationship between the concentration treatment and the decrease in bacterial colonies because the value is more than 0.297.

Table 8 shows that the results of the Simple Linear Regression test showed that the Adjusted R Square (R2) coefficient of determination was 0.660, which means that the contribution of giving Soya durian peel ethanol extract in reducing the number of *E.coli* ATCC 25922 bacterial colonies was 66% while the remaining 34% was due to by other factors not studied.

Table 9 shows that the relationship between changes in the concentration of Soya durian peel ethanol extract and the growth of *E.coli* ATCC 25922 bacterial colonies can be expressed by the formula Y = 245,618 – 29,016 Soya durian peel ethanol extract. This means that without the administration of Soya durian peel ethanol extract, the number of *E.coli* ATCC 25922 colonies produced would increase steadily, namely 245,618 colonies. In other words, every 1% increase in the concentration of Soya durian peel ethanol extract will cause a decrease in the number of bacterial colonies to 29,016 colonies.

DISCUSSION

Secondary metabolism produces approximately 200,000 compounds needed by plants to survive their environmental conditions. Apart from being used to maintain survival, secondary metabolite compounds in plants have many benefits for humans, including as natural medicines and pesticides.[27] It is estimated that around 1,260 types of plants have medicinal properties. One of the compounds that acts as a medicine in plants is their secondary metabolite content.[28] Research results show that there have been 150,000 secondary metabolites identified and an increase of around 4,000 new secondary metabolites every year.[29]

The results of the tests we carried out are in accordance with the results of research on durian skin extract by maceration using 70% ethanol, finding that durian skin contains secondary metabolite compounds consisting of alkaloids, saponins, flavonoids and tannins.[17] Likewise, a study of the results of durian skin extract by maceration using 96% ethanol found that durian skin contains secondary metabolite compounds such as flavonoids, phenolics, alkaloids, steroids and tannins.[30] Different studies also found several secondary metabolite compounds in durian skin such as alkaloids, saponins, flavonoids and tannins.[31] A study also found the same results that the results of phytochemical screening with 96% ethanol filter fluid showed that both simplicia and extracts from durian skin contained compounds in the form of flavonoids, tannins, alkaloids, steroids and triterpenoids.[12]

The antibacterial mechanism of action of phenol compounds can interfere with the peptidoglycan component in bacterial cell walls which usually forms a rigid characteristic in the cell wall so that the synthesis of the bacterial cell wall is disrupted and does not form completely.[32] The results of the research found that the ethanol extract's total phenolic content of the Abelmoschus manihot L fraction influenced the amount of antibacterial activity against Echerichia coli, namely 67%. The phenol content at high concentrations is able to penetrate and disrupt bacterial cell walls and precipitate proteins in bacterial cells. [33] In addition, phenol can cause protein coagulation, change the permeability of bacterial membranes and ultimately cell membranes experience lysis (death).

Flavonoid compounds are easily oxidized at high temperatures and are not heat resistant. As of 2011, there were more than 9000 flavonoids and they had been used as health supplements.[34] The activity of the antibacterial compounds total flavonoids and total tannins using the diffusion method to obtain an inhibition zone ranging from 10 mm - 20 mm at concentrations of 10%, 20% and 25%, shows that Flavonoid and tannin compounds have strong antibacterial power at this concentration.[35] Antibacterial test results show that mango leaf flavonoid isolates produce a larger inhibitory zone diameter against Escherichia coli and Staphylococcus aureus bacteria. This shows that flavonoid compounds play an active role in inhibiting bacterial growth. Falavonoids inhibit bacterial growth by damaging cell walls, deactivating enzymes, binding to adhesins and damaging cell membranes.[36]

Alkaloids are the most important group of secondary metabolites found in plants. The existence of alkaloids in nature never stands alone. This class of compounds is a mixture of several major and several minor alkaloids. Alkaloid compounds have the ability to act as antibacterial compounds and have an inhibitory mechanism by disrupting the peptidoglycan components in bacterial cells, so that the bacterial cell wall layer does not form completely and causes the death of the bacterial cell. Apart from that, alkaloids also inhibit the formation of synthetic proteins so that they can interfere with bacterial metabolism.[37] Tannin compounds have the ability to destroy and kill bacteria by attacking the polypeptide walls of bacterial cell walls so that the cell wall formation becomes less than perfect and then the bacterial cells will die. Tannin compounds can also inactivate bacterial enzymes and disrupt the flow of proteins in the inner layer of bacterial cells.[38] Meanwhile, saponin compounds act as antibacterials by destroying the permeability of cell walls so that they can cause bacterial cell death.[37]

The way secondary metabolite compounds work as antibacterials is by forming a complex with cellular extract proteins, dissolved and with microbial cell walls. In limiting bacterial growth, an extract depends on the concentration of the secondary metabolite compounds used. Several aspects that influence it include biological factors, plant planting period, storage of plant material, age of the plant used and are influenced by chemical aspects such as qualitative and quantitative composition of active compounds, average total content of active compounds, extraction procedures, size, hardness, dryness. materials and solvents used in the extraction process.[39]

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) tests of Soya durian peel ethanol extract on *E.coli* ATCC 25922 bacteria

The purpose of the antimicrobial activity test is to determine the potential of a material or compound to have antimicrobial activity on bacteria. The methods used in each antimicrobial test are the diffusion and dilution methods.[40] The diffusion methods that are often used by researchers are the disk diffusion method, the well method[41] and the antimicrobial gradient method.[42] Meanwhile, the dilution method is divided into two test methods, namely the liquid dilution method and the dilution method. congested.

The use of the liquid dilution method is a test method to determine Minimum Inhibitory Concentrations (MIC) while the solid dilution method is used to determine Minimum Bactericidal Concentration (MBC). The method used in the liquid dilution method is to make a series of dilutions of the antimicrobial agent in a liquid medium and then add the test microbes. Meanwhile, the solid dilution method is carried out by inoculating the test microbes on agar media containing antimicrobial agents. Using one concentration of the antimicrobial agent being tested can be used to test several test microbes which is an advantage of the dilution method.[43]

We carried out the Minimum Inhibitory Concentrations (MIC) test using nine concentrations of Soya durian peel extract in test tubes 1 to 9, namely, 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78% and 0.39% and with two controls, namely a positive control using Escherichia coli ATCC 25922 bacterial suspension and a negative control using Brain Heart Infusion Broth (BHIB) liquid media. From the test results we obtained, it was found that at extract concentrations lower than 3.12%, namely 1.56%, 0.78%, and 0.39%, the tube appeared cloudy or the turbidity was the same as in the positive control tube. Meanwhile, in tubes 1 to 6 or at extract concentrations of 3.12%, 6.25%, 12.5%, 25%, 50% and 100%, the tubes appeared clear or similar to the positive control tube containing BHIB liquid media.

Based on the results of a study, it shows that the Minimum Inhibitory Concentrations (MIC) for ethanol extract of durian peel is at a concentration of 25% with an average number of colonies of 0.6x107 CFU/ml, with the positive secondary metabolite compounds found in durian peel as a result of phytochemical screening being alkaloids. , phenols, flavonoids, tannins and saponins.[44] From the research results it was found that the results of antibacterial testing using the disc diffusion method on 96% ethanol extract of durian peel showed the presence of an inhibitory zone at concentrations of 5% and 10% with the types of secondary metabolite compounds from the results of the phytochemical test of durian peel containing alkaloids, saponins and triterpenoids as anti-bacterial ingredients that have the potential to inhibit bacterial growth.[4]

We carried out the Minimum Bactericidal Concentration (MBC) test using the solid dilution method while still using 9 extract concentrations, namely 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56% , 0.78% and 0.39% and with two controls, namely a positive control using Escherichia coli ATCC 25922 bacterial suspension and a negative control using Brain Heart Infusion Broth (BHIB) liquid media. From the test results we obtained, the MBC value was at a concentration of 6.25% Soya durian skin extract. This was obtained from the results of observing bacterial activity in petri dishes that were incubated for 24 hours. The results of research on Minimum Bactericidal Concentration (MBC) using the solid dilution method did not find any MBC in the 96% ethanol extract of durian peel. It is possible that MBC was not found because the use of durian skin in this study did not use the entire skin but only used the white inner skin, and the concentration of the extract used as the test concentration did not vary.[44] However, research results found that durian leaf extract using the percolation method using 70% ethanol extract liquid showed that the Minimum Inhibitory Concentrations (MIC) was 0.1 mg/ml and the Minimum Bactericidal Concentration (MBC) was at a concentration of 0.25 mg. /ml against P.aeruginosa bacteria and a concentration of 0.1 mg/ml against *E.coli* bacteria.[45] In research by extracting purple leaves (Graptophyllum pictum L. Griff) with 70% ethanol extracting liquid and 96% ethanol extract, the same Minimum Inhibitory Concentrations (MIC) value was obtained for both the 70% ethanol extract and the 96% ethanol extract, namely 3.125% and the value The minimum Bactericidal Concentration (MBC) is also the same, namely 6.25%.[46]

Secondary metabolite compounds such as alkaloids, flavonoids, tannins, saponins and steroids are antibacterial compounds found in plants. These compounds have a very important role in inhibiting the growth or killing bacteria. If no MBC value is found in a test, it could be because the extract concentration is low and needs to be increased to get the kill concentration value, or the plant extract contains more secondary metabolite compounds which are only able to inhibit the growth of bacteria such as tannins and saponins so it is also necessary to increase the concentration of the extract to obtain a concentration that kills bacteria.[47]

CONCLUSION

Based on the results we obtained, the qualitative phytochemical screening of Soya durian skin extract with 96% ethanol solvent contained secondary metabolite compounds with the average compound content being 4.24% alkaloids, 22.95% flavonoids, 1.74% saponins, 57% phenols. .41% and tannin 2.27%. Soya durian peel extract has Minimum Inhibitory Concentrations (MIC) against E. coli ATCC 25922 bacteria at a concentration of 3.12% while the Minimum Bactericidal Concentration (MBC) value for Soya durian peel extract against E. coli ATCC 25922 bacteria is at a concentration of 6.25% . Soya durian skin extract with 96% ethanol solvent has a very strong relationship between treatment of the extract concentration as an antibacterial and a decrease in the number of Escherichia coli bacterial colonies. The higher the extract concentration, the greater the decrease in the number of *E.coli* bacterial colonies. So it is very possible to use Soya durian skin extract as an antibacterial to be used as a water disinfection agent.

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