IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 10 (October 2015), PP. 20-25

Total Phenol and Antioxidant from Seed and Peel of Ripe and Unripe of Indonesian Sugar Apple (*Annona squamosa* L.)Extracted with Various Solvents

Deva Krisna Kadarani[1], Setyadjit[2*], Djarot Sasongko Hami Seno[3], Ermi Sukasih[4]

[1,3](Department of Biochemistry, Bogor Agriculture University, Indonesia)[2,4](Indonesian Center for Agriculture Postharvest Research and Development)[*](Coresponding author)

Abstract : *Study on total phenol and antioxidantactivity ofsugar apple fruits of various solvent, part of fruits, and level of ripening. Solvent extraction used were 80% (v/v) methanol, 50% (v/v) acetone, boiling water, and 50% (v/v) ethanol. Part of fruits thatbeen used for samples were seed and peel which are normally by products of sugar apple processing, level of ripening were unripe, and ripe sugar apple fruits. Total phenol was determined by Folin-ciocalteau method. Total antioxidant was quantified by 1,1-diphenyl-2-picrylhydrazyl(DPPH) method.Therewas a difference in type of solvent, part of fruits, and level of ripeningon total phenol and antioxidant concentration of sugar apple fruits. Seeds have higher total phenol concentration than peels of this fruits. Unripe sugar apple fruits have higher total phenol and antioxidant than ripe fruit. The best solvent for phenol extraction was ethanol 50%butthe best solvent for antioxidant extraction was acetone 50%.*

Keywords –*antioxidant, sugar apple, ripening, solvent, total phenol*

I. INTRODUCTION

Sugar apple (*Annona squamosa* L.) was one of many fruits in Indonesia. The pulp of ripe sugar apple can be processed into juice or other processed products. By products of sugar apple processing are seeds and peels still have many bioactive compounds [1]. The by products utilization are used to zero waste approaches that is environment friendly. Utilization of by product study of oil seeds content from sugar apple fruits showed that it can produce biodiesel by transesterification method [2]. Sugar apple fruits also have some other uses such as cytotoxic, antitumour, antiparasites, pesticide, and immunosuppresive activity [1]. Ethanol and methanol of sugar apple leaves extract have insecticidal activity against *Aedes albopictus* and *Culex quinquefasciatus* [3]. This fruit has a few amino acids such as arginine, glutamine, serine, isoleucine, leucine, methinonine, phenilalanine, tyrosine, and triptophan [5]. Bark of sugar apple has phytochemical compounds such as alkaloid, tannin, protein, saponin, phenolic compound etc. These compounds have antioxidant activity that scavenging free radical and important for pharmacology [6]. In the fabrication of sugar apple extract it is required information such as the best solvent, degree of ripening and the parts have to be used. Solvent normally used for bioactive compounds extraction are water, ethanol, methanol, chloroform, ether, and acetone. Best ratio between powder samples and extraction solvent was 1:10 [7]. The previous study showed solvent for phenolic compounds extraction on pulp of sugar apple fruits from highest into the lowest were acetone, methanol, water, and ethanol, respectively [8].This study purposed to know influences of various solvent, part of fruits, and level of ripeningon total phenol and antioxidant concentration of sugar apple (*Annona squamosa* L.) fruits.

II. MATERIALS AND METHODS

2.1 Plant materials

Fresh fruits of sugar apple (*Annona squamosa* L.) werecollected from Cimanggu, Bogor, West Java Province, Indonesia. Fresh fruits were separated *i.e.*between seeds and peels to be dried with tray dryer at temperature of 55^{o}C constantly for 72 h. Seeds and peels of fruits were used for proximate analysis. Dried seeds and peels werechopped with blender to produce powder samples. The powder samples was packed and stored at temperature of 20^{o}C in the dark storage room beforechemical analyses were done.

2.2 Extraction

Extraction was done with maceration for 24 h at room temperature with various solvents. Comparison between the powder samples and extraction solvent was 1:10 (w/v). The solvent used was 80% methanol (v/v), 50% acetone (v/v), boiling water, and 50% ethanol (v/v). After maceration was done, filtrate was filtered and centrifuged at speeds of 3000 rpm for 15 mins. Supernatant was taken and residues was not used. Supernatant was used for chemical analyses.

2.3Proximate Analysis

Proximate analysis was done by using method of AOAC, 2005.

2.4 Phytochemical Analysis

Alkaloid Test. Samples extract were diluted on chloride acid for filtration and get filtrate to test. The filtrate was added with reagen Wagner (1.27 g iodium powder and 2 g potassium iodide were mixed on 100 mL aquadest). Positive alkaloid showed by brown reddish precipitates on the samples test.

Quinone Test. Samples extract as much as 1 mL were added with 1 mL ofsulfate acid (H_2SO_4) concentrated. Positive quinone showed by red colour on the samples test.

Terpenoid Test. Chloroform as much as 2 mL were added with 1 mL of samples extract and followed by 3 mL sulfate acid (H_2SO_4) concentrated. Positive terpenoid showed by red brownish on samples test.

Saponin Test. Samples extract as much as 1 mL were mixed with 5 mL of aquadest. Mixtures were shaken vertically. Positive saponin showed by stable foam on samples test for 5 minutes constantly.

2.3 Total Phenol Analysis

Analysis of total phenol started by adding 0.1 g of gallic acid into 100 mL of aquadest and it was homogenized. And then, it was diluted to concentrations of 25, 50, 100, 150, and 200 ppm. Sodium bicarbonate (Na_2CO_3) 7% was made with dissolved 7 g of Na_2CO_3 powder in 100 mL of aquadest. Samples extract or standard or blanko (aquadest) as much as 0.5 mL were poured into test tube then followed by addition of 0.5 mL Folin Ciocalteau reagent. After 5 mins, 5 mL of Na_2CO_3 7% was added into the mixture and it was incubated for 30 minutes in the dark room. Then, the absorbance was measured using a spectrophotometer UV-Vis at 750 nm. Total phenol content of samples were expressed as mg gallic acid equivalents (GAE)/g fresh sample.

2.3 Antioxidant Analysis

Analysis of antioxidant activity was started byweighing 0.1 g of ascorbic acid powder as standard in 100 mL of aquadest. It was homogenized then, diluted to concentrations of 50, 100, 150, and 200 ppm. Samples extract or standard or blanko solution as much as 0.1 mL was poured into test tube. Then, it was followed by addition of 5 mL of 0.002% (w/v) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals solution. The mixture was incubated for 30 minutes at the dark room at room temperature. Finally, the absorbance was measured using spectrophotometer Uv-Vis at 517 nm.

2.3 Statistics Analysis

The statistical usedwas Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test which 3 replicates. Significant probability value used was $P<0.05$.

III. RESULTS AND DISCUSSION

3.1 Proximate

Proximate analysis indicates that sugar apple (*Annona squamosa* L.) fruit has high moistures, minerals, amino acids, and antioxidants. High moisture content of fruits cause possibility of microbial or fungal growth on fruit during storage. Ash content determines total mineral on fruits [11]. Level of ripening of fruits affected production of secondary metabolites. Ripe sugar apple fruit have higher amino acids than unripe [Table 1]. The sugar apple fruits have various amino acids such as arginine, glutamine, serine, isoleucine, leucine, methionine, phenilalanine, tyrosine, and triptophan [5]. Leucine, valine, and phenilalanine are the amino acids that are the precursor of secondary metabolites such as flavonoid, alkaloid, etc [9].The concentration of vitamin C (ascorbic acid) showed its ability to scavenge reactive oxygen species and free radicals to prevent tissues damaged [10]. This study showed that the highest concentration of vitamin C was at peels of unripe sugar apple fruits [Table 1].

3.2 Phytocemical

3.2.1 Alkaloid

Alkaloid was the largest secondary metabolites on the high level plant. Alkaloid constitued of few ammonia compounds. Nitrogenous compounds on alkaloid have their roles as pharmaceutical, stimulant, narcotic, and poison [17].Biosinthesis alkaloid from amino acid involved decarboxylase enzymes [4].Some alkaloid were alcohol soluble and the other were water soluble [17]. Previous study reported that type of alkaloid on sugar apple seeds were annonaine and samoquasine A [6].

3.2.2 Quinone

Quinone was secondary metabolites that have colored compounds with two ketone substitutions in aromatic ring. Quinone was reactive phytochemical [18].

3.2.3 Terpenoid

Type of terpenoid on *Annona squamosa* were a kaurene diterpenoid 16ß and reported has anti virus HIV activity. Terpenoid also have other biological activity as anti platelet [6].

3.2.4 Saponin

Saponins were phytochemical who soluble in polar solvent likely alcohol and water. Saponins were therapeutic agents with hypolipidemic and anticancer activity [17].

3.3 Total Phenol

Phytochemicals on sugar apple fruits were alkaloid, protein, amino acids, carbohydrates, phytosterol, tannins, and phenolic compounds [12]. This study has measured of phenolic compounds that expressed as gallic acid equivalent (GAE) by Folin-Ciocalteau method. Gallic acid standard regression was y=0.0047x + 0.0023 with R^2=0.9984. Phenolic compounds are both groups that have both aromatic and non aromatic rings. That groups were hydroxyl (-OH), carboxyl (-COOH), and methoxyl (-OCH_3) [13]. The various solvents used on this study were 80% (v/v) methanol, 50% (v/v) acetone, boiling water, and 50% (v/v) ethanol. Each solvent haswater content with various percentages. Because addition of water in the organic solvent will form a polar medium for best extracting polyphenol compounds. Based on previous study, 60% (v/v) ethanol was optimum solvent to extract polyphenols [14]. This study showed that the highest total phenol concentration was ethanol extract from seeds of unripe sugar apple (*Annona squamosa* L.) [Figure 1]. The solvent that have higher polarity can extract the total phenol better [8]. This study also showed that level of ripeningaffectsconcentration total phenol of samples. Unripe sugar apple fruit has higher total phenol concentration than ripe fruits [Figure 1].

3.4 Total of Antioxidant

Sugar apple (*Annona squamosa* L.) fruits have antioxidant activity because it has phenolic acid, phenol, and its derivatives compounds [12]. Phenolic compound is primary antioxidant and act as free radical terminator [15]. Total antioxidant was expressed as ascorbic acid equivalent (AAE) and quantified by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Ascorbic acid standard regression was y = 0.0016x – 0.0017 with R^2 = 0.9658. DPPH was stable free radical that can delocalization free electron into molecules until the molecules cannot do dimerization with another radical. That reaction formed purple colour. DPPH solution mixed with substances that donates hydrogen atom to reduction and decolourization purple colour becomes yellow due to pycril groups. Radical reacts on stoichiometry and amount of reduction of DPPH molecules was measured as antioxidant activity [16]. Based on this study, the highest total antioxidant was acetone extract from peels of unripe sugar apple (*Annona squamosa* L.). Peels of sugar apple fruits have higher total antioxidant than its seeds. Unripe of sugar apple fruits has higher total antioxidant than ripe[Table 2].

Inhibition of free radicals can be seen on rate on decolorization of purple color to yellow that happens on potent antioxidant substance [16]. Percentage of total inhibition of free radicals based on capability of solvent which arranged from the highest until lowest are methanol, acetone, ethanol, and water, respectively. The highest percentage of inhibition of free radicals based on part of fruits was peels then seed. The highest percentage of inhibition of free radicals based on the level of ripening was unripe fruits, then the ripe one [Figure 2]. This study showed that various solvents, parts, and ripening stage have influenced on percentage of inhibition of free radicals.

IV. FIGURES AND TABLES

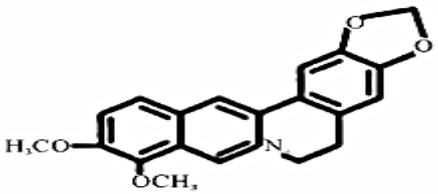

Figure 1 Structures of alkaloides OH [18]

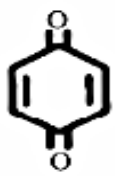

Figure 2 Structures of quinone [18]

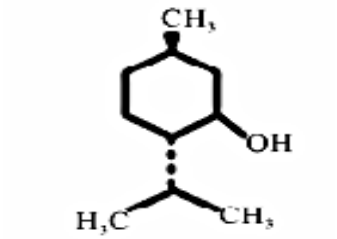

Figure 3 Structures of terpenoides [18]

Table 1 Result of Proximate Analysis (Percentage± Standard deviation)

Samples		Moisture Content (%)	Ash Content (%)	Protein Content (%)	Vitamin C Concentration (mg/100 g samples)
Unripe	Seed	5.98 ± 0.52	0.51 ± 0.40	6.36 ± 0.88	44.00 ± 19.09
	Peel	5.84 ± 0.32	0.70 ± 0.31	4.19 ± 0.05	113.68 ± 9.64
Ripe	Seed	6.82 ± 1.11	0.58 ± 0.36	6.85 ± 0.14	11.65 ± 0.09
	Peel	8.57 ± 0.44	1.35 ± 0.51	5.51 ± 0.02	61.93 ± 6.37

n=2

Table 2 Result of Phytochemical Analysis

Samples		Extract	Alkaloid	Quinone	Terpenoid	Saponin
Unripe	Seed	Methanol 80%	+	+	-	-
		Acetone 50%	-	+	-	-
		Boiling water	-	-	-	+
		Ethanol 50%	-	-	-	-
	Peel	Methanol 80%	-	+	+	+
		Acetone 50%	+	+	+	+
		Boiling water	+	+	+	+
		Ethanol 50%	+	+	+	+
Ripe	Seed	Methanol 80%	-	+	-	-
		Acetone 50%	-	+	-	-
		Boiling water	-	-	-	+
		Ethanol 50%	-	-	-	-
	Peel	Methanol 80%	+	+	+	+
		Acetone 50%	+	+	+	+
		Boiling water	-	+	+	+
		Ethanol 50%	+	+	+	+

n=3

Table 3 Result of AntioxidantActivity Analysis

Samples	Extract	Ascorbic acid equivalent (µg/mL)	
		Unripe	Ripe
Seeds	Methanol 80%	417.69^{b}	254.05^{a}
	Acetone 50%	571.60^{c}	268.12^{a}
	Boiling water	258.53^{a}	194.99^{a}
	Ethanol 50%	318.01^{ab}	210.60^{a}
Peels	Methanol 80%	169.74^{a}	269.05^{a}

Acetone 50%	1365.46^{c}	241.92^{a}
Boiling water	272.48^{a}	231.27^{a}
Ethanol 50%	518.22^{b}	260.67^{a}

n=3, Superscrift characters (a,b,c,d) showed the result significantly different ($p<0.05$)

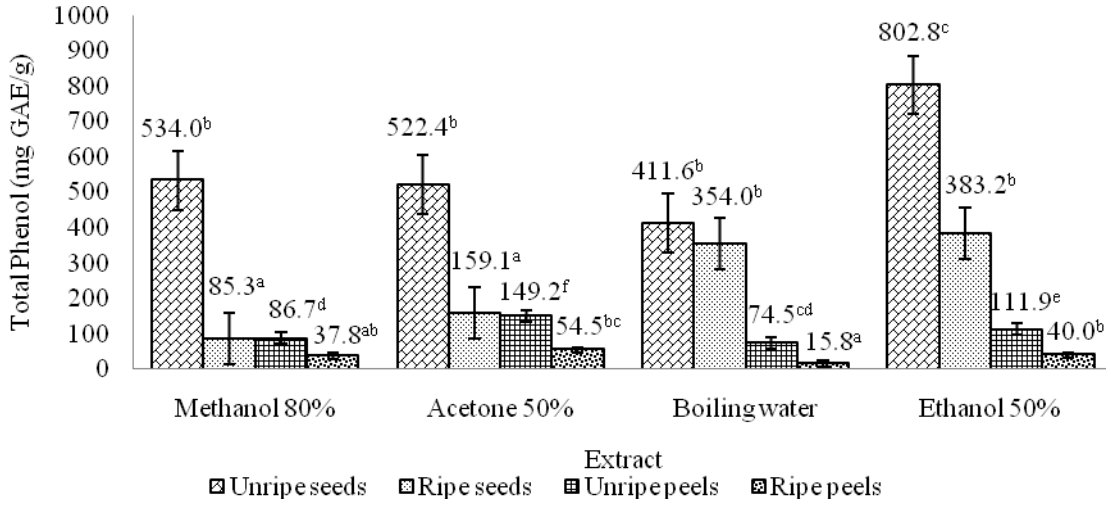

Figure 4 Comparison of total phenol concentrations of samples. Number at aboves bar showed total phenol concentrations and superscript characters (a,b,c,d,e,f) showed the resultsignificantly different ($p<0.05$)

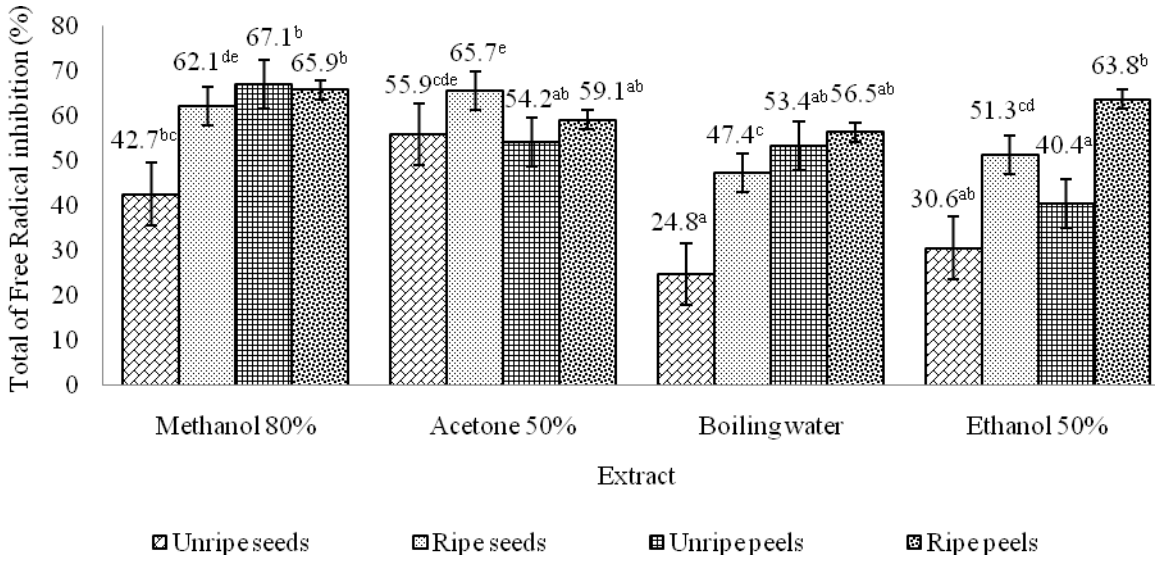

Figure 5 Comparison of total of free radical inhibition samples. Number at aboves bar showed total free radical inhibition and superscript characters (a,b,c,d,e) showed the result significantly different ($p<0.05$)

V. CONCLUSIONS

Based on this study, the difference in solvent, part of fruits, and level of maturity influenced total phenol and antioxidant activity of sugar apple fruits. Seeds have higher total phenol concentration than peels of this fruits. Unripe sugar apple fruits have higher total phenol and antioxidant than ripe fruits. The best solvent for phenol extraction was ethanol 50%, but the best solvent for antioxidant extraction was acetone 50%.

VI. ACKOWLEDGEMENT

We would like to thank to Indonesian Agency for Agriculture Research and Development (IAARD) for providing KKP3N research grant.

REFERENCES

[1] Alali FQ, Liu X, McLaughin JL. Annonaceous acetogenins: recent progress, *J Nat Prod*. 62, 1999, 504-540.

[2] Yathish KV, Omkaresh BR, Suresh R. Biodiesel roduction from custard apple seed *Annona squamosa* oil and its characteristics study, *I J Res Eng Tech.* 2(5), 2013, 31-36.

[3] Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts, *J Vect Borne Dis*. 44, 2007, 145-148.

[4] Richard BH. The biosynthesis of plant alkaloids and nitrogenous microbial metabolites, *Nat Prod Rep*. 20, 2003, 494-508.

[5] Priscila *et al*. Free amino acid composition of *Annona* (Annonaceae) fruit species of economic interest, *Ind Crops Prod*. 45, 2013, 373-376.

[6] Pandey N, Barve D. Antioxidant activity of ethanolic extract of *Annona squamosa* Linn. bark, *I J Res Phar Biomed Sci.* 2(4), 2011, 1691-1697.

[7] Pandey A, Tripathi S. Concept of standarization, extraction, and pre phytochemical screening strategies for herbal drug, *J Pharmaceut Phytochem.* 2(5), 2014, 115-119.

[8] Balkrishna UJ and Anant VB. 2012. Antioxidant activities of various solvent extracts of custard apple (*Annona squamosa* L.) fruit pulp,*Nutrafoods.* 11: 137-144.

[9] Wyllie SG and Fellman JK. Formation of volatile branched chain esters in banana(*Musa sapientum* L.),*J Agr Food Chem.* 48, 2000, 3493-3496.

[10] Himesh S, Singhai AK, Sarvesh S. Quantification of ascorbic acid in leaves of *Annona squamosa, I J Phar Pharmaceut Sci*. 4(3), 2012, 144-147.

[11] Shardul *et al*. Proximate analysis of peel and seed of *Annona squamosa* (Custard apple) fruit, *Res J Chem Sci*. 3(2), 2013, 92-94.

[12] Kumar *et al*. Insecticidal activity of ethanolic extract of leaves of *Annona squamosa* L,*J Chem Phar Res.* 2(5), 2010, 177-180.

[13] Srivastava *et al*. Assessment of phenol and flavonoid content in the plant materials,*J New Bio Reports*. 2(2), 2013, 163-166.

[14] Shashikala *et al*. Studies on extraction of polyphenols from seeds of *Piper ningrum*, *Annona squamosa*, and *Malilkara zappota*,*JEST-M*. 3(2), 2014, 26-30.

[15] Silva *et al*. Chemical composition and antioxidant activity of jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.) flour, *Food Sci Tech Calminas.* 34(3), 2014, 597-603.

[16] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity,*Songklanakarin J Sci Tech.* 26(2),2004, 211-219.

[17] James HD. Phytochemical: extraction methods, basic structures and mode of action as potential chemotherapeutic agents, *Phytochemical-A Global Perspective of Their Role in Nutrition and Health INTECH*. 2012. 1-32.

[18] Singh MG, Shahid A, Masood A, Kangabam SS. Efficacy of plant extracts in plant disease management, *Agr Sci*.3, 2003,425-433.