DETERMINATION OF TOTAL FLAVONOID LEVELS FROM THE N-HEXANE FRACTION OF KIRINYUH LEAF EXTRACT *(Chromolaena Odorata L)* BY UV-VIS SPECTROPHOTOMETRY METHOD

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Abstract

In Indonesia, the plant Chromolaena odorata L, known as kirinyuh, has two different properties. This plant can act as a weed or nuisance plant and is useful as medicines in wound healing to treat headaches, antihypertensive and anti-inflammatory. This study was to analyze the flavonoid content of the n-Hexane fraction of Kirinyuh Leaf Extract (Chromolaena odorata L.) by using the UV-Vis SpectrophotometricMethod. This study uses qualitative analysis to identify flavonoid compounds while quantitative analysis to determine flavonoid levels uses a spectrophotometric method to obtain the value of absorbance entered into a linear regression equation, namely y = bx + a obtained from a comparison calibration curve and the results are expressed in mg/gram units. and percent. The results from the identification of the shinoda color test on kirinyuh leaf extract (chromolaena Odorata L) were positive for red-orange flavonoids. In the Thin Layer Chromatography test In the Thin Layer Chromatography test seen in visible light, UV 254 light and UV 366 nm light were obtained, stain separation was obtained and after evaporation with ammonia was seen a change in yellow stains in visible light and determination of total levels of flavonoids from kirinyuh leaves (chromolaena Odorata L) by 2,6736 mgQE/g or 2,6736 % Conclusions: From this study, it can be concluded that the n-hexane fraction has a flavonoid content of 26,736 mgQE/g or 2,6736 %.

Keyword: (Chromolaena odorata L), N-hexane fraction, UV-VIS Spectrophotometric Method, Total Flavonoid Content.

1. Introduction

In Indonesia the plant *Chromolaena odorata L* known by the name kirinyuh has two different properties. This plant can act as a weed or a nuisance plant that is very detrimental to the surrounding cultivation plants. This is because of its nature as *a competitor* in the absorption of water and nutrients, so it can cause a very high decline in yields in plantation crops, such as rubber, palm oil, coconut, and cashews (Karyati & Adhi, 2018).

This plant can be useful as an organic fertilizer, biopesticide, and medicine that benefits human life. According to Yenti (2012) kirinyuh leaves *(Chromolaena odorata L.)* contain several compounds such as tannins, flavonoids, phenol saponins, and steroids. Qualitative testing of phytochemicals of ethanol extract kirinyuh leaf content against several chemical

compounds proves that kirinyuh leaves contain flavonoids, phenols, alkaloids, tannins, terpenoids, steroids, and saponins (Andika *et al.,*2020).

Kirinyuh plant is declared as a medicinal plant used by the people of Central Kalimantan, especially some people in The Village of Tumbang Koling as a treatment for Hypertension or Decreased Blood Pressure and has a pain relief effect on the joints. How to use leaves is to boil a few strands of leaves then water from the stew is drunk.

Based on the description above, the n-hexane fraction of the kirinyuh leaf plant*(chromolaena odorata L)* has pharmacological activity that is beneficial for human life then analysis determined total flavonoid levels by the UV-Vis Spectrophotometry method.

2. Research methods

The material used this study in Leaves kirinyuh, Aquadest, Aluminum chloride (AlCl3) 10%, acetic acid 5%, Baku quersetin, NaOH 10%, Methanol, Ethanol 70%, concentrated hydrochloric acid metal Mg, HCl 2N, (Mukhriani et al.,2015) and n-hexane : dichloromethane (10:8), (Rudiyansyah Aisyah, 2019).

The tools used in this Mixer rods, porcelain cups, funnels, watch glass, chemical cups, glass kuvets, separator funnels, measuring gourds, drop pipettes, volume pipettes, UV-VIS spectrophotometers, analytical scales, hot plates, ratory evaporators, ovens and glass jars (Mukhriani et al., 2015) and Thin-layeredchrome platygraphs silica gel GF 254,chambers, horn spoons, spatula, filter paper, scissors, reaction tubes, spray bottles and UV lamps.

This research method is observationally decisriptive, this method is a study that aims to analyze the determination of flavonoid levels of the N-Heksana esktrak fraction of Kirinyuh leaves *(Chromolaena odorata L.)* using the UV-Vis Spectrophotometry Method.

Sampling

Sample used from Kirinyuh *(Chromolaena odorata L.)* Obtained from east Kotawaringin Regency precisely in The Village of Tumbang Koling and conducted research in the Laboratory of Pharmaceutical Technology, Department of Pharmacy, Sari Mulia Banjarmasin University.

Material Processing

Samples of Kirinyuh Leaves that have been picked are cleaned from dirt attached to the leaves, washed with running water, the process of reencing, then aerated, in a place that is not exposed to direct sunlight. Once dry, do the extraction process on the sample (Mukhriani et al.,2015).

Extraction

Weigh 9 grams of left-leaf simplisia, put in a glass jar, add 70% ethanol until submerged in all parts of the sample and tightly covered. Leave for 24 hours protected from light, while occasionally stirring. Then strain and separate from the dregs and filtratenya. Then the dregs are re-accelerated using a new ethanol-filtering solvent. Do it 3 times. Ethanol extract obtained is compressed with rotary evaporator (Mukhriani et al.,2015).

n-Hexane Faction

Carried out the gradual extraction of liquids from the condensed extract of the extraction results. The 7g thick extract is put into a split funnel dissolved with an aquadest of 50 mL, then shake until homogeneous. Add the n-hexant 50 mL (1:1), then beat until homogeneous and let

stand until separated. The n-hexant residue is removed, so the n-hexan fraction is obtained. Fractionation with n-hexan is performed three times replication. The n-hexan fraction is further collected and monitored (Rahmati & Lestari, 2018)

Flavonoid compound test

Samples were added with concentrated hydrochloric acid and mg powder. Results obtained if positive contain flavonoids in red-orange (Afriani, et al., 2016).

Thin layer chromatography

Sample Preparation

Extracts on kinyuh leaves are selected using a maceration extraction method and then diffraction with N-Hexane solvent (Wulandari, 2011).

Stationary phase and mobile phase

In the selection of the silent phase for good identification or separation of compounds, the KLT plate of GF 254 gel is good for all compounds and activated in the oven at a temperature of 105 ° C for 30 minutes. The selection of phases of motion or eluen is based on the chemical properties of a secondary metabolite compound that will be studied, namely the level of polarity of the substance. The combination of various phases of motion or eluen gets the result of good identification or separation of substances while the phase of motion or eluen for the identification and separation of a secondary metabolite compound (Wulandari, 2011).

Sample app

The placement of the Kirinyuh Leaf extract sample was carried out by the spot method with a capillary tube. It was chosen using the spot method because it does not need to use tools , is cheap and saves time (Wulandari, 2011) .

Development of TLC plates

Prior to the development of the TLC plate, saturation is required so that the eluent can eluate well in the separation of compounds (Wulandari, 2011).

Chromatography visualization

In kirinyuh leaf extract compounds by spraying the appropriate solvent so that the visualization appears with a specific solvent (Wulandari, 2011)

Documentation

Identification of secondary metabolites that have been visualized is then carried out under UV light 254 and 336. If the TLC plate is visualized, it can be documented before and after derivatization using a digital camera (Wulandari, 2011).

Spectrophotometry UV-VIS

Preparation of 1000 ppm quercetin mother standard solution

Weighed as much as 50 mg of standard quercetin and dissolved with methanol up to 100 mL (Asmorowati & Lindawati, 2019).

Preparation of 100 ppm quercetin working standard solution

The mother standard solution was pipetted as much as 1 mL and the volume was made up to 10 mL with methanol to obtain a concentration of 100 ppm (Asmorowati & Lindawati, 2019).

Preparation of blank solution

Pipette 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid, add n-hexane up to 10 mL (Asmorowati & Lindawati, 2019).

Determination of the Maximum Wavelength (λ) of Quarcetine

The standard working solution of 100 ppm quercetin was taken as much as 1 mL added with 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid. Take readings with UV-Vis spectrophotometry at a wavelength of 370-450 nm. The maximum wavelength was used to measure the absorption of the Kirinyuh Leaf extract *(Chromolaena odorata L)* (Asmorowati & Lindawati, 2019)

Determination of operating time

The standard working solution of 100 ppm quercetin was taken as much as 1 mL added with 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid. The absorbance of the solution was measured at the Maximum Wavelength (λ) of Quarcetine with an interval of 2 minutes until a stable absorbance was obtained. Observing the curve of the relationship between absorbance, time, and determine the operating time (Asmorowati & Lindawati, 2019).

Preparation of quercetin standard curve

1000 ppm quercetin mother standard solution, then 0.1 mL pipette; 0.2 mL; 0.3 mL; 0.4 mL; 0.5 mL; 0.6 mL and added 70% ethanol until the volume is 5 mL so that the concentrations obtained are 20 ppm 40 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm. Each concentration of the standard series of quercetin was pipetted 1 mL, then added 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid, allowed to stand for operating time. The absorbance was determined using the UV-Vis spectrophotometric method at the maximum wavelength obtained (Asmorowati & Lindawati, 2019).

Determination of total flavonoid content in kirinyuh leaves *(chromolaena odorata L)*

Weighed 25 mg of kirinyuh leaf extract *(chromolaena odorata L)* dissolved with n-hexane until the volume was 100 mL. 1 mL of the solution was pipetted and then 1 mL of 10% AlCl3 solution and 8 mL of 5% acetic acid were added. The sample was allowed to stand during the operating time. The absorbance was determined using the UV-Vis spectrophotometric method at the maximum wavelength obtained (Asmorowati & Lindawati, 2019).

3. Results

Extraction

Maceration here uses 9 g of dry simplicia, then extracted using 70% ethanol solvent to produce a thick extract of 7.85 g.

Fractionation

In the fractionation process, 2 layers are formed, the top layer is an n-hexane layer and the bottom layer is an ethanol-water layer. The n-hexane layer was evaporated on a hotplate with a temperature of 50°C to obtain a thick extract of the n-hexane fraction that had been concentrated as much as 228.2 mg.

Flavonoid Compound Test

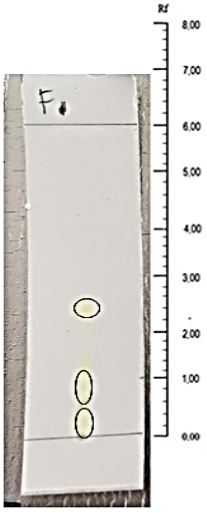
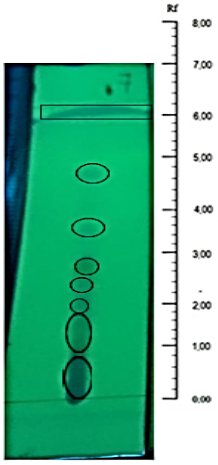
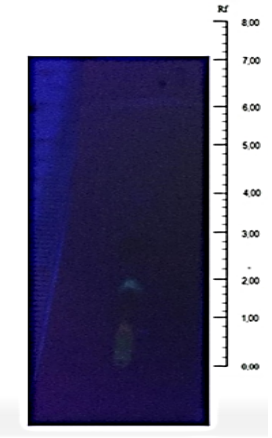
In the test for flavonoid compounds as much as 0.1 mg of extract that has been diluted with 1 ml of n-hexane solvent, 3 drops of concentrated HCL are added and a spoonful of Mg powder is positive for flavonoids with a red-orange color change.

Thin Layer Chromatography

Table 1 Thin Layer Chromatography Test Results of Kirinyuh Leaves

Eluent	Reagents	Detection	Stain Color

n-hexane: dichloromethane (8:12)	Amonia	Visible Light UV 254 UV 366	Yellow-Brown - Blue

Before Evaporation		
Visible Light	**UV 254**	**UV 366**
		

After Evaporation		
Visible Light	**UV 254**	**UV 366**

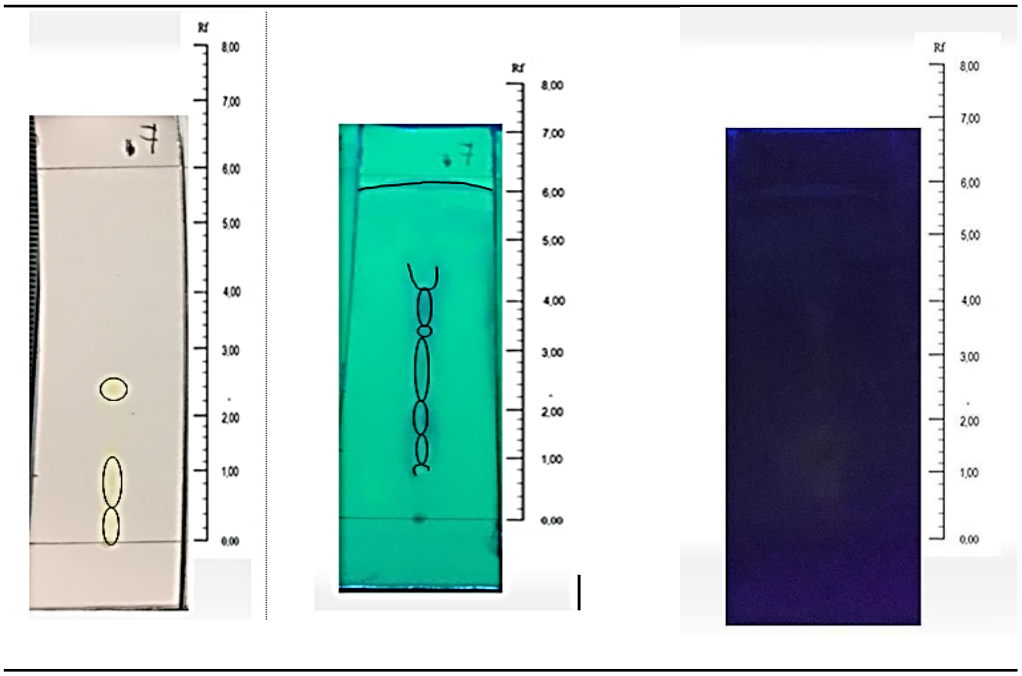


Figure 1 Identification of Flavonoids TLC Method The results of the identification in visible light, UV 254 light and UV light 366 nm obtained stain separation and after evaporation with ammonia seen a yellow stain change in visible light which indicates the presence of flavonoid compounds.

SPECTROPHOTOMETRY UV-VIS

Determination of Wavelength

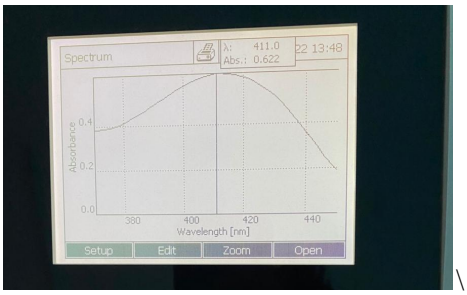


Figure 2 The maximum wavelength obtained with a wave range of 370-450nm on quercetin is 411 nm.

Determination of Operating Time

time	Absorbance
2	0,593
4	0,594
6	0,594
8	0,593
10	0,593
12	0,593
14	0,594

16	0,594
18	0,593
20	0,593
22	0,594
24	0,594
26	0,593
28	0,594
30	0,593
32	0,594
34	0,593
36	0,594
38	0,594
40	0,594
42	0,594
44	0,594
46	0,593
48	0,594
50	0,593
52	0,594
54	0,594
56	0,594
58	0,594
60	0,594

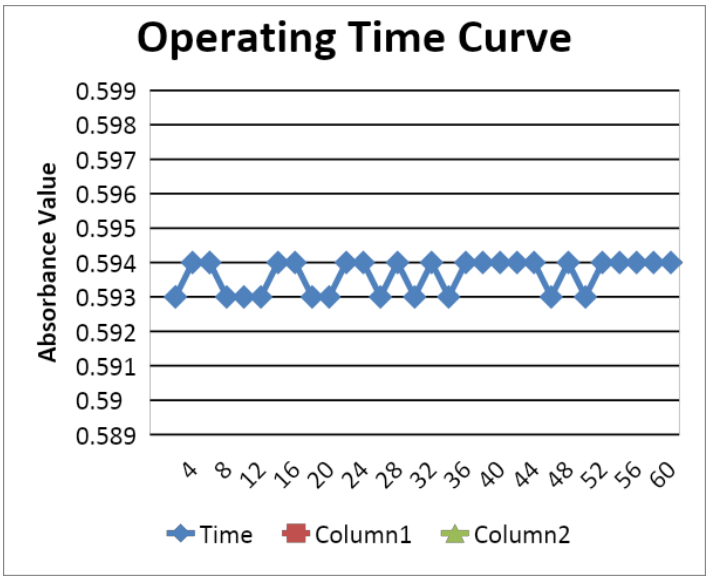


Figure 3 The result of determining the operating time is obtained at the 40th minute. Concentration curve of raw

Absorbance Measurement Results of Quercetin Standard Solution.

Table 2 Absorbance Measurement Results of Quercetin Standard Solution.

	20	**40**	**60**	**80**	**100**	**120**

Concentration (ppm)						
absorbance	0,127	0,274	0,388	0,494	0,605	0,719
	0,127	0,273	0,389	0,495	0,606	0,719
	0,127	0,274	0,389	0,495	0,606	0,720
Average	0,127	0,274	0,388	0,494	0,605	0,719

Standard Curve of Quercetin

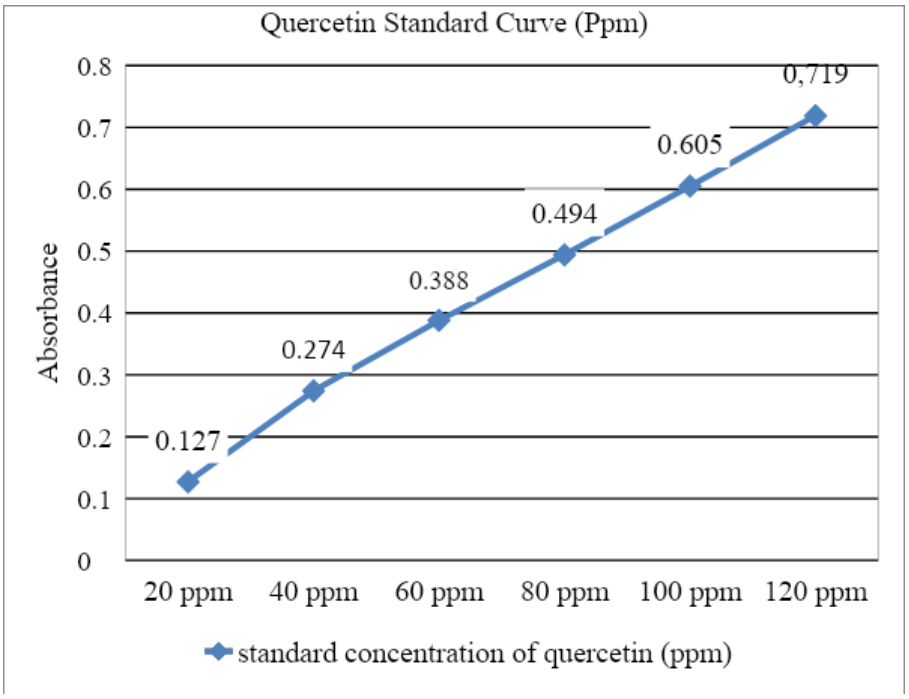


Figure 3 Standard Curve of Quercetin using a wavelength of 411 nm, every increase in concentration, the higher the absorbance value.

Determination of Absorbance Value

Table 3 Determination of absorbance of kirinyuh leaf extract samples

Sample	Absorbance	average
Kirinyuh leaf extract n-hexane fraction	0,182	0,181
	0,182	
	0,181	

Determination of flavonoid levels Kirinyuh leaf extract

Table 4 Determination of Flavonoid Levels in Kirinyuh Leaf Extract

Extract Weight (grams)	Average absorbance (λ)	Equivalent level (ppm)	Total Flavonoid Content (%)
0,025 g	0,181	26,736	2,6735 %

4. Discussion

This study was conducted to determine the total flavonoid content in 70% ethanol extract of kirinyuh leaves *(Chromolaena Odorata L)* using Thin Layer Chromatography (TLC) identification and UV-Vis spectrophotometry. The UV-Vis Spectrophotometry method was chosen because it is a simple, easy, and fast method compared to other methods, besides that it can be used for the analysis of a colored or colorless substance in small levels (Mukhriani, 2014) .

The plant sample used in this study was kirinyuh leaves *(chromolaena Odorata L)* taken from the top of the leaf. The samples that have been dried first are then extracted. The purpose of the extraction process is to extract the chemical components contained in the sample. The extraction method used in this research is maceration. The choice of this extraction method is because it is a simple, easy method, and without going through a heating process, so that the possibility of damage to chemical compound components can be minimized (Mukhriani, 2014) .

Maceration is done by repeating and adding solvent three times or until the desired compound is produced in the sample. In the maceration process, stirring is carried out continuously, it is also called the kinetic maceration process (Heliawati, 2018). While the maceration was carried out at room temperature. The usual way is to place a number of materials placed in a closed container, added with 70% ethanol solvent as much as 3 L, or until the sample is immersed. Leave the sample for 3 days at room temperature protected from direct sunlight with occasional stirring. After that, the liquid is separated and discarded the precipitated part. In the immersion process, the organic compounds contained in the sample will diffuse through the cell wall to dissolve the constituents in the cell and will also spur the solution in the cell to diffuse out (Heliawati, 2018).

The entire filtrate obtained was concentrated using a *rotatory evaporator* with a maximum temperature of 50°C and a speed of 70 rpm. The maximum temperature used is 50°C in order to avoid damage to the active substance due to the influence of high temperature. The purpose of the concentration is to separate the 70% ethanol solvent from the filtrate obtained so that a thick extract is obtained. (Asmorowati & Lindawati, 2019) .

Fractionation of kirinyuh leaf extract *(Chromolaena Odorata L) was* carried out with the principle of different levels of polarity and density between solvents for the fractions used. The first fractionation with n-hexane solvent which is non-polar and with water as polar solvent, n-hexane has a lower specific gravity than water (Rahmati & Lestari, 2018) . The n-hexane fractionation process: water was replicated three times and then concentrated using a *hotplate* to obtain a thick extract of 228.2 mg.

Qualitative analysis was carried out by identifying the flavonoid compound test which was carried out with concentrated HCl and magnesium metal. The identification of these flavonoid compounds is used to detect compounds that have a -benzopyron core (Asmorowati & Lindawati, 2019) . A positive result is indicated by the formation of a red-orange color. The test was carried out by adding 3 drops of HCl and 0.5 mg of Mg powder to a solution of Kirinyuh Leaf *(Chromolaena Odorata L.) extract* . The addition of Mg powder aims to make the flavonoid carbonyl group bind to Mg and the function of adding HCl to form a

red-orange Flavilium salt (Asmorowati). & Lindawati, 2019) Positive results of flavonoids in this study were shown in Kirinyuh Leaf Extract *(Chromolaena Odorata L.)*

The process of identifying flavonoid compounds and knowing the Rf value in Kirinyuh Leaf Extract *(Chromolaena Odorata L)*. performed by thin layer chromatography (TLC). TLC is the simplest chromatographic method that is widely used. The equipment and materials needed to carry out the separation and analysis of samples by the TLC method are quite simple, namely a closed vessel (chamber) containing the solvent and a TLC plate (Wulandari, 2011) .

Identification of flavonoid compounds in Kirinyuh leaves *(Chromolaena Odorata L)* using the thin layer chromatography (TLC) method used from selika gel GF 254 then the TLC plate was activated in an oven at 105°C for 30 minutes. Plate activation is aimed at removing atmospheric moisture that is adsorbed on the plate (Wulandari, 2011) . After activation, make mobile phase and stationary phase, *(n* -hexane: dichloromethane (8:12)).

The principle of separation based on polarity, Separation method based on polarity, compounds separated due to differences in polarity. The affinity of the analyte to the stationary and mobile phases depends on the proximity of the analyte polarity to the stationary and mobile phases *(like dissolves like)* . Analytes will tend to dissolve in phases with the same polarity. Polar analytes will have high affinity for polar solvents and low affinity for non-polar solvents. On the other hand, non-polar analytes will have high affinity for non-polar solvents and low affinity for polar solvents (Wulandari, 2011) . N-hexane has a polarity index value of 0.1 while dichloromethane has a polarity index value of 3.5 (Günzler & Williams, 2008) .

The TLC identification test was carried out by spotting on the TLC plate, then eluted with mobile phase eluent and stationary phase until the eluent rose to the upper limit mark, then dried before being tested on visible light and UV light. Evaporation with ammonia do the test again in visible light and UV light then determine the Rf value obtained in Kirinyuh Leaf Extract *(Chromolaena Odorata L)*. The results of the calculation before evaporation in visible light the first Rf value is 0.05 , the second Rf value is 0.16 rays and the third Rf value is 0.14. UV 254 obtained the first Rf value of 0.08, the second Rf value of 0.23, the third Rf of 0.31, the fourth Rf of 0.38, the fifth Rf of 0.45, the sixth Rf of 0.58, the seventh Rf of 0 .76 and the future Rf value is 0.98. The Rf value after evaporation in visible light, the first Rf value is 0.05 , the second Rf value is 0.16 and the third Rf value is 0.41. In visible light, UV 254 Rf is 0.08, the second Rf value is 0.23, the third Rf is 0.31 the fourth Rf is 0.38, the fifth Rf is 0.45, the sixth RF is 0.58, the seventh Rf is 0. .76 and the future Rf value is 0.98. Based on Figure 4.4 above, the identification seen in visible light, UV 254 light and UV 366 nm light obtained stain separation and after evaporation with ammonia was seen a yellow stain change in visible light which indicated the presence of flavonoid compounds.

Quantitative analysis of flavonoid levels can be done using a UV-Vis spectrophotometer. Ultraviolet and visible absorption spectra are a single useful way to identify the structure of flavonoid compounds. Flavonoid compounds have a conjugated aromatic system and can show strong absorption bands in the UV-Vis region. This method can also be used to perform quantitative tests to determine the amount of flavonoids contained in the extract by measuring the absorbance value. Absorbance value is a quantitative analysis performed based on Lambert-Beer Law. Absorbance with flavonoid levels has a linear relationship, namely the

higher the measured absorbance, the higher the flavonoid content contained in a plant (Gusnedi, 2013) .

The quantitative test using the determination of operating time aims to determine a stable measurement time, namely when the sample reacts perfectly and forms complex compounds (Asmorowati & Lindawati, 2019) . Operating time was carried out using a standard solution of 100 ppm quercetin with an interval of 2 minutes and carried out for 60 minutes. The result of determining the operating time is obtained at the 40th minute.

The maximum wavelength is the wavelength produced by a compound at maximum absorption (Asmorowati & Lindawati, 2019) . Measurement at the maximum wavelength aims to determine the wavelength when it reaches the maximum absorption, but it also has a relatively constant absorption. Determination of the maximum wavelength for quercetin by reading the absorption of the standard working solution of quercetin with a concentration of 100 ppm at a wavelength of 370-450 nm. The results obtained from this study are 411 nm. The next step is to determine the standard curve using a standard solution of quercetin with a concentration series of 20 ppm with an average absorbance value of 0.127, 40 ppm an average absorbance value of 0.274, 60 ppm an average absorbance value of 0.388, 80 ppm an average value absorbance is 0.494, 100 ppm the average value of absorbance is 0.605, and 120 ppm the average value of absorbance is 0.719. Concentration Curve of the standard quercetin standard shows that the concentration is directly proportional to the absorbance value, the greater the concentration of the standard quercetin standard solution , the higher the absorbance value produced .

Absorbance measurements were carried out using a maximum wavelength of 411 and an operating time of 40 minutes. Figure 4. Concentration graph of the standard quercetin standard curve shows that the concentration is directly proportional to the absorbance value, the greater the concentration of the standard quercetin standard solution, the higher the absorbance value produced. The absorbance measurement obtained the quercetin regression equation y = 0.00579x + 0.0286. The results of the linearity value are indicated by the correlation coefficient (r) of 0.998. The value (r) obtained close to 1 indicates that the regression equation is linear, so it can be said that absorbance and concentration have a very strong correlation. (Asmorowati & Lindawati, 2019) .

Determination of total flavonoids in the sample leaves Kirinyuh *(Chromolaena odorata L)* first pass through the sample preparation leaves Kirinyuh *(Chromolaena odorata L)* of each replication is performed three times, with the absorbance value of the first replication of 0.182, second at 0.182 and the third at .181 From the replication level, the average absorbance value was 0.181. Replication was carried out in order to obtain more accurate data. The comparison uses quercetin as a standard solution because quercetin is a flavonoid of the flavonol group which has a keto group at C-4 and has a hydroxyl group at the C-3 or C-5 atom which is neighboring of flavones and flavonols. The total flavonoid content was determined based on a colorimetric reaction after the sample was reacted with AlCl3 in an acid medium (Asmorowati & Lindawati, 2019) . The addition of AlCl3 in the sample can form a complex between aluminum chloride and quercetin so that there is a shift in wavelength towards the visible (visible) and is indicated by the solution producing a more yellow color. The function

of adding acetic acid is to maintain the wavelength in the visible (visible) region (Asmorowati & Lindawati, 2019) .

Based on the data in the study, the 70% ethanol extract of Kirinyuh Leaf *(chromolaena Odorata L)* had an extract weight of n-hexane fraction of 0.025g, equivalent content (ppm) of 26,736 mg QE/g and total flavonoid content of 2,6736%.

5. Conclusion

Based on the results of the research that has been carried out, it can be ascertained that the Kirinyuh leaf extract *(Chromolaena Odorata L)* with the solvent fraction of n-hexane and using the UV-VIS spectrophotometry method, the results of the shinoda color test on kirinyuh leaf extract *(Chromolaena Odorata L)* contain positive flavonoids. which is orange red. In the Thin Layer Chromatography test seen in visible light, UV 254 light and UV 366 light, the stain separation was obtained and after evaporation of ammonia was seen a yellow color change in visible light and determination of total flavonoid content from kirinyuh leaves *(Chromolaena Odorata L)* of 26.736 mg QE/g or 2,6736%.

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