Correlation of Total Phenolic, Flavonoid and Carotenoid Content of Phyllanthus emblica Extract from Bandung with DPPH Scavenging Activities

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Correlation of Total Phenolic, Flavonoid and Carotenoid Content of *Phyllanthus emblica* Extract from Bandung with DPPH Scavenging Activities

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ABSTRACT

Introduction: Many potential compounds have antioxidant activity, such as the flavonoid group, phenolics and carotenoids. Phyllanthus emblica is widespread in Bandung-Indonesia and is a very potent as an antioxidant activity. Antioxidant activity and correlation with total flavonoids, phenolics and carotenoids from Phyllantus extract from Bandung-Indonesia have not been reported. The aim of this research were to determine the antioxidant activity from extract of various parts of P. emblica and its correlation of antioxidant activity with the total flavonoid, phenolics and carotenoid. **Method:** Successive extractions of various part of P.emblica were performed by maceration using differrent polarity solvent n-hexane, ethyl acetate and ethanol. The antioxidant activity of each extracts was performed using DPPH (2.2-Diphenyl-1-Picrylhydrazil) method. The determination of total flavonoids, phenolics and carotenoids were performed by UV-Spectrophotometry. Antioxidant activity was demonstrated by IC50 and its correlation to total flavonoids, phenolics and carotenoids using the Pearson's method. **Result:** The highest antioxidant activity was given by fruit ethyl acetate (BE) extract with IC50 3.032 µg/mL. Etyl acetate extract of stem bark P.emblica (KE) had the highest of total phenol content (12.818 g GAE/100 g), ethanol extract of leaves P.emblica (DO) had the highest of total flavonoid content (3.594 g QE/100 g), and n-hexane extract of leave (DN) had the highest of total carotenoid content (0.759 g BE/100 g). **Conclusion:** According to coeficient correlation Pearson's between P. emblica extract with IC50 of DPPH scavengging activities, suggested that flavonoid and phenolic compound in stem bark extract and leaves extract of P. emblica were contributor major in its antioxidant activity with DPPH methode, and its same with carotenoid content in leaves extract of P. emblica.

Key words: Phyllantus emblica, Antioxidants, Flavonoids, Phenols, Crotenoids

INTRODUCTION

Phyllanthus emblica known as Malacca is a very potent plant as an antioxidant.[1] Malacca is a traditional medicinal plant that has long been used.[2] Research on the biological activity of *P. emblica* has been widely performed, especially in *in vitro*.[3] *P. emblica* plants show a variety of biological activities, ie as anti-inflam- matory, antipyretic, diuretic, and laxative,[4] anticancer,[5] antioxidants, antidiabetes.[6,7]

Chemical compunds of *P. emblica*, including fruit, stem bark, leaves, was known containt of tannins.[1] In addition, chemical content of *P. emblica*, such as alka- loid, phenolics and flavonoids[8] were also found. In one tree, there is the possibility of each part of the plant having the same chemical compounds or vice versa. Chemical compounds can be affected to biologycal activity such as antioxidant activity.

Biological activity and chemical compound in a plant can influenced by the physiological processes in a plant, environmental conditions.[5] such as sunlight condition, air pressure and temperature.[10] Besidethat, the maturity part of plant could be a factor to differences type and quantity secondary metabo- lites.[11,12]

Antioxidants are one of the components needed in the body, to counteract free radicals. Excessive free radicals in the human body can cause several diseases,such as diabetes, heart disease and inflammation.[13] The antioxidant compounds obtained from plants may be phenolic, carotenoid.[14,15] compounds, and flavonoids.[16] This study was conduct the antioxidantactivity of *P. emblica* extract from West Java, Indonesia, and its correlation of chemical compound in *P.emblica* extract.

MATERIAL AND METHOD

Materials

The material used are fruit simplicia, leaf and stem bark of *P. emblica* obtained from District Bale

Endah, Regency of Bandung, Indonesia. DPPH (2.2-diphenyl- 1-picrylhydrazyl) from Sigma-Aldrich (MO, USA), gallic acid from Sigma-Aldrich (MO, USA), quercetin from Sigma Aldrich (MO, USA), Beta carotene obtained from Sigma-Aldrich (MO, USA), methanol P.a,ethanol, ethyl acetate, n-hexane and all the ingredients used in this studyobtained from Merck.

Sample Preparation

Simplicia of fruit, leaf and stem bark of *P. emblica* were authenticated at Herbarium Bandungense, Faculty of Biology, Universitas Padjadjaran,Indonesia. All simplicia were sorted, washed, dried with oven at 40^{o}C, and ground into powder.

Extraction

Each powder simplicia was extracted using a maserator, with increasing gradient polarity solvents (n-hexane, ethyl acetate and ethanol). The n-hexane extract was repeated three times. The remaining residue was extracted three times by ethyl acetate. Finally, the remaining residue was extracted three times with ethanol. So, there were nine extracts, then-hexane extract of fruit (BN), leaf (DN) and stem bark (KN), the ethylacetate extract of fruit (BE), leaf (DE), and stem bark (KE), the ethanolextract of fruit (BO), leaf (DO) and stem bark (KO).

Phytochemical screening

Phytochemical screening performed against all extract (BN, BE, BO, DN, DE, DO, KN, KE, and KO). $FeCl_3$ 10% used for phenolic compound, amyl alcohol for flavonoid compound, gelatin for tannin, dragendorf and mayer for alkaoid, KOH 5% for quinon, vanillin 10% in H_2SO_4 for mono- terpen and seskuiterpen, Lieberman-Buchard for steroid and triterpenoid.[17] Saponins showed by a constant foam ± 10 min in water extracts

Antioxidant activity

The antioxidant activity were performed using DPPH (2.2-Diphenyl- 1-Picrylhydrazil) method, adopted from Blois (1958)[18] with modification. Each sample was made several concentrations in P.a methanol, then into each concentration of sample solution was added DPPH 50 µg/ml solution in ethanol p.a (Volume 1: 1). After that, the mixture was incubated for 30 min in a darkened room. Then measured the absorbance of each mixture using a UV spectrophotometry. Measurements carried out three repetitions. Methanol P.a was used as a blank, DPPH 50 ug/ml solution as control, and ascorbic acid solution as a positive control. IC_{50} DPPH was obtained from the calibration curve of the antioxidant activity of the sample on some sample concentrations in range 10 ppm to 70 ppm.

Determination of Phenolic Content

Determination of henolic content performed by Pourmurad method.[19] using Folin-ciocalteu and absorbance was measured by Spectro UV- Visible at λ 765 nm. Each extract dissolve in methanol Pro analys. Galic acid solutiom used as standar of phenolic compound and to be standar curve. Linier regression equation of standar curve was used for calculating total phenolic content. Total phenolic content expressed as gallic acid equivalent per 100 g of extract (g GAE/100 g).

Determination of total flavonoid content

Determination of total flavonoid performed by Chang methode.[20] modi- fication using $AlCl_3$ and absorbance wae measured by spectro UV-Vis at λ 415 nm. Each extract dissolved in methanol Pro analysis. Quercetinsolution in various concentration used as standar of flavonoid compoundand to be stanadr curve. Linier regression equation of standar curve was used for calculating total flavonoid content. Total flavonoid contentexpressed as quercetin equivalent per 100 g of extract (g QE/100 g).

Detrmination of total carotenoid content

Determination of total carotenoid content performed by Thaipong methode using Spectro UV-Vis. Absorbance was measured at λ 470 nm. each extract was dissolved in n-hexane pro analysis. Beta-caroten solution in various concentration used as standar of catotenoid compound and to be standar curve. Linier regression equation of stanadar curve was used for calcualting total carotenoid content. Total carotenoid content expressed as beta-caroten equivalent per 100 g of extract (g BE/100 g).

Statistical analysis

Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc LSD procedure was done with SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, carotenoid content and antioxidant acivity whiches showed with IC_{50} were conducted using the Pearson's method.[16]

RESULT AND DISCUSSION

Phytochemical screening

Phytochemical screening of extract was showed at Table 1. The result showed their each part of *P.emblica* (fruit, leaf and stem bark) was affected to differences of secondary metabolite compound. Phytochemical screening was the first step to know the group of compounds contained in extracts. All extracts of *P. emblica* have flavonoids and phenolic com- pounds. BN, DN and KN do not have of phenolic compounds. Phenolics and flavonoids are compounds that can cause antioxidant activity in extract. Flavonoids can be classified to phenolic compounds. Flavonoids which unsubstituted OH groups were not phenolic compounds. The presence of OH groups in a compound may cause increased polarity of the compound.

Table 1: Phytochemical screening of *P.emblica* extract.

Compound	Result								
		N-Hexane		Ethyl acetate				Ethanol	
	DN	KN	BN	DE	KE	BE	DO	KO	BO
Alkaloid	-	-	-	+	+	-	+	+	-
Flavonoid	+	+	+	+	+	+	+	+	+
Tannin and Phenol	-	-	-	+	+	+	+	+	+
Ionoterpene and Sesquiterpene	+	+	-	+	+	-	+	+	-
Steroid	+	-	-	+	-	-	-	-	-
Triterpenoid	-	-	-	-	-	-	+	-	-
Quinone	-	-	-	-	-	+	+	-	+
Saponin	-	-	-	-	-	-	+	+	-

Antioxidant activity

Antioxidant activity expressed as IC_{50} value. The result showed, BE had the smallest IC_{50} value than another extract, whereas DE had the highest IC_{50} value than another extract. IC_{50} value of each extract showed at Figure 1. Antioxidant activity of *P. emblica* fruit and leaf extracts has been reported.[22,23] Many reported, antioxidant activity from fruit, leaf and stem bark extracts of *P. emblica* using a solvent with increased polarity (n-hexane, ethyl acetate and ethanol) and antioxidant activity of the stem bark *P.emblica*. The most commonly used method of determining antioxidant activity is the DPPH method because it is a relatively stable and sensitive free radical in determining antioxidant activity.[15] The DPPH method is based on the ability of the antioxidant compounds of the extract to absorb DPPH free radicals shown visibly with a more faded DPPH coloring.[23] The more faded color of DPPH solution, the more DPPH is suppressed by the antioxidant compounds of the extract.

Antioxidant activity of extract were showed with IC_{50} value. IC_{50} value antioxidant activity of extract *P.emblica*. This result indicated linier with previous research of Suaib,[4] that antioxidant activity ethanol and water extract of fruit *P.emblica* had a lower than antioxidant activity of ascorbic acid. According to Blois,[12] potency antioxidant activity of the sample can be of

DPPH scavengging activities was contrasdictinction with percentage of DPPH scavengging activities. It's means, the highest antioxidant activity was indicated by the lowest value of IC_{50}. IC_{50} value of *P.emblica* extract were variated. The environmental conditions.[5] such as sunlight condition,[10] the marurity part of plant and differences part of plant could be a factor to differences type and quantity secondary metabolites.[11,12] The differences and quantity of secondary metabolites of medicinal plant could be causes differences biologycal activity.[24] BE was the lowest IC_{50} value in fruit extract *P.emblica*, DN was the lowest IC_{50} value in leaf categoried to very strong antioxidant which had IC_{50} lower than 50 μg/ml and wich had higher than 50 μg/ml was a weak antioxdant activity. Antioxidant activity of all extract *P.emblica* from Bandung-Indonesia had IC_{50} lower than 50 μg/ml and caould be categoried to very strong antioxidant activity. Antioxidant activity of samples may be suspected containing the compound capable donating proton on free radicals.[25]

The result indicated, BE was the highest antioxidant activity compared to all extract of *P.emblica*. Previous study,[22] stated methanol-water extract of leaf *P.emblica* have 40.24 μg/ml of IC_{50} value. D.sumalatha.[23] showed antioxidant activity was 71.75% at 125 μg/ml ethanol of combine leaf and fruit extract *P.emblica*. Suaib,[4] stated ethanol of fruit extract *P.emblica* had the higher than a water extract of fruit *P.emblica*. IC_{50} of extract *P.emblica* was compared to ascorbic acid of IC_{50} value. IC_{50} value of ascorbic acid was 2.87 μg/ml.

Total phenol content

Total phenol conten in BN, BE, BO, DN, DE, DO, KN, KE, and KO varied from 0.110 to 12.818 g GAE/100 g, and can be seen in Figure 2.Linier regression equation of gallic acid standard curve is $y = 0.0449 + 0.1836$, $R^2 = 0.996$.

Determination total phenolic of *P.emblica* extract varied from 0.110 g GAE/100 g to 12.818 g GAE/100 g. Phenolic compound as major compound in medicinal plant and were caused many biology activity.[28] Phenol is very potent as antioxidant compound.[29] Determinaton of total phenol content was by Folin-ciocalteu reaction.[19] Total phenol content was calculation by galic acid standard curve were $y = 0.044x + 0.185$; $R^2 = 0.996$ and expressed as gallic acid. KE was had the highest of total phenol were 12.818 g GAE/100 g. According previous research, Luqman,[4] stated total phenol of water and ethanol extract fruit *P.emblica* were 336 ± 33.94 and 318 ± 45.25 μg GAE/ mg. Acoording to Naik,[30] total pheno f water extract fruit *P.emblica* was 33% equivalent to gallic acid.

Total flavonoid content

Total flavonoid conten in BN, BE, BO, DN, DE, DO, KN, KE, and KO varied from 0.038 to 3.594 g QE/ 100 g, adn can be seen in Figure 3. Regression linier equation of gallic acid standar curve is $y=0.0342x + 0.0857$, $R^2 = 0.991$. Determination of total flavonoid in. *P.emblica* extract varied at 0.038 gQE/100 g sampai 2,982 g QE/100 g. This result means, part of *P.emblica* plant has production flavonoid in differences quantity.

Total carotenoid content

Total carotenoid conten in BN, BE, BO, DN, DE, DO, KN, KE, and KO varied 0.0004 to 0.7588 g BE/100 g and can be seen in Figure 4. regression linier equation of beta caroten standar curve is $y=0.1061x + 0.0008$, $R^2 = 0.998$. Determination of total carotenoid content at extract *P.emblica* varied under 1 g BE/100 g. This result means, carotenoid compound in *P.emblica* extract as fruit, leaf and stem bark were lower production than phenol and flavonoid. Strong yellow to orange was a color of carotenoid com- pound. That color was caused by double bond conjugated in carotenod compound. DN was more strong yellow to orange color than BN, BE, BO, DE, DO, KN, KE, and KO. Total carotenoid content at *P.emblica* extract calculation used standard curve, $y = 0.105x + 0.0008$; $R^2 = 0.9983$ and expressed as β-caroten. Carotenoid compound were inclanation non polar to young

shoot polar caracter. Becaused that, all of *P.emblica* extract (BN, BE, BO, DN, DE, DO, KN, KE dan KO) soluted in n-hexane solution. BN had the highest of ttoal carotenoid in fruit extract of *P.emblica* as 0.052 g BE/100 g, DN had the highest in leaf extract of P.embica as 0.759 g BE/100 g and KE had the highest in stem bark extract of *P.emblica* as 0.029 g BE/100 g. KO had the lowest of total carotenoid content were compared to all extract *P.emblica* as 0.0004 g BE/100 g. So far, have not been reported about total carotenoid content in *P.emblica* extract.

Correlation between antioxidant activity with total phenol, total flavonoid and total carotenoid of *P.emblica* extract

Correlation between total phenol, flavonoid and carotenoid with IC_{50} of DPPH scavengging activities was expressed with Pearson correlation coeficient (r) and showed in Table 2. Pearson correaltion coefficient of total phenolic, flavonoid, carotenoid content of fruit *P.embilca* extract with IC_{50} of DPPH scavengging activities were r = -0.492, p<0.179; r = 0.510, p<0.161; r = 0.973, p<0.01. Pearson correaltion coefficient of total phenolic, flavonoid, carotenoid content of leaf Phyllantus embilca extract with IC_{50} of DPPH scavengging activities were r = 0.813, p<0.001; r = -0.926, p<0.01; r = -0.621, p < 0.74. While in stem bark of *P.emblica* extract were r = -1.00, p<0.01; r = - 0.843, p<0.01; r = -0,368, p<0.329.

Total phenol, flavonoid and carotenoid of *P.emblica* extract correlation with C-3, 4 oxo, C-5 and or ortho group in C3'-C4'.[31] OH functional flavonoid in C-3, and or C-5 and ortho position in C3'-C4' could be as antioxi- dant activity.[31] In previous study, Dhale,[32] stated ethanol extract of fruit and leaf *P.emblica* have a flavonoid compound. Hasan,[33] stated *P.embica* herba have flavonoid compound as quercetin and luteolin. According to Ghosal,[34] fruit of *P.emblica* extract has flavonoid compound as rutin. OH functional at quercetin, luteolin and rutin could be form complex with $AlCl_3$. BE had the highest of total flavonoid in fruit extract of *P.emblica* as 0.967 g QE/100 g. DO had the highest of total flavonoid in leaf extract *P.emblica* as 3.594 g QE/100 g. Whereas in stem bark extract of *P.emblica*, KE wa the highest of total flavonoid as 1.347 QE/100 g. KN had the lowest of total flavonoid wich compared to all extract of *P.emblica* from Bandung-Indonesia as 0.038 g QE/100 g.

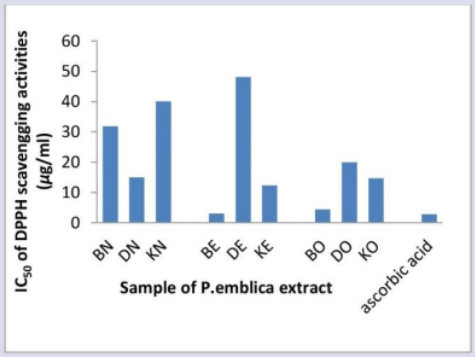

Figure 1: IC_{50} of DPPH scavengging activities of *P.emblica* extract.

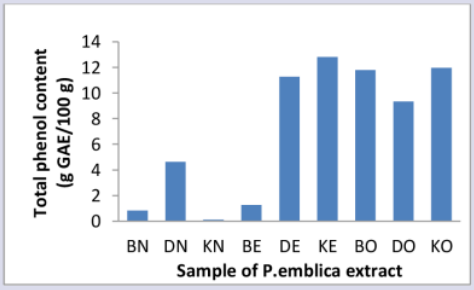

Figure 2: Total Phenol content of *P.emblica* extract.

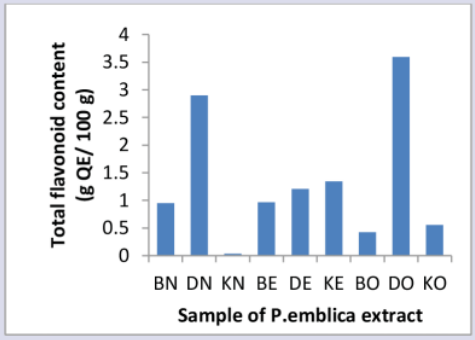

Figure 3: Total flavonoid content of *P.emblica* extract.

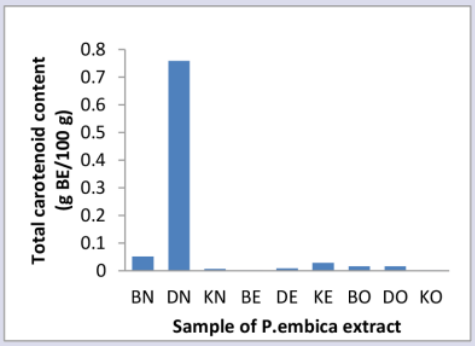

Figure 4: Total carotenoid content of *P.emblica* extract.

Determination correlation between total carotenoid content to IC_{50} of DPPH scavengging activities have showed carotenoid compound were not been mayor group as antioxidant activity contributor compound. Leaf extract of *P.emblica* had the highest as contributor antoxidant compound than stem bark extract and fruit extract. Pearson correlation of leaf extract as $r = -0.621$; $p > 0.05$. Carotenoid compound as beta-carotene and α-tocopherol was the high suspected to antioxidant potency.[35,56] β-carotene was efective as antioxidant compound in human body.[35,36] Much of double bond conjugated in β-carotene, suspected to antoxidant activity.[35,37,38] Besides that, zeaxanthin, astaxanthin and astxanthin- β-glucoside[35,36] can

suspected to antioxidant activity. The previous study,so far have not been reported about correlation total carotenoid content with IC_{50} value of DPPH scavengging activities sed Pearson's correlation. Becaused that, this result not yet compared to previous study.

Table 2 : Pearson's correlation of total phenol, flavonoid and carotenoid content with extract of *P.emblica*.

DPPH Scavenggingactivities	Pearson' Correlation		
	Total Phenol Content	**Total Flavonoid Content**	**Total Carotenoid Content**
IC_{50} of Fruit Extract	-0.492	0.510	0.973
IC_{50} of Leaf Extract	0.813	-0.926	-0.621
IC_{50} of Stem Bark Extract	-1.00	-0.843	-0.368

CONCLUSION

Fruit extract of *P.emblica* had the highest antioxidant activity than leaf extrcat and stem bark extract. Phenol compound in stem bark extract of *P.emblica* had the highest as contributor antioxidant compound than in leaf and fruit extract. Flavonoid and carotenoid compound in leaf extract of *P.emblica* had the highest as contributor antioxidant compound than in fruit extract and stem bark extract of *P.emblica*.

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CONFLICT OF INTEREST

The author declare that there is no conflict of interest, financial or other-wise regarding the publication of this paper.

ABBREVIATIONS USED

DPPH: 2.2-Diphenyl- 1-Picrylhydrazil.

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Correlation of Total Phenolic, Flavonoid and Carotenoid Content of Phyllanthus emblica Extract from Bandung with DPPH Scavenging Activities

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