

Antioxidant Activity Of Various Extracts Of Jarak Tintir (*Jatropha Multifida L.*) Stem, Phenolic And Flavonoid Content

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ABSTRACT

Jarak tintir (*Jatropha multifida L.*) was identified as one of family Euphorbiaceae and used by Indonesian people as traditional medicine. This study aimed to measure IC₅₀ of inhibition DPPH as free radicals and make statistical analysis of flavonoid and phenolic content and correlation with their IC₅₀ of free radicals. Hot continuous extraction was done with Soxhlet apparatus using various polarity solvents (n-hexane, ethyl acetate, and methanol). Methanol extract had the lowest IC₅₀ 32.26 ± 0.37 µg/mL of DPPH inhibition activity. Ethyl acetate extract had highest phenolic and highest flavonoid content with 35.21 ± 1.50 mg TAE/g extract and 49.41 ± 1.65 mg QE/g extract. Flavonoid and phenolic content in extracts had linier correlation with their IC₅₀ of inhibition free radicals.

Keywords: Antioxidant, DPPH, *Jatropha multifida L.*, phenolic, flavonoid

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INTRODUCTION

Jatropha multifida L. (Jarak tintir) is annual shrubs which its sap used as a traditional medicine by Indonesian people (Hutapea 2000). Previous study showed that *J. multifida* has antimicrobial and larvicidal activity that related to phenolic and flavonoid as active compound (Rampadarath, Puchooa, dan Ranghoo-Sanmukhiya

2014; Rampadarath, Puchooa, dan Ranghoo 2014).

Phenolic and flavonoid also have known as antioxidant activity on various extracts of many plants. Phenolic compounds have been reported have many activities such as anti-inflamantory, antibacterial and antioxidant (Cheung, Cheung, dan

Ooi 2003; Fidrianny, Aristya, dan Hartati 2015; Ghasemzadeh, Jaafar, dan Rahmat 2010; Katalinic et al. 2006).

Free radicals (DPPH) inhibition activity has been used to measure antioxidant capacity for a long time ago in many extracts (Molyneux 2004). Simple, fast and accurate become strong point of this method and still used until now in many countries.

The objectives of this study are to measure IC₅₀ of inhibition DPPH as free radicals and make statistical analysis of flavonoid and phenolic content and correlation with their IC₅₀ of free radicals.

METHODS

Sample Preparation. *J. multifida* stem was collected from Manoko Garden, West-Java, Indonesia. Stem was cut into small pieces and dried under morning sun light. The dried stem then grinded to crude drug powder.

Crude Drug Characterization. Crude drug was characterized by loss

on drying content, solvents (water and ethanol) soluble extract content, total, water and acid insoluble ash content (Depkes RI 2000).

Phytochemical Screening of Crude Drug. Phytochemical screening was performed to detect secondary metabolite of crude drug and extract like flavonoids, polyphenols, tannins, quinones, alkaloids, saponins and steroid/triterpenoids (Farnsworth 1966).

Extraction. Hot continuous extraction was done with Soxhlet apparatus using various polarity solvents such as methanol, ethyl acetate, and n-hexane.

IC₅₀ of DPPH Inhibition Activity. DPPH inhibition method was used from Blois's method (Blois 1958) with minor adjustment. Six different concentration samples were mix with 50 µg/mL DPPH solution in equal volume and incubated for 30 min. Analyzed and read absorbances of each samples into UV-Vis spectrophotometer Shimazu UV-1800 at 516-517 nm wavelength in triplicate. IC₅₀ of DPPH scavengin

activity calculated using its calibration curve. Methanol solution was used as blank sample with DPPH solution as control and quercetin was choose as positive standard.

Flavonoid Content. AlCl_3 reagent was used to measure flavonoid content of extract. Analyzed and read absorbances of each samples into spectrophotometer UV-Vis Shimazu UV-1800 413 nm wavelength in triplicate. Quercetin was used as flavonoid standard with six concentrations (30-110 $\mu\text{g/mL}$) to obtain a standard regression curve. Result of sample analysis was stated as mg QE/g extract (Chang, Yang, dan Wen 2002).

Phenolic Content. Folin-Ciocalteu analysis was choose to measure phenolic content of extracts. Analyzed and read absorbances of each samples into spectrophotometer UV-Vis Shimazu UV-1800 726 nm wavelength in triplicate and tannic acid was used as phenolic standard with six concentrations (40-110 $\mu\text{g/mL}$) to obtain standard regression curve. Result of sample analysis was stated as mg TAE/g extract

(Pourmorad, Hosseinimehr, dan Shahabimajd 2006).

Statistical Analysis. Statistical significance level was set at $p < 0.05$ with ANOVA in addition to post-hoc Turkey method. Statistical analysis of flavonoid and phenolic content in correlation with their IC_{50} of free radicals was used Pearson method ($p < 0.05$).

Thin Layer Chromatography Profiles. Each sample was separated by thin layer chromatography using Silica Gel GF₂₅₄ as stationary phase and various organic solvents as mobile phases. After separation, the spot was viewed under UV λ 254 and 366 nm. Then TLC was sprayed by FeCl_3 , AlCl_3 , DPPH and Folin-Ciocalteu spray reagent. (Waksmundzka-Hajnos, Sherma, dan Kowalska 2008).

RESULTS

IC_{50} of DPPH Inhibition Activity.

The IC_{50} of DPPH inhibition activity in extracts of *J. multifida* stem were shown in Fig 1. Each analysis results of each extract were compared to quercetin as positive standard.

Methanol extract as the lowest IC₅₀ of antioxidant activity of extracts. DPPH inhibition has the highest

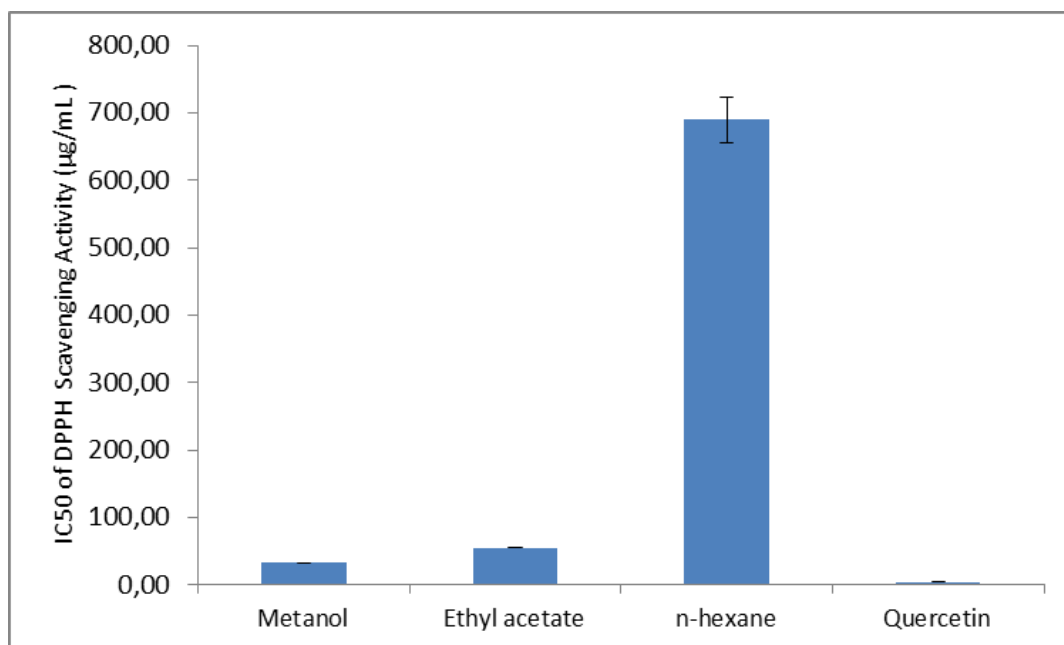


Figure 1. Inhibition Concentration 50% of DPPH in extracts of *J. multifida* stem

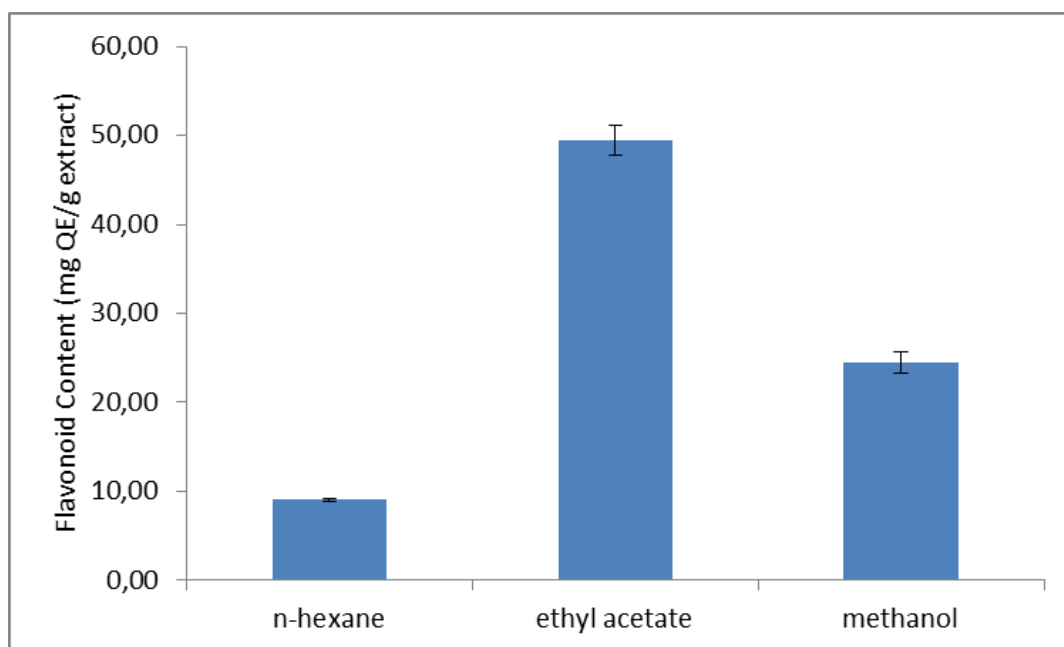


Figure 2. Flavonoid content in various extracts of *J. multifida* stem

Flavonoid Content. Result of as quercetin equivalent with flavonoid content analysis were stated regression equation $y=0.0067x-$

0.0134, $R^2=0.9959$. Extracts analysis of Flavonoid content showed results between 9.05-49.41 mg QE/g extract (Fig 2). Ethyl acetate extract showed the highest (49.41 ± 1.65 mg QE/g extract) and n-hexane the lowest (9.05 ± 0.19 mg QE/g extract) flavonoid content.

Phenolic Content. Phenolic content

was state as tannic acid equivalent with equation of standard regression $y=0.0057x-0.0327$, $R^2=0.994$.

Extracts analysis of phenolic content showed results between 12.23-35.21 mg TAE/g extract (Fig 3). Ethyl acetate extract showed the highest (35.21 ± 1.50 mg TAE/g extract) and n-hexane the lowest (12.23 ± 1.74 mg TAE/g extract) phenolic content.

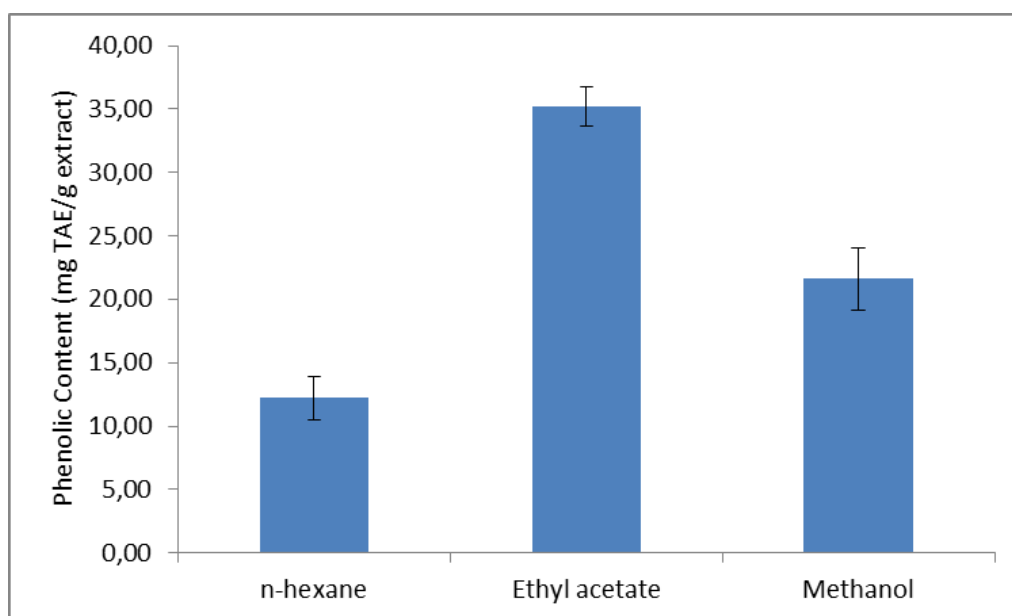


Figure 3. Phenolic content in various extracts of *J. multifida* stem

Table 1. *J. multifida* stem Phenolic and Flavonoid Content in Pearson's Correlation with their IC_{50} of free radicals

	Correlation Coefficient (r)	
	Flavonoid content ($p<0.01$)	Phenolic content ($p<0.01$)
IC_{50} of DPPH	-0,77**	-0,88**

Statistical Analysis. Statistical analysis of flavonoid and phenolic content in correlation showed negative and high with their IC₅₀ of free radicals (Table 1.)

Thin Layer Chromatography Profiles. Thin layer chromatogram profile showed on fig 4-6.

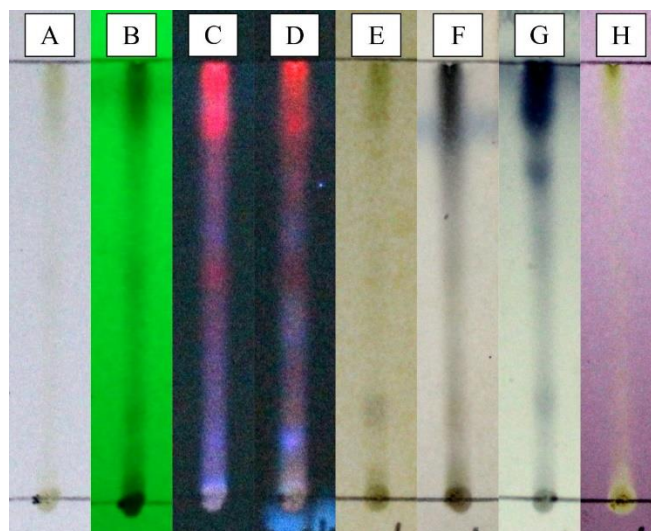


Figure 4. Chromatogram profile of methanol extract of *J. multifida* stem stationary phase silica gel GF₂₅₄, mobile phase butanol 100%, (A) visual (B) under UV light λ 254 nm (C) under UV light λ 366 nm (D) AlCl₃, under UV light λ 366 nm (E) FeCl₃ (F) Vanilin-SO₄ (G) Folin-Ciocalteu (H) DPPH 0,2%

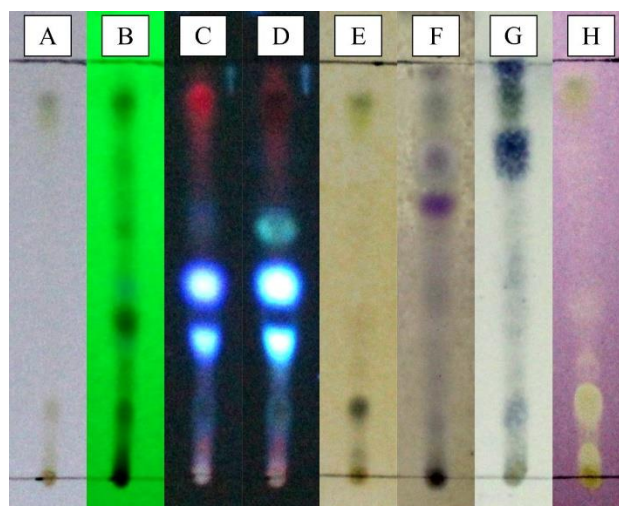


Figure 5. Chromatogram profile of ethyl acetate extract of *J. multifida* stem, stationary phase silica gel GF₂₅₄, mobile phase n-hexane-ethyl acetate (1:1), (A) visual (B) under UV light λ 254 nm (C) under UV light λ 366 nm (D) AlCl₃, under UV light λ 366 nm (E) FeCl₃ (F) Vanilin-SO₄ (G) Folin-Ciocalteu (H) DPPH 0,2%

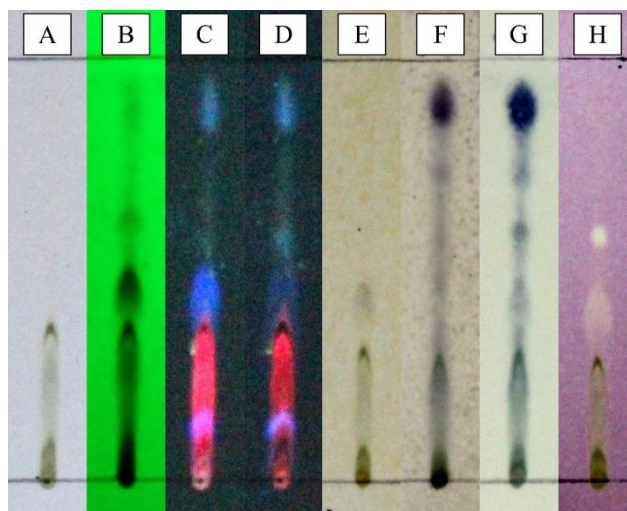


Figure 6. Chromatogram profile of n-hexane extract of *J. multifida* stem stationary phase silica gel GF₂₅₄, mobile phase n-hexane-ethyl acetate (1:1), (A) visual (B) under UV light λ 254 nm (C) under UV light λ 366 nm (D) AlCl₃, under UV light λ 366 nm (E) FeCl₃ (F) Vanilin-SO₄ (G) Folin-Ciocalteu (H) DPPH 0,2%

DISCUSSION

J. multifida previous studies have mentioned that the plant had antioxidant activity (Rampadarath, Puchooa, dan Ranghoo-Sanmukhiya 2014) but there were no antioxidant activity studies of different polarities extracts from Indonesia *J. multifida* stem and their correlation to phenolic and flavonoid content. In previous research reported that *J. multifida* had phenolic, flavonoid, tannins, and coumarins group after testing by phytochemical screening (Syam, Insanu, dan Wirasutisna 2017).

DPPH is the purplish color solution in ethanol or methanol as stable free radicals compounds which show absorption in the range of 515-520 nm wavelength using spectrophotometer UV-Vis. Free radicals scavenging is one of the known as antioxidant mechanism. The DPPH free radicals scavenging activity can be used to evaluate antioxidant activity of extract in a short time an stated as IC₅₀ DPPH (Cheung et al. 2003). IC₅₀ is inhibitory concentration of sampel that can reduce half of maximal DPPH as free radicals. The lowest

IC₅₀ of DPPH inhibition has the highest antioxidant activity of sample (Fidrianny et al. 2015). In this study, showed that IC₅₀ of DPPH inhibition activity of different extracts range from 32-689 µg/mL. Methanol extract has the lowest IC₅₀ 32.26±0.37 µg/mL and quercetin as positive standard gave IC₅₀ 3.96±0.03 µg/mL. Based on IC₅₀ Blois classification showed that methanol extract (32.26±0.37 µg/mL) and quercetin were categorized as very strong antioxidant and ethyl acetate as strong antioxidant (54.77±0.24 µg/mL) (Blois 1958).

Antioxidant activity may be related to flavonoid and phenolic content. In this study, ethyl acetate extract has highest phenolic and highest flavonoid content with 35.21±1.50 mg TAE/g extract and 49.41±1.65 mg QE/g extract respectively.

Pearson's correlation analysis between IC₅₀ of DPPH inhibition that showed antioxidant activity with phenolic and flavonoid content state as linear correlation if described as negative and high (Fidrianny et al. 2015). In this study (Table 1), *J.*

multifida stem of flavonoid and phenolic content in correlation showed negative and high with their IC₅₀ of free radicals. This analysis means by increasing in phenolic and flavonoid content of sample would increasing in IC₅₀ of DPPH inhibition also.

Hydroxy groups in A and/or B ring of flavonoid was also called as phenolic compound. Simple phenolic acid has lower antioxidant activity than flavonoid. Flavonoid compounds that has two hydroxy groups in ortho position (C3'-C4') have the highest impact to antioxidant capacity. Flavonoid in aglycones form would give higher impact to antioxidant activity than flavonoid in glycosides form (Heim, Tagliaferro, dan Bobilya 2002). Figure 4-6 showed that yellowish area in methanol and ethyl acetate TLC chromatogram more contrast than n-hexane chromatogram. Yellowish area showed because inhibition of DPPH generally by hydroxy groups of phenolic and flavonoid compound and double bond of carotenoid compounds with their antioxidant activity (Foot 1976).

CONCLUSION

Methanol extract of *J. multifida* had the lowest IC₅₀ of DPPH scavenging activity with IC₅₀ 32.26 ± 0.37 µg/mL. Ethyl acetate extract had the highest phenolic and flavonoid content (35.21 ± 1.50 mg TAE/g extract and 49.41 ± 1.65 mg QE/g extract, respectively). There were negatively and significant correlation between phenolic and flavonoid content in various extracts with their IC₅₀ of DPPH scavenging activities.

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