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Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

Ni Putu Ermi Hikmawanti[1], Tuti Wiyati[1], M. Abdul Muis[1], Farah Aisyah Nurfaizah[1] and Windy Septiani[1]

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Abstract

Cayratia trifolia leaves contain flavonoids compound that can dissolve well in polar solvents. This study aimed to evaluate the total flavonoids content of polar extracts which extracted sequentially from *C. trifolia* leaves. The dried *C. trifolia* leaves were macerated continually using n-hexane, ethyl acetate, and ethanolic solvent. The total flavonoids content was determined using spectrophotometric methods with a $AlCl_3$ reagent. TLC chromatogram of the extracts were evaluate using chloroform: aceton: formic acid (10:2:1) as a mobile phase and silica gel F_{254} as a stationary phase. The citroborate and ammonia vapour were used as detection spray reagents. The highest total flavonoids content (27.95±0.62 mgQE/g) were found in 70% ethanol extract of *C. trifolia* leaves compared to ethyl acetate extract (17.98 ± 0.89 mgQE/g). The ethanolic extract is found six compound spots, while the ethyl acetate extract found four compound spots which were thought to be a flavonoid group. Thus, it can be concluded that the different polar extracting solvents are able to attract flavonoid compounds with different levels. The flavonoids in *C. trifolia* extract need to be separated to determine the types.

Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

Ni Putu Ermi Hikmawanti*, Tuti Wiyati, M. Abdul Muis, Farah Aisyah Nurfaizah, and Windy Septiani

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Abstract. *Cayratia trifolia* leaves contain flavonoids compound that can dissolve well in polar solvents. This study aimed to evaluate the total flavonoids content of polar extracts which extracted sequentially from *C. trifolia* leaves. The dried *C. trifolia* leaves were macerated continually using n-hexane, ethyl acetate, and ethanolic solvent. The total flavonoids content was determined using spectrophotometric methods with a $AlCl_3$ reagent. TLC chromatogram of the extracts were evaluate using chloroform: aceton: formic acid (10:2:1) as a mobile phase and silica gel F_{254} as a stationary phase. The citroborate and ammonia vapour were used as detection spray reagents. The highest total flavonoids content (27.95±0.62 mgQE/g) were found in 70% ethanol extract of *C. trifolia* leaves compared to ethyl acetate extract (17.98 ± 0.89 mgQE/g). The ethanolic extract is found six compound spots, while the ethyl acetate extract found four compound spots which were thought to be a flavonoid group. Thus, it can be concluded that the different polar extracting solvents are able to attract flavonoid compounds with different levels. The flavonoids in *C. trifolia* extract need to be separated to determine the types.

1. Introduction

In Indonesia, *C. trifolia* is known as galing or lakum. In English, this plant is called Fox grape [1]. The leaves of this plant are used by several people in Indonesia as medicine to cure headaches [2], boils, speed drying of the wound [3], muscle pains, and antidandruff [4]. The polar fraction (ethyl acetate and methanol) of galing leaves has been known to have antioxidant activity [5]. The ethanol crude extract of the galing plant has hepatoprotector properties[6].

Flavonoids are secondary metabolites of polyphenol derivatives with many benefits. Flavonoids function as strong antioxidants and also have the potential to be developed into a nutraceutical source [7]. The good solubility of flavonoids in polar solvents can be used as a basis for guiding the separation procedure for these compounds. Polar solvents that are good for the extraction of flavonoids include alcohol (ethanol, methanol, n-butanol, etc.), ethyl acetate, acetone, or water [8]. Selection of the right solvent will have an impact on the chemical content extracted, the extraction time, the composition of the extracted compound, the ease of handling the extract, and the effectiveness of the extract produced [9].

Plant extracts can be easily identified qualitatively for their flavonoid content using thin-layer chromatography (TLC). Compared to paper chromatography, TLC has a better ability in terms of separating phenolic derivatives, especially in extract samples. The TLC method is also quite

inexpensive and is able to identify several samples at the same time so that it is efficient in terms of using analysis time [10]. TLC can be used to provide an overview of the chromatogram pattern of an extract material. Meanwhile, quantification of total flavonoids is generally carried out by the colorimetric method using a spectrophotometer assay because it is considered simple and fast [10]. Measurement of total flavonoids from plant extracts that have been added with $AlCl_3$ reagent was carried out in the range 410-423 nm [8]. In this study, the total flavonoid levels in *C. trifolia* leaf extract were sequentially extracted using organic solvents and the chromatogram pattern was determined using the TLC method.

2. Material and Methods

Preparation of Sample

The *C. trifolia* leaves were collected from Tuban, East Java, Indonesia. The plant was identified in Conservation Research Center and Botanical Garden, Indonesian Institute of Sciences, Bogor, Indonesia. The *C. trifolia* fresh leaves were dried for a few days at undirect sunlight condition and were made into powder. The 1.5 Kg of dried leaves powder was extracted by cold maceration (2 times, 24 hours) respectively in *n*-hexane, ethyl acetate, and ethanol-water (70:30) according to their increasing strength of polarity. Each filtrate was evaporated using a vacuum rotary-evaporator N-1200 BS series (EYELA, Shanghai, China) at 40 °C. The percentage yield of *n*-hexane, ethyl acetate and ethanolic extracts of *C. trifolia* leaves were calculated [11].

Determination of Total Flavonoids Content (TFC)

The determination of TFC follows the procedure on Chang et al., (2002) with modification [12]. Quercetin as standard. Each extract of *C. trifolia* leaves in methanol (at concentration 1000 ppm) is piped as much as 0.5 mL then 3 mL of methanol is added and 0.2 mL of $AlCl_3$ (10%) reagent is added, 0.2 mL of sodium acetate (1M) and sufficient with aquadest up to 5 mL. The solution was incubated for 60 minutes at room temperature. The absorbance of the extract solution was measured with a UV-Vis Shimadzu UV-1601 Series (Kyoto, Japan) spectrophotometer at a wavelength of 428.5 nm. The absorbance obtained is plotted into the linear line equation. TFC is expressed in mg which is equivalent to quercetin per gram of extract. Each extract was tested for 5 repetitions and reported as mean ± SD.

Analysis of Sampel using Thin Layer Chromatography (TLC)

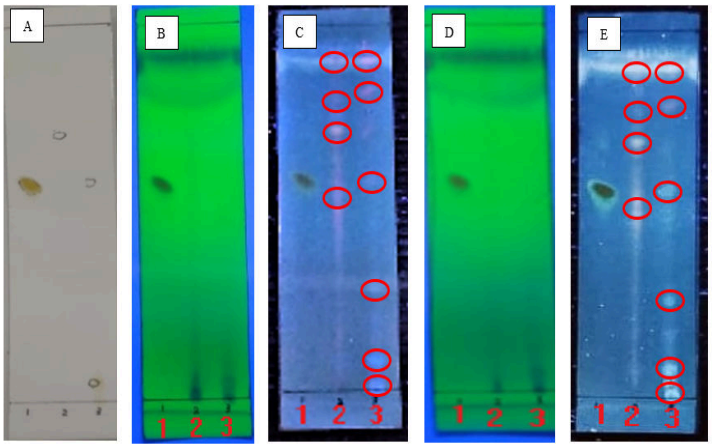
The profile chromatogram of flavonoids in each extract was analysis using TLC. Each extract was dissolved in methanol (at concentration 3%). Chloroform: aceton: formic acid (10:2:1) were used as a mobile phase. Silica gel F_{254} as a stationary phase. The citroborate solution was used as detection spray reagents. The visualisation of spots was performed in visible and UV (UV box, Camag).

3. Results and Discussion

The percentage of yield of the *C. trifolia* sequentially extracts were 1.75% for ethyl acetate extract and 21.30% for ethanolic extract. This means that *C. trifolia* leaves are dominated with polar compounds, either phenolics or polar non-phenolic compounds (such as saponins, glycoside, sugar, etc.). The highest total flavonoids content (27.95±0.62 mgQE/g) was found in ethanolic extract compared to ethyl acetate extract (17.98 ± 0.89 mgQE/g) of *C. trifolia* leaves. The ethanolic extract of *C. trifolia* leaves is found six compound spots, while the ethyl acetate extract found four compound spots which were thought to be a flavonoid group (**Figure 1**).

Flavonoids are structurally very diverse compounds with stable chemical properties in plants and are often used as chemotaxonomic markers. The TLC method is a method that is often used for rapid screening of flavonoids as pharmacological target compounds in plant extracts. Extraction of flavonoids with polar solvents such as ethyl acetate and alcohol usually begin with the removal of unwanted interfering compounds such as fat, chlorophyll using nonpolar solvents such as n-hexane

[13]. Ethanol polarity is caused by the presence of the free OH group, while ethyl acetate is caused by its presence of π electrons and lone-pair electrons present on O [14]. Flavonoid aglycones have a low molecular weight. Its aglycone form is often soluble in less polar solvents than ethanol. Meanwhile, flavonoid glycosides will be extracted more in polar solvents such as alcohol (methanol or ethanol), water, or a mixture of both. [15]. Based on the results obtained, it shows that the extract was extracted using ethanol solvent contains more varied flavonoids and more quantitatively than the extract extracted with ethyl acetate. Further separation accompanied by bioassays is necessary for the purpose of isolating flavonoid compounds that play an active role in a pharmacological activity.



Notes:
1 = Quercetin
2 = Ethyl acetate extract
3 = Ethanolic extract
A: Visible (before spray)
B: UV254 (before spray)
C: UV366 (before spray)
D: UV 254 (after spray)
E: UV 366 (after spray)
Spray reagent:
Citroborate

Figure 1. TLC Chromatogram of the *C. trifolia* extracts are compared with quercetin as standard

4. Conclusion

The different polar extracting solvents can attract flavonoid compounds with different levels. The flavonoids in *C. trifolia* extract need to be separated to determine the types.

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