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

Sani Nurlaela Fitriansyah*, Diah Lia Aulifa, Yessi Febriani, Emi Sapitri

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 Phyllanthus 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 different polarity solvent n-hexane, ethyl acetate and ethanol. The antioxidant activity of each extracts was performed using DPPH (2,2-DiPheynyl-1-Picrylylhydrazil) method. The determination of total flavonoids, phenolics and carotenoids were performed by UV-Spectrophotometry. Antioxidant activity was demonstrated by ICso 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 ICso 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 coefficient correlation Pearson's between P. emblica extract with ICso of DPPH scavenging 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: Phyllanthus 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-inflammatory, antipyretic, diuretic, and laxative,4 anticaner,1 antioxidants, antidiabetes.5,6 Chemical compunds of P. emblica, including fruit, stem bark, leaves, was known content of tannins.1 In addition, chemical content of P. emblica, such as alkaloid, phenolics and flavonoids7 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 compunds can be affected to biological activity such as antioxidant activity.

Biological activity and chemical compound in a plant can influenced by the physiological processes in a plant, environmental conditions.3 such as sunlight condition, air pressure and temperature.10 Beside that, the maturity part of plant could be a factor to differences type and quantity secondary metabolites.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 antioxidant activity 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 of
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 Pa, ethanol, ethyl acetate, n-hexane and all the ingredients used in this study obtained 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°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, the n-hexane extract of fruit (BN), leaf (DN) and stem bark (KN), the ethyl acetate extract of fruit (BE), leaf (DE), and stem bark (KE), the ethanol extract 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). FeCl₃, 10% used for phenolic compound, amyl alcohol for flavonoid compound, gelatin for tannin, drangendorf and meyer for alkaid, KOH 5% for quinon, vanillin 10% in *H₂SO₄* for monoterp and sesquiterpen, Lieberman-Buchard for steroid and triterpenoid. Saponins showed by a constant foam ± 10 min in water extracts.

Antioxidant activity

The antioxidant activity were performed using DPPH (2,2-Diphenyl-1-Picrylhrazil) method, adopted from Blois (1958) 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 Pa was used as a blank, DPPH 50 μg/ml solution as control, and ascorbic acid solution as a positive control. IC₅₀ 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 phenolic content performed by Pourmurad method, using Folin-cioicateu and absorbance was measured by Spectro UV-Visible at λ 765 nm. Each extract dissolve in methanol Pro analysis. Galic acid solution 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. modification using AlCl₃, and absorbance was measured by spectro UV-Vis at λ 415 nm. Each extract dissolved in methanol Pro analysis. Quercetin solution in various concentration used as standar of flavonoid compound and to be standar curve. Linier regression equation of standar curve was used for calculating total flavonoid content. Total flavonoid content expressed as quercetin equivalent per 100 g of extract (g QE/100 g).

**Determination 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 standar curve was used for calculating 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 IC₅₀ were conducted using the Pearson’s method.

**RESULT AND DISCUSSION**

**Phytochemical screening**

Phytochemical screening of extract was showed at Table 1. The result showed their each part of *Pemblica* (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 compounds. BN, DN and KN do not have of phenolic compounds. Phenolics and flavonoids are compounds that can cause antioxidant activity in

<table>
<thead>
<tr>
<th>Compound</th>
<th>N-Hexane</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
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<tr>
<td>Flavonoid</td>
<td>+</td>
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<tr>
<td>Tannin and Phenol</td>
<td>-</td>
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<td>Monoterpene and</td>
<td>+</td>
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<td>Sesquiterpene</td>
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<td>Triterpenoid</td>
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<td>Saponin</td>
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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.

**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. 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 *Pemblica*. 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. 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. 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 of DPPH scavenging activities was contradistinction with percentage of DPPH scavenging activities. It’s means, the highest antioxidant activity was indicated by the lowest value of IC$_{50}$. IC$_{50}$ value of *Pemblica* extract were varied. The environmental conditions, such as sunlight condition, the maturity part of plant and differences part of plant could be a factor to differences type and quantity secondary metabolites. The differences and quantity of secondary metabolites of medicinal plant could be causes differences biological activity.

The differences and quantity of secondary metabolites of medicinal plant could be a factor to differences type and quantity secondary metabolites. The environmental conditions, such as sunlight condition, the maturity part of plant and differences part of plant could be a factor to differences type and quantity secondary metabolites. According previous research, Luqman, was calculation by gallic acid standard curve were $y = 0.044x + 0.185$; R$^2 = 0.996$. Determination total phenolic content of *Pemblica* extract varied from 0.110 g GAE/100 g to 12.818 g GAE/100 g. Phenolic compound as major antioxidant activity. Antioxidant activity ethanol and water extract of fruit *Pemblica* had a lower than antioxidant activity of ascorbic acid.

According to Blois, potency antioxidant activity of the sample can be categorized to very strong antioxidant which had IC$_{50}$ lower than 50 µg/ml and weak had higher than 50 µg/ml was a weak antioxidant activity. Antioxidant activity of all extract *Pemblica* from Bandung-Indonesia had IC$_{50}$ lower than 50 µg/ml and caould be categorized to very strong antioxidant activity. Antioxidant activity of samples may be suspected containing the compound capable donating proton on free radicals. Flavonoid and phenol were compound capable donating proton on free radicals. Besides that, cinamic acid and benzoic acid were compound more higher contributor as antioxidant activity than benzoic acid.

**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. According previous research, Luqman, was calculation by gallic acid standard curve were $y = 0.044x + 0.185$; R$^2 = 0.996$. Determination total phenolic of *Pemblica* extract varied from 0.110 g GAE/100 g to 12.818 g GAE/100 g. Phenolic compound as major antioxidant activity. Antioxidant activity ethanol and water extract of fruit *Pemblica* had a lower than antioxidant activity of ascorbic 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. Determination total flavonoid in *Pemblica* extract varied at 0.038 g QE/100 g sampai 2.982 g QE/100 g. This result means, part of *Pemblica* plant has production flavonoid in differences quantity. Determination total flavonoid used AlCl$_3$ reaction. Total flavonoid content at *Pemblica* extract calculation by standard curve $y = 0.0342x + 0.0857$; R$^2 = 0.991$ and expressed as quercetin. AlCl$_3$ will form omplex with OH functional in...
Correlation between antioxidant activity with total phenol, total flavonoid and total carotenoid of *P. emblica* extract

Correlation between total phenol, flavonoid and carotenoid with *P. emblica* extract were expressed with Pearson correlation coefficient (r) and showed in Table 2. Pearson correlation coefficient of total phenolic, flavonoid, carotenoid content of fruit *P. emblica* extract with IC$_{50}$ of DPPH scavenging activities were $r = -0.492$, $p < 0.179$; $r = 0.510$, $p < 0.161$; $r = 0.973$, $p < 0.01$. Pearson correlation coefficient of total phenolic, flavonoid, carotenoid content of leaf *P. emblica* extract with IC$_{50}$ of DPPH scavenging activities were $r = 0.51$, $p < 0.161$; $r = 0.973$, $p < 0.01$. Pearson correlation coefficient of total phenolic, flavonoid, carotenoid content of stem bark of *P. emblica* extract with IC$_{50}$ of DPPH scavenging activities were $r = 0.813$, $p < 0.001$; $r = 0.973$, $p < 0.01$. While in stem bark extract of *P. emblica* extract were $r = -0.100$, $p < 0.01$; $r = -0.843$, $p < 0.01$; $r = -0.368$, $p < 0.329$.

Total phenol, flavonoid and carotenoid of *P. emblica* extract correlation with IC$_{50}$ value of scavenging DPPH used Pearson method and expressed as Pearson correlation (r). According to Fidrianny,$^{33}$ if (r) value = $0.61 \leq r \leq 0.97$, that means positive and high correlation, and if $r = -0.61 \leq r \leq -0.97$, that means negative and high correlation. Negative and high correlation it was showed correlation between total phenol, flavonoid and carotenoid compound with IC$_{50}$ of scavenging DPPH. This result means, the greater of total phenol, flavonoid and carotenoid content was the smaller value IC$_{50}$ of DPPH scavenging activities.

Stem bark extract of *P. emblica* had negative and high correlation to total phenol content ($r = -1.00$; $p < 0.01$). This result means, phenolic compound in stem bark extract of *P. emblica* has a mayor group wiches suspected antioxidant activity. Phenolic compound in fruit and leaf extract of *P. emblica* have not been major group compound wiches suspected antioxidant activity, Phenolic compound in fruit extract of *P. emblica* more been play role to antioxidant activity than phenolic compound in leaf extract of *P. emblica*.

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

<table>
<thead>
<tr>
<th>IC$_{50}$ of DPPH Scavenging activities</th>
<th>Pearson Correlation</th>
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<tbody>
<tr>
<td>Total Phenol Content</td>
<td>Total Flavonoid Content</td>
</tr>
<tr>
<td>IC$_{50}$ of Fruit Extract</td>
<td>-0.492</td>
</tr>
<tr>
<td>IC$_{50}$ of Leaf Extract</td>
<td>0.813</td>
</tr>
<tr>
<td>IC$_{50}$ of Stem Bark Extract</td>
<td>-1.00</td>
</tr>
</tbody>
</table>
Leaf and stem bark extract of *Pemblica* had negative and high correlation to total flavonoid content as (r = -0.926; p < 0.01, r = -0.843; p < 0.01). This result means, the greater the total flavonoid content was indicated the smaller value IC<sub>50</sub> of DPPH scavenging activities. Flavonoid compound in leaf and stem bark extract of *Pemblica* was a major group compound with suspected to antioxidant activity. OH functional in flavonoid compound can suspected antioxidant activity. OH functional at C-3' and ortho position at C-3 and C-4 will increased antioxidant activity. Ortho OH position at C-3' and C-4' will more increase antioxidant potency than OH functional at C-3. Besides that, o xo group at C-4' and double bond between C-2 and C-3 can be a high suspected to antioxidant activity. This result means, flavonoid compound in leaf and stem bark riches increased antioxidant activity were has OH functional at C-3, ortho position at C-3' and C-4', or has o xo functional at C-4. So far, study of correlation stem bark extract of *Pemblica* to total flavonoid content used Pearson correlation have not been reported. Determination correlation between total carotenoid content to IC<sub>50</sub> of DPPH scavenging activities have showed carotenoid compound were not been mayor group as antioxidant activity contributor compound. Leaf extract of *Pemblica* had the highest as contributor antioxidant 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 alpha-tocopherol was the high suspected to antioxidant potency. Much of double bond conjugated in beta-carotene, suspected to antioxidant activity. Besides that, zeaxanthin, astaxanthin and asxanthin-beta-glucoside can be suspected to antioxidant activity. The previous study, so far have not been reported about correlation total carotenoid content with IC<sub>50</sub> value of DPPH scavenging activities sed Pearson's correlation. Becaused that, this result not yet compared to previous study.

**CONCLUSION**

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

**ACKNOWLEDGEMENT**

We would like to thank Sekolah Tinggi Farmasi Indonesia (Yayasan Hazanah) for funding and supporting this research.

**CONFLICT OF INTEREST**

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

**ABBREVIATIONS USED**

DPPH: 2,2-Diphenyl-1-Picrylhydrazil.

**REFERENCES**

Cite this article: Fitriansyah SN, Aulifa DL, Febriani Y, Sapitri E. Correlation of Total Phenolic, Flavonoid and Carotenoid Content of Phyllanthus emblica Extract from Bandung with DPPH Scavenging Activities. Pharmacog J. 2018;10(3):447-52.