

ANTIOXIDANT CAPACITIES FROM VARIOUS LEAVES EXTRACTS OF FOUR VARIETIES MANGOES USING DPPH, ABTS ASSAYS AND CORRELATION WITH TOTAL PHENOLIC, FLAVONOID, CAROTENOID

IRDA FIDRIANNY^{1*}, IRA RAHMIYANI¹, KOMAR RUSLAN WIRASUTISNA¹

¹School of Pharmacy, Bandung Institute of Technology, Indonesia. Email: irda@fa.itb.ac.id, irdafidrianny@gmail.com

Received: 26 Jun 2013 Revised and Accepted: 19 Aug 2013

ABSTRACT

Objectives: The objectives of this research were to study antioxidant activity of leaves extract from four varieties of mangoes using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) and correlation of total phenolic, flavonoid and carotenoid content in various extracts of four varieties mangoes with DPPH and ABTS scavenging activities.

Methods: Extraction was performed by reflux using gradient polarity solvent. The extracts were vaporated using rotavapor. Chromatogram pattern on each extracts were observed by thin layer chromatography (TLC). Then, antioxidant activity of each extracts using DPPH and ABTS assays, IC₅₀ of DPPH and ABTS scavenging activities and determination of total phenolic, flavonoid and carotenoid content and its correlation with DPPH and ABTS scavenging capacities.

Results: GD2 (ethyl acetate extract of gedong mango leaves) had the highest DPPH scavenging capacity (98.70 %), while AR3 (ethanolic extract of arumanis mango leaves) was the highest ABTS capacity (70.55 %). AR2 (ethyl acetate extract of arumanis mango leaves) contained the highest total flavonoid (37.57 g QE/100 g), GD2 had highest phenolic content (30.73 g GAE/100 g), while the highest carotenoid 16.28 g BET/100 g was given by GL1 (n-hexane extract of golek mango leaves).

Conclusions: There were positively high correlation between total phenolic content in four varieties mangoes leaves with their antioxidant activity using ABTS and DPPH assays. ABTS scavenging activities in various leaves extracts of four varieties mangoes had positively high correlation with their DPPH scavenging activities.

Keywords: Antioxidants, DPPH, ABTS, Mangoes leaves, Flavonoid, Phenolic, Carotenoid.

INTRODUCTION

Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity [1]. Many studies had revealed that phenolic content in plants could be correlated to their antioxidant activities. Plants contained phenolic and polyphenol compounds can act as antioxidant [2].

Some of antioxidant methods such as ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and DPPH (2,2-diphenyl-1-picrylhydrazyl) were used to predict antioxidant capacity of fresh fruits, beverages and food [1]. In previous study [3] [4] revealed that DPPH and ABTS methods could be used to determine antioxidant activity in many plants extracts.

Study by Ling and Palanisamy [2] demonstrated that IC₅₀ of DPPH and ABTS scavenging capacities in ethanolic extract of mango leaves was lower than their water extract. The other study stated that IC₅₀ of DPPH scavenging capacities in methanol extract of mango leaves was higher than its water extract, while IC₅₀ of ABTS scavenging activities of methanol extract lower than its water content [5].

The objective of this research were to study antioxidant activity of various extracts (n-hexane, ethyl acetate and ethanol) of four varieties mangoes (gedong mango, golek mango, apel mango and arumanis mango) leaves using simple methods of antioxidant testing DPPH and ABTS assays and correlations of their activity with total flavonoid, phenolic, and carotenoid contents in each extracts.

MATERIALS AND METHODS

Materials

Leaves of four varieties mangoes ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, quercetin, beta carotene was

purchased from Sigma-Aldrich (MO, USA), potassium persulfate, methanol, ethanol. All other reagents were analytical grades.

Preparation of sample

Leaves of four varieties mangoes (*Mangifera indica* L.) were collected from Tasikmalaya that were gedong mango (GD), golek mango (GL), apel mango (AP) and arumanis mango (AR) were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder.

Extraction

Three hundred grams of powdered samples were extracted by reflux using increasing gradient polarity solvents. The n-hexane extract was repeated three times. The remaining residue was then extracted three times with ethyl acetate. Finally the remaining residue was extracted three times with ethanol. So there were four n-hexane extracts (namely GD1, GL1, AP1 and AR1), four ethyl acetate extracts (GD2, GL2, AP2 and AR2) and four ethanolic extracts (GD3, GL3, AP3 and AR3).

ABTS scavenging capacity

Preparation of ABTS radical solution were adopted from Li *et al.* [6] and Pellegrini *et al.* [7] method with minor modification. ABTS diammonium salt aqueous solution and potassium persulfate aqueous solution were prepared. Each solutions allowing to stand in the dark room for 12-18 hours. Each extracts 50 µg/mL was pipetted into ABTS solution 50 µg/mL (1:1) to initiate the reaction. The mixture was diluted in ethanol. The absorbance was read at wavelength 734 nm without incubation time using spectrophotometer UV-Vis Hewlett Packard 8435. Ethanol (95%) was used as a blank and ABTS solution 50 µg/mL was used as standard. Analysis was done in triplicate for standard and each extracts. Antioxidant capacity of each extracts were determined based on the reduction of ABTS absorbance by calculating percentage of antioxidant activity [8].

DPPH scavenging capacity

Preparation of DPPH solution were adopted from Blois [9] with minor modification. Each extracts 50 µg/mL was pipetted into DPPH solution concentration 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 516 nm by using spectrophotometer UV-Vis Hewlett Packard 8435. Methanol was used as a blank and DPPH solution 50 µg/mL as standard. Analysis was done in triplicate for standard and each extracts. Antioxidant activity of each extracts were determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity [8].

Total phenolic determination

Total phenolic content were measured using the modified Folin-Ciocalteu method adapted from Pourmorad [10]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extracts. Standard solutions of gallic acid with concentration 60-150 µg/mL were used to obtain a standard curve. The total phenolic content was reported as percentage of total gallic acid equivalents per 100 g extract (g GAE /100 g).

Total flavonoid determination

Total flavonoid content was measured using adapted method from Chang *et al* [11]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration 40-100 µg/mL were used to obtain a standard curve. The total flavonoid content was reported as percentage of total quercetin equivalents per 100 g extract (g QE/100 g).

Total carotenoid determination

Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al* [1]. Each extracts were diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extracts. Standard solutions of beta carotene with concentration 10-80 µg/mL were used to obtain a standard curve. The total carotenoid content was reported as percentage of total beta carotene equivalents per 100 g extract (g BET/100 g).

RESULTS

Antioxidant capacity of various leaves extracts of four varieties mangoes using ABTS and DPPH assays

The antioxidant capacity using ABTS and DPPH assays for various leaves extracts of four varieties mangoes were shown in Table 1, Table 2, Table 3. In ABTS method, antioxidant activities in various leaves extracts of four varieties mangoes (*Mangifera indica*) in the range of 5.87- 70.55 %. AR3 leaves extract (ethanolic extract of arumanis mango leaves) had the highest ABTS scavenging activity (70.55 %), while the lowest activity (5.87 %) was given by GL1 leaves extract.

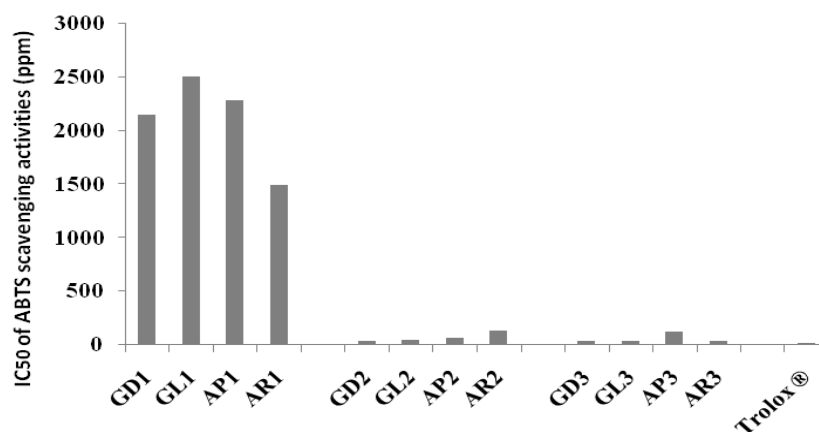


Fig. 1: IC50 of ABTS scavenging capacities in various leaves extracts of four varieties mangoes

Table 1: DPPH and ABTS scavenging activities of n-hexane leaves extracts

Sample	ABTS scavenging activity (%)	DPPH scavenging activity (%)
GD1	8,77 ± 0,32 (a)	10,11 ± 0,07 (a)
GL1	5,87 ± 0,21 (b)	10,67 ± 0,20 (a)
AP1	6,95 ± 0,09 (c)	8,33 ± 2,09 (a)
AR1	21,27 ± 0,48 (d)	9,19 ± 2,01 (a)
P value	< 0.05	< 0.05

Note: a -d = means within a column with the same letter were not significantly different (p=0.05)

Table 2: DPPH and ABTS scavenging activities of ethyl acetate leaves extracts

Sample	ABTS scavenging activity (%)	DPPH scavenging activity (%)
GD2	65,68 ± 1,98 (a)	98,70 ± 0,05 (a)
GL2	48,95 ± 1,09 (b)	98,31 ± 0,43 (a)
AP2	47,19 ± 1,16 (b)	91,52 ± 0,80 (b)
AR2	27,53 ± 0,5 (c)	76,77 ± 2,04 (c)
P value	< 0.05	< 0.05

Note: a -c = means within a column with the same letter were not significantly different (p=0.05)

Table 3: DPPH and ABTS scavenging activities of ethanolic leaves extracts

Sample	ABTS scavenging activity (%)	DPPH scavenging activity (%)
GD3	66,99 ± 0,52 (a)	94,95 ± 0,14 (a)
GL3	60,86 ± 3,55 (b)	95,46 ± 0,08 (a)
AP3	36,50 ± 0,88 (c)	61,14 ± 1,95 (b)
AR3	70,55 ± 1,31 (a)	90,79 ± 0,87 (c)
P value	< 0.05	< 0.05

Note: a -c = means within a column with the same letter were not significantly different (p=0.05)

In the DPPH method, free radical scavenging activities of various extracts of *Mangifera indica* leaves from four varieties mangoes ranged from 8.33 to 98.70 %. GD2 (ethyl acetate extract of gedong mango leaves) had the highest DPPH radical scavenging activity (98.70%), while AP1 leaves extract (8.33%) had the lowest DPPH antioxidant activity.

IC50 of ABTS and DPPH scavenging capacity

The IC50 of DPPH and ABTS scavenging activities in various extracts of mango leaves using DPPH and ABTS assays were shown in Fig 1 and Fig 2. IC50 of ABTS scavenging activities of each extracts were compared to IC50 trolox @ standard, while IC50 of DPPH scavenging capacities were compared to IC50 ascorbic acid standard.

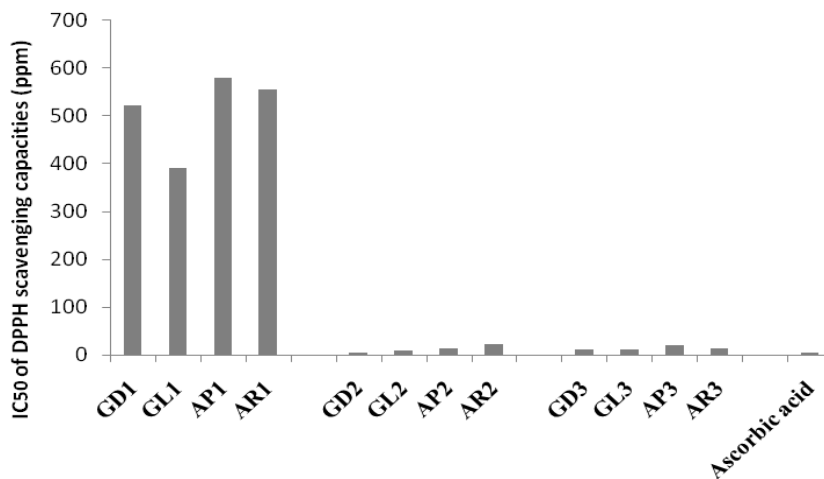


Fig. 2: IC50 of DPPH scavenging capacities in various leaves extracts of four varieties mangoes

Total flavonoid in various leaves extracts of four varieties mangoes

The total flavonoid contents among the different varieties were expressed in term of quercetin equivalent using the standard curve equation $y = 0.005x - 0.008$, $R^2 = 0.994$. The total flavonoid content in various leaves extracts of different varieties of mangoes showed different result in the range of 6.34 - 37.57 g QE/100 g (Fig 3). AR2 (ethyl acetate extract of arumanis mangoe leaves) had the highest total flavonoid contents (37.57 g QE/100 g) and the lowest (6.34 g QE/100 g) for AP3 leaves extract.

Total phenolic in various leaves extracts of four varieties mangoes

The total phenolic contents among the different varieties were expressed in term of gallic acid equivalent using the standard curve equation $y = 0.004x + 0.993$, $R^2 = 0.993$. The total phenolic content in various leaves extracts of four varieties mangoes showed different result ranged from 6.13 to 30.73 g GAE/100 g. GD2 leaves extract (ethyl acetate leaves extract of gedong mangoe) had the highest phenolic contents (30.73 g GAE/100g) (Fig 4).

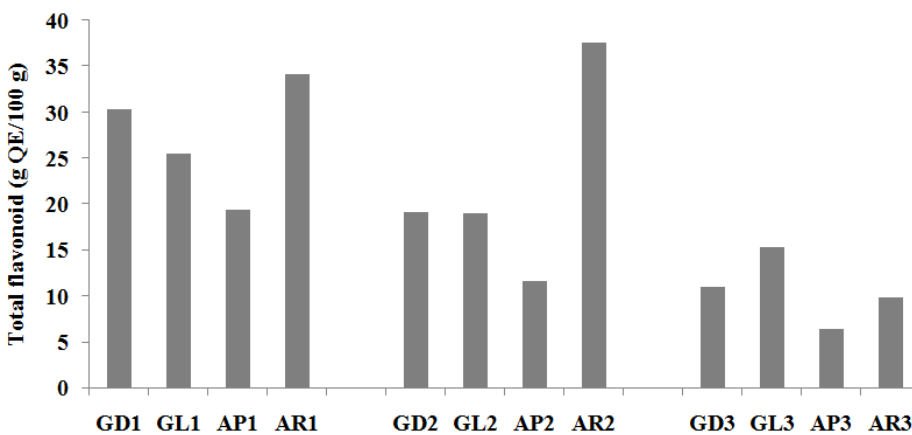


Fig. 3: Total flavonoid content in various mangoes leaves extracts

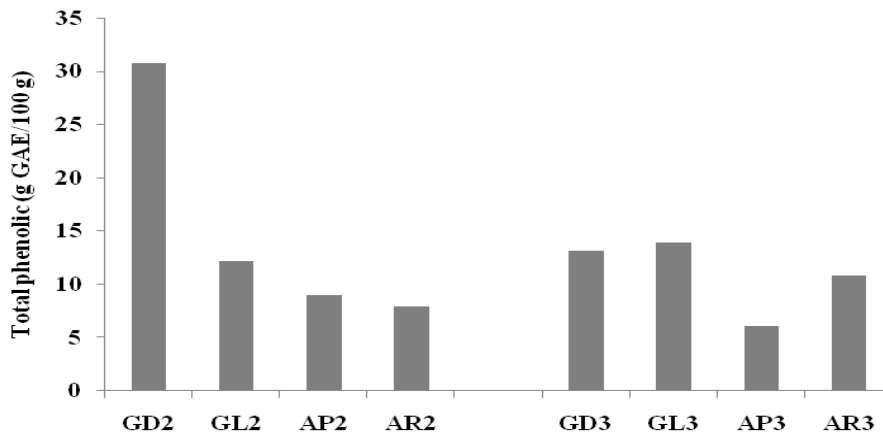


Fig. 4: Total phenolic content in various mangoes leaves extracts

Total carotenoid in various leaves extracts of four varieties mangoes

The total carotenoid contents among the different varieties were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.022x - 0.008$, $R^2 = 0.997$. The total carotenoid content in various leaves extracts of four varieties mangoes showed different result in the range of 0.20 - 16.28 g BET/100 g (Fig 5). The highest carotenoid contents (16.28 g BET/100 g) for GL1 leaves extract, while the lowest carotenoid (0.20 g BET/100 g) for AP3 leaves extract.

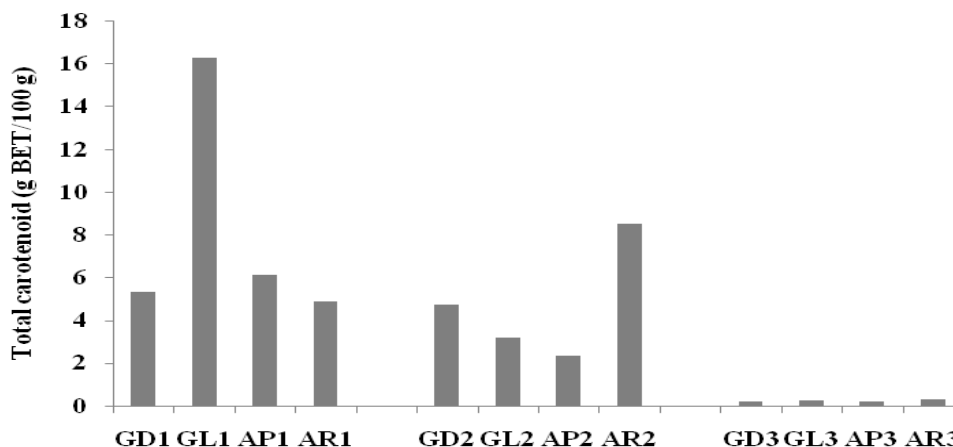


Fig. 5: Total carotenoid content in various mangoes leaves extracts

Table 4: Pearson's correlation coefficient of total flavonoid, phenolic, carotenoid of leaves extract from four varieties mangoes and DPPH, ABTS scavenging activities

	Total Flavonoid	Total Phenolic	Total Carotenoid	DPPH GD	DPPH GL	DPPH AP	DPPH AR
ABTS GD	-0,913**	0,815**	-0,773*	0,718*			
ABTS GL	-0,984**	0,993**	-0,979**		0,993**		
ABTS AP	-0,783*	0,998**	-0,811**			0,969**	
ABTS AR	-0,972**	0,789*	-0,839**				0,998**
DPPH GD	-0,893**	0,842**	-0,560 ^{ns}				
DPPH GL	-0,920**	0,990**	-0,997**				
DPPH AP	-0,712*	0,998**	-0,744*				
DPPH AR	-0,538 ^{ns}	0,993**	-0,225 ^{ns}				

Note: ABTS = ABTS scavenging activity, DPPH = DPPH scavenging activity, GD = sample GD, GL = sample GL, AP = sample AP, AR = sample AR, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

DISCUSSION

In previous study by Ling and Palanisamy [2] demonstrated that some of tropical plants included mango leaves had antioxidant capacity by DPPH assays. The research showed that antioxidant activity of ethanolic extract of mango leaves was higher than its water extract. There were no study regarding antioxidant activity of three different polarity extracts (which were n-hexane, ethyl acetate and ethanol) from leaves of four varieties mangoes using DPPH and ABTS assays.

Both of ABTS and DPPH are stable free radicals which dissolve in methanol or ethanol, and their colors show characteristic absorption at wavelength 734 nm or 516 nm, respectively. Colors ABTS and DPPH would be changed when the free radicals were scavenged by antioxidant [6] [12]. In ABTS method, the highest antioxidant activities was given by sample AR3 (ethanolic extract of arumanis mango). The result of present study was similar with research by Ling *et al.* [2] which showed antioxidant activity of ethanol extract of mango leaves was higher than its water extract.

The ABTS scavenging capacities among n-hexane leaves extract demonstrated that GD1, GL1, AP1 and AR1 were significantly different from each other ($p < 0.05$), while DPPH scavenging activities of four samples were not significantly different from each other.

Correlations between total phenolic, flavonoid, carotenoid content and DPPH, ABTS scavenging capacities in various leaves extracts of four varieties mangoes

Pearson's correlation coefficient was positively high if $0.68 \leq r \leq 0.97$ [1]. The highest and positive correlation between total phenolic content and ABTS scavenging capacity ($r = 0.998$, $p < 0.01$) was given by sample AP, followed by sample GL ($r = 0.993$, $p < 0.01$) (Table 4). The highest and positive correlation between total phenolic content and DPPH scavenging activity ($r = 0.998$, $p < 0.01$) for sample AP, followed by sample AR ($r = 0.993$, $p < 0.01$)

Statistical analysis of ABTS scavenging activity among ethyl acetate leaves extract indicated that GD2 and AR2 were significantly different from each other ($p < 0.05$). Sample GL2 and AP2 were not significantly different and both of them were significantly different with GD2 and AR2 ($p < 0.05$). DPPH scavenging radical activity among ethyl acetate leaves extracts illustrated that AP2 and AR2 were significantly different ($p < 0.05$). Sample GD2 and GL2 were not significantly different and both of them were significantly different with AP2 and AR2 ($p < 0.05$).

Statistical analysis of DPPH scavenging activity among ethanolic leaves extract showed that AP3 and AR3 were significantly different ($p < 0.05$). Sample GD3 and GL3 were not significantly different and both of them were significantly different with AP3 and AR3 ($p < 0.05$). The ABTS scavenging capacity exposed that GL3 and AP3 were significantly different from each other ($p < 0.05$). Sample GD3 and AR3 were not significantly different and both of them were significantly different with GL3 and AP3 ($p < 0.05$).

Concentration of sample that could scavenge 50 % free radical (IC_{50}) was used to determine antioxidant capacity of sample compared to standard. Sample that had $IC_{50} < 50$ ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with $IC_{50} > 150$ ppm [9]. The

lower IC50 would give the higher antioxidant activity. Ethyl acetate and ethanolic extracts leaves extract of four varieties mangoes gave IC50 of DPPH scavenging activity < 50 ppm, while ascorbic acid standard gave IC50 5.35 ppm. Based on IC50 of DPPH scavenging activity, ethyl acetate extract and ethanolic extract of mangoes leaves were very strong antioxidant, while n-hexane extracts were weak antioxidant. Potency antioxidant of ethyl acetate extract of sample GD2 (5.02 ppm) was similar with potency of ascorbic acid (5.35 ppm).

ABTS scavenging activity showed that various leaves extracts gave varieties in results. Ethanolic extract of sample GD, GL and AR (GD3, GL3 and AR3) were very strong antioxidant, while AP3 was medium antioxidant. Ethyl acetate extract GD2, GL2 and AP2 were strong antioxidant, while AR2 was medium antioxidant. Potency of sample AR3 (ethanolic leaves extract of arumanis mango) using ABTS assays (34.20 ppm) which was around a half of potency antioxidant of trolox® (17.50 ppm). The previous study by Kawpoomhae [5] revealed that IC50 of DPPH scavenging activity of water extract of mango leaves was lower than its methanol extract, while IC50 of ABTS scavenging activity of methanol extract of mango leaves was lower than its water extract.

The presence of total phenolic might contribute to antioxidant activity of *Mangifera indica* leaves [2]. GD2 leaves extract (ethyl acetate leaves extract of gedong mango) had the highest phenolic contents (30.73 g GAE/100 g). The previous research by Ling *et al* [2] demonstrated that ethanolic extract of mango leaves had higher phenolic content than water extract.

The data in Table 4 exposed that there were positively high correlation between total phenolic content in various extracts of four varieties of mangoes and antioxidant capacities using two methods ABTS and DPPH assays ($p < 0.01$). Based on this data it could be concluded that antioxidant activities of various leaves extracts of gedong mango, golek mango, apel mango and arumanis mango with ABTS and DPPH assays might be estimated indirectly by determining their total phenolic content. Phenolic acid might contributed in antioxidant activity, while cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid [13].

Pearson's correlation coefficient above showed that total flavonoid in various leaves extract of four varieties mangoes had negative correlation with their antioxidant activities by ABTS assays. The result illustrated that higher flavonoid content in mangoes leaves extracts would gave lower antioxidant capacities in ABTS. The similar results were given by DPPH assays, there were negative correlation between total flavonoid and their antioxidant capacities, except in arumanis mango that was not significant correlation.

Flavonoid not always be phenolic compounds its depending on position of OH in flavonoid. Phenolic compound included tannins, flavonoid, phenolic acid and other compounds that had phenolic structure. Flavonoid that had OH in A ring and or B ring would be called as phenolic groups. Phenolic acid had the lower antioxidant activity than flavonoid [13]. Flavonoid would give antioxidant activity which had OH in ortho C 3',4', OH in C 3, oxo function in C 4, double bond at C 2 and C 3. The OH with ortho position in C3'-C4' had the highest influence to antioxidant activity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides [13]. Based on the data correlation of total phenolic, flavonoid of mangoes leaves extracts and their antioxidant activities above it can predicted that many flavonoids in mangoes leaves were flavonoid that had no OH in ortho C 3',4', OH in C 3, oxo function in C 4, double bond at C 2 and C 3. There were predicted that flavonoid in mango leaves had OH in other position, example in C5, C7, or C3' only, or C4' only, or C3 only without oxo function in C4, that had no and low antioxidant activities.

Total carotenoid in various leaves extract of four varieties mangoes (gedong mango, golek mango, apel mango and arumaanis mango) showed negative correlation with antioxidant activities in ABTS assays. The data illustrated that higher total carotenoid in mangoes leaves extracts would gave lower antioxidant activities in

ABTS assays. The same results could be seen in DPPH assays, except in gedong mango and arumanis mango there were not significant correlation. Carotenoid had antioxidant activity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical activity [14]. Carotenoid that consisted of maximum 7 double bonds gave lower scavenging radical free capacity than more double bonds [15]. In previous study by Kobayashi and Sakamoto [16] stated that increasing in lipophilicity of carotenoid would increase scavenging radical capacity. Beta carotene was used as standard because of it had conjugation double bonds due to its ability to scavenge free radicals [17]. Based on the above data could be seen that many carotenoid in mango leaves extracts contained lower than 7 double bonds, that had no or low antioxidant capacity.

ABTS and DPPH methods had the same mechanism reaction that was electron transfer assays [18], but the results of the present study showed that ABTS scavenging capacity not always linear with DPPH scavenging activity. The Pearson's correlation coefficient of various leaves extracts of four varieties mangoes indicated that there were positively and high correlation between ABTS activities and DPPH capacities. It could be seen that antioxidant activities various mangoes leaves extracts in ABTS assays were linear with DPPH assays.

CONCLUSION

Leaves of four varieties mangoes had antioxidant activities, thus as the potential source of antioxidant. Ethyl acetate extract of gedong mango leaves had the highest DPPH scavenging capacities and ethanolic extract of arumanis mango had the highest ABTS scavenging activities. The positively high correlation between total phenolic content with DPPH and ABTS scavenging activities were given by four varieties mangoes. Antioxidant activity using ABTS and DPPH assays in gedong mango, golek mango, apel mango and arumanis mango leaves extracts might be estimated indirectly by using total phenolic content. Phenolic compounds were the major contributor to antioxidant activity in gedong mango, golek mango, apel mango and arumanis mango leaves. Antioxidant capacities in various extract of four varieties mangoes using ABTS and DPPH were linear.

REFERENCES

1. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH . Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal 2006; 19: 669-675.
2. Ling, LT and Planisamy, UD. Review: Potential antioxidants from tropical plants, In: Valdez, B., editor, Food industrial processes-methods, Kuala Lumpur: In Tech; 1999. p.64-72.
3. Ling, LT, Radhakrishnan AK, Subramaniam T, Cheng HM, Palanisamy UD. Standardised *Mangifera indica* extract is an ideal antioxidant. J Food Chem 2009; 113(4): 1154-1159.
4. Luis A, Gil N, Amaral ME, Duarte AP, Antioxidant activities of extract from *Acacia melanoxylon*, *Acacia dealbata*, *Olea europaea* and alkaloids estimation. Int J Pharm Pharm Sci 2012, 4(1), 225-231.
5. Kawpoomhae K, Sukma M, Ngawhirunpat T, Opanasopit P, Sripattanaporn A. Antioxidant and neuroprotective effects of standardized extracts of *Mangifera indica* leaf. Thai J Pharm Sci 2010; 34: 32-43.
6. Li XC, Wang XZ, Chen DF, Chen SZ. Antioxidant activity and mechanism of protocatechuic acid in vitro. Journal of Functional Food in Health and Disease 2011; 1: 232-244.
7. Pellegrini N, Serafini M, Colombi B, Rio DD, Salvatore S. Total antioxidant capacity of plant food, beverages and oils consumed in Italy assessed by three different in vitro assays. J Am Coll Nutr 2003; 133: 2812-2819.
8. Bedawey AA. Characteristics of antioxidant isolated from some plants sources, Cairo: Shubin El-Kom; 2010, p. 1-11.
9. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958; 181: 1199-2000.
10. Pourmorad F, Hosseinimehr SJ, Shahabimajid N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. Afr J Biotechnol 2006; 5(11): 1142-1145.

11. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; 10: 178-182
12. Apak R, Kubilay G, Birsen D, Mustava O, Saliha EC, Burcu B et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 2007; 12: 1496-1547
13. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 2002; 13: 572 -584.
14. Foote, CS. Free radicals in biology. 3rd ed. New York: Academic Press; 1976.
15. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. *Methods Enzymol* 2000; 319: 226-241.
16. Kobayashi M, Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. *Biotech Lett* 1999; 21: 265-269.
17. Charles DJ. Antioxidant properties of spices herbs and other. London: John Willey; 2013.
18. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 2005; 53: 1841 -1856.