International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.2, pp 243-246, 2014-2015

Determination of Phenolic and Flavonoid Contents of Ethanolic Extract of Kanunang Leaves (*Cordia myxa* L.)

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Abstract: *Cordia myxa* L. (*C. myxa*) leaves is included in family Boraginaceae which has been used as traditional medicine in Indonesia. This study aims to determine of total phenolic and flavonoid content of Kanunang leaves extract (*C. myxa*). They were extracted by maceration method with ethanol 70%. The level of phenolic and flavonoid content were determined by spectrophotometer UV-Vis with gallic acid and rutin as standard. It were obtained 25 gram extract from extracting 1380,00 gram powdered leaves, the rendament shows 7,14%. Determination of phenolic content by Folin-Ciocalteau method shows 8,45% GAE (Gallic acid equivalnet), while flavonoid content determined by colorimetric methode $AlCl_3$ is 1,202% RE (rutin equivalent).
Keyword : *Cordia myxa* L., Phenolic, Flavonoid, Kanunang.

Introduction

Kanunang (*C. myxa)* is one of the species of genus *Cordia,* family Boraginaceae. *C. myxa* has some pharmacological effect. In some studies, *C. myxa* has been reported that may act as hepatoprotective, anti-inflammatory, anti-diabetic, dispepsia, some degenerative diseases, antimicrobial, and antioxidant[1].

The potency of this plant in order to use as traditional medicine is related to its chemical compounds e.g phenolic, alkaloid, and terpenoid.

In addition, according to Artanti *et al.,* (2006)[2] that some plants that contain phenolic and flavonoid has been reported have some pharmacological activities, e.g anti-diabetic, antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer. Moreover, flavonoid has been reported to have some biological activities such as anti-inflammatory, antibacterial, antiviral, anti-allergic, antitumor, neurodegenerative and vasodilatory effect.

Besides that, flavonoid is also known may inhibit lipid-peroksidase, platelet aggregation, capillary blood vessels permeability and activity of enzyme cyclooxigenase and lipoxygenase, through its antioxidant activity, free radical scavenging, cation divalent chelators. Furthermore, it has been reported that may inhibit some hydrolase enzyme e.g hyalouronidase, alkali phosphatise, arylsulphatase, cAMP-fosfodiesterase, lipase, kinase and α-glucosidase[3].

Based on those information, it needs further investigation for instance determination of phenolic and flavonoid content of Kanunang *C. myxa* leaves extract in order to develop the potency of this plant become traditional medicine.

Method

Material

The powdered of kanunang *C. myxa* leaves, ethanol 70%, Folin-Ciocalteau (Sigma, Aldrich), Sodium hydroxide 1%, gallic acid, rutin, methanol Pa, $AlCl_3$ 10%, potassium acetate.

Extraction

The powdered of *C. myxa* leaves were extracted with methanol 70% by using maceration method. The powdered leaves is added with ethanol 70%, incubated for 3-5 days and stir frequently[4]. Then filtered, and maceration is continued until the clear solution was obtained. The resulting solution is concentrated by using *rotary vacuum evaporator* (Rotavapor) at temperature 50^o C.

Determination of phenolic content

The solution test (1,0 mL) is added 5,0 mL Folin-Ciocalteau in volumetric flask. The resulting solution is incubated for 8 minutes, then added with 4 mL NaOH 1%, and incubated for 1 hour. The absorption was measured at wavelength 730 nm. The calibration curve is made of gallic acid. The level of phenolic content is presented by gallic acid equivalent (GAE)[5,6].

Determination of flavonoid content

100 mg extract were dissolved with 10 mL methanol pa. 1,0 mL resulting solution is added with 3 mL methanol, 0,2 mL $AlCl_3$ 10%, 0,2 mL potassium acetate, and 5,6 mL sterile distilled water, the resulting solution is incubated for 30 minutes in dark place at room temperature, the absorption was measured with spectrofotometricUV-Vis at wavelength 415 nm. The level of flavonoid total is performed with rutin equivalent(RE)[7].

Result and Discussion

The result of extraction of kanunang *C. myxa* leaves with ethanol 70% by maceration method were obtained 25 g crude extract with the percentage of rendament is shown in Table 1.

Table 1. Result of extraction

No	Name	weight (g)	Rendament (%)
1	Wet weight	2900,0	-
2	Dry simplisia	1380,0	-
3	Extracted simplisia	350,0	-
4	Dry Extract	25,0	7,14
5	Total solvent	13,7 (L)	

The determination of phenolic content by folin-ciocalteau method with gallic acid as standard with linear regression (Figure 1), with percentage of phenolic total of 500 ppm sample is 8,43% GAE (Table 2).

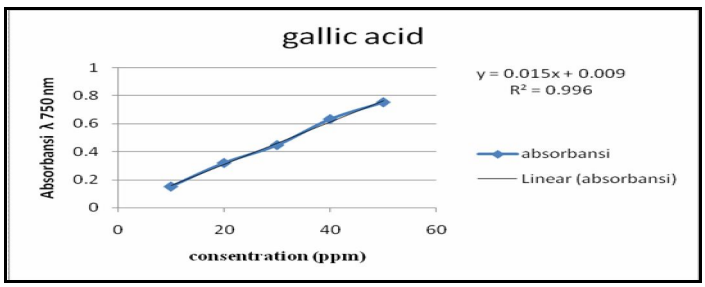
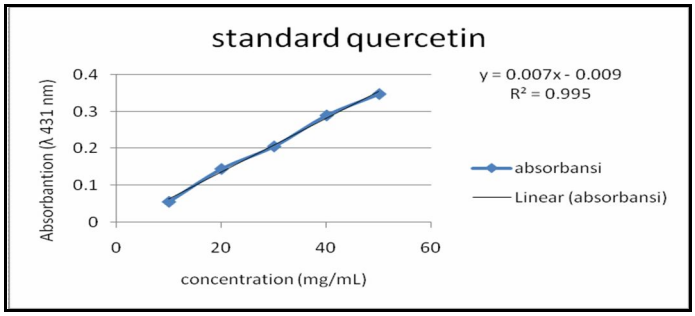


Figure 1. gallic acid curve

Tabel 3. Persentage of phenolic total

Replication	consentration (ppm)	Absorbantion (745 nm)	% phenolic content GAE (b/b)
1		0,602	
2		0,623	
3	5000	0,706	8,43
4		0,652	
5		0,662	

The determination of flavonoid total of ethanolic extract of kanunang (*Cordiamyxa*L.) leaves with rutinas standard curve (figure 2) andthe percentage of level flavonoid is 1,202% RE (Table 3).



Gambar 2. Standard quercetin curve

Tabel 4. Result of determination of Flavonoid total

Replication	concentration (ppm)	Absorbantion (745 nm)	% level of flavonoid QE (b/b)
1		0,460	
2	5000	0,408	1,202
3		0,521	
4		0,478	

Kanunang *C. myxa* leaves were obtained from state ofEnrekang, South Sulawesi Selatan and identified from Herbarium Bogoriense LIPI-Cibinong. *C. myxa* leaves were powdered in order to extract more chemical compound[8]. The maceration method was used in this study due to its simplicity, and without heat[4], which more safety for the compounds that unstable with heat. Ethanol 70% was used as solvent since it can dissolve some chemical compounds, some secondary metabolites[9].

The powdered of *C. myxa* leaves were extracted with methanol 70% by using maceration method. The powdered leaves is added with ethanol 70%, incubated for 3-5 days and stir frequently[4]. Then filtered, and maceration is continued until the clear solution was obtained. In this study, the powdered leaves(1380 g) with ethanol(13,7 L) were used and were obtained 25 g crude extract, with the percentage of rendament 7,14%.

The potency of extracts to be traditional medicine depends on their chemical compounds (secondary metabolite). Phenolic and flavonoid occur widespread in some plants. Some studies have been reported the biological activities of those compounds. Thus, the determination of phenolic and flavonoid total is need to conduct.

Phenolic that occurs in extract is analyzed by using spectrophotometric UV-Vis[9]. According to Chun *et al*, 2003 dan Depkes RI, 2011, Folin-Ciocalteau was used for this method. Folin-Ciocalteaucauses bathochromic wavelength movement around 750 nm which is showed by the color change from yellow to blue after incubated. Its color change occur due to the reduction which leads to make complex between phenolic group and the tungsten and molibdat.

Then it is plotted to curve (concentration vs absorption) so as to obtain linear regression y = 0,0151x + 0,009 and yield R^2 = 0,9962 which indicates good linearity. Subsequently, it then be used to determine of

phenolic level from 500 ppm sample compare and the yield is 8,43%. The result shows that this yield is more than the other extracts that presented in Farmakope Herbal Indonesia.

Flavonoid content analyzing was conducted by colorimetric method refers to Chang, *et al.* (2002) procedure which validates by Mujahid (2011) with rutin as a standart, the one of flavonoid compounds that occurs in numerous plants, as comparison. For measuring flavonoid total, it needs to add $AlCl_3$ 10 % into extract solution in order to lead to complexity form between flavonoid and $AlCl_3$ which indicates with color changing into yellow at maximum wavelength 415 nm.

The yield of absorption of standard rutin then was plotted to curve of rutin concentration (x) and absorption (y) and was obtained linear regression $y = 0.0073x - 0.0097$ with $R^2 = 0.9953$, the R^2 value shows the good linearity. Therefore, it can be used then to determine the level flavonoid total. There was obtained that ethanolic extract of *C. myxa* leaves at concentration 500 ppm contains phenolic which equal with rutin (1,202%).

Conclusion

The result extract was obtained is 25,0 g with percentage of rendament 7,14%. The determination of phenolic and flavonoid is 8,45% GAE and 1,202% RE, respectively.

Acknowledgment

We acknowledge to Direktorat Pendidikan Tinggi (DIKTI) Ministry of education and culture of Indonesia for giving a funding of this research.

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