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Study of Antioxidant Profile and Phytochemical Content of Different Organs Extracts of *Morinda citrifolia* L.

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Abstract

Fruits of noni have been known as natural antioxidant that can scavenge free radical of DPPH. The other organs of noni might contain similar components and are expected to have similar activity. The aims of this research were to determine antioxidant profile of three different organs of noni (leaves, twigs and fruit) by DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) and also its phytochemical content. Antioxidant activities, total phenolic content (TPC) and total flavonoid content (TFC) were performed by uv-visible spectrophotometric method. Correlation of TPC and TFC with their IC_{50} of DPPH and EC_{50} of FRAP and also correlation of two methods were performed by Pearson's method. Ethyl acetate and ethanol extracts of three organs of noni were categorized as very strong antioxidant by DPPH method. Waste products of noni (leaves and twigs) were potential antioxidant. Flavonoid compounds were the major contributors in antioxidant activities of leaves, twigs and fruit extracts of noni by FRAP method. Antioxidant activities of leaves and twigs extracts of noni were linear by two antioxidant testing methods.

Keywords: antioxidant, DPPH, FRAP, noni, leaves, fruits, twigs

INTRODUCTION

Noni (*Morinda citrifolia* L.) have many pharmacological activities such as breast cancer [1-3], cervical cancer [4], antidiabetic [5], antimicrobial activity [6] and antioxidant [7]. Many compounds have been isolated from noni such as americanin A, narcissoside, asperuloside, asperulosidic acid, borreriagenin [8], isoscopoletin, aesculetin and quercetin [9].

Free radicals are very reactive due to containing unpaired electrons in their outer orbitals. The excessive of free radical are related with many degenerative diseases such as cardiovascular and hypercholesterolemia [10]. Many plants included vegetables and fruits contain many phenolic and flavonoid compounds which are expected to prevent degenerative diseases such as cancer, heart disease [11-13], and other pharmacological activities such as anti-inflammatory [14], and antioxidant activity [15-19]. Quercetin is one of phenolic and flavonoid compounds in noni fruits which have antioxidant activity [9].

Antioxidant activities of methanol extract of noni fruits in different maturity stages and also their phenolic and flavonoid content have been studied [20]. Comparison of antioxidant activities using two methods and phytochemical content of three organs of noni using three polarities solvent have not been exposed yet. Leaves and twigs are the waste products of noni, which might have similar potential antioxidant with their fruit. The goals of this research were to observe antioxidant activity by two antioxidant testing methods (DPPH and FRAP) and phytochemical content of different organs of noni using three different polarity solvents.

MATERIALS AND METHODS

Chemicals. Gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tripyridyl-S-triazine (TPTZ) were purchased from Sigma-Aldrich (MO, USA). Other chemicals used were analytical grade.

Sample preparation. Noni was collected from Subang, West Java-Indonesia and determined in Herbarium Bandungense - School of Life Science and Technology- Bandung Institute of Technology, presented as noni (*Morinda citrifolia* L.). Organs of noni which were used: leaves namely as LV, twigs as TW, and fruits as FR then selected, washed, dried and grinded into powder.

Sample extraction. Extraction was done triplicate by reflux for each solvent. Sample 300 g was extracted using different polarity solvent: n-hexane, ethyl acetate and ethanol, consecutively. There were three n-hexane extracts (namely LV1, TW1 and FR1), three ethyl acetate extracts (LV2, TW2 and FR2), and three ethanol extracts (LV3, TW3 and FR3).

DPPH assay. Each extract and ascorbic acid as standard were prepared in various concentrations. DPPH 50 µg/ml was used as control and methanol as a blank. Modification of Blois's method [21] was carried out in this research. Briefly, two ml DPPH 50 µg/ml was added by extract 2 ml, then absorbance was observed by UV-Vis spectrophotometer at wavelength 515 nm after incubation 30 min. Analysis was performed in triplicate for standard and each extract. Antioxidant capacity was presented by determining inhibitory concentration 50 % (IC_{50}) of DPPH scavenging activity using its calibration curve.

FRAP assay. FRAP solution was prepared in acetate buffer pH 3.6. Ascorbic acid was used as standard. Ascorbic acid and each extract were prepared in various concentrations. Antioxidant capacity by FRAP method was conducted using modification of Benzi's method [22]. Two ml FRAP solution 50 µg/ml was added by two ml extract to initiate the reaction. FRAP 50 µg/ml was used as control and acetate buffer as a blank. After 30 min incubation, the absorbance was observed at wavelength 593 nm. Analysis was performed in triplicate for each extract and standard. Calibration curve was used to calculate its antioxidant activity which reported by EC_{50} (exhibitory concentration 50 %) of FRAP capacity.

Total phenolic content (TPC). Folin-Ciocalteu reagent (which diluted 1:10 with aquadest) was used to calculate total phenolic content. Gallic acid 40-120 µg/ml was used as standard. Folin-Ciocalteu reagent 5 ml was put into gallic acid 0.5 ml, then added by 4 ml sodium carbonate 1 M. The mixture was allowed to stand 15 min at room temperature, then absorbance was read at wavelength 765 nm. Sample was carried out by the same procedure. TPC in each sample was demonstrated as gallic acid equivalent (GAE) per 100 g extract (g GAE /100 g) [23].

Total flavonoid content (TFC). Quercetin 36-104 µg/ml was used as standard. TFC was calculated using modification of Chang's method. Quercetin solution 0.5 ml was diluted by adding 1.5 ml methanol, 0.1 ml aluminium (III) chloride 10%, 0.1 ml sodium acetate 1M and 2.8 ml aquadest. The same procedure was performed for sample. Absorbance was observed at wavelength 415 nm after incubation 30 min. TFC was investigated using calibration curve of quercetin and reported as quercetin equivalent (QE) per 100 g extract (g QE/100 g) [24].

Statistical Analysis. ANOVA-post hoc Tukey ($p<0.05$) by SPSS 16 for Windows was used in statistical analysis. Meanwhile Pearson's method were conducted to analyze correlations between TFC, TPC and their antioxidant activities and also between two antioxidant testing methods. All of results are means ± standard deviation at least triplicate experiments.

RESULTS AND DISCUSSION

Three different polarities solvents such as n-hexane, ethyl acetate and ethanol consecutively were used to separate most nonpolar compound, semi polar compound and polar compound. Fruits of noni have been known had antioxidant activity [25]. The other organs in a plant might have the similar components, therefore the other organs of noni, might also give the similar activity with its fruits.

Density of each extract was presented by 1% extract, because it was difficult to put the thick extract into pycnometer. The similarity density among extracts was important point, because higher density extract may give higher activity and or phytochemical content than lower density extract. The density of each 1% extract of noni organs in the present study gave similarity density around 0.67 - 0.90 g/ml.

In the present study antioxidant activity by DPPH method was demonstrated as IC_{50} of DPPH, meanwhile the other study presented by percentage of DPPH scavenging activity [20, 25]. Lower percentage of DPPH scavenging activity was not always given by the lower concentration of sample, and higher percentage of DPPH scavenging activity was not always given by the higher concentration. The linear result will be presented by some concentration only. Previous research [26] exposed that percentage of DPPH scavenging activity of methanol peel extract of pineapple 100 µg/ml 95.74% was higher than DPPH scavenging activity 200 µg/ml (95.17%) and 400 µg/ml (94.96%). Extract contained many compounds which not all compounds in extract had antioxidant activities. Some compounds in extract act as antioxidant and the other compounds act as antagonist of antioxidant. In methanol peel extract 100 µg/ml antagonist antioxidant have not reach their effective minimum concentration yet, meanwhile in 200 µg/ml they reached this concentration, therefore antagonist of antioxidant will be appeared and against the antioxidant activity, then reduced the percentage of DPPH.

The previous study revealed that IC_{50} DPPH scavenging activity of commercial noni juice was 5.85 mmol/l, meanwhile its TPC was 91.90 mg GAE/ 100 ml juice [27]. Fermented noni fruit juice which was extracted by petroleum ether, ethyl acetate and n-butanol solvent demonstrated that the ethyl acetate extract had higher antioxidant activity than ascorbic acid, and the other extracts. The phenolic compounds which were isolated from the ethyl acetate extract also showed potential antioxidant [9]. It was contrary with the present study which expressed that ethanol fruit extract of noni (FR3) gave the highest antioxidant activity by DPPH assay (IC_{50} DPPH 1.51 µg/ml) compared to n-hexane fruit extract (FR1) with IC_{50} DPPH 237.31 µg/ml and ethyl acetate fruit extract (FR2) IC_{50} DPPH 2.57 µg/ml (Figure 1). Leaves and twigs are the waste products of noni. All leaves extracts of noni (LV1, LV2 and LV3) presented IC_{50} DPPH ranged from 4.87 to 17.34 µg/ml, meanwhile all twigs extracts of noni (TW1, TW2 and TW3) had IC_{50} DPPH in the range of 1.04 – 10.84 µg/ml. Based on the results it can be concluded that leaves and twigs extracts of noni can be classified as very strong antioxidant by DPPH assay (IC_{50} DPPH < 50 µg/ml), meanwhile in fruits extract of noni only FR2 and FR3 can be categorized as very strong antioxidant by DPPH assay. The n-hexane extract of noni (FR1) was classified as weak antioxidant (IC_{50} DPPH > 150 µg/ml).

The second antioxidant testing method in the present study was FRAP. EC_{50} of FRAP of all organs extract from noni (leaves, twigs and fruits) varied from 162.83 to 350.64 µg/ml (Figure 2). In generally, those all extracts can be categorized as weak antioxidant by FRAP method. Ethyl acetate leaves extract of noni (LV2) exposed the highest antioxidant activity by FRAP assay (EC_{50} FRAP 162.83 µg/ml), while the lowest antioxidant activity was given by ethanol twigs extract of noni (TW3) with EC_{50} FRAP 350.64 µg/ml.

The other research presented that water extract of fresh noni fruits gave higher TPC 31.67 ± 1.6 mg GAE/g than noni slices which was dried on 50°, 60°, 70° C (25.28 ± 1.1, 22.76 ± 3.0, 22.97 ± 2.3 mg GAE/g, respectively). Meanwhile their antioxidant activities revealed that percentage of DPPH scavenging activities of water extract of fresh noni fruits (93.25%) was not significant different to noni slices which dried on 50° and 60 °C [25]. Methanol extract of noni fruits during development process (10-130 days after fruits initiation) was investigated by Singh et al. [20]. Methanol extract of noni fruits with 110 days after fruit initiation gave higher polyphenolic content (76.9 mg/100 g), tannin content (213.3 mg/100 g) and flavonoid content (321.4 mg/100 g) than the other development process, while the higher antioxidant activity was shown by methanol extract of noni fruits with 80 days after fruit initiation which gave higher percentage DPPH scavenging activity (81.3%) than the other development process.

Table 1. Phytochemical content in noni organs

Sample	Phenolic content (g GAE/100 g)	Flavonoid content (g QE/100 g)
LV1	2.26 ± 0.16	2.85 ± 0.83
TW1	1.87 ± 0.08	1.70 ± 0.49
FR1	0.9 ± 0.05	1.04 ± 0.13
LV2	2.83 ± 0.17	10.61 ± 0.04
TW2	3.47 ± 0.07	1.98 ± 0.48
FR2	2.37 ± 0.09	0.93 ± 0.21
LV3	3.08 ± 0.15	2.06 ± 0.45
TW3	2.02 ± 0.03	0.27 ± 0.06
FR3	1.34 ± 0.02	0.20 ± 0.01

Table 2. Correlation of phenolic and flavonoid content with antioxidant activities

Antioxidant parameter	Pearson's correlation coefficient (r)	
	Phenolic content	Flavonoid content
IC_{50} DPPH LV	0.919^{**}	-0.150^{ns}
IC_{50} DPPH TW	-0.307^{ns}	-0.873^{**}
IC_{50} DPPH FR	-0.722^{*}	0.586^{ns}
IC_{50} ABTS LV	0.582^{ns}	-0.710^{*}
IC_{50} ABTS TW	-0.215^{ns}	-0.841^{**}
IC_{50} ABTS FR	-0.549^{ns}	-0.823^{**}

**= significant at p<0.01, * = significant at p<0.05, ns= not significant

Table 3. Correlation of DPPH and FRAP methods

Antioxidant parameter	Pearson's correlation coefficient (r)		
	EC_{50} FRAP LV	EC_{50} FRAP TW	EC_{50} FRAP FR
IC_{50} DPPH LV	0.789^{**}		
IC_{50} DPPH TW		0.994^{**}	
IC_{50} DPPH FR			-0.159^{ns}

** = significant at p<0.01, ns= not significant

Benzoic acid has higher antioxidant activity than cinnamic acid [28]. In the present study the highest TPC was given by TW2 (3.47 g GAE/100 g) and the highest TFC was seen by LV2 (10.61 g QE/100 g) (Table 1), but the highest antioxidant activity by DPPH method which shown by the lowest IC_{50} was presented by TW1 (IC_{50} DPPH 1.04 µg/ml). It exposed that the extract with the highest TPC and or TFC did not always give the highest antioxidant activity. TPC in FR1 (0.9 g GAE/100 g) was similar to TPC in FR3 (1.34 g GAE/100 g), but IC_{50} DPPH of FR3 (1.51 µg/ml) which categorized as very strong antioxidant and IC_{50} DPPH of FR1 (237.31 µg/ml) as weak antioxidant. Americanin A

(a neolignan) which was isolated from n-butanol fraction of methanol fruit extract of noni [8] was potent antioxidant by DPPH assay. Based on the results above it can be predicted that most of phenolic compounds in FR1 had low antioxidant activity, meanwhile in FR3 had high antioxidant activity.

TFC in TW3 (0.27 g QE/100 g) was similar to TFC in FR3 (0.20 g QE/100 g). The TW3 had lower antioxidant activity by DPPH assay (IC_{50} DPPH 10.84 µg/ml) than FR3 (IC_{50} DPPH 1.51 µg/ml). Flavonoid which has di-OH in C3'and C4', double bond in C2 and C3, OH in C3 and keto in C4 will give high antioxidant activity. Substitution di-OH in C3' and C4' influence to give higher antioxidant activities [28]. Based on the results it can be suggested that most of flavonoid compounds in FR3 had high antioxidant activity. Isoscopoletin, aesculetin and quercetin have been isolated from ethyl acetate fruit extract of noni [9] and leaves, fruit and root of noni contained kaempferol, rutin, quercetin and anthraquinone [29]. Quercetin have di-OH in C3'and C4', double bond in C2 and C3, OH in C3 and keto in C4 which fulfill to have high antioxidant activity. It can be suggested that there were many quercetin in FR3.

LV2 gave the highest TFC (10.61 g QE/100 g), also exposed the highest antioxidant capacity by FRAP assay (EC_{50} 162.83 µg/ml), while TW3 showed the lowest TFC (0.27 g QE/100 g) also presented the lowest antioxidant capacity (EC_{50} FRAP 350.64 µg/ml). Based on the results it can be predicted that most of flavonoid compounds in LV2 had redox potential lower than 0.77 V the potential redox of Fe^{3+}/Fe^{2+}, so sample will be oxidized and Fe^{3+} will be reduced to Fe^{2+}, then Fe^{2+} -TPTZ will give blue color and the absorbance was determined at wavelength λ 593 nm.

Correlation between TPC, TFC in all noni organs extracts (n-hexane, ethyl acetate and ethanol) and its antioxidant activities by DPPH and FRAP methods have been determined in the present study (Table 2). The expected correlation between TPC and TFC with IC_{50} DPPH or EC_{50} FRAP was significant and negative correlation [30], because the higher TFC and TPC will show lower IC_{50} DPPH and EC_{50} FRAP which exposed higher antioxidant activities. Pearson's correlation coefficient (r) was significantly negative if $-0.61 \leq r \leq -0.97$ and significantly positive if $0.61 \leq r \leq 0.97$ [31]. In generally there were significantly negative correlations between TFC in all organs extracts of noni (leaves, twigs and fruits) with their antioxidant activities by FRAP method ($r = -0.710$, $p < 0.05$; $r = -0.841$, $r = -0.823$, $p < 0.01$, respectively), so it can be concluded that flavonoid compounds in all organs extracts of noni were the major contributor in their antioxidant activities by FRAP assay.

Correlation between antioxidant activities of all noni organs extracts using two antioxidant testing method have been observed (Table 3). IC_{50} DPPH of leaves and twigs extracts of noni was positively correlation with their EC_{50} FRAP, therefore DPPH and FRAP methods gave linear results in antioxidant activities of leaves and twigs extracts of noni.

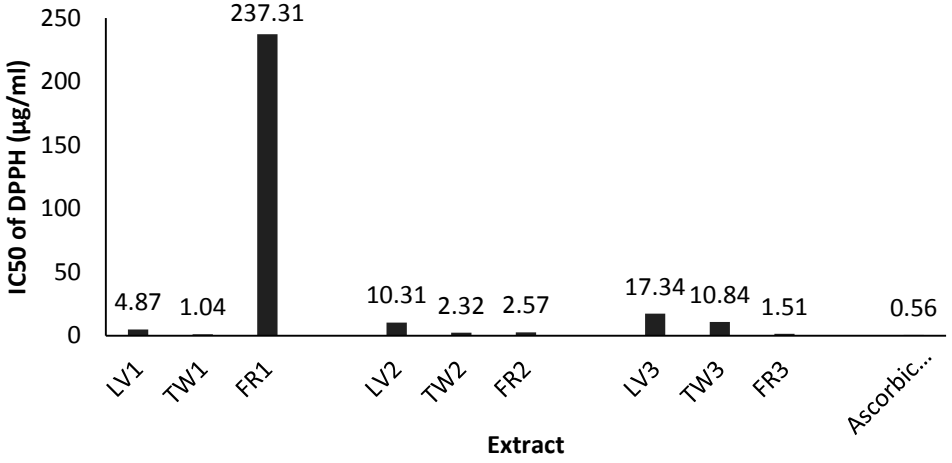

Figure 1. Antioxidant activities of noni organs by DPPH assay

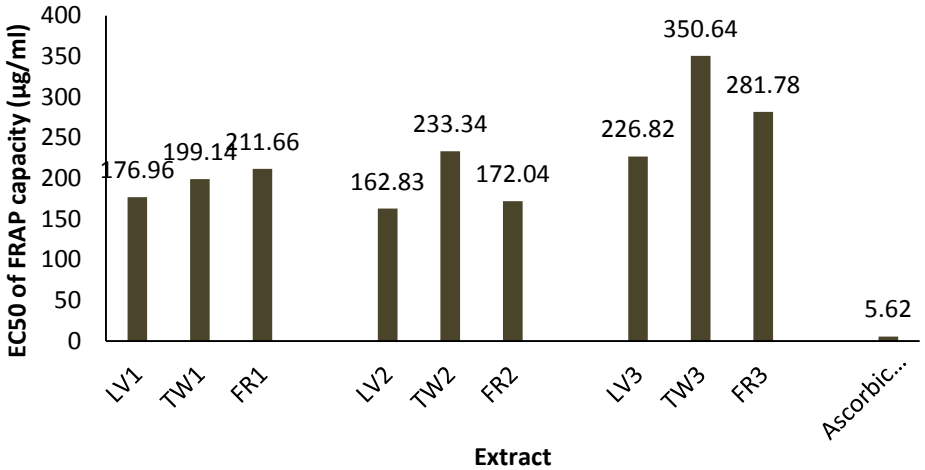

Figure 2. Antioxidant activities of noni organs by FRAP assay

CONCLUSIONS

Leaves and twigs which were waste products of noni had antioxidant activities. All leaves and twigs extracts of noni can be categorized as very strong antioxidant, using DPPH assay. The higher TPC and or TFC did not always show the higher antioxidant activities. Flavonoid compounds in leaves, twigs and fruit of noni were the major contributor in their antioxidant activities by FRAP assay. Noni leaves and twigs extracts gave linear result in two antioxidant testing methods (DPPH and FRAP). Waste products of noni (leaves and twigs) have potential to be developed as sources of further natural antioxidant.

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