
ANALYSING LEVELS OF FLAVONOID, SAPONIN, AND TANNIN OF MANGO (*Mangifera indica* L.) PEEL WASTE AS AN ANTIBACTERIAL AGENT AGAINST *Escherichia coli*

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Abstract

Escherichia coli bacteria are a microorganism that is needed in the digestive system, but if the amount is excessive, it will be pathogenic and cause diseases, including diarrhea. Antibacterial can be used as an inhibitor of the development of microorganisms that are capable of causing disease and death. The focus of this study is to identify the phytochemical compounds contained in it, as well as how the bark of mango arumanis has antibacterial properties that fight *Escherichia coli* by using soxhletation extraction methods, uv-vis spectrophotometry, gravimetry, titrimetry and disc diffusion. Phytochemical compounds in the ethanol extract of Arumanis mango peel (*Mangifera indica* L.) are flavonoids, saponins, alkaloids, and tannins. The ethanol extract of Arumanis mango peel (*Mangifera indica* L.) contains flavonoids at 2.27%, saponins at 51.62%, and tannins at 3.2553%. Furthermore, the antibacterial activity test of the ethanol extract of Arumanis mango peel (*Mangifera indica* L.) resulted in an inhibition zone of 46%, categorized as less effective.

Keywords: Antibacterial, Arumanic Mango Peel, Phytochemicals

1. Introduction

Diarrhea is an infectious disease with a high mortality rate that occurs in all countries, both developing and developed countries, including Indonesia (Muttaqin, et al. 2016). Diarrhea sufferers in 2023 in Indonesia with all ages at 2%, among toddlers as much as 4.9%, and among babies as much as 3.9%. The number of diarrhea sufferers among toddlers in Central Java is ranked 15th in Indonesia at 27.6% (Ministry of Health, 2024). According to the Semarang City Health Office (2022), from 2018 to 2021, those suffering from diarrhea decreased. Meanwhile, in 2022 it has

increased from 2021. Diarrhea patients have increased from 21,765 in 2021 to 32,488 people in 2022 (Semarang Health Department, 2022).

Changes in texture in stools that were initially solid to soft three times a day are signs of diarrhea (WHO, 2016). The cause of diarrhea can be due to factors that are not clean and healthy, the food consumed, and even due to the presence of *Escherichia coli* bacteria in the human body (Halim, et al. 2017). Most of the Indonesian people in overcoming diarrhea use chemical drugs.

However, this has become a problem in the health sector because it is ineffective and unsafe (Permana & Emelia, 2022). The use of these drugs for a long period of time will cause resistance to the body. This is due to the presence of chemicals contained in medicines. In order to reduce the use of chemical drugs, traditional medicines are made to overcome diarrhea (Supiana, 2022).

Indonesia is rich in natural resources, both biological and non-biological resources. The biological wealth can be taken from plants as the basic ingredients for producing traditional medicines that have been distributed in various countries which contain several active compounds in them (Rahayu, et al. 2021). The choice of using traditional medicine because it has low side effects, is easy to obtain, and can be made by everyone. The World Health Organization says that as many as 80% of humans still consume traditional medicines that can be obtained from plants (Ningsih, 2016).

According to Hanifa, et al. (2022) arumanis mango leaves have flavonoid compounds, tannins, and saponins that can act as antimicrobial agents against *Escherichia coli* bacteria. These active compounds are not only found in the leaves of the arumanis mango, but also found in the skin of the arumanis mango. According to Zulhipri, et al. (2011) arumanis mango seeds have phenolic, flavonoid and terpenoid compounds that are high in phenolic compounds. Methanol extract of arumanis mango seeds is able to inhibit the growth of *e-coli* and *Staphylococcus aureus* bacteria. Antibacterial itself can be used as an inhibitor of the development of microorganisms that are able to cause disease and death. This is due to the performance of antibacterials that are able to inhibit microorganisms in the metabolic system that are pathogenic (Rahmadhany, 2022).

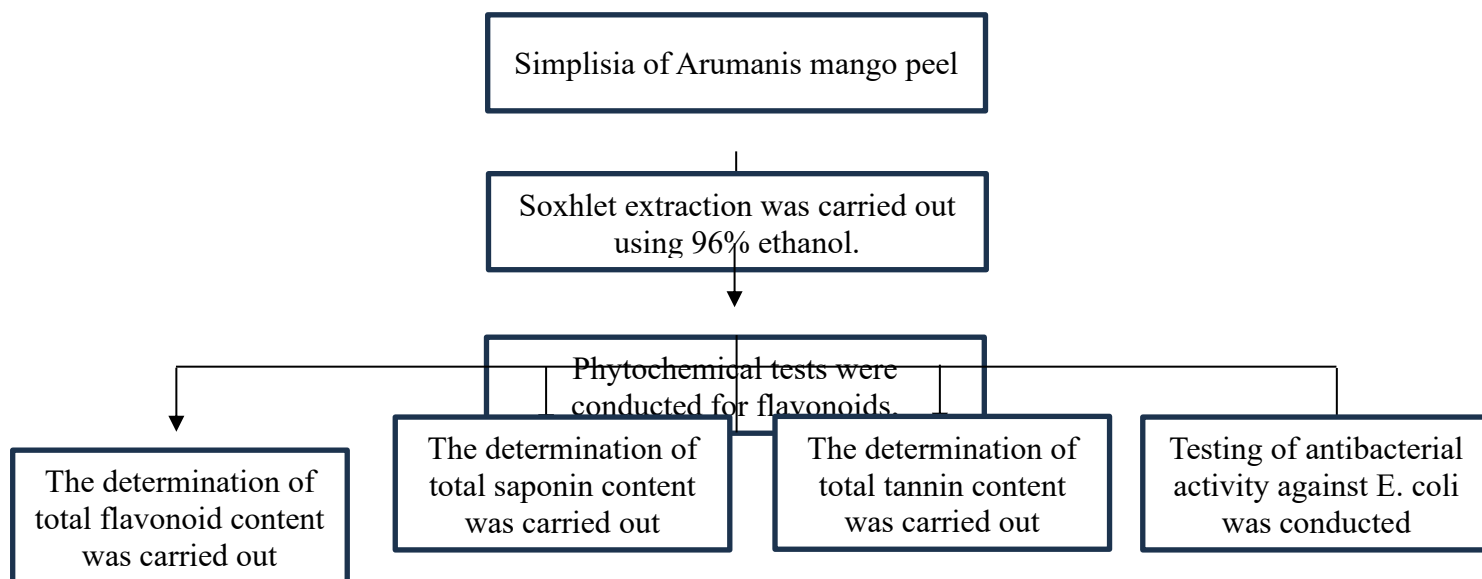
Based on this background, a study was conducted on the Analysis of Flavonoids, Saponins and Tannins with the Utilization of Mango Arumanis Peel Waste (*Mangifera indica* L.) as an antibacterial against *Escherichia coli*. The focus of this research is to identify phytochemical compounds, as well as flavonoids, saponins, and tannins. In addition, to find out the antibacterial properties of mango arumanis peel against *Escherichia coli*.

2. Methods

2.1. Material

Arumanis mango peel, ethanol 96%, HCl 5N, amyl alcohol, aquades, HCl 1%, FeCl₃, chloroform, petroleum ether, methanol, NH₃ 10%, H₂SO₄, anhydrous acetate, AlCl₃, quercetin, CH₃COOH, ethanol p.a, ethyl acetate, n-butanol, diethyl ether, indigokarmine powder, alcohol 70%, Nutrient Agar, NaCl 0.9%, DMSO 10%, siproflacssin and *Escherichia coli* bacteria.

2.2. Method



3. Result and Discussion

3.1. Sample Preparation

Arumanis mango fruit is obtained from a garden in Kalikondang Village, Demak District, Demak Regency. Then the skin is taken and dried in the sun for 4 days. This aims to remove moisture content in the skin of the arumanis mango which can cause the skin of the mango arumanis to become moldy and damaged. The dried arumanis mango skin will turn blackish-green with a hard mango skin texture as shown in figure 3.1. After drying, the arumanis mango peel is mashed with a blender so that 250 grams of fine powder of arumanis mango peel are obtained.

Figure 1.

Sample Preparation



3.2. Extraction Process

The extraction used uses the soxhletation method because the soxhletation method is an easy-to-use method and does not take a long time. In soxhletation extraction, 96% ethanol is used as a solvent because the substance to be tested is included in polar compounds, so the suitable solvent for use is polar solvent. When soxhletation extraction is carried out, 50 grams of the measured sample is wrapped in filter paper and then closed tightly so that when extraction occurs the sample used is not contaminated with solvents so that it will cause blockage in the chamber, damage the extraction system, and even the weight of the sample used is reduced.

The temperature used during the extraction of soxhletation of the bark of the arumanis mango is in the range of 50-55°C because 96% ethanol solvent has a boiling point of 78.32°C (Rosita, et al. 2017). The use of high temperatures when soxhletation extraction occurs includes the indirect heat method. This is so that volatile compounds do not suffer damage. Extraction is carried out until it is colorless in order to obtain the desired extract to the maximum extent (Rosita, et al. 2017). In this study, soxhletation extraction was carried out for up to 10 cycles.

During the extraction process, osmosis will occur and will cause the cell to become dehydrated so that the plasma membrane will be detached from the cell wall. In addition, the state of osmosis can also cause the cell wall to break and compounds in the cytoplasm to dissolve with their solvents (Rosita, et al. 2017). This extraction produces a thick extract of arumanis mango peel. Extraction was carried out 3 times with 15.4107 grams, 14.2985 grams, and 15.2706 grams of arumanis mango peel ethanol extract with a total yield of 29.87% respectively. After that, the ethanol extract of the arumanis mango peel is evaporated with a waterbath at 65°C to evaporate. Evaporation with a waterbath produces a thick ethanol extract of arumanis mango peel. The results of soxhletation extraction of ethanol extract of mango arumanis peel can be seen in Table 1

Table 1.

Results of Ethanol Extract of Mango Arumanis Peel






Solvent	Sample Weight (grams)	Extract Weight (grams)	Yield (%)	Extract color
Etanol 96%	50,0527	15,4107	30,78	Deep greenish brown
	50,4994	14,2985	28,31	
	50,0120	15,2706	30,53	

3.3. Phytochemical Screening

Phytochemical screening is carried out to identify the active compounds present in the sample. The phytochemical compounds tested were flavonoids, saponins, alkaloids, tannins, and steroids. Flavonoids. The results of the phytochemical screening of the ethanol extract of arumanis mango peel can be seen in Table 2.

Table 2.

Phytochemical screening results of ethanol extract of Arumanis mango peel

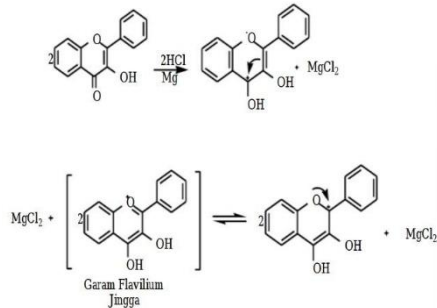
Active Compound	Ethanol Extract of Arumanis Mango Peel	Result	Picture
Flavonoid	+	Orange color on the amyl alcohol layer + brown precipitate	
Saponin	+	Produces foam	
Tannin	+	Turn into a dark bluish-black color	
Alkaloid	+	There is a white sediment underneath it	
Steroid	-	Does not change to blue	

Identification of flavonoid compounds by adding magnesium powder and HCl after which they are reacted with amyl alcohol to the sample. The final result obtained in the sample changed color from brown to orange after being reacted with amyl alcohol on the amyl alcohol layer accompanied by brown deposits underneath.

Flavonoid compounds are made up of 15 carbon atoms with two benzene rings bonded to propane chains. When ethanol extract is added magnesium powder and HCl and reacted with amyl alcohol, the solution is orange. This allows that the flavonoid compounds in it are flavanon, flavanonol, and flavanols (Noer, et al. 2018). Figure 2 shows the reaction that occurs between flavanone compounds and magnesium.

Figure 2.

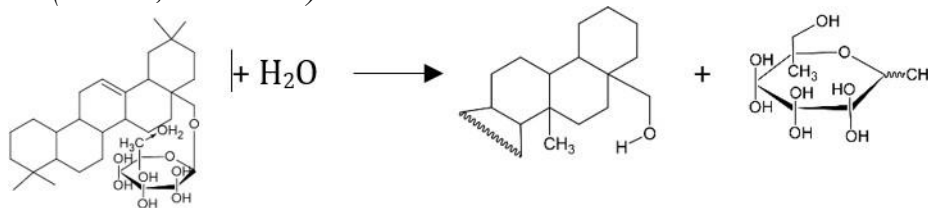
Flavonoid Reactions with Mg and HCl Metals (Pirdina, et al. 2021)



Mango arumanis peel extract still has foam in it after being reacted with 1% HCl as much as 1 drop. This proves that the extract of mango arumanis peel contains saponin compounds in it. Saponin compounds have the role of nonpolar groups and have steroids and triterpenoids which will form micelles when shaken using water. Inside the micellar structure consists of an inward-facing non-polar group and an outward-facing polar group. This results in a situation such as foam in the sample being tested (Rohmah, 2021). The reaction to saponin compounds is shown in figure 3.

Figure 3.

Saponin Reaction (Surtina, et al. 2019)

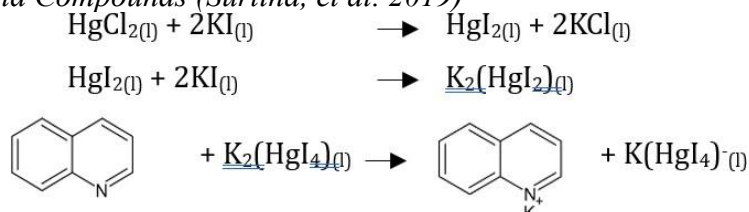


In the identification of alkaloid compounds, ethanol extract of mango arumanis peel was directed with chloroform and 10% NH₃ which produced a brown color. After that, it is added with H₂SO₄ 2N and the solution separates into 2 phases with the top and bottom layers brown. Where the top layer is taken then reacted with a Mayer reagent and produces a white color. This shows that the ethanol extract of mango arumanis peel has alkaloid compounds in it. The sample used was reacted with 10% NH₃ with the aim of returning the alkaloid salts to be insoluble in the solution that still has a water content, in addition to the use of chloroform functions so that the alkaloids

undergo a transfer from the water phase to the organic phase. The addition of H₂SO₄ 2N solution functions to separate separate alkaloid compounds from salts that are alkaline and are able to produce sediments that will later be tested with several reagents, such as Mayer reagents, wagner, and dragendorff reagents. In addition, the addition of mayer reagents to the sample also serves to identify the alkaloid compounds contained in it (Fadhila & Ethics, 2023).

Figure 4.

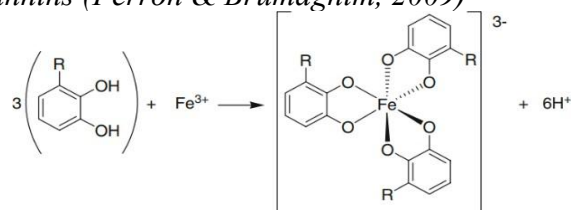
Reactions to Alkaloid Compounds (Surtina, et al. 2019)



Identification of tannin compounds in the ethanol extract of arumanis mango bark was carried out by reacting using FeCl₃ until the sample changed color to blackish-blue. This shows that in the ethanol extract of the bark of mango arumanis there are tannin compounds. The occurrence of this color change is a reaction between tannins and Fe³⁺ ions so that complex compounds are formed. The tannin reaction with FeCl₃ is shown in figure 3.5.

Figure 5

Reaction of FeCl₃ with Tannins (Perron & Brumaghim, 2009)



Steroid compounds are secondary metabolite compounds obtained from terpene derivative reactions in which there are three cyclohexane rings and one cyclopentane ring. (Melati & Parbuntari, 2022). In this identification, the Liebermann-Buchard reagent was used to analyze the presence of steroid compounds in the sample of the mango arumanis peel extract used. The reaction begins with acetylation in the hydroxy group caused by anhydrous acetate. The acetyl group then forms a double bond resulting in the release of the hydrogen and electron groups. The release of the hydrogen group with its electrons results in resonance and carbocation. In addition, the release of hydrogen will undergo an electrophilic addition reaction which will undergo prolonged conjugation (Qomaliyah, et al. 2023). As for the study, there were no steroid compounds in it.

3.4. Determination of Total Flavonoid Levels

The use of UV-Vis Spectrophotometer in the analysis of flavonoid levels is because there are conjugated aromatics in flavonoid compounds and resulting in strong absorption bands in the ultraviolet and visible light spectrum regions (Aminah, et al. 207). The use of quercetin as a standard solution in testing total flavonoid levels with UV-Vis Spectrophotometer is due to quercetin being a flavono-derived flavonoid compound that is widely distributed in plants. This is because when reacted with $AlCl_3$, the most stable complex compounds will be formed with the presence of orthohydroxyl groups in the dalma (Qomaliyah, et al. 2023).

In the flavonoid level test, an absorbance result of 0.199 A with a wavelength of 421 nm was obtained in a standard quercetin solution of 40 ppm. This is evidenced by research that quercetin is able to absorb light in the range of 400-500 nm (Uebel, et al. 2016). The wavelength results were used in measuring the total flavonoid levels in the ethanol extract sample of mango arumanis peel Then absorbance readings were carried out in the quercetin raw solution of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm with a wavelength of 421 nm shown in table 3.3 and figure 3.6. The total level of flavonoids in the plant is expressed in QE (Quercetin Equivalent) units where the number of milligrams of quercetin in 1 gram of the sample extract tested.

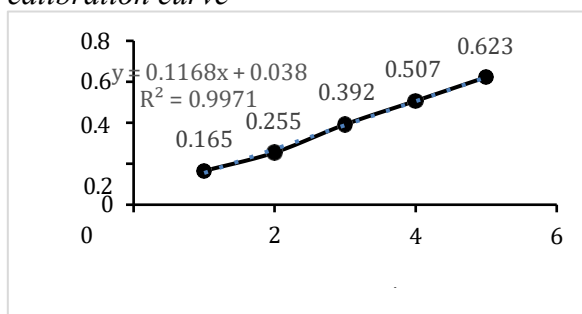
Table 3.

Absorbance Results of Quercetin Raw Curve

No	Concentration	Absorbance
1	20 ppm	0.165
2	40 ppm	0.255
3	60 ppm	0.392
4	80 ppm	0.507
5	100 ppm	0.623

Figure 6.

Standard quercetin solution calibration curve



Based on the calibration curve of the standard quercetin solution, a linear regression equation was obtained $y = 0.1168x + 0.038$ with an R^2 (correlation coefficient) of 0.9971. Sample measurements were carried out as many as 2 replications with the aim of ensuring that the

absorbance of the sample obtained was accurate. The mg/ml concentration of the sample was measured using a Uv-Vis spectrophotometer with a wavelength of 421 nm. Sample 1 obtained an absorbance of 0.302 A while sample 2 obtained an absorbance of 0.305A. The total flavonoid content of mango arumanis peel extract is 2.27%.

3.5. Determination of Total Saponin Levels

Determination of saponin levels contained in mango arumanis peel extract using the gravimetric method. The use of the gravimetric method is because this method is an easy and efficient method to do where this method does not require other comparative substances (Noviyanty, et al. 2020). This is evidenced by the determination of the mass of the substance used directly. The use of reflux with a temperature of 60-80°C for 30 minutes in the gravimetric method works by heating the analyzed compounds and the extracts used have a coarser texture and are easier to withdraw. The non-polar compounds contained in the extract are attracted with petroleum ether which is used as a solvent. When the petroleum ether solvent is at room temperature, it is necessary to separate the petroleum ether from the sample extract so that the non-polar compounds contained in the sample are lost (Adawiyah R., 2017).

The remaining residue is dissolved with ethyl acetate so that the semipolar compounds contained in the extract are absorbed. As for removing the semipolar compounds, separation is carried out with an ethyl acetate solution. Saponins are polar compounds with a number of hydroxyl groups or glycosides that are easily soluble in n-butanol. Then the polar n-butanol is added to the remaining residue and evaporated with a waterbath to produce a more concentrated extract. The resulting extract is dissolved with methanol and diethyl ether by stirring. The addition of diethyl ether to form a deposit of saponin compounds that are insoluble in diethyl ether (Adawiyah R., 2017). The resulting saponin deposits are separated from other impurities by filtering using a filter paper whose mass has been known. The sediment is dried and then weighed until a fixed weight is obtained. The saponin content of the ethanol extract of mango arumanis peel is 51.63%. The results of saponin levels of ethanol extract of mango arumanis peel can be seen in table 4.

Table 4.

Determination of Saponin Levels of Mango Arumanis Peel Extract

Replication	Extract weight (grams)	Saponin content (%)
1	2,1518	51.62
2	2,1502	51.64
3	2,1514	51.64
Average saponin levels		51.63

3.6. Determination of Total Tannin Levels

The determination of tannin levels of ethanol extract of arumanis mango bark was carried out using the permanganometry method in which a reduction and oxidation reaction occurred using KMnO₄ as a standard solution with the solution used as the primary standard, namely oxalic acid. The use of KMnO₄ solution as a standard solution is because it is easy to find and is included in

the strong oxidizer (Ryanata, 2015). KMnO_4 standard solutions are standardized with a 0.1N oxalic acid solution first before use. This is to determine the concentration of KMnO_4 solution used in titration (Maulida, et al. 2020). The presence of a change in light color when titration occurs indicates that there has been a titration endpoint (TAT). After standardization, the concentration of KMnO_4 solution is 0.10926 as shown in table 5.

Table 5.
Normality Determination Results of KMnO_4

No	Solution H_2SO_4	Solution KMnO_4	Normality
1		9 mL	0.1111 N
2	10 mL	8 mL	0.125 N
3		10.9 mL	0.0917 N
Average Normality			0.10926 N

Then the determination of tannin levels in the ethanol extract of mango arumanis peel was carried out using a KMnO_4 solution that has been standardized as a titran. A total of 0.2239 grams was dissolved with aquades and then heated so that the analyzed tannin compounds were soluble in water. After 30 minutes of heating, the solution is cooled and then filtered and carried out continuously until the resulting residue is not blue when FeCl_3 is dripped in. This is so that tannin compounds are completely filtered (Ryanata, 2015). A filtrate of 2.5 mL was dissolved in aquades and indigokarmine indicators were added and titrated with a standardized KMnO_4 solution. The indicator used is the indigokarmine indicator to be able to detect the occurrence of TAT with a change in color to golden yellow. When it reaches the titration endpoint (TAT), the titration is stopped. Based on the data in table 6, the tannin content of mango arumanis peel tannin extract was obtained at 3.2553% from 3 replications.

Table 6.
Determination of Tannin Levels of Ethanol Extract of Mango Arumanis Peel

No	Sample Weight	Titran Volume (V)	Blank Volume (V_0)	Normality KMnO_4	Tannin Levels
1	0.2239 gram	24.4 mL	9 mL		3,0817%
2	0.2117 gram	24,8 mL	9 mL	0.10926	3,3622%
3	0.2077 gram	24.4 mL	9,2 mL		3,3220%
Average (%)					3,2553%

3.7. Antibacterial Activity

In antibacterial testing, ciproflaxacin is a fluoroquinolone class antibiotic that has a broad spectrum of both gram-positive and gram-negative. To ensure that the negative control does not

affect the antibacterial activity test, the negative control is tested using a 5% DMSO solution (Hanifah, et al. 2022). In addition, DMSO solution has the ability to dissolve polar and nonpolar compounds without changing the results obtained (Permatasari, 2021). The extract used in testing the antibacterial activity of *Escherichia coli* is ethanol extract of arumanis mango peel. Antibacterial activity testing was carried out 3 times with the same concentration to ensure that the resulting inhibition was appropriate. The results of the inhibition zone can be seen in table 7. The inhibition zone in the antibacterial test that has been carried out obtained an average inhibition yield of 46% which is in the less effective category.

Table 7.

Results of Inhibition Zone of Ethanol Extract of Mango Bark Arumanis

Ekstrakt	Diameter Inhibition Zone	Effectiveness (%)	Effectiveness Levels
Replication 1	15 mm	42	Less effective
Replication 2	15 mm	42	Less effective
Replication 3	19 mm	54	Less effective
siproflaksasin	35 mm	100	Effective
DMSO 5%	0 mm	0	

The presence of flavonoid compounds, saponins, and tannins in the ethanol extract of mango arumanis peel involving ethanol extract of mango arumanis peel has the potential for antibacterial activity of *Escherichia coli*. In addition, there is also an influence of the polarity of a compound in the solvent. This is because each compound contained in the extract is able to cause different effects to inhibit the growth of bacteria. Different synergistic effects in secondary metabolite compounds have morphological properties of bacteria that can result in differences in antibacterial activity (Hanifa, et al. 2021)

4. Conclusion

Based on the research conducted, the following conclusions were obtained:

1. Phytochemical compounds in the ethanol extract of mango arumanis peel (*Mangifera indica* L.) are flavonoids, saponins, alkaloids, and tannins.
2. Ethanol extract of mango arumanis peel (*Mangifera indica* L.) has a flavonoid content of 2.27%, a saponin content of 51.62%, and a content of tannin compounds of 3.2553%.
3. The results of the antibacterial activity test of the ethanol extract of the bark of mango arumanis (*Mangifera indica* L.) resulted in an inhibition zone area of 46% with the less effective category.

5. References

- Adawiyah, R. (2017). Analisis Kadar Saponin Ekstrak Metanol Kulit Batang Kemiri (*Aleurites moluccana* (L.) Willd) dengan Metode Gravimetri. Makassar: Universitas Islam Negeri Alauddin Makassar.
- Fadhila, D., & Etika, S. B. (2023). Skrining Fitokimia Ekstrak Metanol Daun Cemara Sumatera (*Taxus sumatrana*). Jurnal Pendidikan Kimia Universitas Riau Vol. 8 No. 1, 66-73.
- Hanifa, H. N., Kurniasih, N., Rosahdi, T. D., & Rohmatulloh, Y. (2022). Antibacterial Effect of Ethanol Extract of Arumanis Mango Leaves (*Mangifera indica* L.) Against *Escherichia coli*. National Chemistry Seminar 2021 (pp. 70-76). Bandung: Sunan Gunung Djati State Islamic University.
- Khaerunnisa, H. (2021). Skrining Fitokimia Biji Pepaya (*Carica papaya* L.) Di Pulau Lombok. Karya Tulis Ilmiah. Mataram: Program Studi DIII Farmasi Fakultas Ilmu Kesehatan Universitas Muhammadiyah Mataram.
- Maulida, S., Hakim, A. R., & Mohtar, M. S. (2020). Analysis of Tannin Content in Ethanol Extract of Candle Nut (*Aleurites moluccana* (L.) Willd) Stem Bark Using Titrimetric Method. Journal of Pharmaceutical Care and Science Vol. 1 No. 1 , 85-93.
- Melati, & Parbuntari, H. (2022). Screening Fitokimia Awal (Analisis Qualitative) pada Daun Gambir (*Uncaria Gambir Roxb*) Asal Siguntur Muda. Chemistry Journal of Universitas Negeri Padang Vol. 11. No. 03, 88-92.
- Permatasari, C. (2021). Uji Aktivitas Antibakteri Terhadap Bakteri *Escherichia coli* dan *Staphylococcus* serta Uji Toksisitas Ekstrak Air Daun Kelor (*Moringa oleifera* L.) Hasil Sonikasi. SKRIPSI. Malang: Universitas Islam Negeri Maulana Malik Ibrahim.
- Ramadhani, N., Samudro, A. G., & Pratiwi, L. W. (2020). Analysis of Flavonoid Content Determination in Kalamansi Orange Extract (*Citrofortunella microcarpa*) Using the UV-VIS Spectrophotometer Method. Jurnal Mandala Pharmacon Indonesia Vol. 06. No. 01, 53-58.
- Rohmah, B. L. (2021). Identifikasi Senyawa Metabolit Sekunder dan Uji Aktivitas Antioksidan Ekstrak Daun Sangket (*Basilicum polystachyon* L. Moench). Skripsi. Semarang: Program Studi Kimia Fakultas Sains dan Teknologi Universitas Islam Negeri Walisongo.
- Ryanata, E. (2015). Penentuan Jenis Tanin dan Penetapan Kadar Tanin dari Kulit Buah Pisang Masak (*Musa paradisiaca* L.) secara Spektrofotometri dan Permanganometri. Jurnal Ilmiah Mahasiswa Universitas Surabaya Vol. 04. No. 01, 1-16.
- Qomaliyah, E. N., Indriani, N., Rohma, A., & Islamiyati, R. (2023). Phytochemical Screening, Total Flavonoid Content, and Antioxidant Activity of Cocor Bebek Leaves. Current Biochemistry Vol. 10. No. 01, 1-10.
- Waluyo, L. (2018). Teknik dan Metode Dasar dalam Mikrobiologi. Malang: Universitas Muhammadiyah Malang.