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Original Article

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 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 different 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 IC_{50} 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 IC_{50} 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 IC_{50} 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.

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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] 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 alkaloid, 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] 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

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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 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). $FeCl_3$ 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 monoterpen 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] modification using $AlCl_3$ and absorbance wae 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 stanadr 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).

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 compounds. BN, DN and KN do not have of phenolic compounds. Phenolics and flavonoids are compounds that can cause antioxidant activity in

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	-	-	-	+	+	+	+	+	+
Monoterpene and Sesquiterpene	+	+	-	+	+	-	+	+	-
Steroid	+	-	-	+	-	-	-	-	-
Triterpenoid	-	-	-	-	-	-	+	-	-
Quinone	-	-	-	-	-	+	+	-	+
Saponin	-	-	-	-	-	-	+	+	-

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.[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 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 extract of *P.emblica*, and KO wa the lowest IC_{50} in stem bark extract of *P.emblica*. BE was the lowest whereas comared to all extract of *P.emblica*. 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. This result means, antioxidant activity of ascorbic acid had a higher than

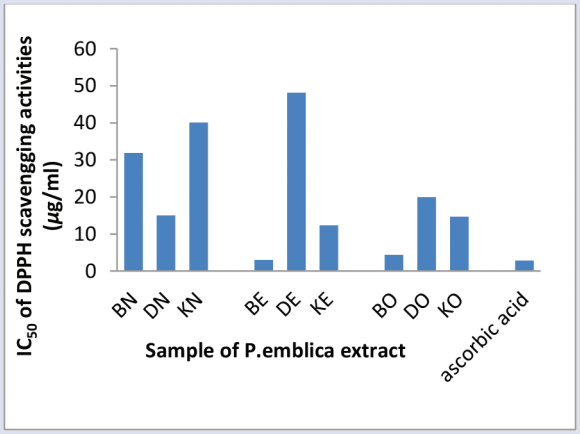

Figure 1: IC_{50} of DPPH scavengging activities of *P.emblica* extract.

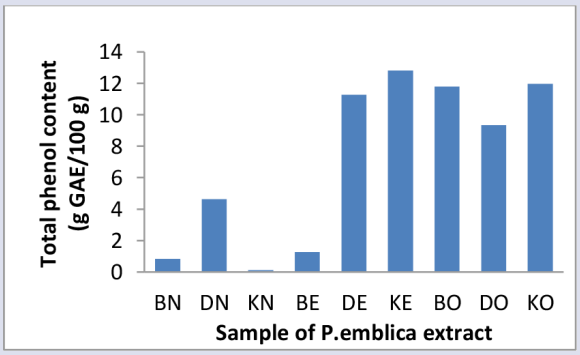

Figure 2: Total Phenol content of *P.emblica* extract.

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 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] Flavonoid and phenol were compound capable donating proton on free radicals. Besides that, cinamic acid and benzoic acid were compound capable donating proton on free radicals.[26,27] Cinamic acid more higher contributor as antioxidan activity than benzoic acid.[26,27]

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 g QE/100 g sampai 2,982 g QE/100 g. This result means, part of *P.emblica* plant has production flavonoid in differences quantity. Determination total flavonoid used $AlCl_3$ reaction.[20] Total flavonoid content at *P.emblica* extract calculation by standard curve y = 0.0342 + 0.0857; r^2 = 0.991 and expressed as quercetin. $AlCl_3$ will form omplex with OH functional in

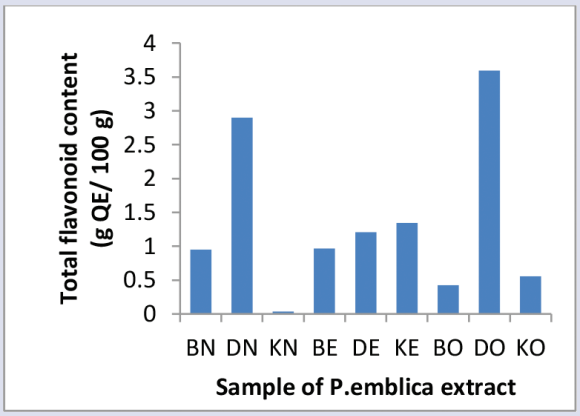

Figure 3: Total flavonoid content of *P.emblica* extract.

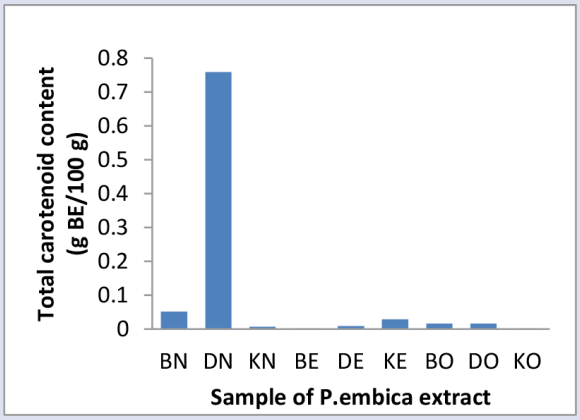

Figure 4: Total carotenoid content of *P.emblica* extract.

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 antioxidant 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.

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 compound. 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 IC_{50} value of scavengging DPPH used Pearson method and expressed as Pearson correlation (r). According to Fidrianny,[25] if (r) value = 0.61≤ r ≤0.97, that means positive and high correlation, and if r = -0.61 ≤ r ≤ -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 scavengging DPPH. This result means, the greater of total phenol, flavonoid and carotenod content was indicated the smaller value IC_{50} of DPPH scavengging activities.

Stem bark extract of *P.emblica* had negative and high correlation to total phenol conten (r = -1.00 ; p < 0,01). This result means, phenolic compound in stem bark extrac of *P.emblica* has a mayor group wiches suspected antioxidant activity. Phenolic compound in fruit and leaf extract of *P.emblica* have not been mayor group compound wiches suspected antioxidant activity. Phenolic compound in fruit extract of P.embica more been play role to antioxidant actvity 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*.

IC_{50} of DPPH Scavengging activities	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

Leaf and stem bark extract of *P.embica* 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 of total flavonoid content was indicated the smaller value IC_{50} of DPPH scavengging activities. Flavonoid compound in leaf and stem bark extract of *P.emblica* was a mayor group compound wich suspected to antioxidant activity. OH functional in flavonoid compound can suspected antioxidant activity. OH functional at C-3[25] and ortho position at C-3' and C-4' will increased antioxidant activity.[26] Ortho OH position at C-3' and C-4' will more increase antioxidant potency than OH functional at C-3. Besides that, oxo group at C-4[26] and double bond between C-2 and C-3 can be a high suspected to antioxidant activity.[13,25] This result means, flavonoid compound in leaf and stem bark wiches increased antoxidant activity were has OH functional at C-3, ortho positon at C-3' and C-4', or has oxo functional at C-4. So far, study of correlation stem bark extract of *P.emblica* to total flavonoid content used Pearson correlation have not been reported.

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.

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 otherwise regarding the publication of this paper.

ABBREVIATIONS USED

DPPH: 2.2-Diphenyl- 1-Picrylhydrazil.

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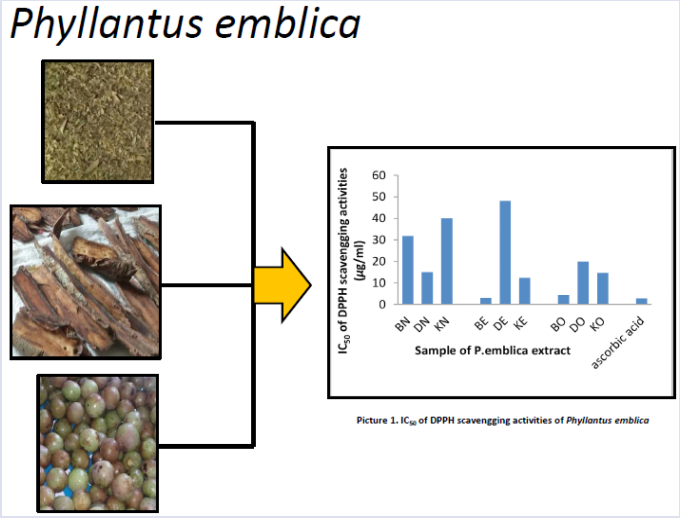
GRAPHICAL ABSTRACT



Picture 1. IC_{50} of DPPH scavengging activities of *Phyllantus emblica*

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