Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 4:176-181

Flavonoids content in extracts secang (Caesalpinia Sappan L.) maceration method infundation analysis and visible ultraviolet spectrophotometer

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ABSTRACT

Secang (Caesalpinia sappan L) has a component of a chemical compound essential oils, flavonoids, benzopyran, sapanin, brazilin, caesalpin S, caesalpin P, sapanon A, sapanon B. Flavonoids are chemical components that can be used in medical and industrial fields. This study aimed to determine the levels of flavonoids contained in extracts secang results maceration and extraction methods infundation. This study used quantitative and descriptive research design. This study using purposive sampling with two sample of Caesalpinia sappan L extract result of infundation and maceration. Analysis using univariate analysis. This study shows that there are types of flavonoids flavones, flavonols and flavanols on extracts of Caesalpinia sappan L results maceration method, and there are flavonoid types of flavones, flavonols and flavanols on extracts of Caesalpinia sappan L results infundation method. Flavonoid content test results showed that the levels of flavonoids in extracts of Caesalpinia sappan L results maceration 0.0539% whereas the levels of flavonoids in the extract of Caesalpinia sappan L results infundation methods 0.1902%. Necessary standardization of botanicals Caesalpinia sappan L to determine the levels of flavonoids using ultraviolet visible spectrophotometer, and the analysis of the levels of flavonoids in the extract of Caesalpinia sappan L using different solvents. As a follow-up is necessary to design the manufacture of dosage formulations of the active ingredient flavanoid compounds from the extract of Caesalpinia sappan L.

Key Words: Flavonoids, Extract, Caesalpinia sappan L

INTRODUCTION

Indonesia is a country that has a biodiversity that is big enough to be developed mainly for traditional medicine that is an ingredient or ingredients in the form of plant material, animal material, mineral materials, preparation sarian or galenic, or mixtures of these materials, which for generations has been used for treatment based on experience.

One of the medicinal plants used for the treatment of a disease and the maintenance and improvement of health is a cup (Caesalpinia sappan L). Rina[10] explained that (Caesalpinia sappan L) is one kind of medicinal plants as a potential source of natural antioxidants. There have been many studies on the efficacy of the plant Caesalpinia sappan L, both as antimicrobial, antioxidant, and natural dyes. Components of bioactive compounds contained in Caesalpinia sappan L flavonoid, brazilin, brazilein, sappanone, chalcone, sappancalchone and other common components, such as amino acids, carbohydrates and palmitic acid which amount is relatively very small [10].

One of the factors that influence the efficacy of medicinal plants is how to process so that the active substance contained in the plants are not damaged or diminished. Extraction is a method of making the active ingredient, and to attract a compound using a suitable solvent. Soluble compound from the cell via diffusion, depending on the location of the compound in the cell and the cell wall permeability of the material to be extracted. Selection of the extraction method is one of the factors that influence the levels of substances that will be taken. Intake of flavonoids in general from natural materials using the basic principle of extraction, there are two kinds of extraction methods used by solvent extraction using cold extraction method using a solvent by means of heat. But to isolate the

flavonoid extraction method is used in a way cool because flavonoids are not heat resistant and damaged at high temperatures, for example maceration. Maceration using immersion techniques while infundation method using a temperature of 90 °C for 15 minutes. Maceration and infundation is a method by means of a simple and easy to be applied. This study determine the levels of flavonoids in extracts Caesalpinia sappan L through maceration and infundation degan Ultraviolet Visible spectrophotometer.

MATERIALS AND METHODS

This research is descriptive. The population in this study extract Caesalpinia sappan L. This study uses a single variable, namely levels of flavonoids contained in extracts of Caesalpinia sappan L. maceration and infundation with Ultraviolet Visible spectrophotometer analysis. The research sample for qualitative tests using extracts Caesalpinia sappan L results maceration of 0.1 g and yield of 0.1 g infundation method. The research sample for quantitative assay using extracts Caesalpinia sappan L results maceration of 25 mg and infundation as much as 25 mg. Implementation research begins with raw material preparation, followed by extracting raw materials, identification of flavonoids with the qualitative test, and then determining the levels of flavonoids in each extract. Qualitative test is used to identify the presence of flavonoids in extracts Caesalpinia sappan L crimped maceration and infundation. Preliminary test flavonoid compounds do with color test using a reagent concentrated HCL, Mg powder, 2N HCl, 2N NaOH, and FeCl3. Each extract maceration results and outcomes as much as 0.1 g infundation put into a test tube, add 70% ethanol 5 ml, then put in 4 test tubes. Analysis of studies using univariate analysis.

RESULTS

1.1 Extraction Process with maceration method and infundation

This study uses a maceration extraction methods and infundation. At the maceration is done by soaking the powder simplia Caesalpinia sappan L in liquid penyari be water, whereas the method infundation done by wetting the powder Caesalpinia sappan L with solvent water as much as ten times the weight of the material, and then heated in a water heater as much as 15 minutes, calculated from the temperature in the pot reached 90 °C, while stirring. Extraction by maceration method and infundation can be seen in table 1 below.

Table 1. Extraction Results Caesalpinia sappan L with maceration method and infundation

Number	Extraction Method	Powder Waight (gr)	Extract Weight (gr)	The yield (%)
1	Maceration	50	3,595	7,19
2	Infundation	50	1,942	3,88

Extraction by maceration method of 3595g, and the extraction method infundation of 1.942 g.

1.2 Qualitative Test Flavonoid Compounds

Qualitative test performed using reagents flavonoid compound Mg powder and concentrated HCl, 2N HCl, 2N NaOH, and FeCl3. Flavonoid Compounds Qualitative Assay results are shown in Table 2 below:

Table 2. Qualitative Test Results Flavonoid Compounds

Number	Extracts were tested	Reagent	Positive results	Experimental results	Information
1	Extract Caesalpinia sappan L with maceration method	Mg – HCl concentrated	Pink - Red strong	Pink	(+) Flavon
		HCl 2N	Brown yellow	Brown yellow	(+) katekin (flavonol)
		FeCl3	Black Blue	Bluish black	(+) Flavanol (galokatekin)
		NaOH 2N	Yellow / Brown Orange	Red	(-)
2	Extract Caesalpinia sappan L Method infundation	Mg – HCl concentrated	Pink - Red strong	Pink	(+) Flavon
		HCl 2N	Brown yellow	Red	(-)
		FeCl3	Black Blue	Bluish black	(+) Flavanol (galokatekin)
		NaOH 2N	Yellow / Brown Orange	Red	(-)

Qualitative test flavonoid extract of Caesalpinia sappan L results maceration and infundation showed positive results contain flavonoids. The experimental results in maceration and infundation indicate the types of flavonoids contained in the extract are flavones, flavanols and flavonols.

1.3 Determination of Levels of flavonoids with Ultraviolet Visible Spectrophotometer

Determination of flavonoids with the spectrophotometer is done by making a standard solution, using a standard solution with a concentration of flavonoids routine 0, 10, 20, 30, 40, and 50 ppm at a wavelength of 326 nm. The results of the standard absorbance measurement at a wavelength of 326 nm can be seen in Table 3 below:

Table 3. Absorbance Measurement Standards

Number	Concentration (ppm)	Absorbance
1	0	0
2	10	0,193
3	20	0,394
4	30	0,566
5	40	0,766
6	50	1,034

Absorbance measurement results of standard solutions at various concentrations of the calibration curve of standard solution of flavonoid compounds obtained routinely intercept value of -0.0256 and slop at 0.02054 so that the equation obtained is y = 0.02054 x - 0.0256, with the y is the value absorbance and x is a flavonoid (ppm). The equation used for comparison in the analysis of quantitative measurements are routinely extract flavonoids Caesalpinia sappan L results and infundation maceration method. Based on the results of measurements on a sample extract Caesalpinia sappan L results infundation maceration method and the data obtained as follows:

Table 4. Results of absorbance measurement at the sample extract

No	Samples	Absorbance
1	Extract Caesalpinia sappan L results maceration R1	0,768
2	Extract Caesalpinia sappan L results maceration R2	0.700
3	Extract Caesalpinia sappan L results maceration R3	0,829
4	Extract Caesalpinia sappan L results maceration R4	1,793
5	Extract Caesalpinia sappan L results maceration R5	1.185
6	Extract Caesalpinia sappan L results maceration R6	1.500

Absorbance the sample measurement then calibrate the linear regression equation of the standard concentration curve of absorbance versus standard by the equation y = 0.02054 x - 0.0256, so the concentration of flavonoids in the extract obtained Caesalpinia sappan L results and infundation maceration method. The results of the measurement of the concentration of the sample extract calculation Caesalpinia sappan L results and infundation maceration method can be seen in Table 5 below:

Table 5. Sample Concentration Measurement Results

Number	Samples	Absorbance	Concentration (ppm)
1	Extract Caesalpinia sappan L results maceration R1	0,768	39,513
2	Extract Caesalpinia sappan L results maceration R2	0,700	35,326
3	Extract Caesalpinia sappan L results maceration R3	0,829	41,606
4	Extract Caesalpinia sappan L results maceration R4	1,793	88,539
5	Extract Caesalpinia sappan L results maceration R5	1,185	58,938
6	Extract Caesalpinia sappan L results maceration R6	1.500	74.274

The concentration obtained from the equation y = 0.02054 x - 0.0256

DISCUSSION

This study uses materials such as Caesalpinia sappan L obtained from Boyolali area, Caesalpinia sappan L used are Caesalpinia sappan L were clean and there was no contamination of objects. Caesalpinia sappan L first made powder using a blender which aims to increase the surface Caesalpinia sappan L in order to simplify the process of making cider, and then the process of making cider by maceration method and infundation thus obtained extracts of Caesalpinia sappan L. The extract sampled to know how many levels of flavonoids from each of the extract. Selection of the sample must be considered carefully to avoid the chemical content of the samples were not representative.

The process of making cider in this study using the method of maceration and infundation, different methods are selected based on the level difference in temperature. Anggraini[1] explain the difference in temperature, which enables the amount of active substance that the most important will be different. Maceration method chosen because it has the advantage that the workmanship and the equipment used is simple and easily cultivated. This study uses a powder Caesalpinia sappan L 50 g solvent soaked into the water. Selection of water as a solvent for polar water has properties that correspond to the characteristics of chemical compounds that will be researched in this study are flavonoids [6]. The process of soaking and stirring carried out for the water to penetrate the cell wall Caesalpinia sappan L powder and enter into the cell cavity containing flavonoids. Stirring serves to flatten the concentration of pollen grains simplicia outside, so that the degree of the concentration difference between the solution in the cell with the solvent will remain intact. Flavonoid concentration difference between the solution inside the cell and outside the cell resulted in the displacement of flavonoids future of cell cavities Caesalpinia sappan L toward penyari fluid is water, occurs repeatedly, so in getting maserat [6]. Maserat evaporated with a water bath to extract the condensed because the water content which contains most of flavonoid compounds in maserat Caesalpinia sappan L evaporate.

Infundation method in this study using powder Caesalpinia sappan L of 50 g with a solvent such as water. Selection methods infundation in this study due to the warming will facilitate the release of chemicals contained in botanicals [1]. Infusion obtained is then evaporated with a water bath to extract the condensed because the water content which contains most of flavonoid compounds in the infuse Caesalpinia sappan L yawn.

The extract obtained in this study, there are two types of extracts and infundation results maceration method, both the extract has a different weight, the extraction by maceration method has greater weight than the extract produced by the method infundation. The difference in weight of the extract caused by factors that affect the temperature of the evaporation process. Temperatures in the extraction process by maceration method only through one heating process, whereas the method infundation through two stages of heating so that the liquid evaporates penyari have a lot, so the weight of the extract of the methods infundation less.

Extract infundation results maceration method and then conducted a qualitative test to ensure that there are flavonoid compounds contained in the resulting extract. Flavonoids are compounds containing a carbon-carbon bonds in the core base, which can be described as a sequence of compound C6-C3-C6 means karbonya framework consists of clusters C6 (substituted benzene ring) which is connected by a three-carbon chain aliphatic [4]. In plants, flavonoids typically bind to sugar called aglycone (Riyanto, 1989). Flavonoids can be classified into Anthocyanins, Proantosianin, Flavonols and flavones, Glikoflavon, Biflavonil, Khalkon and Auron, Flavonon, Isoflavones [5]. Flavonols and flavones are compounds that are widespread on all the yellow plant pigments [5].

Qualitative test to extract flavonoids Caesalpinia sappan L results maceration and infundation performed using reagents that Mg powder pekat- HCL, HCL 2N, 2N NaOH, and FeCl3 with the result of the change in color [11].

The results of qualitative tests showed that an extract of Caesalpinia sappan L maceration method results and positive infundation contains flavonoid. Extract the results of maceration reacted with concentrated HCl - powder Mg produces a pink color indicates flavones, reacted with HC l 2 N a brown color yellow indicates flavonol, reacted FeCl3 produces black color bluish indicate the presence of flavanols, reacted with NaOH 2N produce a red color has a negative result does not contain flavonol.

Extract the results infundation reacted with concentrated HCl - powder Mg produces a pink color indicates flavones, reacted with HCl 2N produce a red color which is negative containing flavonol, reacted with FeCl3 produces black

color bluish indicate the presence of flavanols, reacted with NaOH 2N produce sorrel negative means it contains flavonols.

The results showed the extract Caesalpinia sappan L using maceration method positive for flavonoid class of flavones, flavonols, and flavanols, while the extract positive results infundation method contains flavonoids flavones and flavanols groups.

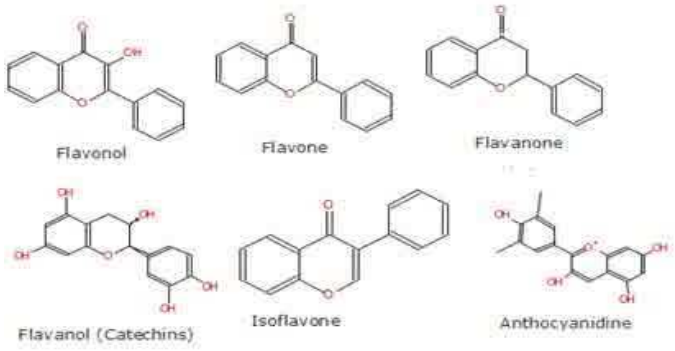

Figure 1. Chemical structure of flavones, flavonoids and flavonoid

Flavones and Flavonols a type of flavonoid in the form of O-glycosides that act as antioxidants. The major difference between flavones and flavonols are flavonols have a hydroxy group at C3. Flavon consists of apigenin and luteolin, whereas flavonol consisting of quercetin, kaempferol, myricetin [9]. Flavonoids are chemical content in plants that are found in some fruits, vegetables, tea and chocolate [8]. Flavanol are chemical content in plants that are found in some fruits, vegetables, tea and chocolate [2].

Analysis of flavonoid levels in extracts of Caesalpinia sappan L results maceration and infundation done using Ultraviolet Visible spectrophotometer. Before taking measurements levels, first performed the maximum wavelength scanning. This study uses the wavelength of 326 nm. This is because the wavelength of each compound may differ if specified conditions and different tools. Goal to find the wavelength is in order to get maximum sensitivity to changes in absorbance for each unit of concentration is the greatest and the risk of error is smaller when do repeated measurements [3].

This study uses a standard solution with a concentration of flavonoids routine 100 ppm then dilakukam dilution with various concentrations were 0, 10, 20, 30, 40, and 50 ppm. Dilution function is to minimize errors, as Beer's law applies to the aqueous solution so that the solution easily penetrated by light. The concentration of 0 ppm is the concentration of the blank form of pure methanol. The purpose of making the reference solution is to determine the absorption by substances not analat (solution to be analyzed). Extract Caesalpinia sappan L results maceration and infundation each dilution at a concentration of 500 ppm with 3 replication to minimize errors.

Absorbance measurement standard solution with varying concentrations of flavonoids routinely produce a calibration curve equation y = 0.02054 x - 0.0256, the equation is used as a benchmark in the analysis of quantitative measurements are routinely extract flavonoids Caesalpinia sappan L results and infundation maceration method. The calculation result obtained at different flavonoid extract Caesalpinia sappan L results and infundation maceration. The content of flavonoid compounds in the extract on average 23 478 results maceration or 0.0162%, whereas in the extract Caesalpinia sappan L results infundation method is 73 917 ppm, or 0.19026%. The content of flavonoids in infundation outweigh the resulting extract of maceration caused by the heating process in infundation method will facilitate the release of the content of flavonoid compounds in botanicals.

CONCLUSION

Extract Caesalpinia sappan L maceration method positive results contain flavonoids. The extract showed positive results maceration method contains flavonoid class of flavones, flavonols, and flavanols. Extract Caesalpinia sappan L infundation method positive results contain flavonoids. The extract showed positive results infundation method

contains flavonoid class of flavones, flavonols and flavanols. Levels of flavonoids using ultraviolet visible spectrophotometer in extracts of Caesalpinia sappan L maceration method results showed lower levels of flavonoids of 0.0162%. Levels of flavonoids using a spectrophotometer Ultraviolet Visible on the extract Caesalpinia sappan L infundation method results showed levels of 0.19026% flavonoids. Need to analyze the levels of flavonoids in extracts of Caesalpinia sappan L using different solvents. Need to do the analysis on the levels of flavonoids extract of Caesalpinia sappan L uses material simplisia Caesalpinia sappan L from various regions.

Acknowledgements
The author would like to thank the team of researchers. This paper is not possible without the work of innovative and high dedication to developing science.

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