

Qualitative And Quantitative Analysis Of Chemical Compounds In Ethanol, Acetone, Hexane Extracts Of Malur Fruit (Bruceajavanica(L.) Merr)

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ABSTRACT: Research on the qualitative and quantitative analysis of the content of chemical compounds from ethanol, acetone, and hexane extracts from malur fruit (*Bruceajavanica*(L.) Merr) has been carried out. The purpose of this study was to determine the type and content of secondary metabolites from ethanol, acetone, and hexane extracts. The extraction method used was maceration in ethanol, acetone, and hexane solvents. Determination of the secondary metabolite level of malur fruit (*Bruceajavanica*(L.) Merr) using gravimetric and spectro photometric methods. The results of the qualitative analysis of the ethanol extract contained alkaloid, flavonoid, and tannin chemical compounds, acetone extract contained flavonoid chemical compounds, hexane extract contained alkaloid and phenol chemical compounds. The quantitative results of the total alkaloid compound in the ethanol and hexane extract were 4.0156% and 3.5293%, the flavonoid levels in the ethanol and acetone extract were 6.03% and 6.5266%, respectively, the total phenol content in the hexane extract was 6.1446%, the tannin content in the ethanol extract was 1.1225%. From the research results, it can be concluded that the variation of solvent based on the degree of polarity for malur fruit extract (*Bruceajavanica* (L.) Merr) contains different secondary metabolites, where ethanol solvent attracts more secondary metabolites than acetone and hexane solvents.

KEYWORDS: Qualitative; Quantitative; Fruit malur (*Bruceajavanica*(L.) Merr)

I. INTRODUCTION

Medicinal plants have long been utilised by various levels of society. The use of medicinal plants in Indonesia is used in efforts to cure and prevent diseases, increase power endurance body as well as restore fitness and as a treatment [3]. One of the plants empirically utilised as traditional medicine by the Dayak, Chinese and Malay ethnic

inland communities is malur fruit (*Brucea javanica* (L.) Merr).

In general, the organ of the malur plant (*Brucea javanica* (L.) Merr) used is the old black fruit which is used by the community to treat diseases such as amoba dysentery, diarrhoea, malaria and swelling. Malur fruit is consumed directly by grinding 10-15 fruits until smooth and then putting them into capsules. It is taken three times a day for 7-10 days [12].

Based on the results of research by Jacob and Rumklak (2020), stated that in ethanol and hexane extracts secondary metabolite compounds identified from (*Brucea javanica* (L.) Merr) from the island of Timor were tannins, terpenoids, alkaloids and saponins. According to the results of research from Pandiangan (2015), the chemical content contained in malur fruit (*Brucea javanica* (L.) Merr) has the potential to have the ability as an anticancer with the contents that play a role are alkaloids, flavonoids, and triterpenes. According to the results of research by Ifora (2019), showed that bioactive compounds from malur fruit contained in ethanol extracts such as steroids, alkaloids, flavonoids, phytosterols and fatty oils that have an effect on reducing total cholesterol levels, triglycerides, LDL, VLDL, and increasing HDL levels in the blood of white mice. The results of research from Udin (2010), showed that ethanol extract of malur fruit has breast anticancer bioactivity shown by LC50= 2.69 µ/mL against T747D breast cancer cells.

The solvent used is a solvent that can extract most of the desired secondary metabolites in simplisia [6]. Ethanol is an extracting solvent that has a good ability to attract almost all compounds. Hexane and acetone is a solvent that is very easy to remove oils contained in volatile seeds and leaves. Acetone dissolves many hydrophilic and lipophilic compound components from plants. Acetone can mix with volatile water and has low toxicity, used to extract phenolic compounds, tannins, and saponins

[19].

Based on the description above, the use of malur fruit extracts has been widely carried out but no research has been carried out on qualitative and quantitative analysis tests of ethanol, acetone and hexane extracts of malur fruit (*Brucea javanica* (L.) Merr). Therefore, researchers are interested in conducting qualitative and quantitative analysis research of ethanol, acetone and hexane extracts of malur fruit (*Brucea javanica* (L.) Merr).

II. EXPERIMENTATION

Tools

UV-Vis double beam spectrophotometer (Shimadzu UV-1800), analytical balance (Precisa), rotary evaporator (Buchi R 200), Microscope, ash free filter paper, volumetric flask (Iwaki), Wooden Tongs, weighing bottle (Iwaki), measuring cup (Iwaki) waterbath (Mammert), maceration vessel (Pyrex), oven (Mammert), Furnest (Carbolite-CWF1200), measuring pipette (Iwaki), sonicator (Branso 1800).

Material

Materials used in this study, malur fruit, ethanol 70 % (C₂H₅OH) (PT.Brataco), hexane (C₆H₁₄) (PT.Brataco), distilled water (H₂O) (PT Bratachem), hexanes, hydrochloric acid (HCl) (Merck), Chloroform (CHCl₃) (Merck), iron ammonium sulphate (Merck), ethyl acetate (C₄H₈O₂) (Merck), methanol (CH₃OH), gallic acid (C₆H₈O₇) (PT.Bratachem), sodium hydroxide (NaOH) (Merck), folic acid (C₆H₈O₇) (PT.Bratachem), (C₁₀H₅NaO₅S) (PT.Bratachem), acetone (C₃H₆O) (PT.Brataco), catechin (C₁₅H₁₄O₆) (PT.Bratachem), quercetin (C₁₅H₁₀O₇) (PT.Bratachem), zinc (Zn) powder (Merck), magnesium (Mg) powder (Merck), sodium acetate (CH₃COONa) (Merck), aluminium chloride (AlCl₃) (Merck).

Research procedures

The samples used in this study were malur fruit (*Brucea javanica* (L.) Merr) used in the plant, namely the fruit parts taken as much as 3 kg in the Jalan Telanai Pura area, Surau Gadang Village, Nanggalo District, Padang City, West Sumatra.

Process for Making Malur Fruit Simplicia

The preparation of malur fruit simplicia according to the Ministry of Health of the Republic of Indonesia (1985) is as follows:

- a. Material collection: ripe malur fruits, which are black in colour, are collected. Then washed with water until clean and drained to separate the washing water.
- b. Making simplicia: After properly cleaning and free residual of malur fruit under running water to get rid of any impurities, the malur fruit is drained, sliced into smaller pieces, and kept damp. Finally, the malur is pureed in a blender until simplicia powder is achieved.

Simplicia Standardization

The standardization of malur fruit (*Brucea javanica* (L.) Merr) simplicia according to the Ministry of Health of the Republic of Indonesia (2010) is as follows:

1. Organoleptic test
2. Microscopic test
3. Dry shrinkage
4. Total ash and insoluble acid ash
5. Water soluble simplicia
6. Ethanol soluble simplicia

Extract Preparation

The extract was made by maceration as much as 50 g of malur fruit simplicia was put into the macerator and added 70% ethanol solvent as much as 500 mL. then soaked for the first 6 hours while occasionally stirring, then allowed to stand for 18 hours. Maserat while separated by filtration using filter paper at least twice with the same type and amount of solvent. All the macerates are collected and evaporated with a vacuum evaporator or low pressure evaporator until a thick extract is obtained. Calculate the yield obtained, namely the weight percentage (b / b) between the yield and the weight of the simplicia powder used in weighing. Do the above with acetone and hexane solvents [5].

Qualitative Analysis with Phytochemical Screening

After obtaining malur fruit (*Brucea javanica* (L.) Merr) extracts from three solvents (ethanol, acetone and hexanes), Phytochemical Screening tests were carried out as follows:

1. Alkaloid Test

Extract 0.5 g add 1 mL of 2 N hydrochloric acid and 9 mL of water, heat on a water bath for 2 minutes, cool and filter. Transfer 3 drops of filtrate on a watch glass, add 2 drops of Bouchardat LP brown to black precipitate, then there is a possibility of alkaloids, 3 drops of filtrate add with mayer LP formed white or yellow clotted precipitate [6].

2. Phenol Test

1 g of malur extract was added to a solution of vanillin P 10% b/v in ethanol (90%) P add 1 drop of hydrochloric acid P the portion containing the phenol derivative is intensively coloured red [6].

3. Tannin Test

1 g of malur extract is added to iron (III) ammonium sulphate LP and a green or blue to black green colour is formed [6].

4. Flavonoid Test

Extract 0.5 g of the examined malur fruit was added with 10 mL of methanol P, heated using a counter-cooling device (reflux) for 10 minutes. Filter while hot through filter paper. Dilute the filtrate with 10 mL of water. After cooling, add 5 mL of petroleum ether, shake carefully, let stand. Take the methanol layer, evaporate at 40°C under pressure. The remainder is dissolved in 5 mL of ethyl acetate, filter. Evaporate to dryness 1 mL of the experimental solution, the remainder is dissolved in 1 mL to 2 mL of ethanol (95 %) P, add 0.5 g of zinc powder P and 2 mL of 2 N hydrochloric acid, let stand for 1 min. Add 10 drops of concentrated hydrochloric acid P, if within 2 to 5 minutes an intensive red colour occurs, indicating the presence of flavonoids[6].

After conducting phytochemical analysis tests on ethanol, acetone, hexane extracts of malur fruit, positive results were obtained containing flavanoid compounds, alkaloids, phenols, tannins contained in each extract. Then the determination of levels is carried out, as follows:

Determination of Phenol Content

Determination of phenol compound levels was carried out using the Folin- Ciocalteu method. Standard test solution was made in various concentrations obtained by dilution of gallic acid mother solution. Determination of total phenol content was measured using a UV-Vis spectrophotometer [7].

a. Comparison solution

Carefully weighed 10 mg of gallic acid, dissolved in a 100 mL flask with P methanol solvent, the volume was sufficient to the limit, so that a parent concentration of 100 µg/mL was obtained.

b. Determination of the maximum wavelength

Measurement of gallic acid solution concentration 40 µg/mL: pipetted 4 mL of 100 µg/mL gallic acid mother liquor put into 10 mL

flask add methanol P until the limit mark then homogenised, pipetted 1 mL put into vial add 5 mL Folin- Ciocalteu LP, keep still for 8 minutes, add 4 mL NaOH 1% incubate for 1 hour. Measure the absorbance with UV-Vis spectrophotometer at a concentration of 40 µg/mL and determine the maximum wavelength of gallic acid.

c. Determination of calibration curve

From the parent solution of gallic acid 100 µg/mL, various concentrations of 20; 30; 40; 50; 60 mL were made by pipetting the parent solution as much as 2; 3; 4; 5; 6 mL into a 10 mL flask, adding methanol P until the limit mark and then homogenised. From each concentration pipetted 1 mL put into the vial add 5 mL Folin- Ciocalteu LP (7.5% in water), let stand for 8 minutes, add 4 mL of 1% NaOH incubate for 1 hour. Measure the absorbance at the wavelength of gallic acid with a UV-Vis spectrophotometer and draw a calibration curve so that the linear regression equation can be calculated.

d. Test solution

Weighed carefully 10 mg of hexane extract, dissolved in a flask 10 mL with P methanol solvent, the volume is sufficient to the limit mark, so as to obtain a parent concentration of 1000 µg/mL. Pipetted from the test solution 1 mL put into a vial add 5 mL Folin- Ciocalteu LP (7.5% in water), let stand for 8 minutes, add 4 mL NaOH 1% incubate for 1 hour. Measure the absorbance at the maximum wavelength of gallic acid with a UV-Vis spectrophotometer.

Determination of Alkaloid Content

Determination of total alkaloid levels was carried out by the Gravimetric method [7,8].

Weigh approximately 2 g of acetone, ethanol extracts of malur fruit using 100 mL of methanol P and 10 mL of ammonia P, heat on a water bath for 2 hours.

30 minutes, filter. Repeat 2 times using the same type and amount of solvent. Add 50 mL of 1 N hydrochloric acid LP and collect the filtrate, evaporate to a volume of approximately 25 mL, filter into a separatory funnel. Saturate the filtrate with ammonia P to pH ± 10 using pH indicator, extract 3 times with 25 mL chloroform P. Collect and evaporate the chloroform phase at 50 °C, then dry at 100 °C until the weight remains. Calculate the drying residue as total alkaloids.

Determination of Flavonoid Content

Determination of total flavonoid content was measured using a UV-Vis spectrophotometer according to [8]

a. Comparison solution

Weigh 10 mg of quercetin comparator, then dissolve it with 80% ethanol until the limit mark in a 10 mL volumetric flask to obtain a concentration of 1000 µg/mL.

b. Determination of the maximum wavelength

Measurement solution quercetin 50 µg/mL concentration: Pipette 0.5 mL of 1000 µg/mL quercetin mother liquor into a 10 mL flask add 80% ethanol to the mark. limit then homogenised, pipetted 0.5 mL into the vial, add 1.5 mL ethanol, 0.1 mL 10% P aluminium chloride, 1 mL 1 M sodium acetate, and 2.8 mL distilled water. Let stand for 30 minutes. Measure the absorbance with a UV-Vis spectrophotometer at a concentration of 50 µg/mL and determine the maximum wavelength of quercetin.

c. Determination of calibration curve

From the mother liquor of 1000 µg/mL concentration, various concentrations of 30, 40, 50, 60, and 70 (µg/mL) were prepared by pipetting the parent solution as much as 0.3; 0.4; 0.5; 0.6; 0.7 mL put into a 10 mL flask add 80% ethanol until the limit mark then homogenised. From each concentration, 0.5 mL was pipetted into different vials, then add 1.5 mL of ethanol P, 0.1 mL of aluminium chloride, .1 mL of sodium acetate. 1 M and 2.8 distilled water. Stand still for 30 min. Measure the maximum wavelength absorbance of quercetin with a UV-Vis spectrophotometer.

d. Test solution

Weigh 10 mg of extract, then dissolve it with 80% ethanol until the limit mark in a 10 mL volumetric flask so that a concentration of 1000 µg/mL is obtained. 0.5 mL of test solution was pipetted into the vial, add 1.5 mL of ethanol P, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate and 2.8 distilled water. Stand still for 30 minutes. Measure the maximum wavelength absorbance of quercetin with a UV-Vis spectrophotometer.

III. RESULT AND DISCUSSION

Simplicia Standardization

Macroscopic examination of the test results obtained is where the malur fruit simplicia has a

powder form, very bitter taste, brownish colour, slightly sour smell microscopic examination of the results obtained the results of cells containing prism-shaped calcium oxalate, mesocarp, wood vessels. ±Drying shrinkage of malur fruit (Bruceajavanica(L.)Merr) simplicia was 2.6605% ± 0.2003%. Total ash content of malur fruit (Bruceajavanica(L.) Merr) simplicia was 1.7340% ± 0.00866%. The water soluble compound content of malur fruit (Bruceajavanica(L.)Merr) simplicia was 22.5534% ± 0.6485%. The content of compounds soluble in ethanol of malur fruit simplicia (Bruceajavanica(L.)Merr) was 27.7397% ± 0.0142%. The yield of malur fruit (Bruceajavanica(L.)Merr) simplicia extract. The ethanol extract yield obtained was 62.7602%, the acetone extract yield was 37.1024% and the hexane extract yield was 26.6984%.

The results of qualitative analysis on ethanol extract of malur fruit showed positive results containing flavonoid compounds, tannins, alkaloids. The hexane extract of malur fruit (Bruceajavanica(L.) Merr) showed positive results containing phenols and alkaloids. From the results of research on the content of chemical compounds in ethanol and acetone extracts of malur fruit (Bruceajavanica(L.) Merr), namely flavonoids, alkaloids and tannins, this is indicated by flavonoid testing showing positive results with a positive result.

Red or orange-yellow colour changes indicate the formation of flavylum salts upon addition of magnesium powder and hydrochloric acid [18].

While the alkaloid test, the extract solution is made then added 1 mL of HCl 2 N and 9 mL of water. The purpose of adding HCl 2 N to attract alkaloid compounds in the extract because alkaloids are alkaline so with the addition of acids such as HCl will form salts, so that alkaloids will separate with other components of plant cells that participate in the extract by distributing them to the acid phase [11]. After that, heating for 2 minutes on a water bath then cooled and filtered and then pipetted three drops of filtrate and entered in a test tube and then reacted with Mayer and Bouchardat reagents.

In testing alkaloids with reagent Bouchardat obtained positive results are characterised by the formation of a brown to black precipitate. Based on what is described by Fansworth (1966), that the addition of Bouchardat reagent will show a brown to black precipitate. So it can be said that ethanol extract and hexane extract

of malur fruit (*Bruceajavanica*(L.) Merr) contain alkaloid compounds.

The phenol test showed positive results marked by the formation of an intensive red colour with the addition of vanillin P 10%. In the tannin test, positive results were obtained with the addition of iron (III) ammoniusulfate. Marked with the formation of green or blue to black colour.the formation of intensive red colour with the addition of vanillin P 10%. In the tannin test, positive results were obtained with the addition of iron (III)

ammoniusulfate. marked with the formation of green or blue to black colour.

Quantitative Analysis of Ethanol,Acetone, Hexane Extracts of Malur Fruit (*Bruceajavanica*(L.)Merr)

Determination of Phenol Content

Determination of Maximum Absorption WavelengthSolutionAcidGallic acid solutionwith a concentration of 40 µg/mL can be seen in **Figure 1**.

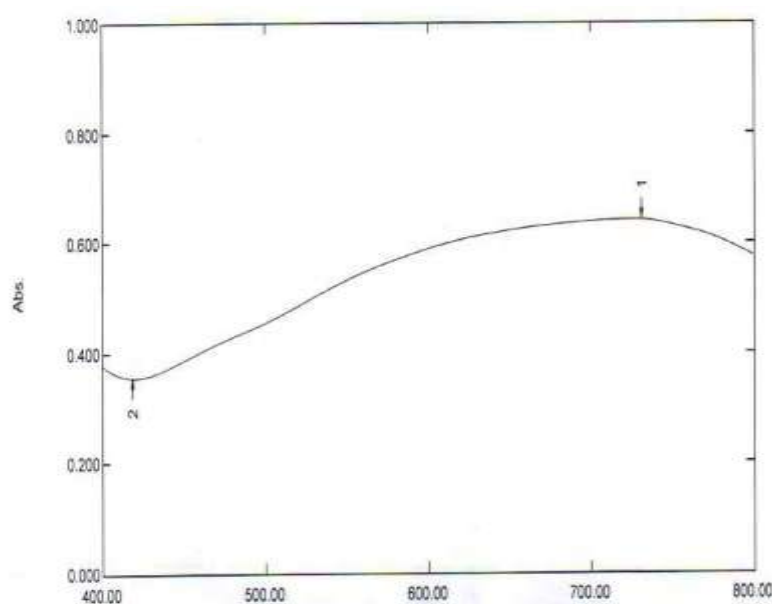


Figure 1.Maximum Wavelength Spectrum of Gallic Acid with a Wavelength of 733.50 nm.

Concentration and absorbance data of Gallic Acid with a wavelength of 733.50 nm can be seen in **Table 1** and **Figure 2**

Concentration (µg/mL)	Absorbance
20	0,247
30	0,361
40	0,475
50	0,578
60	0,698

Concentration and Absorbance Data of Gallic Acid with a Wavelength of 733.50 nm.

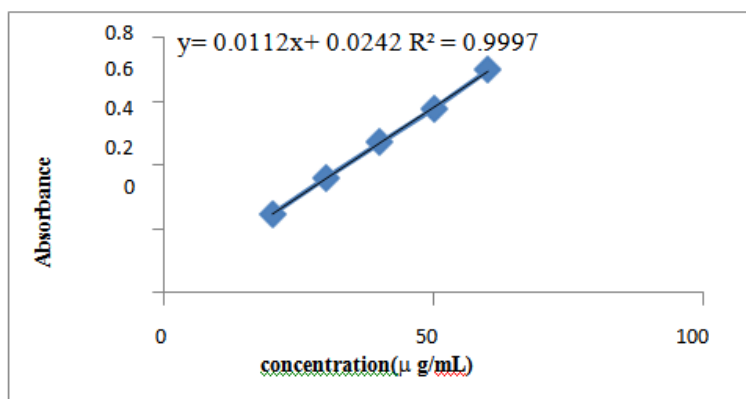


Figure 2.Gallic acid curve

Determination of phenol content in hexane extract was carried out using UV-Vis spectrophotometer method used in this study is double beam spectrophotometry. In the determination of phenol content using Folin-Ciocalteu reagent. Folin-Ciocalteu reagent is used because phenolic compounds can react with Folin to form a coloured solution that can be measured absorbance. The principle of measuring phenolic content with Folin-Ciocalteu reagent is the formation of a blue complex compound that can be measured at a wavelength of 733 nm. This reagent oxidises phenolics (alkali salts) or the phenolic-hydroxy group reduces the heteropoly acid (phosphomolybdate-phosphotungstate) contained in the Folin- Ciocalteu reagent into a molybdenum-tungsten complex.

Phenolic compounds react with Folin-Ciocalteu reagent only in an alkaline atmosphere in order to dissociate protons on phenolic compounds into phenolic ions. To create alkaline conditions used NaOH 1%. The blue colour formed will be more intense, equivalent to the concentration of phenolic ions formed; meaning that the greater the

concentration of phenolic compounds, the more phenolic ions that will reduce heteropoly acid (phosphomolybdate-phosphotungstat) into molybdenum-tungsten complex so that the blue colour produced is more intense [2].

Gallic acid is used as a measurement standard because gallic acid is a derivative of hydroxybenzoic acid which is classified as a simple phenolic acid [16].

In the results of determining the total phenol content in the hexane extract of malur fruit, with gallic acid as a comparison. Results were obtained at a wavelength of 733 nm at a concentration of 40 ppm with an absorbance of 0.424 (Figure 1). And calibration curve of gallic acid solution with FolinCiocalteu reagent and absorbance of gallic acid solution with various concentrations can be seen in (Figure 2, Table 1).

Measurement of total phenol content was carried out by UV-Vis spectrophotometry which was expressed as gallic acid with the regression equation $Y = 0.01119X + 0.0242$ so that the total phenol content of hexane extract was 6.1366%.

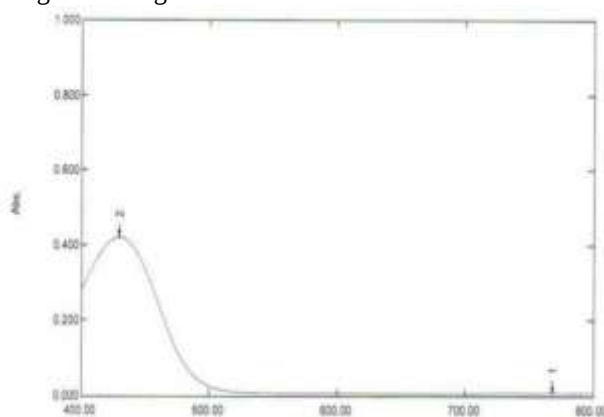


Figure 2.Maximum wavelength spectrum of quercetin with a wavelength of 429.50 nm.

Concentration and Absorbance Data of Quercetin with a Wavelength of 429.50 nm can be seen in **Table 2** and **Figure 3**.

Concentration (µg/mL)	Absorbance
30	0,256
40	0,361
50	0,455
60	0,561
70	0,664

Concentration and Absorbance Data of Quercetin with a Wavelength of 429.50 nm.

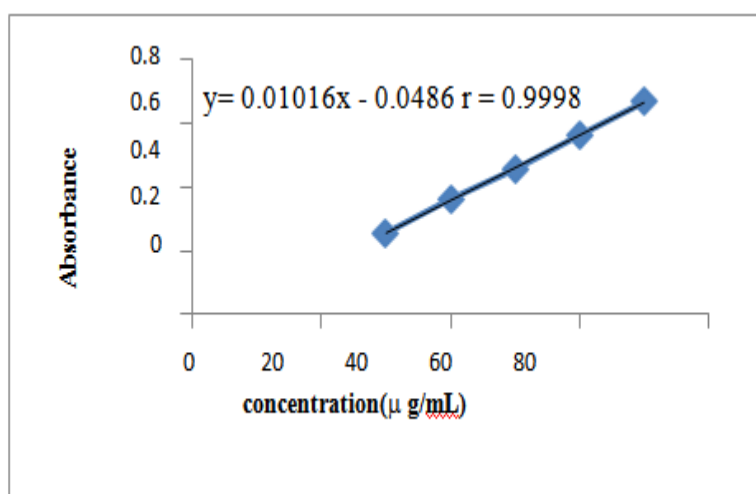


Figure 3.Quercetin Calibration Curve.

Determination of total flavonoid levels in malur fruit using UV-Vis spectrophotometric method calculated as quercetin. With the principle of measuring light absorption by molecules that are directly proportional to the concentration of the sample solution [9]. The addition of ethanol P serves as an enhancer of solubility, aluminium chloride P which serves to provide a batokramik effect by shifting towards longer wavelengths, thus changing the wavelength of the longer wavelength, so as to change the wavelength of the standard quercetin to enter into the UV-Vis wavelength range, resulting in also hyperchromic effect or increase in the intensity of the standard solution of quercetin produces a more yellow colour [4].

From the results of determining the total flavonoid content of each malur fruit extract with quercetin as a comparison, a wavelength of 429.50 nm was obtained. at a concentration of 50 ppm with an absorbance of 0.419 (Figure 3) with the regression equation $Y = 0.01016X + 0.0486$ and the calibration curve of quercetin solution in ethanol 80% and absorbance of quercetin solution with various concentrations can be seen in (Figure 3, Table 2).

Measurement of total flavonoid levels using UV-Vis spectrophotometry expressed in quercetin with the regression equation $Y = 0.01016X + 0.0486$ obtained results for ethanol extract of malur fruit is 6.03% and for acetone extract 6.5266%.

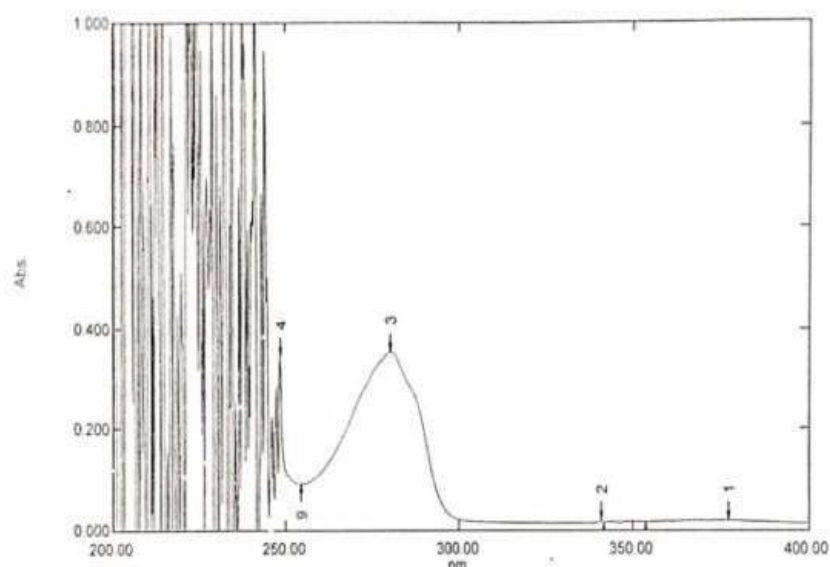


Figure 4.Maximum wavelength spectrum of catechins with a wavelength of 280 nm.

The maximum wavelength of catechins can be seen in **Table 3**

No.	Maximum Wavelength (nm)	Uptake
1.	280 nm	0,421

Table 3.Maximum wavelength of catechins

Determination of tannin content was carried out by UV-Vis spectrophotometric method using catechin standard compounds. To determine the total tannin content. In this method, there was no determination of operating time and making of calibration curve because the determination of tannin content was not carried out.

The maximum wavelength of catechin is only based on the comparison of the absorbance of standard catechin, blank solution and test sample. From the measurement of the test solution, the maximum wavelength was 280 nm with an absorbance of 0.421. So that the total tannin content in ethanol extract is 1.1225%. The results of the spectrum of catechin solution in ethyl acetate can be seen in (Figure 4, Table 3).

Determination of Alkaloid Content

The determination of alkaloid levels using the gravimetric method is based on the measurement of weight changes after the heating process [21].

This method is very simple and easy to do, also the results obtained are specific and accurate [1]. Determination of alkaloid content is calculated from the results dried at a fixed temperature.

The addition of ammonia aims to release the alkaloid bond with the acid so that the alkaloid

is back in free condition because ammonia will bind to hydrochloric acid which forms a salt that dissolves in water while alkaloids in free condition are alkaline and not dissolved in water. With the addition of chloroform, two layers will be formed, namely the top layer of acid while the bottom layer is chloroform.

Alkaloids in the extract will react with NH_3 and attract H^+ and form free alkaloids in chloroform while ammonia will separate in another phase and then evaporated to produce a residue which is the total alkaloid. Based on the calculation of hexane extract of malur fruit, the alkaloid content is $3.5293\% \pm 0.0678$ and the alkaloid content of ethanol extract of malur fruit (*Bruceajavanica*(L.) Merr) is 4.0156%.

IV. CONCLUSION

Based on the research that has been done on qualitative and quantitative analysis of the content of chemical compounds from ethanol, acetone, hexane extracts of malur fruit, it can be concluded that:

1. The content of secondary metabolite compounds contained in the ethanol extract of malur fruit contains positive compounds, namely alkaloids, tannins and flavanoids. Acetone extract contains flavonoid

compounds. The hexane extract contains positive compounds, namely alkaloids and phenols.

2. Result Alkaloid content of hexane extract of malur fruit (*Bruceajavanica*(L.) Merr) was $3.5293\% \pm 0.00678$ and the alkaloid content of ethanol extract of malur fruit (*Bruceajavanica*(L.) Merr) was $4.0156\% \pm 0.0336$. The average phenol content of hexane extract of malur fruit (*Bruceajavanica*(L.) Merr) was $6.1366\% \pm 0.0757$. The average flavonoid content of ethanol extract of malur fruit (*Bruceajavanica*(L.) Merr) was $6.03\% \pm 0.1783$ and the average flavonoid content of acetone extract of malur fruit (*Bruceajavanica*(L.) Merr) was $6.5266\% \pm 0.7744$. The tannin content of ethanol extract of malur fruit (*Bruceajavanica*(L.) Merr) was $1.1225\% \pm 0.10028$.

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