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Literature Review: Standardization of Rutin Compounds in Several Plant Types

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ABSTRACT

Rutin is a compound derived from flavonoids. Rutin is a quercetin glycoside with a disaccharide consisting of glucose and rhamnose. Rutin is known to have activity as an antioxidant, anti-radical, estrogenic, anti-inflammatory, antiviral, antitumor, cytotoxic and protects against organ damage. The manufacture of herbal medicines must first go through a standardization process. This review was conducted to identify the rutin contained in an extract by means of Visible Light, KLT, HPLC, and LCMS. This research uses the literature review method by collecting and identifying relevant research data sources or articles published online, through the Google Scholar web database and Science Direct.

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1. INTRODUCTION

Indonesia is a country rich in medicinal plants, and among the thousands of medicinal plants, there are still many plants whose properties are not known. Many plants that can be cultivated because of its usefulness for humans in medicine. The chemical content in plants has many benefits, so that people can use these plants to be used as medicine. In the manufacture of herbal medicines must first go through a standardization process. Standardization means a series of processes that will involve various methods of chemical analysis based on pharmacological data, will also involve physical and microbiological analysis based on general safety or toxicological criteria for a natural extract. Standardization is also carried out to improve product quality and safety, which is expected to further increase confidence in the benefits of drugs derived from natural ingredients, quality that meets standard requirements (chemical, biological and pharmaceutical), including guarantees (limits) of general stability as drugs. Standardization consists of specific parameters and non-specific parameters [1].

Rutin is a compound derived from flavonoids. Rutin is a quercetin glycoside with a disaccharide consisting of glucose and rhamnose [2]. Rutin is a pale yellow fine powder and contains not less than 95% and not more than 100.5% C27H30O16 calculated to the anhydrous substance. Rutin is odorless, rutin crystals bind three water molecules which melt at 185°C and 192°C and decompose at 211°C and 215°C. Drying at a temperature of 95–97°C will remove one H2O molecule, if heating is carried out at 100°C for 12 hours and a pressure of 10 mmHg, anhydrous rutin is obtained [3].

Rutin is known to have activity as an antioxidant, anti-radical, estrogenic, anti-inflammatory, antiviral, antitumor, cytotoxic and protects against organ damage. Rutin is widely distributed in fruits and vegetables such as apples, green asparagus and tea [4].

2. METHOD

The method used in making this literature review is by collecting literature, identifying relevant data sources or articles published online. Literature search via Google Scholar and Science Direct web

databases. Various terms or keywords used in the literature search included "standardization of routine compounds", "routine compounds" and "standardization of routine compounds". A literature search was carried out using data inclusion criteria in the form of filtering articles with the last 10 years of publication (ie, 2012-2022) and the selected literature is available in full-text.

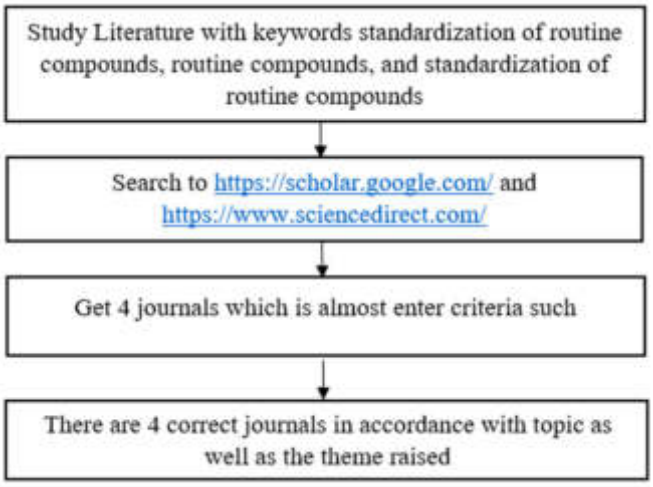

Figure 1. Research Method

3. RESULTS

Table 1. Results of Standardization of rutin

REFERENCES	SAMPLE	Metode Ekstraksi	STANDARDIZATION METHOD	RESULTS
[5]	Cassava Leaves (Manihot Esculenta Crantz))	Maceration Method (70% Ethanol)	Visible Spectrophotometry	The results showed that the levels of flavonoids obtained from cassava leaves (Manihot esculenta Crantz) were 4.987% where these levels were calculated as routine flavonoid levels found in cassava leaves (Manihot esculenta Crantz).
[6]	Binahong leaves (Anredera cordifolia (Ten.) Steenis)	Digestive Method (Ethanol 96%)	High Performance Liquid Chromatography (HPLC) mobile phase: mobile phase 1: methanol, acetonitrile, and aquabides with a ratio of 20 : 20 : 60, mobile phase 2: methanol, acetonitrile, and aquabides with a ratio of 30 : 10 : 60.	This study determined the levels of rutin in the ethanol extract of binahong leaves using high-performance liquid chromatography as a standardization process for natural product extracts with rutin as a marker. The routine level obtained was 0.014 ± 0.000704 mg/g extract and the coefficient of variation was 5.17%.
[7]	Crown of Gods Fruit (Phaleria	Maceration and Percolation	KLT eluent (mobile phase): n-butanol : acetic acid :	Mahkota dewa contains several active compounds, one of which is flavonoids. .

	macrocarpa [Scheff] Boerl)	Methods (Methanol)	water in a ratio of 9:2:6 (v/v).	the similarity in the color of the spots between routine reference standards and the results of percolation and maceration extraction that appeared on the TLC plate, which was bright yellow in color, indicated that the percolate and macerate samples were positive for containing flavonoid active substances.
[8]	Majapahit Plant Stem Bark (Crescentia cujete)	Maceration Method (Ethanol 96%)	LCMS	Based on the detection results using LCMS, it is known that there are around 88 compounds detected, including 12 flavonoid compounds. One of the flavonoid compounds found is rutin.

DISCUSSION

Rutin is a class of flavonoid compounds that are widely distributed in plants but are only found in a small number of plant species. Rutin has various pharmacological activities, namely as an antioxidant, anti-rheumatoid arthritis, anti-inflammatory, anti-radical, prevents platelet aggregation, estrogenic, antiviral, antitumor, cytotoxic and vasodilator [9]. There are several types of plants used as samples for standardization of routine compounds, namely Cassava Leaves (Manihot Esculenta Crantz), Binahong Leaves (Anredera cordifolia (Ten.) Steenis, Mahkota Dewa Fruit (Phaleria macrocarpa [Scheff] Boerl), and Stem Bark of the Majapahit Plant (Crescentia cujete).

Before carrying out standardization, the plant must be extracted so that the process is easier. The extraction method used in making extraction from each of these plants can be done by hot and cold way. In the hot method, maceration and percolation and in the cold method with digestion. Maceration is a simple extraction of simplicia by immersing simplicia in the solvent. Maceration is carried out because the process is easier, does not require special treatment, does not require heat so that it can prevent damage to thermostable substances due to high temperatures and because the samples used are in the form of leaves [10]. Percolation is a way of extracting simplicia by passing the appropriate solvent slowly through the simplicia in a percolator. The advantage of percolation is that the flow of the filter fluid causes a change of solution and the space between the grains of the simplicia powder forms a capillary channel where the filter liquid flows. Digestion is kinetic maceration (continuous stirring) using weak heating, namely at a temperature of 40-50°C [11].

The type of solvent used in the manufacture of plant extraction also varies, some use 96% ethanol, 70% ethanol, and methanol. Ethanol solvent is relatively less toxic than other organic solvents, as a universal solvent capable of dissolving almost all substances, both polar, semipolar and nonpolar [10]. The ethanol used is 70% ethanol because the sample used is a dry sample which has relatively little water content. 96% ethanol is used because it can dissolve more antioxidant compounds than water, so it can produce higher antioxidant activity as well. 70% ethanol is a more polar solvent than 96% ethanol so that the polar flavonoid compounds tend to dissolve more in 70% ethanol. Differences in the concentration of ethanol solvent affect the degree of polarity of a solvent. Methanol solvent is used to explore the types of secondary metabolites contained in the methanol extract, which are polar and some are non-polar so that it is very good at extracting the secondary metabolites contained in the sample.

If you want to standardize routine compounds from extractions that have been made, then this can be done in various ways, such as the Visible Spectrophotometry (UV-Vis) method, High

Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), and Liquid Chromatography-Mass. Spectrometer (LCMS). Visible Spectrophotometry includes the measurement of light energy by a chemical system at a specific wavelength. Ultraviolet (UV) light has a wavelength of 200-4400 nm while visible light has a wavelength of 400-750 nm [12]. HPLC works by separating and determining the levels of more than one active substance in the preparation with optimal results. The advantage of this method is that it provides fast, efficient, and high-resolution separations [13]. Thin layer chromatography is a simple analytical method used to confirm the chemical compounds contained in plants in addition to phytochemical screening [14]. Liquid Chromatography-Mass Spectrometer (LCMS) is an analytical method that combines the physical separation capabilities of liquid chromatography with the detection specificity of mass spectrometry [15].

Cassava leaf extract in the determination of routine flavonoid levels using the colorimetric method with AlCl3 reagent. The principle of this method is that AlCl3 forms stable complexes with C-4 keto groups, then with C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition to aluminum chloride it forms stable acidic complexes with the ortho hydroxyl groups on the A or B ring of the flavonoids. Routine standard solutions were used as a comparison because most of the flavonoids most commonly found in plants are in the form of glycosides such as quercetin 3-rutinoside. In determining the levels of routine flavonoids, 80% ethanol is added to increase the concentration, while the addition of 10% AlCl3 is used to provide a bathochromic effect by shifting towards a higher wavelength so that it changes the wavelength of the routine standard solution to enter the wavelength range. UV-Vis 400-800 nm. The bathochromic effect results in a more yellow color. After that, the addition of 1M sodium acetate served as a stabilizer, then added distilled water and allowed to stand for 30 minutes so that the reaction between the routine standard solution and the added reagents could take place perfectly.

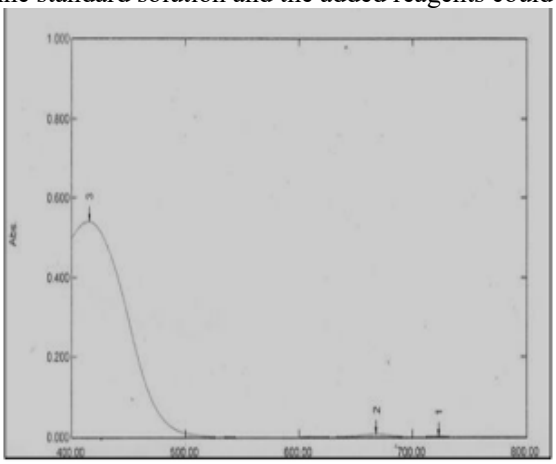

Figure 1. Absorption spectrum of routine flavonoids in cassava leaf extract (Manihot esculenta Crantz) 2nd repetition

The results of measuring the flavonoid content in cassava leaf extract were 4.979 g/100 g, 4.992 g/100g, 4.992 g/100g, the average levels were 4.987 g/100g where these levels were calculated as routine flavonoid levels found in cassava leaves (Manihot esculenta Crantz). The results of measuring the content of flavonoids obtained % levels, namely 4.979%, 4.992%, 4.992% with an average value of 4.987%.

The 96% ethanol extract of binahong leaves has been tested for maximum wavelength optimization. The maximum wavelength was determined by scanning a routine standard solution in three different concentrations (0.5 ppm, 1 ppm, and 1.5 ppm) with a wavelength range of 200-400 nm using a UV double beam spectrophotometer.

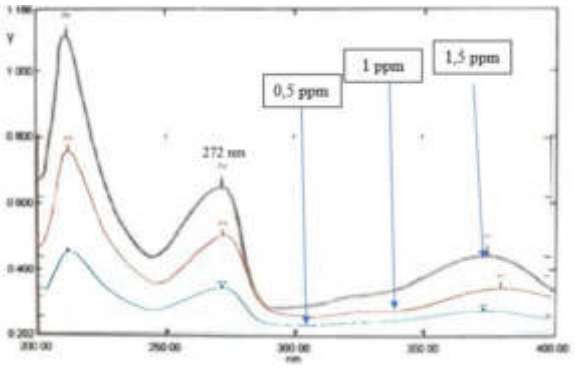

Figure 2. Spectra 3 Series of Routine concentrations at a Wavelength of 200-400 nm

information:

x-axis = wavelength,
y-axis = absorbance

Routine compounds can produce absorbance at a maximum wavelength of 272 nm, namely the presence of chromophore and auxochrome groups in rutin compounds and based on Woodward Fieser calculations. This wavelength value can also increase in the presence of auxochrome groups. This will certainly affect the absorbance produced. Determination of routine levels in the ethanol extract of binahong leaves can be carried out using HPLC under mobile phase conditions which have a methanol: acetonitrile : aquabides ratio of 30 : 10 : 60 with a flow rate of 0.7 mL/minute. obtained routine levels of 0.014 ± 0.000704 mg/g extract and a coefficient of variation of 5.17%.

The methanol extract of Mahkota dewa obtained an average yield value of the extract of the Mahkota Dewa fruit in the maceration extraction method obtained 17.3264% and percolation was 15.3864%. Things that can affect the yield value are the type of solvent, temperature, extraction time, extraction principle, particle size, extraction volume. Qualitative test results for flavonoid compounds using Shinoda reagent. This causes a color change to orange. Thin Layer Chromatography (TLC) analysis yielded the Rf value of the extract. Spots have been found on the TLC plate from the two methods, namely the maceration and percolation methods. There is a similarity in the color of the spots between the routine reference standard and the extraction results which appear on the bright yellow TLC plate which indicates that the sample contains positive flavonoids. The results of determining the absorbance of standard solutions are obtained from routine standard calibration curves (Figure).

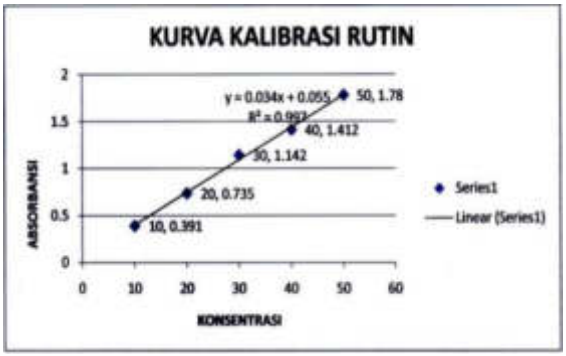

Figure 3. Routine Calibration Curve

Determination of absorbance of routine standard solution with λmax 254 nm. Quantitative test results on the average levels of flavonoids in the crown of the god fruit extract by maceration of 2.184 mg and by percolation of 2.060 mg. Statistical tests did not produce significant differences, yield values or levels of flavonoids between the maceration and percolation extraction methods.

The 96% ethanol extract of the stem bark of the Majapahit plant has been tested quantitatively for flavonoid compounds with a UV-Vis spectrophotometer using a wavelength of 435 nm.

The standard curve regression results obtained a linear equation y = 156.07x - 2.0274 with an R2 value of 0.9922, which means that there is a correlation between the concentration value and the absorbance value. Measuring the concentration of total flavonoids in the ethanol extract of stem bark as much as 206 mg using a wavelength of 435 nm, obtained a total of 99.097 mgQE/g of total flavonoids. The results of identification using LCMS found that there were 88 compounds contained in the ethanol extract of the stem bark of the Majapahit plant. There are 12 compounds identified as suspected flavonoids, including acetoin, quercetin, kaempferol-3-O-rhamnoside, acacetin 7-rutinoside, fortunellin, kaempferol 3-[6"- (3-hydroxy-3-methylglutaryl)glucoside], didymin, diosmin, hesperidin, rutin, narirutin 4'glucoside, kaempferol 3-[6"-(3-hydroxy-3- methylglutaryl) glucoside]-7-glucoside. In routine compounds, the total retention time (Rt) was 35.517 and the composition value was 3.530%. The flavonoid compound with the highest amount of composition from the ethanol extract of the stem bark of the Majapahit plant was kaempferol-3-O-rhamnoside of 4.07%, which appeared at a retention time of 21.429 minutes. Rutin is known to have the ability as an anticancer. Lung (A549) and colon cancer cell lines (HT29 and Caco-2) are known to reduce cell attachment and migration, resulting in inhibited proliferation and reduced ROS production. The component of the flavonoid compound in the ethanol extract of the bark of the Majapahit plant in general has the potential as an anti-cancer

4. CONCLUSION

Rutin is a compound derived from flavonoids which includes quercetin glycosides with disaccharides consisting of glucose and rhamnose. Rutin is known to have activity as an antioxidant, anti-radical, estrogenic, anti-inflammatory, antiviral, antitumor, cytotoxic and protects against organ damage. Methods for obtaining routine compounds in plants can use visible spectrophotometry, TLC, HPLC, and LCMS methods. In the four journals that have been reviewed, there are routine compound contents in the plants Cassava Leaves, Binahong Leaves, Mahkota Dewa Fruit, Stem Bark of the Majapahit Plant.

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