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Quantitative Measurement of Flavonoid Content of Kecapi Leaves *(Sondoricum koetjape Merr.)*

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Abstract
The kecapi leaf plant *(Sondoricum koetjape Merr.)* is a plant that has efficacy as a traditional medicine such as trating roundworms reducing fever. The kecapi leaf plant has been known to contain flavonoid, phenol, tannin and saponin compounds with properties as free radical scavengers. This study aims to determine the total flavonoid content of kecapi leaf extract. This study is experimental in nature. The sample used was kecapi leaves originating from Ckalahi Village, Cinangka Anyer District, Serang Regency, Banten. Determination of total flavonoid content was carried out using a UV-Vis Spectrophotometer. The result of this study obtained flavonoid levels in mg/L units of 5.4845 mg/L, while the flavonoid concentration in percent (%) units was obtained of 10.97% and in mg QE/g units the total flavonoid obtained were 109.6899 mg QE/g, while the qualitative test indicated that it contained flavonoid, phenol, tannin and saponin compounds.

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INTRODUCTION

Flavonoids are natural coumpounds with varying phenolic structures found in bark, stems and leaves. Flavonoids are also considered essential components in various application, such as pharmaceuticals, medicine and cosmetics. This is due to their antioxidant, anti inflammatory, anti mutagenic and anti carcinogenic properties, as well their capacity to modulate the function of key cellular enzymes (Panche dkk., 2016).

One of the plants utilized by the community is the kecapi leaf *(Sondoricum koetjape Merr.)*, the bark of which is used in the treatment of round-worms, while a decoction of the leaves is believed to be effective in reducing fever. This pharma-cological potential is closely related to its phyto-chemical content (Taibatul dkk., 2022) in addition to flavonoids containing strong aromatic groups in spectrophotometry (Natanael dkk., 2025).

Based on research (Susy Saadah dkk., 2020) kecapi leaves have various pharmacological activities supported by the presence of phytochemical compounds such as flavonoids, phenols, tannins, triterpenoid alkaloids, alkaloids, saponins and quinones. In the qualitative test of flavonoids, phenols, tannins, saponins, alkaloids, triterpenoids were indicated while the alkaloid and quinone tests were not indicated and the total phenol content in kecapi leaves was 3.1469 mg/g and the stems were 1.4155 mg/g but until now, there has been no research that measures flavonoid levels quantitative analysis of the flavonoid content.

The flavonoids used in this study are the largest flavonol group quercetin, which accounts for 60%-75% of the total flavonoids. Quercetin is believed to protect the body from several types of degenerative diseases. Quercetin has been shown to prevent the oxidation process of Low Density Lipoproteins (LDL) by scavenging free radicals. Scientists have conducted research on the flavonoid content of various plants, such as onion and broccoli, as well as fruits such as cherries, apples ad berries (Rahmah dkk., 2024)

METHOD

This research was conducted experimentally in the laboratory. The kecapi leaves used in this

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study were leaves that were not damaged or had holes due to past attacks. The kecapi leaves were first washed, then dried for 14 days and extracted using the soxhletation method. This extraction method was chosen to produce a larger amount of extract and is efficient in solvent use because it requires a smaller volume, in addition, the extraction process is faster and the simple can be extracted optimally because the solvent is continuously applied (Eva dkk., 2018).

Equipment Used

Complete set of Soxhlet extraction equipment (two-neck flask, heating mantle, condenser stand, clamp, hoses for inlet and outlet water lines, T-tube, Erlenmeyer flask), thermometer, test tube, dropper, 50 mL volumetric flask, 5 mL volumetric pipette, 1 mL syringe pipette, rotary evaporator, 100 mL volumetric flask, analytical balance, spray bottle, 10 mL cuvette, UV-Vis spectrophotometer (Metler Toledo UV 5), plastic spoon, 100 mL beaker glass.

Materials Used

Ethanol 96% p.a Merck, $FeCL_3.6H_2O$ p.a. Supelco, kecapi leaves, distilled water, quercetin p.a. Sigma Alderich, $AlCl_3$ p.a Merck and CH_3COONa p.a Merck.

Making Kecapi Leaf Extract Using the Soxhlet Method

20 g of kecapi leaf powder was weighed, wrapped in filter paper and the ends were tied to be inserted into a Soxhlet tube to ensure proper extraction. This Soxhlet extraction process was carried out for 3 days until the resulting extract solution was clear, indicating that the active compounds from the kecapi leaves had been maximally extracted.

Qualitative Phytochemical Testing

Based on the method (Purnamasari dkk., 2022)

Flavonoid Test

The kecapi leaf extract was added to 2 mL of 2% NaOH solution and then 10 drops of H_2SO_4. If flavonoids are present, a color change to colorless will occur.

Phenol and Tannin Test

The kecapi leaf extract was added to 2 mL of 3% $FeCl_3$ solution. If phenols and tannins are present, a color change from blue green to black will occur.

Saponin Test

The kecapi leaf extract was added into 5 mL of distilled water, then shaken vigorously. Stable foam will form in saponins present.

Quantitative Flavonoid Testing

Preparation of Quercetin Standard Solution

A quercetin stock solution with a concentration of 1000 ppm was prepared by dissolving 0.05 g of quercetin in 96% ethanol to a volume of 50 mL. This was then diluted to 40 ppm, 80 ppm, 120 ppm, 160 ppm and 200 ppm with 0.5 mL of each concentration. Next, 1.5 mL of 96% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa and 2.80 mL of distilled water were added. The mixture was homogenized and allowed to stand for 10 minutes, after which it was analyzed using a UV-Vis spectrophotometer (Cheng dkk., 2002).

Determination of Maximum Wavelength for Flavonoid Analysis

One prepared standard solution concentration was used to determine the maximum wavelength. The concentration chosen was 120 ppm because it is in the middle range. The maximum wavelength was determined by measuring at a wavelength of 400-500 nm using a UV-Vis spectrophotometer. The maximum wavelength indicates a high level of absorbance (Handayani., 2024).

Determined Optimal Incubation Time for Flavonoid Analysis

The solution components to be tested were the same as those used for determining the maximum wavelength, but five measuring tubes were prepared with an incubation time of 30 minutes. The wavelength used was the maximum wavelength obtained from the previous step (Hanafis dkk., 2023).

Quercetin Standard Curve Preparation

The standard curve was created by connecting the absorbance measurements at the maximum wavelength (absorbance on the ordinate and quercetin concentration on the abscissa) with the concentration of the standard solution (Handayani., 2024).

Quantitative Analysis of Total Flavonoids

The analysis used a calorimetric method with aluminum chloride according to the procedure (Cheng dkk., 2002) 10 mg ethanol extract of kecapi leaves was weighed and dissolved in 10 mL of ethanol p.a. (1000 ppm). Then, 0.5 mL of ethanol pa, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.80 mL of distilled water were added to the pipette. After the mixture was shaken until homogeneous, mixed thoroughly, and allowed to stand for 30 minutes. Then, an ultraviolet-visible (UV-Vis) spectrophotometer with a maximum wavelength was used to measure the absorbance.

Each sample was tested in triplicate. The standard curve equation of quercetin was used to determine the sample concentration in the total flavonoid test based on the absorbance obtained.

RESULTS AND DISCUSSION

Preparation of Kecapi leaf Kecapi Leaf Extract Using the Soxhlation Method

Soxhlation is a heat extraction method where the extraction is continuous, involving refrigeration using a temperature based on the solvent's boiling point. In this soxhlation method, the separation of components in herbal medicine involves repeated filtration. The end point of the Soxhlet method is when the extract drops become colorless, indicating that all compounds in the extract have been maximally extracted.

The purpose of this soxhlation method is to help extract compounds that are insoluble at room temperature, thus freeing low molecular weight compounds from high molecular weight polymer molecular subunits, maximizing the extraction activity of the compounds during the extraction process (Taufiqurrahman., 2017). In this study, the Kecapi leaf extraction process used the soxhlation method for 3 days until the resulting extract solution was clear, as shown in Table 1.

Table 1. Preparation of Kecapi Leaf Extract by Soxhlation

Before Circulation	After Circulation	Information
		Day 1 The extract obtained was 100 mL.
		Day 2 The extract obtained was 225 mL.
		Day 3 The extract obtained was 175 mL.

The heat process in this soxhlation does not make the compound easily evaporate, because it uses an indirect heat process, where the solvent in the flask will experience evaporation and then go to the condenser where the condensation process occurs. The resulting extract is concentrated using a rotary evaporator to obtain a thicker extract. The evaporation process on this kecapi leaf extract produces 47 ml of thick extract which is carried out at a temperature of 46 °C according to the boiling point of ethanol to ensure that the active compounds are not damaged by high temperatures. After the soxhletization process was completed, the kecapi leaf extract was

evaporated using a rotary evaporator to separate the ethanol solvent and obtain a thicker extract. The evaporation process was carried out at 46 °C, adjusted to the boiling point of ethanol to prevent the active compounds from being damaged by the high temperature. The result of this process was a thick extract of 47 mL.

Quantitative Flavonoid Testing of Kecapi Leaf Extract

The maximum wavelength in this study was used to determine the wavelength at which flavonoid compounds produce the highest absorbance. A standard quercetin solution with a concentration of 120 ppm was used because it is in the middle of the standard series and is considered representative of the other standard series. This solution was reacted with 1.5 mL of 96% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa and 2.80 mL of distilled water, then incubated for 30 minutes. Measurements were carried out using a UV-Vis spectrophotometer in the wavelength range of 400–550 nm (Handayani., 2024). The measurement results showed that the quercetin solution provided maximum absorbance at a maximum wavelength (λ) of 428 nm. The results of the absorbance measurement of the standard quercetin solution are presented in Table 2 then used to create a standard series curve of quercetin which is shown in Figure 1.

Table 2. Standard Series Data of Quercetin

No.	Concentration (ppm)	Absorbance
1.	40	0.4843
2.	80	0.7389
3.	120	1.1041
4.	160	1.4657
5.	200	1.8366

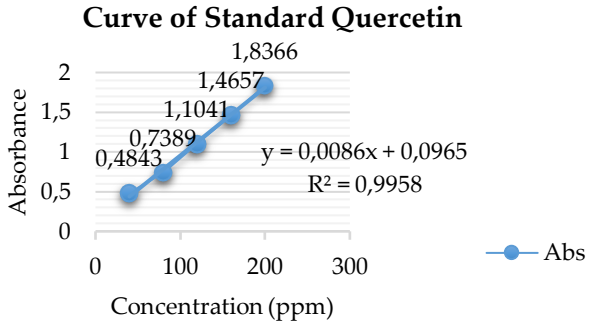


Figure 1. Quarcetin Standard Curve

This standard curve was then used as a reference for determining flavonoid levels in the samples. The measurement data for the kecapi leaf extract samples are presented in Table 3.

Table 3. Standard Series Data of Quercetin

Repeat	Absorbance	Mean Absorbance	Standard Deviation
1.	0.1327		
2.	0.1479	0.1437	0.00958
3.	0.1504		

The formula used in measuring the absorbance of quercetin solutions in units of mg/L, % and mg QE/g refers to (Din dkk., 2021) as follows.

$$\text{Flavonoid}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{(y - a)}{b}$$

$$\text{Flavonoid}\ (\text{mg}\frac{\text{QE}}{\text{g}}) = \frac{\text{C}\ (\frac{\text{mg}}{\text{L}})\ \text{x V x Fp}}{\text{W}}$$

$$\text{Flavonoid}\ (\%\frac{\text{b}}{\text{b}}) = \frac{\text{C}\ (\text{mg}\frac{\text{QE}}{\text{g}})}{10}$$

The results of the absorbance measurements of the quercetin solution and the concentration calculated in mg/L units are described in Table 4 in mg QE/g units.

Table 4. Total Flavonoid Analysis in mg/L

Repeat	Concentration (mg/L)	Average Concentration (mg/L)
1.	4.2093	
2.	5.9767	5.4845
3.	6.2674	

In units of mg QE/g the total flavonoid results are described in Table 5.

Table 5. Total Flavonoid Analysis in mg QE/g

Repeat	Concentration (mg/L)	Average Concentration (mg/L)
1.	84.1860	
2.	119.5349	109.6899
3.	125.3488	

The percentage units (% w/w) of the total flavonoid analysis results are described in Table 6.

Table 6. Total Flavonoid Analysis in %(w/w)

Repeat	Concentration (mg/L)	Average Concentration (mg/L)
1.	8.42	
2.	11.95	10.97
3.	12.53	

Based on the standard series curve of quercetin shown in Figure 1, a linear regression equation is obtained y = 0.0086x (slope) (b) + 0.0965 (intercept) (a) with a coefficient of determination value of R^2 = 0.9958. The R^2 value approaching 1 indicates a good linear relationship between quercetin concentration and absorbance value so that the standard curve is suitable for use in calculating total flavonoid levels in kecapi leaf extract. The absorbance measurements of the kecapi leaf

extract were carried out three times, and the absorbance values are described in Table 3, with absorbance values of (0.1327); (0.1479); (0.1504). Based on these data, the average absorbance value was 0.1437 with a standard deviation of 0.0095. A standard deviation value <1 indicates that the resulting curve is increasingly linear, so the standard deviation obtained is quite precise (Handayani., 2020).

The absorbance values of the kecapi leaf extract are shown in Table 4. The flavonoid concentrations in mg/L were (4.2093); (5.9767); (6.2674) with an average concentration value of 5.4845 mg/L. The variation in concentration obtained was influenced by differences in absorbance values. The flavonoid concentrations were converted into mg QE/g units, as d in Table 5, obtained as (84.1860); (1 (125.3488) with an average value of 109. QE/g while in percent units (% w/w) desc Table 6 obtained as (8.42%); (11.95%); with an average concentration value in (%) of 10.97%. The total flavonoid value c leaf extract in mg QE/g units is greater than the flavonoid value in torch ginger leaf extract which is 88.11 mg QE/g (Renditya., 2025) while, in Ficus deltoidei leaf extract the total flavonoid value is 761.32 mg QE/g (Din dkk., 2021) which is greater than the flavonoid value of kecapi leaf extract.

Based on the results obtained from the data above, the extraction of kecapi leaves was carried out using the soxhlet method for 3 days until the extract that came out of the soxhlet apparatus was clear, indicating that the active compounds contained in kecapi leaves had been maximally extracted, then the extraction results were evaporated using a rotary evaporator to obtain a thick extract so that 47 ml of kecapi leaf extract was obtained.

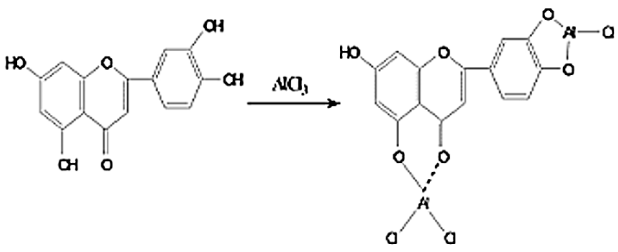


Figure 2. Flavonoid-$AlCl_3$ Complex Formation Reaction (Aliffia dkk., 2021)

The quantitative flavonoid test was obtained at 5.4845 mg/L; 10.97%; 109.6899 mg QE/g of flavonoid content in kecapi leaves was carried out quantitatively using the UV-Vis Spectrophotometer method, namely using $AlCl_3$ solution as a stable complex former with C-4 keto groups, C-3 or C-5 hydroxyl groups of flavones and flavanols (Aliffia dkk., 2021) as described in Figure 2.

Qualitative Flavonoid Testing of Kecapi Leaf Extract

A qualitative flavonoid test is performed by adding NaOH, which produces a yellow color. This yellow color occurs because flavonoid compounds, especially flavones or flavanols, react with the base, causing their structure to break down. This process causes the bonds in the isoprene structure to break, resulting in the formation of derivative compounds such as acetophenone, which gives the yellow color (Aliffia dkk., 2021) as shown in Figure 3.

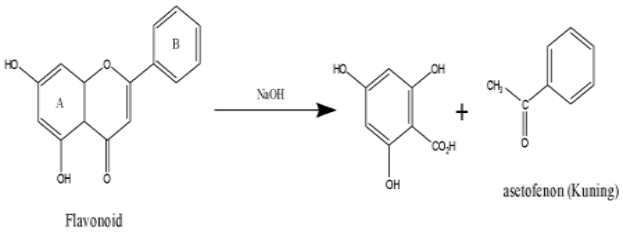


Figure 3. Reaction of Flavonoids with NaOH (Aliffia dkk., 2021)

The flavonoid test for the kecapi leaf extract showed a color change from brown to pale yellow after the addition of the reagent. This color change indicates a positive result, indicating that the kecapi leaf extract contains flavonoid compounds (Purnamasari dkk., 2022) as shown in Table 7.

Table 7. Flavonoid Level Test in Kecapi Leaf Extract

Before adding the reagent	After adding the reagent
	

Flavonoids have a low molecular weight based on the 2-phenylchromone nucleus which is biosynthesized from acetic acid or phenylalanine derivatives using the shikimate pathway. Flavonoids are classified by the degree of oxidation, the annularity of the C ring and the connection position of the B ring. The following classification of flavonoids is shown in Figure 4 as follows (Kaur dkk., 2021).

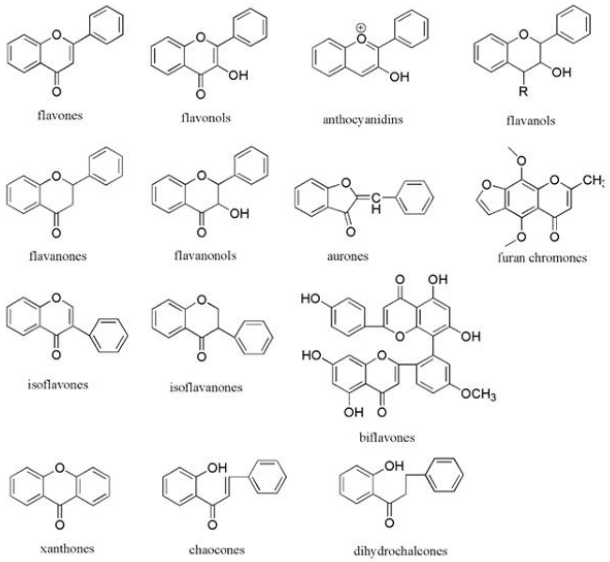


Figure 4. Flavonoid Classification (Arifin., 2018)

The pharmacological activity of kecapi leaf extract is influenced by the presence of active compounds, flavonoids and several other phenolic compounds. Flavonoids also act as antioxidants by donating hydrogen atoms or through their ability to chelate metals. They exist in the form of glucosides containing glucoside side chains, or in their free form, called aglycones (Sari dkk., 2022).

Phenol and Tannin Test on Kecapi Leaf Extract

The phenol test uses $FeCl_3$ reagent to detect the presence of phenolic groups in the sample. The results of the phenol and tannin tests on kecapi leaf extract showed a color change to black after the addition of $FeCl_3$ reagent, resulting in a positive (Purnamasari dkk., 2022). The black color occurs because the compounds in the extract react with Fe^{3+} ions to form a complex compound, as shown in Figure 5.

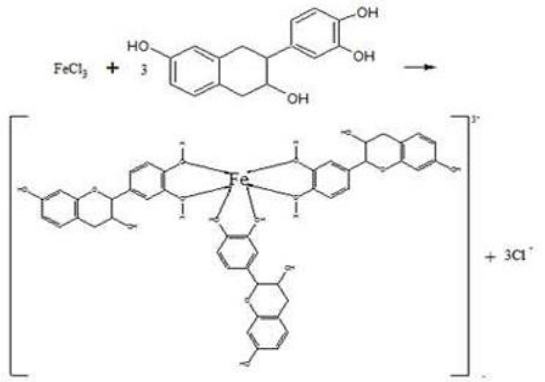


Figure 5. Reaction between Tannin and FeCl (Elsa., 2021)

If the $FeCl_3$ test shows a positive result, the sample likely contains phenolic compounds, including tannins, which are part of the polyphenol group. Furthermore, kecapi leaf extract contains polyphenolic compounds suspected to be tannins. Tannins have oxygen atoms with lone electron pairs, allowing them to bond with the Fe^{3+} ion as the central atom. In this reaction, one Fe^{3+} ion can bind to three tannin molecules. The oxygen atoms in the dihydroxy groups at positions 4' and 5' act as the most stable electron donors, thus forming a coordinate bond with Fe^{3+}, this interaction causes the formation of a black complex compound (Elsa., 2021). The color change indicates a positive result, that the kecapi leaves contain phenol and tannin compounds as shown in Table 8.

Table 8. Results of Phenol and Tannin Tests on Kecapi Leaf Extract

Before adding the reagent	After adding the reagent
	

Based on research (Susy Saadah dkk., 2020) the total phenolic content in kecapi leaves was found to be 3.1469 mg/g using a colorimetric method with Folin-Ciocalteu reagent. In this procedure, phenolic cosmpounds react to producing a blue complex, which is then measured using a spectrophotometer at a wavelength of 730 nm. The structure of this phenol is shown in Figure 6

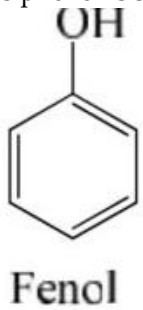


Figure 6. Phenol Structure (Aisam., 2017)

Tannins are phenolic compounds with large molecules consisting of hydroxyl groups that effectively form strong complexes with proteins and several macromolecules. Tannins cause some plants to have a bitter taste. Chemically, condensed tannins occur due to polymerization

reactions between flavonoids. The structure of tannins is shown in Figure 7 (Hidjarawan., 2018).

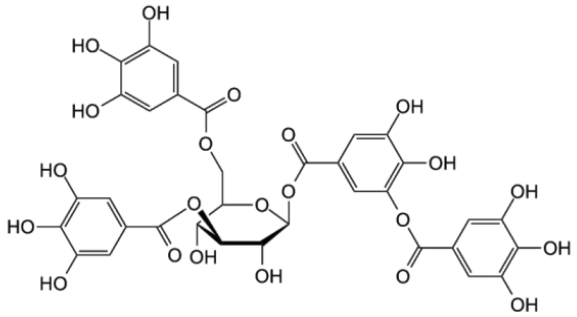


Figure 7. Tannin Structure (Hidjarawan., 2018)

Qualitative Saponin Test on Kecapi Leaf Extract

The results of the saponin test on kecapi leaf extract showed a color change and the formation of stable foam after shaking (Purnamasari dkk., 2022). The formation of this foam indicates the presence of glycosides, which can form foam in water and hydrolyze into glucose, as shown in Figure 8 (Hadi & Intan., 2019)

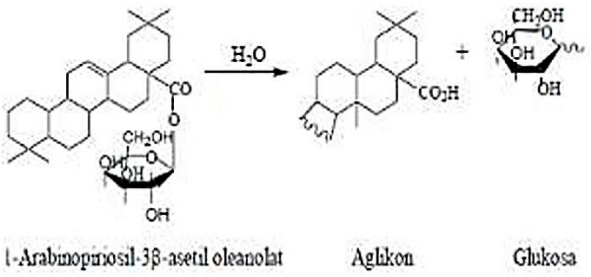


Figure 8. Hydrolysis Reaction of Saponin with Water (Hadi & Intan., 2019)

These changes indicate positive results indicating that the kecapi leaf extract contains saponin compounds as shown in Table 9.

Table 9. Saponin Test Results on Kecapi Leaf Extract

Before adding the reagent	After adding the reagent
	

The name saponin is derived from the Latin word "Sapo," meaning soap. Saponins are a complex group of natural compounds with large molecular weights, as shown in Figure 9. The structure of saponins can act as soaps, making them known as natural surfactants (Calabria., 2008).

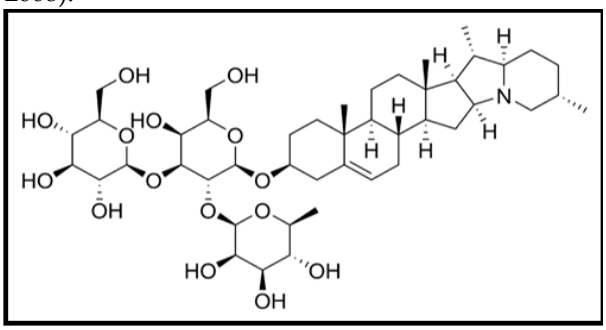


Figure 9. Saponin Structure (Budi., 2016)

CONCLUSION

From the research that has been done, it can be concluded that the standard series curve of quercetin obtained a linear regression equation y = 0.0086x + 0.0965; R^2 determination of 0.9958; the average absorbance of kecapi leaf extract was 0.1437; the standard deviation was 0.00958 so that the concentration of flavonoids of kecapi leaf extract was obtained in mg/L units of (4.2093), (5.9767), (6.2674) with an average concentration value of 5.4845 mg/L while, the concentration of flavonoids in percent units (%w/w) was obtained at (8.42), (11.95), (12.53) with an average concentration in percent (%) of 10.97% and in mg QE/g units the total flavonoids were obtained at (84.1860); (119.5349); (125.3488) with an average of 109.6899 mg QE/g.

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