Analysis of Total Phenolics and Flavonoids from the Root Bark of *Flacourtia rukam*

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Keywords: Phenolic, Flavonoid, *Flacourtia rukam*

Abstract: *Flacourtia rukam* have been used in the to folk medicine. The roots was used by women after giving birth as antiseptic and the stem bark was used to antihypertensive in the Regency of Musi Banyu Asin South Sumatera Indonesia. Total phenolic and flavonoid content were determined for methanol, ethyl acetate and n-hexane extracts from the root bark of *F. rukam*. Each extracts were determined antioxidant activity using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. The results of the study showed that phenolic higher in methanol extract (71.8868 mg GAE/g) compared with ethyl acetate extract (56.6094 mg GAE/g), while n-hexane extract no detection. For flavonoids content showed, methanol extract (2.3116 mg QE/g) and ethyl acetate extract (2.3304 mg QE/g) where the flavonoid content is not significantly different, while n-hexane extract only contained flavonoid 0.8129 mg QE/g. The antioxidant activity the methanol, and ethyl acetate extracts showed IC_{50} 52.91µg/mL and 86.92 µg/mL and in categorized as active antioxidant, while n-hexane extract obtained IC_{50} values 268.02 µg/ml in category weak antioxidant.

1 INTRODUCTION

Phenolic compounds is antioxidant properties and antiradical activities that are beneficial to health. Phenolic compound also showed activity as anti-inflammatory, enzyme inhibition and induction, detoxification and process of skin rejuvenation or the premature aging process through stimulate collagen production (Fuad *et al*., 2016). Antioxidant activity of phenolic pompounds in small concentrations can protect, prevent or induction the level of oxidative damage to biomolecules. One of the plants used as traditional medicine is *Flacourtia rukam*.

This plant in the south of Sumatra the stem bark is used as an antihypertensive drug. Besides that the fruit is used to treat diarrhea and dysentery, the leaves are used to treat swollen eyelids, and the roots are used by women after giving birth (Yustian *et al.*, 2012. The biological activity of a plant extract is closely related to the secondary metabolites it contains, including flavonoid, phenol, terpenoid, steroid and alkaloid. Secondary metabolites can be utilized in the field of pharmacology (Mustarichie *et al*., 2013), including as an antioxidant, antibiotic, anticancer, blood anticoagulant, inhibiting carcinogenic effects. Besides that secondary metabolites can also be used as environmentally friendly pest controllers (Samsudin and Khoiruddin, 2008).

Some information on the chemical content and biological activity of *F. rukam* has been reported. Saree *et al*., (1998) reported that the *Flacourtia rukam* contained stigmastan-3,6-dione and friedelin. Besides that, Ikram *et al*., (2009) reported that the fruit contained phenolic and showed antioxidant activity with a value of % inhibition of 70.9%. From the fruit section 5 compounds have been reported, namely monogalactosyl diacylglycerol, β-sitosterol-3β-glucopiranoside-6β-fatty acid esters, β-sitosterol, triacylglycerol, and chlorophyll a (Ragasa *et al*., 2016). Monogalaktosildiasilgliserol compound is active as anti-inflammatory (Imbs *et al*., 2013). Meanwhile for the parts of the stem bark of *Flacourtia rukam* was reported to contain friedelin, poliothrysoside, and β-sitosteryl-3β-glucopyranoside (Muharni *et al*., 2018). The phytochemical test of the *Flacourtia rukam* plant has been shown contain triterpenoid, steroids, flavonoids and phenolic compounds (Muharni *et al*., 2016). In this paper we will report the analysis of total phenolic and

Muharni, ., Elfita, ., Julinar, . and Yohandini, H.
Analysis of Total Phenolics and Flavonoid from the Root Bark of Flacourtia rukam.
DOI: 10.5220/0008839200300035
In *Proceedings of the 1st International Conference on Chemical Science and Technology Innovation (ICOCSTI 2019)*, pages 30-35
ISBN: 978-989-758-415-2

flavonoids from the root extracts of *Flacourtia rukam*

2 MATERIALS AND METHODS

2.1 Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), quercetin, gallic acid, Folin-Ciocalteu, dimethylsulfoxide, sodium carbonat, aluminum chloride, potassium acetate (reagent were obtained from Sigma Aldrich, USA), methanol, ethyl acetate, n-hexane, and aquadest. The solvents is analytical grade and were destilation before used.

2.2 Preparation of Sampel and Identification

The root bark of plant of *F. rukam* was collected at month of january 2018 2018 from Musi Banyuasin, South Sumatera, Indonesia. The taxonomic identity of the plant was confirmed by Dr. Laila Hanum (number specimen VIC 2702) at of the Botanical laboratory Departement of Biology University of Sriwijaya. The plant materials (1Kg) was cleaned from the pollutants and the materail plants were cut into smaller sizes and then to air dried at room temperature until the weight constant. The dried sample was powdered busing a grinding machine and obtained (300 g) and used for further studies.

2.3 Extraction

Powdered of root bark *F. rukam* (300 g) was place in bottle and extracted with organic solvents with step gradien polarity: using n-hexane, ethyl acetate, and methanol and placed at room temperature. Maceration was done for 24 h and then were filtered using paper filter. The extraction was repeated three times for each solvent, and extraction has been complete. The maserate obtained was concentrated by rotary vacuum evaporator, at temperature 55 ºC and then air dried so that we obtained eachs crude extract.

2.3.1 Yield Precentage of Extracts

The extraction yield precentage is the amount of extract to obtained compared with the amount of root plant used. Yield precentage (%) was determined for each solvent with equations:

$$\% \text{ Yield} = Wa / Wb \; x \; 100 \; \% \qquad (1)$$

Wa = weight of crude extract
Ws = weight of sample

2.4 Analysis of Total Phenolic

Determination of the total phenolic content of each extracts of obtained used spectrometrically analysis to the Folin-Ciocalteu method. (Santoso *et al*., 2012). The Standard gallic acid solution of 1000 µg/mL was prepared by dissolving 5 mg gallic acid in 2 mL ethanol and the volume made up to a volume of 5 mL with aquadest. The standard gallic acid with a concentration series of 5, 10 , 15, and 20 µg/mL were preparated with multilevel dilution from a standard solution of 1000 µg/mL . 100 µL of each standard gallic acid add 1 mL of 5% Na_2CO_3 solution. The mixture incubated for 5 min, then added 1 mL Folin-Ciocalteu reagent 50%, incubation to continued for 1 hour at room temperature, then measured absorbance at λ_{maxs}765 nm. The calibration curve is made through the relationship between the concentration of gallic acid (µg/mL) and absorbance.

The total phenolic in the sample was determined by means of 5 mg of the each extracts were dissolved in 2 mL ethanol and the volume made up to a volume 5 mL with distilled water. Then each sample 1 ml were added 1 mL sodium carbonat solution (5%) 0.5 mL, and incubated for 5 minutes, then add folin ciocalteu's reagent 50%) and incubated for 1 hour. Absorbance was recorded at λ_{maxs} 765 nm used spectrofotometer UV-Vis. The total phenolics were expressed as gallic acid equivalents (GAE). Base on the calibration curve total phenolic calculations used the following formula:

$$\text{Total phenolic} = cxv\text{x Fp} / m \qquad (2)$$

note :
c = equivalent levels of gallic acid (mg GAE / L)
v = volume of extract solution (mL)
m = mass of extract (g)
Fp = Dilution factor

2.5 Determining of Flavonoid Contents

The Determining of flavonoid contents eachs extracts were according to the method described (Nugroho *et al*., 2013). The standard concentration of quercetin 1000 µg/mL 25 mL was prepared by means of 0.025 g quercetin dissolved in aquadest in a 25 mL flask. The quercetin standard was prepared with a concentration of 50, 100, 150 and 200 µg/mL by piping 0.5 mL; 1 mL; 1.5 mL; and 2 mL of a

standard solution of quercetin 1000 µg/mL into a 100 ml volumetric flask and added distilled water. 500 µL each standard solution were added 1.5 mL of methanol, 0.1 mL of $AlCl_3$ 10%, 1 mL of $NaNO_2$ 0.5 M and 2.8 mL of aquadest, then homogenized the mixture by means of the vortex, and incubation to continued until 30 min and followed the addition of 1 mL of NaOH (1 M). After that absorbance was measured at at the λ_{maxs} 415 nm.

The flavonoid content in the extract is determined by means of the sample 1000 µg/mL was prepared by means of 5 mg dissolved in aquadest in a 5 mL flask. Sample solution (500 µL) of different extract wereadded 1.5 ml of methanol, 0.1 mL of $AlCl_3$ 10%, 1 mL of $NaNO_2$ 1M and 2.8 mL of aquadest, incubating for 30 min at room temperature and then to continued with addition of 1 mL of NaOH (1 M). As the blank using test tube containing 2.5 mL of aquadest. The sample containing of flavonoid was show by pink color to the read with UV-spectrophotometrically at λ_{maxs} 415 nm. For the determining quantification of flavonoid content of sampel used Quercetin compound as the standard. This experiment with with triplicates and the results were determined in quercetin equivalents (QE). Flavonoid content calculations use the following formula

$$\text{Flavonoid content} = cxvx\ \text{Fp} / m \qquad (3)$$

note :
c = equivalent levels of quercetin (mg QE / L)
v = volume of extract solution (mL)
m = mass of extract (g)
Fp = Dilution factor

2.6 *In vitro* Antioxidant with DPPH Method

The radical DPPH methd was used to measure antioxidant activity (Nataraj *et al*., 2013). 200 µL the samples at various concentrations (62.5, 125, 250, 500, and 1000 µg/mL of extracts with respective organic solvents) were taken and added 3.8 mL of a 0.05 mM. As a negative control used . 200 µL of methanol in 3.8 ml of DPPH solution. The reaction mixtures were incubated for 30 min at room temperature. Antioxidant activity determined as inhibition of DPPH by the samples with reduced content of DPPH radical characterized by decreased absorbance. against the blank (methanol) and was measured at λ_{maxs} 517 nm.

3 RESULT AND DISCUSISON

3.1 Yield Percentage

The dry powder of plant roots *F. rukam* (300 g) were extracted successively using solvent with increased polarity of n-hexane, ethyl acetate and methanol and after concentrating the extracts of each extract were obtained. The choice of maceration method has many advantages compared to other methods. such as procedures and tools that are used simply, do not use heating so that the compounds contained therein will not be damaged or decomposed, the solvents used are also less than the other cold methods of percolation. The yield percentage showed in Table 1. Each extracts after concentrating to obtained n-hexane extract (1.101 g), ethyl acetate fraction (3.221 g) and methanol fraction (17.701 g). Extract yield percentage of methanol (5.90%) higher compared ethyl acetate (1.07%) and n-hexane extract (0.36%). Among the different solvent extractions, methanol solvent found to have higher ecovery over other.

Table 1: Yield percentage of extracts

Solvent	weight of crude extract (g)	yield percentage (%)
n-Hexane	1.101	0.36
Ethyl acetate	3.221	1.07
Methanol	17.701	5.90

3.2 Determining of Total Phenolic, and Flavonoid Contents

The total phenolic contents of different extracts were analyzed with used curva standard gallic acid. The gallic acid standard curve with 4 series of standard quercetin solutions in ethanol with folin ciocalteu and sodium carbonate solution. The results are shown in Table 2.

Table 2: Effect of concentration on the absorbance value of gallic acid

Concentration (µg/mL)	Absorbance
0	0
5	0.226
10	0.445
15	0.739
20	1.003

The coefficient correlation of the gallic acid standard curve based on the regression equation y = 0,050x - 0,021 R^2 = 0.996, is greater than 0.98, this shows that the curve linearity obtained is very good. The total phenol content of each extract was determined with standard gallic acid calibration

curve, were stated in mg gallic acid in each g of extract (Mg QE/g) and presented in Table 3. In this study, methanol extract (71.8868 mg/g) shows higher total phenolic content compared ethyl acetate extract (56.6094) and n-hexane extract phenolic compound was no detection. The methanol solvent was more effective in extracting phenolic constituents.

Table 3: Total phenolic contents of the root of *F. rukam*

Extract	Total phenolics (mg GAE/g
n-hexane	-
Ethyl acetate	56,6094
Methanol	71,8868

Analysis of flavonoid content was determined based on the quercetin standard curve. Standard curve was done by reacting 4 series of standard quercetin solutions in ethanol and the results are shown in Table 4.

Table 4: Effect of concentration on the absorbance valueof quercetin

Concentration (µg/mL)	Absorbance
0	0
0.5	0,032
1	0.063
2	0.138
5	0.346

Based on the regression equation, y = 0,069x - 0,002, R^2 = 0,999 the correlation coefficient value is approaching 1. This shows that the curve has a good linearity value. The flavonoid content of each extract was determined based on the quercetin standard curve regression equation. Flavonoid content is expressed in mg qercetin in each gram of extract (Mg QE/g) as shown in Table 5. This data show, methanol extract (2.3116 mg QE/g) and ethyl acetate extract (2.3304 mg QE/g) shows The flavonoid content is not significantly different, Meanwhile n-hexane extract shows lower flavonoid content than other extracts. Base on this data methanol and ethyl acetate solvent having the same effectiveness were used to isolate flavonoid constituents.

Phenolic and flavonoids compounds have been know to have multiple biological effects oxidative stress related disorders such as antioxidant, anticancer, antidiabetic, anticholesterol, hypertence, and anti-inflammatory properties (Amarowicz, 2007). Phenolic and flavonoid constituents of root bark of *F. rukam* allegedly plays a role in the used of this plant traditionally for the treatment of diseases related to oxidative stress related disorders.

Table 5: Flavonoid contents of the root bark of *F. rukam*

Extract	Flavonoid content (mg QE/g)
n-hexane	0.8129
Ethyl acetate	2.3304
Methanol	2.3116

3.3 *In vitro* Antioxidant Activity by DPPH Method

The DPPH method, has been widely used for determining the antioxidant activity of plant extracts. The results of all extract show decrease Inhibition percentage with decrease concentration test (Table 6) .

Table 6: The effect of concentration on % inhibition values each extracts

concentration (µg/ml)	Percentage Inhibition extrcts		
	n-hexane	Ethyl acetate	Metanol
1000	71.96	98.20	93.53
500	68.94	78.71	72.86
250	53.30	69.06	68.10
125	44.42	59.08	57.57
62.5	25.76	39.28	39.33

To determine the IC_{50} value of each extracts, base on a linear regression equation between the % inhibition value eachs extracts and the concentration variation. Based on the regression equation obtained IC_{50} values for n-hexane, ethyl acetate, and methanol extract 268.02, 86.92, and 52.91 µg/mL respectively. The IC_{50} value which is getting lower, the extract is stated to be more active. Based on this data, the methanol extract showed higher antioxidant activity compared to other extracts (Table 7).

Table 7.: IC_{50} value eachs extracts

Extracts	IC_{50} (µg/ml)
n-hexane	268.02
Ethyl acetate	86.92
Methanol	52.91

According to Molyneux (2004) extract is categorized as active as an antioxidant if it has an IC_{50} of less than 200 µg/mL. If the IC_{50} value is found at around 200 to 1000 µg/mL in category weak antioxidant and if above 1000 µg/mL is categorized as in active. Base on data we conclusion that methanol and ethyl acetate extract in category active antioxidant and n-hexane extract category weak antioxidant. Based on this study, the search for antioxidant compounds from root bark of *F.*

rukam plants can be carried out on ethyl acetate and methanol extracts. This data also proves that the used of *F. rukam* as traditional medicine for the treatment of diseases related to degenerative diseases can be explained by the presence contained of antioxidant compounds found in *F. rukam*. Antioxidant compounds are generally phenolic groups which are soluble in semi-polar solvents or polar solvents. However, the discovery of the antioxidant properties of non-polar n-hexane fraction is thought to be due to the presence of flavonoid compounds extracted into n-hexane solvents. according to the results of previous studies where in the n-hexane extract was found to contain flavonoid compounds. Fidrianny *et al*., (2014) also found flavonoid compounds as antioxidant from n-hexane extract *Tamarindus indica* L. Atioxidant activity was also reported from the n-hexane fraction *Euphorbia tirucalli* (Mawadah *et al*., 2016).

Antioxidant activity is proportional to the total phenolic content and flavonoids of the extract (Esmaeilli *et al*., 2015). At the tested extracts found the significant correlation between the antioxidant activity of extracts with value total phenolic and flavonoid content. According to the findings, the extract of methanol possesses a comparable antioxidant activity to the total phenolic and flavonoid content.

4 CONCLUSIONS

The result of the study showed that phenolic higher in methanol extract compared with ethyl acetate extract while n-hexane extract no detection. For flavonoids content showed, methanol extract and ethyl acetate extract, the flavonoid content was not significantly different, while n-hexane extract only contained flavonoid very less. The antioxidant activity the methanol, and ethyl acetate extractin category active antioxidant, while n-hexane extract in category in weak antioxidant.

ACKNOWLEDGMENTS

The authors are thankful to ministry Ristekdikti Republic of Indonesia, who have funded this research through grant Basic Research superior university periode of 2019. Thank you also to the department of chemistry University of Sriwijaya and Basic Laboratory of University on providing facilities for the research work.

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