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To cite this article: M Suzery *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **292** 012064

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Determination of Quercetin and Rutin in Red Galangal Rhizomes (*Alpinia purpurata*) and White Galangal (*Alpinia galanga*) with High Performance Liquid Chromatography Method

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Abstract. Research of flavonoid determination (quercetin and rutin) in *Alpinia purpurata* and *Alpinia galanga* rhizomes using HPLC method has been conducted. This study aims to determine the qualitative and quantitative presence of rutin and quercetin compounds in *Alpinia purpurata* and *Alpinia galanga* rhizomes. Fractions of ethyl acetate and n-butanol have been obtained by TLC, followed by total flavonoid analysis with UV - Vis spectrophotometer method and quercetin and rutin determination with HPLC. The results obtained in this study are as follows: Total flavonoid from ethyl acetate fraction obtained is 46,48 mg EQ/ gram in *Alpinia purpurata* and 70,60 mg EQ/ gram in *Alpinia galanga*, and total flavonoid from n-butanol fraction obtained is 68,50 mg EQ/ gram for *Alpinia purpurata* and 103,80 mg EQ/ gram for *Alpinia galanga*. The ethyl acetate fraction of *Alpinia purpurata* detected quercetin content at 5469.64 mg / kg and rutin at 4955.59 mg / kg, while *Alpinia galanga* detected quercetin at 5764.10 mg / kg and rutin at 5327.93 mg / kg. From n-butanol fraction, quercetin are only detected at 6737.14 mg / kg in *Alpinia purpurata* and 9098.74 mg / kg in *Alpinia galanga*. From these results, it can be concluded that HPLC method can be used to detect the presence of flavonoid quercetin and rutin in *Alpinia purpurata* and *Alpinia galanga* rhizomes.

Keywords: *Alpinia purpurata*, *Alpinia galanga*, Quercetin, Rutin, HPLC.

1. Introduction

Alpinia is the largest genus in *Zingiberaceae* family and comprises of 200 species [1], two of which are *Alpinia purpurata* (Viell). K. Schum and *Alpinia galanga* (L) Willd. In pharmacological tests, galangal extract exhibits promising potentials, which has a reduced blood pressure effect [2], antioxidant activity, anticancer [3], antibacterial, antiviral, antifungal, antiparasitic [4], anti-inflammatory [5], antitumor, analgesics, and antifatulence [6]. Phytochemical test results from red galangal ethanol extract contain alkaloid compounds, phenols, flavonoids, and tannins [5]. Red galangal essential oil containing *1,8-cineole*, *chavicol*, *β-caryophyllene* and *α-cellinene* has antimicrobial potential [7]. Whereas in white galangal contains essential oils such as cineole, methyl cinnamate, *myrecene*, and methyl eugenol and also contains many flavone compounds such as galangin, alpinin, kaempferide, and 3-dioxy-4-methoxy flavones [8].



Previous research reports that some species of the *Zingiberaceae* family have antioxidant activity influenced by the presence of flavonoids such as rutin, quercetin, alpinetin, and kaempferol in the genus *Alpinia* [9][10]. Flavonoids are naturally contained in natural materials as a form of flavonoids, aglycones and flavonoids glycosides [11].

At present, High Performance Liquid Chromatography is defined as the easiest method used in the separation and identification of flavonoids with various detection systems [12]. The High Performance Liquid Chromatography method was developed for qualitative and quantitative analysis of flavonoids in various natural materials [13]. Tao (2006) evaluated the quality of *A. officinarum* Hance for the assessment of two main bioactive flavonoids: galangin and 3-O-methyl galangin [14]. Research conducted by Victorio showed that ethyl acetate and n-butanol fractions of *Alpinia purpurata* (red galangal) leaves detected kaempferol-3-O-glucoronide and rutin flavonoids using HPLC [2]. In this study, the identification and quantification of flavonoid compounds (quercetin and rutin) will be carried out in red galangal and white galangal rhizomes using HPLC method. This research will be important for future use on identification and quantitative analysis of flavonoids in natural materials.

2. Research Methods

2.1. Sample preparation

Samples consisted of red galangal and white galangal rhizomes were obtained from Banyumanik, Semarang, Central Java. The samples were then cleaned from impurities, thinly sliced and aerated until dried and then mashed to their powder forms.

2.2. Preparation of extracts and fractions

Each powder was macerated with methanol solvent. The filtrate obtained was concentrated using Buchii rotary evaporator at 500°C to obtain thick extract.

Fractionation of red galangal and white galangal extract were carried out using vacuum column chromatography (KKV) method. Silica gel 60 G F254 was used as absorbent in TLC. Each 30 grams of methanol extracts were eluted with n-hexane solvent followed by dichloromethane, ethyl acetate and n-butanol. Ethyl acetate fraction from fractionation result would be used for further analysis.

Each ethyl acetate fraction of red galangal and white galangal were tested by TLC using silica gel GF254 with n-hexane : ethyl acetate eluent with a ratio of 3: 2 (v / v). Chromatograms were observed in the spectrum of 254 nm and 365 nm.

2.3. Determination of total flavonoid

Determination of total flavonoid were based on Zou method [15]. 0.05 grams of ethyl acetate and n-butanol fraction dissolved in 10 ml ethanol. About 2 ml of distilled water and 0.15 ml of 5% NaNO₂ were added to 0.5 ml of extract solution. After being left for 6 minutes, AlCl₃ 10% was added to solution and left for another 6 minutes. The solution was then added with 2 ml of 4% NaOH and diluted to 5 ml of volume. The mixture was shaken out and left for 15 minutes and has its absorbance measured with UV-Vis spectrometer at a wavelength of 510 nm. The standard quercetin calibration curve was used with concentrations of 633, 792, 1079, 1255, 1427, and 1584 mg / L respectively. Flavonoid content were calculated as mg equivalent quercetin / gram sample.

2.4. Preparation for standar flavonoid

Standard flavonoid of quercetin (98%) (sigma aldrich) and rutin (94%) were used for HPLC (sigma Aldrich). Each quercetin and rutin standard solution was prepared with concentration ranging from 50, 100, 200, 400, 800 to 1000 mg / L.

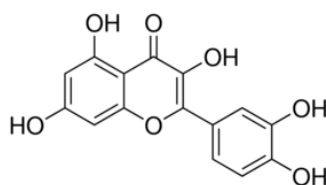


Figure 1. Quercetin Structure (Sigma Aldrich)

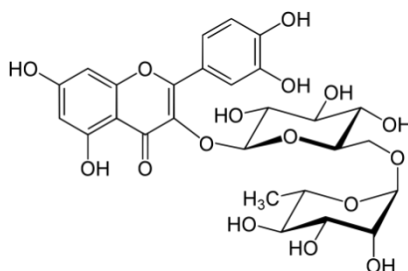


Figure 2. Rutin Structure (Sigma Aldrich)

2.5. Quercetin and rutin analysis using HPLC methods

Each ethyl acetate and n-butanol fraction of red galangal and white galangal were weighed 0.05 grams and dissolved with methanol p.a to reach 10 ml of solution. Standard from each quercetin, rutin, ethyl acetate fraction, and n-butanol fraction of red galangal and white galangal were injected into HPLC under the following conditions: Purospher® STAR C18 column (250 mm x 4.0 mm, 5 µm), Methanol mobile phase: Acetonitrile: Aquabidest (60: 20: 20), Flow rate 1.1 ml / min, Column Temperature 25 °C, UV-Vis Detector (254 nm), Injection Volume 10 µl, Running Time 15 min. Quercetin and rutin were detected based on retention time and the content levels were calculated as mg flavonoid / gram samples.

3. Results and Discussion

3.1. Extraction of red galangal and white galangal

Each of 15 ginger red and white galangal rhizomes weighing 15 kg was dried and mashed to produce a powder of 2.0 kg respectively. The powder was extracted by maceration using methanol solvent for 5x24 hours. Red galangal and white galangal filtrate was then concentrated and the thick brown extract was obtained. In red galangal, thick extract of 75.91 grams with a yield of 3.80% b / b was obtained. In white galangal, the thick extract was obtained at 87.51 grams with a yield of 4.38% b / b.

3.2. Fractionation of red galangal and white galangal extract

Each red galangal and white galangal thick extract was fractionated with KKV method and eluted with n-hexane, dichloromethane, ethyl acetate and n-butanol. From fractionation results, the ethyl acetate fraction of red galangal was obtained with a weight of 5.5072 grams and 5.951 grams in white galangal. Whereas a thick fraction of 8.10 grams in red galangal and 8.04 grams in white galangal were obtained from n-butanol fraction.

3.3. Determination of total flavonoid

TLC results showed that the spot pattern in ethyl acetate fraction from red galangal was observed only on the 2nd and 7th fractions. The chromatogram pattern gave three fluorinated spots which produce orange, green, and blue colors at 365 nm, while spot pattern in ethyl acetate fraction of white galangal was observed from the 2nd to 22nd fractions. At 365 nm, the wavelengths identified two spots capable of fluorescing to produce orange and green colors. Given the TLC results, it can be concluded that red galangal has different compound components as compared to white galangal.

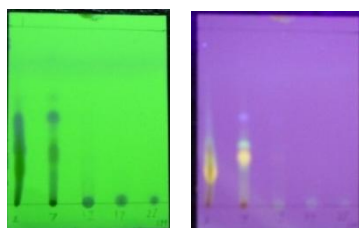


Figure 3. TLC of Red Galangal in Ethyl Acetate Fraction

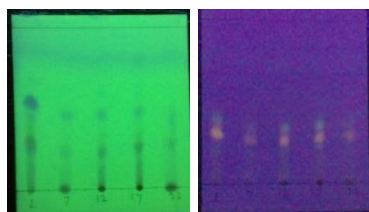


Figure 4. TLC of White Galangal in Ethyl Acetate Fraction

3.4. Quercetin calibration curve

Quercetin was used as standard for determining the total flavonoid content in red and white galangal rhizomes. From quercetin standard calibration curve analysis, the straight line equation $y = 0,0004x - 0,1025$ with correlation coefficient (R^2) price of 0,9942. The regression equation stated a systematic relationship between quercetin concentration and its absorbance measurements using UV-Vis spectrophotometer, whereas correlation coefficient (R^2) price defined the strong correlation between concentration (x-axis) and absorbance (y-axis). The price of R^2 approaching number 1 states that the regression equation is linear and the concentration affects the absorbance at 99% [16].

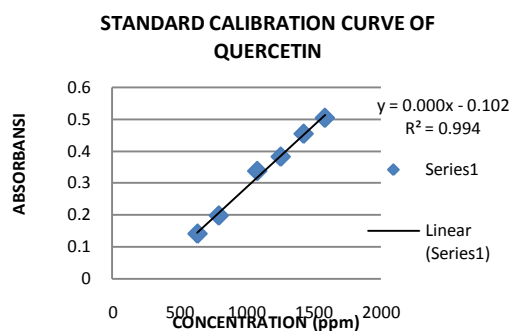


Figure 5. Standard Quercetin Calibration Curves

Table 1 Total Flavonoid Content in Red Galangal and White Galangal Rhizomes.

Sample	Total Flavonoid (mg equivalent quercetin / g sample*)
F _{EA} Red Galangal	46,48
F _{EA} White Galangal	70,60
F _{n-butanol} Red Galangal	68,50
F _{n-butanol} White Galangal	103,80

*Sample = ethyl acetate and n-butanol fraction

As can be seen from the results above (Table 1), total flavonoid content in white galangal were greater than in red galangal for both ethyl acetate and n-butanol fractions.

3.5. Quercetin and rutin analysis using HPLC method

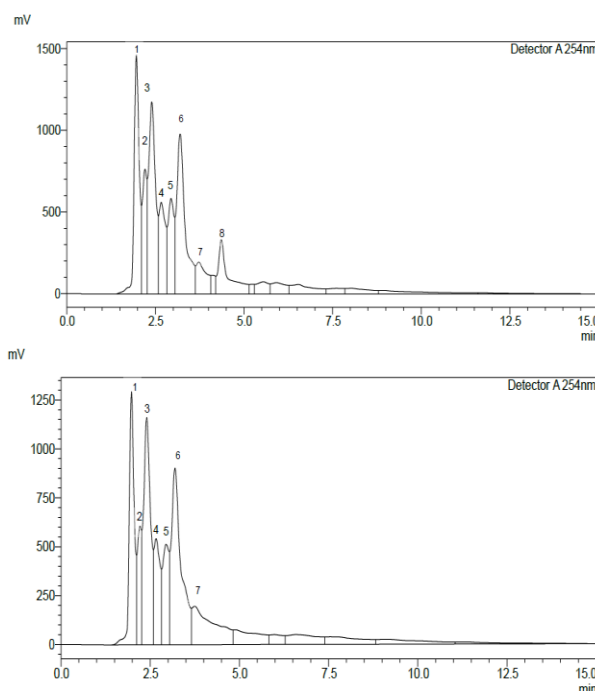


Figure 6. HPLC Chromatography Profiles of Red Ethyl Acetate Fraction (A) And White Galangal (B), Quercetin (4) Rutin (5)

HPLC results above showed that quercetin and rutin flavonoid were able to be detected in both species although both were not the main compounds of red galangal and white galangal. Quercetin was detected at retention time of 2.66 minutes, while rutin was detected at 2.94 minutes. There were three maximum peaks that belong to main compounds in both species, namely in retention time of 1.9 minutes, 2.1 minutes and 3.1 minutes. The peaks similarity in ethyl acetate fraction of red galangal and white galangal can be seen. A prominent difference was detected by the presence of a peak from an unknown compound at retention time of 4.36 minutes in red galangal. From these results, it can be concluded that red galangal and white galangal has different composition of compounds.

Table 2 Flavonoid analysis results in Ethyl Acetate Fraction of Red Galangal and White Galangal

Flavonoid	TR (minute)	Red Galangal (mg/g sample*)	White Galangal(mg/g sample*)
Quersetin	2,67	5,41	4,88
Rutin	2,94	4,90	4,51

*Sample = fraksi etil asetat dan n-butanol

The results in table 2 showed that quercetin compounds in ethyl acetate fraction of red galangal were 5.41 mg / gram, while those in white galangal were 4.90 mg / gram. Rutin analysis detected 4.88 mg / gram in red galangal and 4.51 mg / gram in white galangal. Therefore the quercetin and rutin content of red galangal is greater than in white galangal.

In addition to that, quercetin and rutin analysis were carried out on n-butanol fraction to further compare the results with the quercetin and rutin content of ethyl acetate fraction.

From HPLC results (figure 9), only quercetin flavonoid was detected in n-butanol phase of red galangal and white galangal. Quercetin was detected at 2.6 minutes of retention time. There was one maximum peak believed to be the main compound in both species at 1.9 minute of retention time with the largest area occurred in n-butanol fraction of white galangal. The similarity of peaks in n-butanol fraction of red galangal and white galangal were evident. A significant difference was detected by the presence of a peak from an unknown compound at a retention time of 4.36 minutes in red galangal.

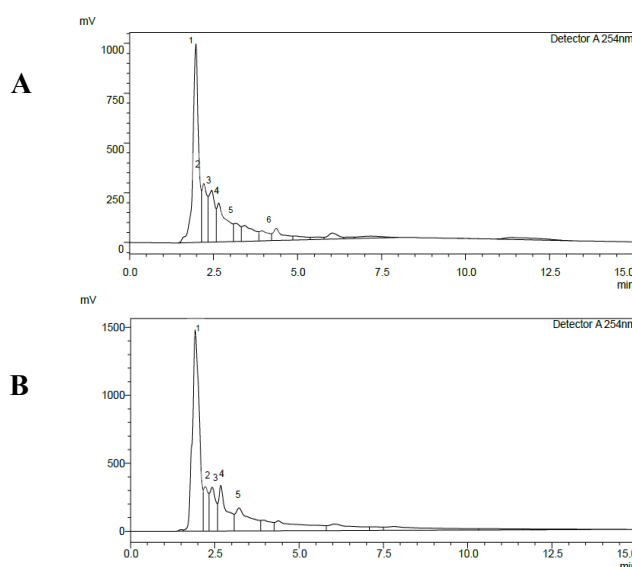


Figure 7. Profiles of HPLC Chromatography in N-butanol fraction of Red Galangal (A) and White Galangal (B), Quercetin (4) Rutine (5)

Analysis of n-butanol fraction in red galangal and white galangal (table 3), observe greater levels of quercetin in white galangal compared to red galangal. Rutin flavonoid was not detected in red galangal because these compounds have completely dissolved into ethyl acetate fractions.

Table 3 Flavonoid Analysis Results in n-Butanol Fraction of Red Galangal and White Galangal

Flavonoid	TR (Minute)	Red Galangal (mg/g sample*)	White Galangal (mg/g sample*)
Quercetin	2,6	2,97	4,22
Rutin	-	ND**	ND**

*Sample = ethyl acetate and n-butanol fraction

**ND = not detected

The n-butanol fraction detected quercetin levels at 2.97 mg / gram and 4.22 mg / gram dry samples for red galangal and white galangal respectively. From these results, it can be seen that white galangal contains abundant amount of quercetin compounds.

This study confirmed HPLC as a method capable of performing qualitative and quantitative analysis for quercetin and rutin compounds in red and white galangal rhizomes. In addition, this method is able to determine the distinctive compounds between red galangal and white galangals with the compounds type are yet to be known.

4. Results and Discussion

To summarize, the largest total flavonoid content comes from n-butanol fraction of white galangal, while measurements of quercetin and rutin using HPLC method found the most abundant flavonoid content in n-butanol fraction of white galangal.

5. Acknowledgement

This project was supported by a research grant from DIPA FSM Undip

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