

THE WASTE PRODUCTS OF *Mangifera indica* L. 'ARUMANIS': IN VITRO ANTIOXIDATIVE ACTIVITIES AND PHYTOCHEMICAL CONTENT

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ABSTRACT

The objectives of this study were to determine antioxidative activities of waste products (peel and seed) and flesh of arumanis mango (*Mangifera indica* L. 'Arumanis') using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Cupric Reducing Antioxidant Capacity (CUPRAC) methods and to analyze the correlation between total phenolic content (TPC) and total flavonoid content (TFC) with their antioxidative activities. TPC, TFC and antioxidative activities were observed by UV-Vis spectrophotometry. Pearson's method was used to elaborate the relationship between phytochemical content with their antioxidative activities. The ethanol seed extract gave the top antioxidative activity using DPPH and ethyl acetate flesh extract by CUPRAC assay. The highest TPC was showed by ethyl acetate seed extract, meanwhile, ethanol peel extract gave the highest TFC. The TFC in arumanis mango peel, flesh, and seed extracts had a significant and positive correlation with their AAI (antioxidant activity index) CUPRAC. In general, the ethyl acetate and ethanol extracts of waste products of arumanis mango were greatly potent antioxidants by DPPH and CUPRAC assays. Flavonoid compounds in arumanis mango peel, flesh and seed were the primary contributors in antioxidative activities by CUPRAC method. The waste products (peel and seed) of arumanis mango fruit were potential sources as natural antioxidants.

Keywords: Antioxidant, Arumanis Mango, Flavonoid, Phenol

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INTRODUCTION

The surplus free radicals are correlated with substantial degenerative diseases, which can be scavenged by antioxidants. Plentiful plants included fruits and vegetables that contained phenolic and flavonoid compounds which were related to antioxidative activity,¹⁻¹⁰ antibacterial, tyrosinase inhibitory and antidiabetic activities.¹¹⁻¹⁴

Many methods such as CUPRAC, FRAP, DPPH, ABTS, ORAC can be used for determining antioxidative activity.¹⁵⁻²⁰ *Mangifera indica* L. 'Arumanis' belong to Anacardiaceae is one of the mango cultivars and the most popular in Indonesia. The flesh is the edible parts of mango fruit, and the other parts (peel and seed) are categorized as waste products. The previous study demonstrated that leaf extracts of some cultivars of mango (gedong mango, apple mango, golek mango, and arumanis mango) had antioxidative activities using DPPH and ABTS assays.²¹ The other recent research showed the DPPH scavenging activity of mango flesh extract.²² Peel of mango contained ascorbic acid, tocopherol, carotenoids, and polyphenols.²³ Different parts of the plant contained similar compounds, therefore they might be had similar activity. Only a few papers discussed the antioxidative activity of mango peel and no study presented the antioxidative activity of the waste products of arumanis mango (peel and seed). The antioxidative activity of the waste products can be their added value for development in future research.

Many compounds in arumanis mango such as nonpolar, semi polar, and polar compounds may act as an antioxidant. There was no investigation regarding the antioxidative activity of the waste products (peel and seed) and flesh of arumanis mango (*M. indica* L. 'Arumanis') using increasing polarity solvents (n-hexane, ethyl acetate, and ethanol) and determined using DPPH and CUPRAC assays. This research

aimed to observe the antioxidative activities in different polarities (n-hexane, ethyl acetate, and ethanol) extracts from the waste products (peel and seed) and flesh of arumanis mango grown in Cirebon, West Java-Indonesia using DPPH and CUPRAC assays, and to analyze the correlations of total phenolic and flavonoid content with their antioxidative capacities.

EXPERIMENTAL

Materials

Gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and neocuproin were purchased from Sigma-Aldrich (MO, USA), parts of arumanis mango fruit such as peel, flesh, and seed.

Collection and Preparation of Sample

Arumanis mango fruit was separated between waste products (peel and seed) and edible parts (flesh). The parts of the arumanis mango were peel namely as PEE, flesh as FLE and seed as SEE were washed, dried and milled into a powder. Arumanis mango fruits were collected from Cirebon, West Java-Indonesia and determined in Herbarium Bandungense-School of Life Science and Technology-Bandung Institute of Technology, stated as arumanis mango (*M. indica* L. 'Arumanis').

Extraction

Reflux method with increasing polarity solvents was utilizing for every sample. Sample (300 g) was extracted three times using n-hexane. The residual was extracted three times by using ethyl acetate, and then the remaining residual was extracted using ethanol. There were nine extracts, which were n-hexane peel extract (PEE1), n-hexane flesh extract (FLE1), n-hexane seed extract (SEE1), ethyl acetate peel extract (PEE2), ethyl acetate flesh extract (FLE2), ethyl acetate seed extract (SEE2), and ethanol peel extract (PEE3), ethanol flesh extract (FLE3), and ethanol seed extract of (SEE3).

Total Phenolic Content (TPC)

Gallic acid 40-110 µg/ml was used as a standard. Folin-Ciocalteu reagent was used to determine total phenolic content.²⁴ Gallic acid 0.5 ml was added by 5 ml Folin-Ciocalteu reagent (which diluted 1:10 with aquadest) and 4 ml sodium carbonate 1 M. The solution was kept 15 min at room temperature, then absorbance was read at wavelength 765 nm. The same procedure was performed for all samples. TPC of the samples was calculated using the calibration curve of gallic acid and reported as gallic acid equivalent (GAE) per 100 g extract (g GAE/100 g).²⁵

Total Flavonoid Content (TFC)

Quercetin 36-104 µg/ml was used as a standard. Chang's method with minor modification was performed to determine TFC.²⁶ Quercetin solution 0.5 ml was added by 1.5 ml methanol, 0.1 ml aluminum (III) chloride 10%, 0.1 ml sodium acetate 1M and 2.8 ml aquadest. The sample was conducted by the same procedure. Absorbance was read at λ 415 nm after 30 min incubation. TFC was exposed by g quercetin equivalent (QE) per 100 g extract (g QE/100 g).²⁷

Antioxidant Activity Index by DPPH

Several concentrations were set for every extract. Blois's method with slight modification was implemented to determine DPPH scavenging activity.²⁸ Ascorbic acid was utilized as standard. The control was DPPH 50 µg/ml and methanol as a blank. Extract 2 ml was added into 2 ml DPPH 50 µg/ml. After incubation 30 min, the absorbance was monitored at wavelength 517 nm by UV-Vis spectrophotometer. Standard and each extract were analyzed in triplicate. The calibration curve was used to investigate IC₅₀ (inhibitory concentration 50%) of DPPH scavenging activity. The antioxidant activity index (AAI) can be calculated by formula:²⁹

$$\text{AAI DPPH} = [\text{Final concentration of DPPH}] / [\text{IC}_{50} \text{ of DPPH}] \quad (1)$$

Antioxidant Activity Index by CUPRAC

Ammonium acetate buffer pH 7 was used to prepare the CUPRAC solution. Various concentrations of every extract were set. Antioxidative capacity by CUPRAC was done using Apak's method with minor modification.¹⁵ Ascorbic acid was implemented as standard. The blank was ammonium acetate buffer and CUPRAC 100 µg/ml as a control. Two ml of extract was added into 2 ml CUPRAC 100 µg/ml. After incubation 30 min, the absorbance was observed at wavelength 450 nm. The concentration of sample or standard that can exhibit 50 % of CUPRAC capacity is exhibitory 50 % (EC₅₀) of CUPRAC capacity, which was presented antioxidative capacity and determined using a calibration curve. Then the AAI of each sample was evaluated by the equation:

$$\text{AAI CUPRAC} = [\text{Final concentration of CUPRAC}] / [\text{IC}_{50} \text{ of CUPRAC}] \quad (2)$$

Data Analysis

Data were statistically analyzed by SPSS 22 for Windows. All the exposed results at least three independent experiments are means $\pm$ standard deviation. Statistical significance was investigated using one-way ANOVA-post hoc Tukey ($p < 0.05$). Pearson's method was used to elaborate the relation between TFC, TPC and their antioxidative activities, also between two antioxidative testing methods.

RESULTS AND DISCUSSION

Three different polarities solvents such as n-hexane, ethyl acetate and ethanol were used to separate based on the polarity of compounds in crude drug. N-hexane and ethyl acetate were selected solvents, therefore mainly of the nonpolar compounds will be extracted in n-hexane and semi-polar compounds in ethyl acetate. Meanwhile in ethanol will find most of the polar compounds.

Table-1: Density of Arumanis Mango Extracts

Sample	Density of Extract 1% (g/ml)		
	n-Hexane	Ethyl Acetate	Ethanol
Peel	0.663	0.890	0.798
Flesh	0.664	0.891	0.798
Seed	0.664	0.890	0.799

Extract with higher density might give higher phytochemical content and activity. Therefore, to compare the activity and phytochemical content among the extracts, the density of each extract should be similar. The density of extract was investigated as density 1%, because of the difficulty to place 100% thick extract into a pycnometer. The density of each extract was expressed in Table-1.

The recent study investigated 11 mango cultivars and revealed that 70% ethanol fruit extract of Guire No. 82 gave the highest TPC (141.36 ± 1.76 mg GAE/100 g fresh weight) and TFC (2.25 mg RE/100 fresh weight) compared to the other cultivars.³⁰ Study by Meneses et al.³¹ presented that ethanol peel extract of mango contained quercetin (480.8 ± 4.38 mg GAE/kg dry mango peel) and kaempferol (77.2 ± 2.42 mg GAE/kg dry mango peel), meanwhile the research demonstrated that mango pulp extract from Uba showed the highest TPC (208.70 mg/100 g).³² The other study presented that methanol kernel extract of mango from Sewe found the highest TPC (546 ± 1 mg GAE/g) compared to leaves, stem bark and root extracts. It was similar to the Bouka regions which kernel extract demonstrated the top TPC (489 ± 3 mg GAE/g) compared to the other parts of mango.³³

Research by Jain et al. expressed that aqueous ethanol leaves extract of mango (*M. indica*) had higher TPC and TFC than *Nicotiana tabacum*.¹⁷ The TPC and TFC of 80% ethanol peel and flesh extracts of unripe and ripe fruit of mango have been investigated. The peel and flesh extracts of unripe fruit had higher TPC (92.6 ± 3.40 mg GAE/g and 27.8 ± 2.21 mg GAE/g) than the ripe one (70.1 ± 4.61 mg GAE/g and 26.9 ± 3.76 mg GAE/g). The TFC of peel and flesh extract of ripe fruit (22.2 ± 3.32 mg GAE/g and 8.15 ± 1.54 mg GAE/g) higher than the ripe fruit (21.2 ± 2.47 mg GAE/g and 3.30 ± 0.79 mg GAE/g).²² It was in line with the present investigation which expressed that the TFC in ethanol peel extract of arumanis mango (8.61 ± 1.92 g QE/100 g) higher than TFC in ethanol flesh extract (4.34 ± 0.08 g QE/100

g) (table 2). The other recent study stated that water peel extract of mango from Sidhura region gave higher TFC (15.6 ± 0.23 mg QE/g) and TPC (87.38 ± 0.43 mg GAE/g) than the other regions.³⁴

Table- 2: Total Phenolic and Flavonoid Content in Arumanis Mango Extracts

Sample	TPC	TFC
	(g GAE/100 g)	(g QE/ 100 g)
PEE1	2.61 ± 0.06	8.10 ± 1.05
FLE1	1.92 ± 0.05	6.71 ± 0.14
SEE1	2.14 ± 0.02	1.8 ± 0.15
PEE2	5.58 ± 0.03	2.28 ± 0.07
FLE2	11.54 ± 0.11	7.31 ± 0.36
SEE2	22.45 ± 0.11	3.74 ± 0.63
PEE3	1.50 ± 0.17	8.61 ± 1.02
FLE3	11.79 ± 0.11	4.34 ± 0.08
SEE3	20.56 ± 0.13	6.22 ± 0.03

PEE = peel, FLE = flesh, SEE = seed, 1 = n-hexane extract, 2 = ethyl acetate, 3 = ethanol extract

Some recent studies exposed the DPPH scavenging activity in percentage and its comparison to standard. But greater concentration does not constantly give a greater percentage of DPPH scavenging activity. The methanol peel extract of pineapple 100 µg/ml gave a bigger percentage DPPH scavenging activity (95.74%) than 200 µg/ml (95.17%) and 400 µg/ml (94.96%).¹² Based on the information above, it can be reviewed the percentage of DPPH scavenging activity does not demonstrate the real antioxidative activity. The other research presented that DPPH scavenging activity was exposed as µmol Trolox Equivalent (TE)/g, so the higher value will show the higher antioxidative activity. Methanol kernel extract of mango from 2 regions (Sewe and Bouka) gave higher DPPH scavenging activities (4980 ± 50 µmol TE/g and 5510 ± 6 µmol/TE) than the other parts (leaves, stem bark and root) of mango. It was different from ORAC assay which found methanol leaves extract of mango from the two regions had a higher ORAC value (6635 ± 176 µmol/TE and 5310 ± 61 µmol/TE) than the methanol stem bark, root and kernel extracts. Meanwhile, by iron (II) chelating activity the methanol stem bark extract of mango from Sewe showed a higher value (10593.0 ± 4.0 µg EDTAE/g) than the other parts.³³ The previous research stated that the ethanol peel extract of mango fruit had DPPH scavenging activity around 850 µMol TE/g dP.³¹ The method which was used in determining antioxidative activity should be verified by using a standard, such as ascorbic acid, alpha-tocopherol, Trolox, BHT (butylated hydroxyl toluene) and BHA (butylated hydroxyl anisole). In the present research, verification was conducted using ascorbic acid. The method was valid if the standard gave a positive result with the assay.

In the current study, antioxidative activity was presented as AAI of DPPH and AAI of CUPRAC. The IC₅₀ DPPH and EC₅₀ CUPRAC were determined using a calibration curve. The linear results will be found in certain concentrations only. After IC₅₀ and EC₅₀ of the sample were calculated, then continued with the evaluation of AAI of DPPH and AAI of CUPRAC of each sample. The higher AAI presents a higher antioxidative activity. The value of IC₅₀ DPPH and or EC₅₀ CUPRAC was dependent on the concentration of DPPH or CUPRAC that be implemented in research. If one sample X be tested using DPPH 50 µg/ml and DPPH 25 µg/ml, the different IC₅₀ DPPH will be found. IC₅₀ DPPH of X that be tested with DPPH 50 µg/ml will give a higher value than DPPH 25 µg/ml. The usage of AAI to present antioxidative activity will be fixed, because the AAI value is based on the final concentration of DPPH solution and IC₅₀ of the sample.

AAI of DPPH of ethanol seed extract of arumanis mango (12.26) > peel extract (6.88) > flesh extract (4.56). In ethyl acetate extract, AAI DPPH of seed extract > flesh extract > peel extract, while in n-hexane extract, AAI DPPH of peel extract > flesh extract > seed extract (Fig.-1). It was different from the CUPRAC assay which displayed that AAI CUPRAC of n-hexane peel extract > seed extract > flesh extract. In ethyl acetate extract revealed that AAI CUPRAC of flesh extract > seed extract > peel extract,

meanwhile in ethanol extract exhibited that AAI CUPRAC of seed extract > peel extract > flesh extract (Fig.-2). A study by Umamahesh et al. reported antioxidative activity by IC_{50} of DPPH and IC_{50} of ABTS.³⁴ The water peel extract of mango from Sindhura had lower IC_{50} of DPPH ($65.34 \pm 0.62 \mu\text{g/ml}$) and IC_{50} of ABTS ($28.29 \pm 0.43 \mu\text{g/ml}$) than the other regions.

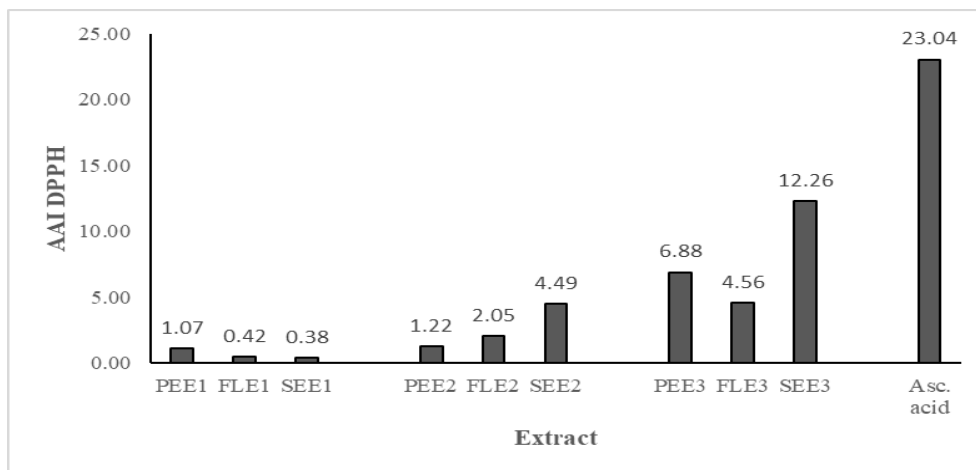


Fig.-1: AAI of Arumanis Mango Extracts by DPPH Assay

A study by Li et al.³⁰ investigated the antioxidative capacity of water extract of mango fruit using FRAP assay and presented FRAP value as mmol of $\text{FeSO}_4/100 \text{ g}$ fresh weight. The result stated that the water fruit extract of Guire NO. 82 mango cultivar had a higher FRAP value than the other cultivars. The other research exposed the antioxidative activity by the percentage of DPPH and ABTS scavenging activities, which revealed 80% ethanol peel extract 50 $\mu\text{g/ml}$ of unripe fruit gave higher percentage DPPH and ABTS scavenging activities than ripe fruit.²²

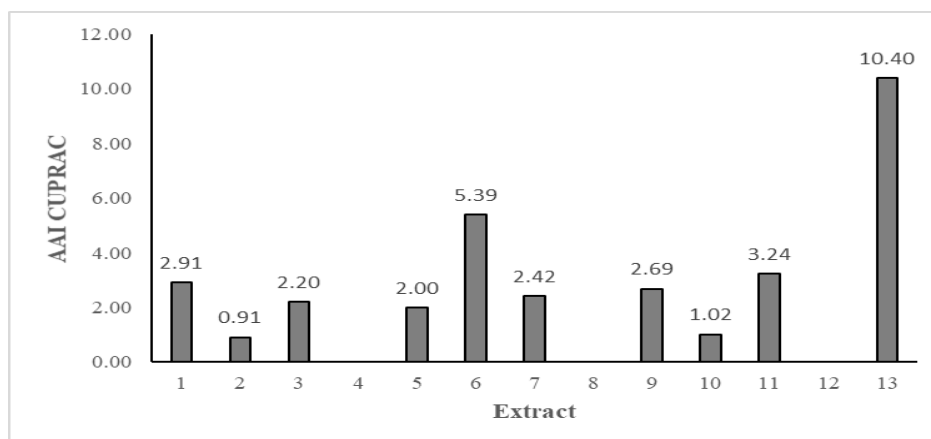


Fig.-2: AAI of Arumanis Mango Extracts by CUPRAC Assay

ns= not significant, * = significant at $p < 0.05$, **= significant at $p < 0.01$, PEE = peel, FLE = flesh, SEE = seed

Table-3 exposed the relation between TPC and TFC in arumanis mango fruit extracts with their antioxidative activities (such as between TPC in peel extracts and AAI DPPH of peel extracts). This correlation can be investigated by preparing a curve between TPC or TFC and AAI DPPH or AAI CUPRAC. The good correlation will be figured by a higher R^2 value. In the present research, the correlation was observed by using Pearson's method. The higher antioxidant will be shown by higher AAI DPPH and AAI CUPRAC, so the TPC and or TFC will be correlated with AAI DPPH or AAI CUPRAC, if higher TPC or TFC gave positive and significant correlation with their AAI DPPH or AAI

CUPRAC. In Table-3, it can be seen there were significant and positive correlation between TPC in flesh extract and AAI DPPH ($r = 0.812$, $p < 0.01$); TFC and TPC in seed extract with AAI DPPH ($r = 0.979$, $p < 0.01$; $r = 0.706$, $p < 0.05$, respectively); TFC in peel, flesh and seed extracts with AAI CUPRAC ($r = 0.934$, $p < 0.01$; $r = 0.638$, $p < 0.05$; $r = 0.926$, $p < 0.01$, respectively). Based on Table-3, it can be concluded that phenolic compounds in flesh extract were the main contributor in antioxidative activity using DPPH assay, flavonoid compounds were the main contributor in the antioxidative capacity of peel, flesh and seed extracts by CUPRAC assay. Meanwhile, phenolic and flavonoid compounds contributed together in the antioxidant of seed extract by DPPH assay.

Table- 3: Relationship of TPC and TFC with its antioxidant activities index

Antioxidant parameter	Pearson's correlation coefficient (r)	
	TFC	TPC
AAI DPPH PEE	0.532 ^{ns}	-0.691 [*]
AAI DPPH FLE	-0.819 ^{**}	0.812 ^{**}
AAI DPPH SEE	0.979 ^{**}	0.706 [*]
AAI CUPRAC PEE	0.934 ^{**}	-0.827 ^{**}
AAI CUPRAC FLE	0.638 [*]	0.498 ^{ns}
AAI CUPRAC SEE	0.926 ^{**}	0.577 ^{ns}

Table 4: Correlation Pearson of DPPH and CUPRAC Methods

Antioxidant Parameter	Pearson's Correlation Coefficient (r)		
	AAI CUPRAC PEE	AAI CUPRAC FLE	AAI CUPRAC SEE
AAI DPPH PEE	0.251 ^{ns}		
AAI DPPH FLE		-0.102 ^{ns}	
AAI DPPH SEE			0.971 ^{**}

ns= not significant, ** = significant at $p < 0.01$, PEE = peel, FLE = flesh, SEE = seed

The correlation between two antioxidant testing methods has also been performed (Table-4). The different assays will give a different result. Two methods with different mechanisms might give no linear result. DPPH is a combination of hydrogen transfer and electron transfer, meanwhile CUPRAC electron transfer only. Two methods will give linear results when they show positive and linear correlation. The result demonstrated that AAI DPPH of seed extract gave positive and significant correlation with their AAI CUPRAC ($r = 0.971$, $p < 0.01$). Therefore, it can be reviewed that DPPH and CUPRAC showed linear results in the antioxidative activity of arumanis mango seed extract.

A sample can be classified as a poor antioxidant when AAI smaller than 0.5, moderate antioxidant between 0.5-1, potent antioxidant between 1-2, and very potent antioxidant AAI greater than 2.²⁹ In CUPRAC assay, a sample will act as an antioxidant when it has reduction potential smaller than the reduction potential of $\text{Cu}^{2+}/\text{Cu}^+$ (1.59V). TFC in n-hexane flesh extract of arumanis mango (FLE1) 6.71 ± 0.14 g QE/100 g was similar to TFC in ethanol seed extract (SEE3) 6.22 ± 0.03 g QE/100 g (table 2), but AAI CUPRAC of SEE3 (3.24) was higher than FLE1 (0.91) (Fig.-2). So, the SEE3 can be categorized as a greatly potent antioxidant, while FLE1 is a moderate antioxidant. It can be suggested that most of the flavonoids in SEE3 had reduction potentials smaller than 1.59 V, while only a little bit of flavonoid in FLE1 lower than 1.59 V.

TPC in ethyl acetate flesh extract (FLE2) 11.53 ± 0.11 g GAE/100 g was in line with TPC in ethanol flesh extract (FLE3) 11.79 ± 0.11 g GAE/100 g (table 2), but AAI DPPH of FLE2 (2.05) was lower than FLE3 (4.56) (Fig.-1). It can be supposed that most phenolic compounds in FLE3 had higher antioxidative activities, which had many hydroxyl substituents in their structure.

Flavonoid compounds will give high antioxidant power through transfer hydrogen mechanism when it has ortho di-OH in C3'-C4', C2-C3 double bond, OH in C3 and oxo in C4. Ortho di-OH in C3'-C4' had the highest effect on antioxidant activity.³⁵ TFC in n-hexane peel extract (PEE1) 8.10 ± 1.05 g QE/100 g higher than TFC in ethyl acetate peel extract (PEE2) 2.29 ± 0.07 g QE/100 g, but AAI DPPH of PEE1

(1.07) was similar with PEE2 (1.22). Based on the result it may be predicted that most of the flavonoids in PEE2 had great antioxidative activities, while PEE1 contained flavonoid compounds with low antioxidative activities.

CONCLUSION

All sample extracts of arumanis mango (*M. indica* L. 'Arumanis') showed a variety of antioxidative activity. In general, the ethyl acetate and ethanol extracts of waste products of arumanis mango fruit (peel and seed) can be categorized as a greatly potent antioxidant by DPPH and CUPRAC assays. The higher antioxidative activity did not always obtain from the higher phenolic and or flavonoid content. Flavonoid compounds in peel, flesh and seed extracts were the primary contributor in their antioxidative activities by CUPRAC method. Two antioxidant testing DPPH and CUPRAC assays gave linear results in antioxidative activities of arumanis mango seed extract. The waste products (peel and seed) of arumanis mango fruit have added value and potential to be developed as natural antioxidant sources.

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