Total Flavonoid Content Test Of N-Hexane Fraction Of Leaf Of Rubbish Kebo (Ficus Elastica Roxb Ex.Hornem) Using Uv-Vis Spectrophotometric Method

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Abstract. Kebo rubber plant (Ficus elastica roxb ex.hornem) is a plant that has been known to contain flavonoid compounds that can be used as traditional medicine. This study aims to determine the total flavonoid content of the n-hexane fraction of the rubber kebo leaves (Ficus elastica roxb ex.hornem) and what compounds are contained therein. The extraction process in this study used the maceration method and then fractionated. The 70% ethanol extract was fractionated with n-hexane solvent and carried out qualitative phytochemical identification then tested the total flavonoid levels using the UV-Vis Spectrophotometric method at a wavelength of 430 nm, as a comparison used quercetin standards. The results of phytochemical identification were secondary compounds of alkaloids, flavonoids, saponins and tannins. In the n-hexane fraction of rubber kebo leaves, the total flavonoid content was 13.711 ± 1.111%. The n-hexane fraction of rubber kebo leaves contains alkaloids, flavonoids, saponins and tannins. The n-hexane fraction of kebo rubber leaves contained a total flavonoid content of 13.711 ± 1.111%.

Key words: [Kebo rubber leaves (Ficus elastica roxb ex.hornem), n-hexane fraction UV-Vis spectrophotometry]

INTRODUCTION

The rubber tree (Ficus elastica roxb ex.hornem) is a traditional medicinal plant. This plant grows well in tropical and subtropical climates (Saifudin & Rahmi, 2019). One part of the rubber tree (Ficus elastica roxb ex.hornem) that can be used as a traditional medicine is the leaves, because traditionally the leaves of this plant are used for medicine to lower blood pressure, lower cholesterol, stroke and reduce joint pain (Warisno, 2003). According to Hari, Kumar & Devi (2011) the rubber tree (Ficus elastica roxb ex.hornem) contains secondary metabolite compounds flavonoids, alkaloids, organic acids, and triterpenes.

Flavonoids are phenolic compounds found in nature and possess free radical scavenging properties. Flavonoids also act as antioxidants (Pourmourad, 2006). The antioxidant effect of these compounds is due to the capture of free radicals through the donation of hydrogen atoms from the flavonoid hydroxyl group (Sofnie, *et al*., 2003). Flavonoids have a number of hydroxyl groups or a sugar, so flavonoids are called polar compounds. Generally, flavonoids are quite soluble in polar solvents such as water. The presence of sugars bound to flavonoids makes them more soluble in water, thus a mixture of these solvents with water is a better solvent for glycosides. Conversely, less polar aglycones such as isoflavones, flavanones, flavones, and methoxylated flavonols tend to be more soluble in solvents such as ether, chloroform, ethyl acetate, and n-hexane (Markham, 1988).

One analytical method that can be used to determine flavonoid levels is UV-Vis spectrophotometry. Ultraviolet and visible absorption spectra are the most useful single methods for identifying flavonoid structures. Flavonoids contain conjugated aromatic systems and can exhibit strong absorption bands in the UV-Vis region (Mukhriani *et al*., 2015).

Extraction factors in secondary metabolites can affect the polarity of the solvent (Arifianti, *et al*., 2014). The choice of solvent type used for extraction is an important factor. Based on its polarity, solvents are divided into three, namely polar, semipolar and nonpolar solvents. This is in accordance with the principle of like dissolves like where a solvent will tend to dissolve compounds that have the same level of polarity (Suryani *et al*., 2015). Inappropriate solvents may allow the desired active compounds to not be attracted properly and perfectly (Kristianti *et al*., 2008). In this study, the solvents used were 70% ethanol and n-hexane with the maceration extraction method then fractionated.

Ethanol 70% is widely used because it has a low boiling point, has high polarity and is not dangerous. Ethanol has a boiling point of 70°C so that the temperature used in extraction can attract all the components contained in the simplex, in addition, ethanol also has an OH- group with polar properties and a CH_2CH_3 group with nonpolar properties (Azis *et al*., 2014). Thus, ethanol can dissolve

both polar and nonpolar. N-hexane is a straight chain alkane hydrocarbon that has 6 carbon atoms with the molecular formula C_6H_{14}. Hexane isomers are not reactive and are used as inert solvents in organic reactions because hexane is very nonpolar (Tamzil *et al*., 2009).

Based on this background, a study was conducted on the Total Flavonoid Content Test of the N-Hexane Fraction of Rubber Kebo Leaves (Ficus elastica roxb ex.hornem) using the UV-Vis spectrophotometry method. In this study, fractionation of 70% ethanol extract of rubber kebo leaves using n-hexane, phytochemical screening and total flavonoid content testing will be carried out.

METHODS

Research Type and Design

This study employed a quantitative, experimental approach. Extracts were prepared using the maceration method, followed by fractionation, and phytochemical screening and UV-Vis spectrophotometry tests.

Research Location and Timeline

The phytochemical screening test was conducted in the Microbiology and Pharmacognosy Laboratory, while the total flavonoid content test was conducted in the ITEKES Cendekia Utama, Kudus. The study period was from April to June 2024.

Research Population and Sample

The population in this study was rubber tree leaves collected from Panti Rahayu Hospital, Purwodadi District, Grobogan Regency. The samples used in this study were fresh, dark green rubber tree leaves (Ficus elastica roxb ex.hornem).

Tools and Materials

Analytical balance, stirring rod, measuring cylinder (Pyrex), porcelain cup, beaker glass (Pyrex), glass funnel (Pyrex), glass jar, flask, flannel cloth, blender, oven, rack and test tube (Pyrex), mask, dropper pipette, micropipette (100-1000 µl), sieve no. 44, cuvette and UV-Vis spectrophotometer (Biobase). Rubber tree leaves (Ficus elastica roxb ex.hornem), 70% ethanol, n-hexane, distilled water, 1% NaOH, $FeCl_3$, 2N HCl, magnesium powder, concentrated HCl, distilled water, ethanol p.a., sodium acetate 1 M, $AlCl_3$ 10%, quercetin, Mayer's reagent, Wagner's reagent.

Plant Determination

Plant determination was conducted in the Ecology and Biosystems Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, by submitting intact plant samples.

Extraction and Fractionation of Rubber Leaves

1. Maceration

   100 grams of dry powder was weighed, ground, and placed in a maceration container. 70% ethanol was added at a ratio of 1:10 until the entire sample was submerged. The mixture was then covered, stirred, and left to stand for 3 x 24 hours until saturated. The macerate was filtered using flannel. The filtrate was then macerated again with 70% ethanol three times (Octaviani *et al*., 2016). The resulting extract was then concentrated in an oven at 40ºC.

2. Fractionation

   The ethanol extract was fractionated using n-hexane. Five grams of the ethanol extract was placed in a beaker, dissolved in 25 ml of ethanol, and 25 ml of distilled water was added, while the mixture was ground and stirred until homogeneous. After that, 50 ml of n-hexane was added and then placed into a separating funnel. The mixed solution was shaken slowly and waited for several minutes until the solution separated and the n-hexane fraction was obtained (Nuari *et al*., 2017). The resulting fraction was then pic petted and concentrated using an oven at 40°C.

Phytochemical Screening

a. Alkaloid Test

   Performed using the Mayer and Wagner method. A 1 mg sample was placed in a porcelain dish, then 3 drops of 2N HCl and 3 drops of distilled water were added, then heated in a water bath for 1 minute. The sample was cooled to room temperature and filtered. The filtrate was divided into three parts, A, B, and C. Filtrate A served as a blank, filtrate B was added with Mayer's reagent, and filtrate C with Wagner's reagent. A brown precipitate formed, indicating a positive reaction (Agustina *et al*., 2016).

b. Flavonoid Test

1 mg sample was dissolved in ethanol. 1 mg of magnesium was added to the solution and 3 drops of concentrated HCl were added. If the color changes to orange, the extract was positive for flavonoids (Harborne, 1996). A 1 mg sample was dissolved in ethanol. 1-3 drops of 1% NaOH were added to the solution. Afterward, shake it, and if a yellow color appears and becomes clear when dilute acid is added, the extract is positive for flavonoids (Lukmandaru *et al*., 2014).

c. Saponin Test

1 mg sample is placed in a test tube, 2 ml of water is added, and stirred. It is then heated for 3 minutes, then filtered through filter paper and shaken. After shaking, if foam is seen that persists for 10 minutes and remains stable after the addition of 2 N HCl, the extract contains saponins (Lukmandaru *et al*., 2014).

d. Tannin Test

1 mg sample is added with 1-3 drops of $FeCl_3$ solution. The formation of a bluish-green black color indicates the presence of tannins (Harborne, 1996).

Determination of total flavonoid content

a. Preparation of 1,000 ppm quercetin stock solution: Weigh 50 mg of quercetin into a 50 ml volumetric flask, then dissolve it in ethanol p.a. to the mark (Puspitasari and Prayogo, 2016).
b. Determination of maximum wavelength: This was performed using UV-Vis spectrophotometry by pipetting 1 ml of 1,000 ppm quercetin stock solution, 1 ml of 10% quercetin, and 1 ml of 1M sodium acetate. The resulting solution was pipetted into a cuvette. The absorbance was measured at a wavelength of 400-500 nm (Alwi, 2017).
c. Determination of operating time: carried out by pipetting 1 ml of 1000 ppm quercetin stock solution then adding 1 ml of 10%, 1 ml of 1M sodium acetate after which ethanol p.a. was added up to 10 ml. The obtained solution was pipetted into a cuvette and the stability time was sought in the time range of 0-20 minutes with 1 minute intervals. The absorbance was measured at the Maximum wavelength to determine the stability time.
d. Preparation of quercetin standard solution: Weighed 50 mg of quercetin standard and dissolved it in 50 ml of ethanol p.a. From the 1000 ppm quercetin standard solution, 1 ml was taken, then several concentrations were made, namely 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. From each concentration of quercetin standard solution, 1 ml of $AlCl_3$, 1 ml of 1M sodium acetate and ethanol p.a. were added up to 10 ml. After that, it was incubated for 20 minutes at room temperature and the absorbance was measured using UV-Vis spectrophotometry at the maximum wavelength.
e. Preparation of the quercetin standard curve: The standard curve was created by correlating the concentration of the standard solution with the absorbance obtained from measurements using UV-Vis spectrophotometry at the maximum wavelength.
f. Preparation of the sample solution: Weighed 0.01 mg of the ethanol extract of the n-hexane fraction of rubber tree leaves and dissolved it in 10 ml of ethanol p.a. From the stock solution, pipetted 1 ml of sample, 0.1 ml of 10%, 0.1 ml of 1M sodium acetate, and added ethanol p.a. to the 10 ml volumetric flask. After that, it was incubated for 10 minutes at room temperature and the absorbance was measured using UV-Vis spectrophotometry at a wavelength of 430.0 nm. The sample solution was prepared in three replicates (Chang et al., 2002).

Data analysis

The data obtained from this study are presented in tabular form, then data processing is carried out for data analysis using the linear regression standard curve method (y = b.x+a) created based on data on the area under the curve and the concentration of the standard solution.

$$y = bx + a$$

Description:
y = absorbance value
x = quarsetine equivalence (ppm)
a = slope of the standard series absorbance
b = intercept of the standard series absorbance

Made based on data on the area under the curve and the concentration of the standard solution. Analysis results:

$$F = \frac{c \times v \times f \times 10^{-6}}{m} 100\%$$

Description:
F = Total flavonoids (%)
C = quercetin equivalence (ppm)
V = total extract volume (ml)
f = dilution factor
m = sample weight (gram)

RESULTS AND DISCUSSION

Results

Table 1. Phytochemical Screening Results

No	Active substance	Reagent	Conclusion	Description
1.	Alkaloid	+ HCL 2N + aquadest + mayer + wagner	Positif	Formation of brown sediment.
2.	Flavonoid	Uji pertama + etanol + 1mg magnesium + HCl pekat Uji kedua + etanol + NaOH 1% + asam encer	Positif	First test Orange Second test Yellow and clear
3.	Saponin	+ Air + HCl 2N	Positif	There is stable foam.
4.	Tanin	$FeCl_3$	Positif	The formation of a bluish green black color.

Source: Processed primary data (2024)

Table 2. Absorbance Measurement Results of Quercetin Standard Solution

Kadar kuersetin (ppm)	Absorbansi	Persamaan regresi linear
20	0,074	
30	0,109	
40	0,169	y = 0,0047 x - 0,0228
50	0,209	r^2 = 0,9951
60	0,257	

Source: Processed primary data (2024)

Table 3. Results of Determination of Total Flavonoid Content of the N-Hexane Fraction of Rubber Leaves

Concentration	Absorbance	Flavonoid concentration (ppm)	Total flavonoids (%)	X ± SD
1000 ppm	0,565	125,067	12,506 %	13,711 ± 1,111 %
	0,668	146,978	14,697 %	
	0,632	139,319	13,931 %	

Source: Processed primary data (2024)

Discussion

Plant determination is the initial stage carried out before the research. Plant determination is carried out to determine the accuracy of the simplicia used in this study and errors in taking plant materials. Based on the results of plant determination, it proves that the plant used is the Ficus elastica roxb ex.hornem species with the local name karet kebo. The macerated rubber leaf powder produces a thick blackish brown extract with a thick form, and has a distinctive odor of 5.5 grams with a yield of 5.5%. The maceration process is carried out using 70% ethanol. The next extraction process is fractionation using n-hexane solvent. Fractionation of 5 grams of ethanol extract produces 2.2 grams of n-hexane fraction with a yield of 2.2%, blackish brown in color, thick in shape and has a distinctive odor.

Phytochemical tests are used to identify the content of active compounds found in the sample. The secondary metabolite groups tested in this study include alkaloids, flavonoids, saponins and tannins. Based on the results of the phytochemical screening test that has been carried out, it can be seen that the n-hexane fraction contains alkaloids, flavonoids, saponins and tannins. In the examination of alkaloid compounds in the n-hexane extract of rubber kebo leaves, a brown precipitate was formed so that it was declared positive for containing alkaloids (Agustina *et al*., 2016). In the examination of flavonoid compounds in the n-hexane extract of rubber kebo leaves, an orange color was formed (Harborne, 1996). And the examination of flavonoid compounds in the rubber kebo leaf extract formed a yellow color after being shaken and became clear (Lukmandaru *et al*., 2014). So it was declared positive for containing flavonoids therefore two experiments were carried out to confirm it. In the examination of saponin compounds in the n-hexane extract of rubber tree leaves, there was foam or foam so it was declared positive for containing saponins (Lukmandaru *et al*., 2014). In the examination of tannin compounds in the n-hexane extract of rubber tree leaves, a bluish-green black color was formed so it was declared positive for containing tannins (Harborne, 1996).

Total Flavonoid Level Test using UV-Vis spectrophotometry method. The determination of the maximum wavelength is carried out to determine at what wavelength produces the maximum absorption value in the sample, so that the measurement results are accurate. The maximum wavelength is carried out by making a quercetin solution, 50 mg of quercetin is weighed and then dissolved with ethanol p.a up to 50 ml, resulting in a solution concentration of 1,000 ppm. The 1,000 ppm stock solution is taken as much as 1 mL and added ethanol p.a up to 10 mL in a 10 mL volumetric flask, resulting in a solution concentration of 100 ppm. From the 100 ppm quercetin standard solution, several concentrations are then made, namely 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. The determination of the maximum wavelength is carried out on the quercetin stock solution with a concentration of 40 ppm to ensure that there is a true wavelength where maximum absorbance occurs. The maximum wavelength was determined in the 400-500 nm range. The maximum wavelength obtained was 430 nm. Standard absorbance measurements of the n-hexane fraction of rubber tree (Ficus elastica roxb ex.hornem) leaves were performed using this wavelength.

The operating time determination aims to determine the measurement time for a compound that provides stable absorbance and no decrease in absorbance. This determination also minimizes measurement errors. The operating time was achieved at 33 minutes with an absorbance value of 0.101, consistent with 1-minute intervals.

The standard curve determination began with the preparation of a series of quercetin standard solutions at concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. Quercetin was chosen as a reference solution because it is a flavonoid compound that can react with $AlCl_3$ to form a complex that produces a yellow color. Measurements of the series of quercetin standard solutions were performed using UV-Vis spectrophotometry at a wavelength of 430 nm for an operating time of 33 minutes. The absorbance values for each concentration were 0.074; 0.109; 0.169; 0.207; 0.256, as shown in Table 8. A standard curve graph was then created between concentration (x) and absorbance (y), resulting in a linear regression equation of $y = 0.0047x - 0.0228$, with a correlation coefficient (r) of 0.9951. A good correlation coefficient is close to 1. The higher the correlation coefficient, the greater the linearity.

Flavonoids are secondary metabolites with antioxidant activity. Flavonoids act as antioxidants because they can donate hydrogen atoms from hydroxyl groups to free radicals (Sofnie, Ros & Chairul, 2003). The free radical-inhibiting ability of the n-hexane fraction of rubber tree leaves is related to the chemical compounds they contain, namely flavonoids. Therefore, in research, testing total flavonoid levels is crucial to determine the flavonoid levels in the plant and its potential use in traditional medicine.

In this study, the determination of total flavonoid content of n-hexane fraction of rubber tree leaves (Ficus elastica roxb ex.hornem) was carried out by making a test solution of n-hexane fraction of rubber tree leaves (Ficus elastica roxb ex.hornem) with a concentration of 1,000 ppm. The test solution of n-hexane fraction with a concentration of 1,000 ppm was taken as much as 1 mL, added 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate and added ethanol p.a to the boundary mark in a 10 mL measuring flask then left for 33 minutes before being measured using UV-Vis spectrophotometry at a wavelength of 430 nm. The sample was replicated 3 times. From the results of the sample measurements in UV-Vis spectrophotometry, calculations were carried out with quercetin equivalence with a formula that had been determined from the linear regression equation, namely $y = 0.0047x - 0.0228$ where y is the absorbance and x is the concentration. The correlation coefficient is 0.9951. From the calculation results, the total flavonoid content of the n-hexane fraction of rubber tree leaves (Ficus elastica roxb ex.hornem) is 13.711 ± 1.111%.

CONCLUSION

There are alkaloid, flavonoid, saponin and tannin compounds in the n-hexane fraction of rubber tree leaves. The total flavonoid content of the n-hexane fraction of rubber tree leaves (Ficus elastica roxb ex.hornem) is 13.711 ± 1.111%.

Further research is needed to determine the levels of specific pure compounds contained in the n-hexane fraction of rubber tree leaves (Ficus elastica roxb ex.hornem). Further research is needed to test the total flavonoid levels of the n-hexane fraction of rubber tree leaves (Ficus elastica roxb ex.hornem) using other solvents such as ethyl acetate.

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