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Determination of Antioxidant Activity and Phenolic Compounds of Methanolic Extract of Java Plum (*Syzygium cumini* Linn.) Seed

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Abstract

Methanolic extract of Java plum (*Syzygium cumini* L.) seed (MEJS) is a potential source of natural antioxidant. As indicated by several *in vitro* measurements, the extract had strong DPPH (1,1 diphenyl, 2–picryl hydrazyl) and ABTS (2,2-azinobis, 3-ethylbenzothiazoline-6-sulphonate) radical scavenging activity, strong Ferric Reducing Antioxidant Power (FRAP) and moderate inhibition activity of linoleic acid oxidation. This study aimed to determine antioxidant activity and phenolic compound of Java Plum seed (*Syzygium cumini* L.) methanolic extract fractions. Phenolics compound identification using Thin Layer Chromatography (TLC) showed that all fractions (polar, semi-polar, and hydrolyzed semi-polar fraction) contained gallic acid, tannic acid and flavonol's rutin. HPLC-DAD analysis showed that its polar fraction contained 25 ppm flavonol's quercetine and 55181 ppm flavonol's (+)-catechin, ethyl acetate fraction contained 54 ppm flavonol's rutin and 528 ppm (+)-catechine, while hydrolyzed ethyl acetate fraction contained 404 ppm Rutin and 28692 ppm (+)-catechine.

Keywords: antioxidant (+)-catechine, Java Plum seed, methanolic extract

Introduction

Duwet (*Syzygium cumini* L.) is local name for Java plum, widely grown in many countries whose seed has been used for traditional medication, particularly to treat type-2 diabetes mellitus (Helmstadter, 2008; Kumar et al., 2010; Swami et al*.,* 2012; Tupe et al., 2015). In several places in Indonesia, the seeds of subvar *Genthong* are roasted, ground, brewed and served as "Duwet coffee" for diabetes traditional treatment. Various in vitro study showed that Java plum seed extract exhibit strong antioxidant capacity as 1,1 diphenyl, 2–picryl hydrazyl (RSA-DPPH) free radical scavenger and ferric radical reducing agent (Vasi and Austin, 2009; Rydlewsky et al*.,* 2013; Saha et al*.,* 2013; Rohadi et al*.,* 2016), strong scavenger against *2,*2-azinobis, 3-ethylbenzothiazoline-6-sulphonate cation and nitro-oxide ($^{\bullet}$NO) radicals, as well as strong inhibition activity on lipid oxidation and α-glucosidase (Vasi and Austin, 2009). Java plum seed is the potential natural antioxidant source (Vasi and Austin, 2009;

Saha et al., 2013; Rydlewsky et al., 2013; Rohadi et al., 2016). Phenolic compounds are believed to be responsible for its strong antioxidant properties.

Java plum seeds shape is oblong, ovoid, with average weight of 1.67±0.31 g/seed, 2.18±0.15 cm long, about 1.03±0.05 cm in diameter and 0.5±0.05 cm wide. Covered in red to pink fruit flesh, the seed is glossy white–pink at respective color scale (*L, *a, *b) of 63.92, 2.67, 9.88. It contains 6.63% (db) protein, lower than the flesh of 10.7% (db). It also contains 0.66% lipid, 3.28% ash, 75.4% total carbohydrate, 1.32% insoluble dietary fiber, and several sugars such as 3.25% (db) fructose, 2.60% (db) glucose, 0.19% (db) sucrose, 0.09% galactose, and small amount of raffinose (<128 ppm). Its mineral content is predominantly potassium (K) of 8.812 ppm, 2.161 ppm magnesium, 136.8 ppm iron (Fe), 115 ppm sodium (Na), 86 ppm calcium (Ca), 35 ppm phosphor (P), and 5 ppm copper (Cu) (Rohadi et al., 2016).

Previous researches showed that the seed contains glycoside, hoursbolin (anti-mellin), ellagic acid, alkaloid (Quisumbing, 1951; Parmar et al., 2010), gallic acid, galloylglucose, 3-galloylglucose, 3,6-hexa-hydroxy diphenoylglucose, 4,6 hexa-hydroxydiphenoylglucose and β-sitosterol (Helmstadter, 2008; Swami et al., 2012; Rydlewsky et al., 2013). A research reported kaempferol and epicatechin claimed as first publication reported epicatechin content of Java plum seed (Rydlewsky et al., 2013). The seed for several years has attracting food researches to further study its functionality possibility (Rohadi et al., 2016).

Materials and Methods

Chemicals

This research used methanol, ethyl acetate, Folin Ciocalteu reagent, gallic acid, tannic acid (Sigma Chemical Co. St. Louis USA) (+)-catechine, rutin, quercetin (Waco Pure Chemical Industry-Osaka Japan), butylated hydroxyanisole/BHA (Sigma Chemical Co.), aqueous Na_2CO_3, Tween-40, HCl, buffer phosphate pH 7, ferrous chloride ($FeCl_2$), ferric chloride ($FeCl_3$), ammonium thiocyanate, $K_3Fe(CN)_6$, trichloroacetic acid (TCA), tungsto-phosphoric acid, 1,1-diphenyl-2-picryl hydroxyl radical (DPPH) and linoleic fatty acid, all in analytical grade. It also used rotary vacuum evaporator (IKA-RV 10 basic), vortex, incubator, water-bath shaker (Julabo SW 22), a UV-Visible spectrophotometer (UV-1601 Shimadzu) and HPLC-DAD Hitachi Auto sampler L-2200 (Berkshire, UK).

Plant Authentication and Extracts Preparation

A dry powder of Java plum seed (*Syzygium cumini* Linn.) subvar "Genthong" (≤14% moisture content, 80 mesh) was obtained from optimum ripe fresh fruit harvested from several trees belong to residents of Semarang city, Jawa Tengah, Indonesia. The sample was authenticated and taxonomically identified by Laboratory of Plant Taxonomy, Faculty of Biology, Universitas Gadjah Mada. Approximately 50 g dried powder of Java plum was extracted using 50% methanol at material:solvent ratio of 10:1 (v/v), macerated in 100 rpm water-bath shaker (Julabo SW 22) at room temperature (28±2^{o}C) and filtrated using Whatman paper. This process was repeated 3 times. Resulted extract was separated from the solvent using a rotary vacuum evaporator, to obtain a methanol free extract, which then subjected to partition fractionation using ethyl acetate solvent. A half volume of ethyl acetate fraction was subsequently hydrolyzed using 7% H_2SO_4 (10:1, v/v) by reflux condenser for 4 h. Ethyl acetate faction was separated and taken using separating funnel to obtain 3 fractions *i.e*. water fraction, ethyl acetate fraction, and hydrolyzed-ethyl acetate fraction.

Determination of Total Phenolic Compounds and Flavonoid Content

Total phenolic content of MEJS was measured using Folin-Ciocalteu reagent (FCR) colorimetric method using spectro-photometer at λ 765 nm according to the procedure applied by Ebrahimzadeh et al., (2008) with gallic acid as standard (g GAE/100 g extract). Total flavonoid content was measured using spectrophotometer at λ 415 nm using protocols based on previous researches (Ebrahimzadeh et al., 2008; Vasi and Austin, 2009) with Quercetine as standard (g QE/100 g extract). Total tannin was also measured using spectrophotometer at λ 725 nm based on the method applied by Palici et al., (2005) with tannic acid as standard (g TAE/100 g extract).

DPPH Radical Scavenging Activity Assay

Measurement of radical scavenging activity (RSA)-DPPH of MEJS according to procedure applied by Vasi and Austin (2009). Approximately 0.5 mL MEJS at various concentrations (50-400 ppm) in 50% ethanol added with 0.5 mL 2,2-diphenyl-1-picryl hydroxyl radical (DPPH)-100 µM then incubated in the dark at room temperature (37±2^{o}C) for 30 min, in triplicate for all samples. Its absorbance was read at λ 517 nm using UV-Vis spectrometer (UV-1601 Shimadzu). Vitamin C, BHA, and Quercetin was used as a comparison. DPPH radical scavenging activity can be calculated as follows refereed (Vasi and Austin, 2009).

$$\text{RSA-DPPH (\%)} = \left[\frac{\text{1 - sample absorbance}}{\text{control absorbance}}\right] \text{x100\%}$$

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric (Fe^{+3}) reducing antioxidant power (FRAP) was used as an indicator of electron transfer activity of phenolic compounds (Vasi and Austin, 2009). As much as 2.5 mL MEJS various concentrations (50-400 ppm) in ethanol was added with 2.5 mL buffer phosphate (0.2 M, pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$, incubated at 50^{o}C for 20 min. Subsequently, 2.5 mL of 10% trichloroacetic acid (TCA) was added to stop the reaction. After centrifugation using a vortex at 3.000 rpm for 10 minutes, 2.5 mL supernatant was taken and added with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). Reducing activity of the sample was measured at λ 700 nm, with vitamin C as a positive control. Increasing absorbance is an indicator of higher reducing power. The reduction percentage of the sample compared to control was calculated using the formula below:

$$\text{\%reducing activity} = \left[\frac{\text{1 - sample absorbance}}{\text{control absorbance}}\right] \text{x100\%}$$

Lipid Peroxidation in Linoleic Acid Emulsion Model System

To measure peroxidation inhibition activity on linoleic acid, the analysis was carried out according to the method by Jayaprakasha et al., (2001). Initially, the emulsion was made by adding 0.28 g Tween-40 and 50 mL buffer phosphate (0.2 M pH 7.0) into 0.28 g linoleic acid, mixed and homogenized. As much as 0.5 mL MEJS solution (Met-OH 60%) in various concentrations (50-400 ppm) were mixed with 2.5 mL linoleic acid emulsion and 2.5 mL buffer phosphate (0.2 M pH 7.0) then incubated at 37^{o}C for 120 h (5 days). Control was prepared as above without MEJS. From incubated solution, 0.1 mL was taken in 24 h interval, mixed with 5 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 20 mM ferrous chloride ($FeCl_2$) in 3.5% HCl, then incubated in room temperature for 3 min. After color changed to brick red, its absorbance was measured at λ 500 nm. BHA and Quercetine were used as a comparison.

Antioxidant activity was calculated using Jayaprakasha et al., (2001) formula as follows:

$$\text{Inhibition (\%)} = 100 - \left[\frac{\text{sample absorbance}}{\text{control absorbance}}\right] \text{x} 100\%$$

TLC Analysis of Tannic Acid

As much as 100 mg was taken from each extract, added with 1 mL of 50% ethanol, homogenized for 2 minutes using vortex and then centrifuged for 3 minutes. The liquid phase was taken using a micropipette, 10 µL was spotted on silica gel 60 F254 plate. The plate was put into a saturated chamber with 150 mL ethyl acetate–acetic acid–formic acid–water (100-11-11-27, v/v/v/v) as the mobile phase. Elusion was done to upper-end limit and the plate was dried under UV ray. Staining was done using ferric chloride.

TLC Analysis of Flavonoid Rutin

Into the flask, 50 mg of extract and 10 mL of 4 N hydrochloric acid were put for reflux hydrolysis using a condenser for 30 minutes. The mixture was cooled before extraction using 5 mL diethyl ether. Diethyl ether phase was taken prior to solvent removal using nitrogen spray. Sample spotting of 10 µL was done on cellulose plate. Rutin was also spotted as a comparison. The plate was put into saturated chamber using n-butanol:acetic acid:water (3:1:1, v/v/v) as the mobile phase. Elusion was done to upper-end limit and the plate was dried and observed under UV ray. Staining was done using aluminum chloride.

TLC Analysis of Gallic Acid

From each extract, 100 mg was taken and added with 1 mL methanol, homogenized using vortex for 2 min then centrifuged. The liquid phase was spotted for 20 µL on silica gel 60 F254 plates before putting into a saturated chamber with methanol–10% formic acid (97:3, v/v) as the mobile phase. Elusion was done until the upper-end limit of the plate before drying and observation under UV ray. Staining was done using ferric chloride.

Determination of Gallic Acid, Catechine, and Rutin Content

RP-HPLC Hitachi L-2200 (Berkshire, UK) equipped with LC-Diode Array Detector (DAD), auto sampler and column oven was used in this stage. Separation system consists of 5 µm Hypersil ODS RP 18 Symmetry column (150 x 4.6 mm). Acetonitrile–50 mM phosphate buffer set at pH 2.5 was set as elusion gradient system. Elusion gradient was made from variation between solvent A (acetonitrile): solvent B (50 mM phosphate buffer) at 1 mL/min rate. Mobile phase composition was initiated by 100% solvent B (2 min), followed by gradual increasing of solvent A to 60% and decreasing solvent B to 40% for 32 min. Subsequently, the sample was re-equilibrated for 3 min similar to initial part (100% solvent B). Thus, 35 min was needed per injection. This running was also equipped with mobile phase 0.45 µm filtration system and compound detector at λ 210 nm (Santagati et al*.,* 2008)**.**

Determination of Quercetine Content

RP-HPLC Hitachi L-2200 (Berkshire, UK) equipped with Phenomenex Luna C_{18} column (ø 4.6 mm×250 mm) and 5 µm particle size was used. The mobile phase consisted of methanol:acetonitrile:water mixture (40:15: 45, v/v/v) contained 1% acetic acid, previously filtered using 0.45 µm filter paper (Millipore). The flow rate and volume of injection were set at 1 mL/min and 10 µL, respectively, using ultraviolet detection at λ 368 nm. Quantification of the target compound was conducted based on UV spectrum peak area ratio of the standard compound with the sample at similar tR (retention time) **(**Phani et al*.,* 2010).

Determination of (+)-Catechine Content

RP-HPLC Hitachi L-2200 (Berkshire, UK) equipped with Phenomenex Luna C_{18} column (ø 4.6 mm×250 mm) and 5 µm particle size was used. The mobile phase consisted of methanol:acetonitrile:water mixture (40:15: 45, v/v/v) contained 1% acetic acid, previously filtered using 0.45 µm filter paper (Millipore). The flow rate and volume of injection were set at 1 mL/min and 10 µL, respectively, using ultraviolet detection at λ 279 nm. Quantification of the target compound was conducted based on UV spectrum peak area ratio of the standard compound with the sample at similar tR (Zu et al., 2006).

Statistical analysis

Experimental results are expressed are as mean ±SD. All measurement were conducted in triplicate, analyzed using analysis variance ($p<0.05$). A significant difference was measured using by Duncan's Multiple range tests.

Results and Discussion

Preliminary phytochemical analysis of the methanolic extracts revealed the presence of phenolic compounds, reducing sugars, proteins, insoluble dietary fiber and some macro minerals such as potassium, magnesium, sodium and ferrum. Total phenolic referred to standard curve y= 7.144x-0.034 (r^2=0.986) was 45.99±0.25% (g GAE/100 g dry extract). Total flavonoid contents of 2.28±0.07% (g-Quercetin equivalent/100 g dry extracts) was referred to standard curve y = 1.364x+0.006 (r^2 = 0.996). Total tannins of 26.9±0.07% (g-Tannic acid equivalents/100 g-dry extract) was referred to tannin standard curve y = 7.441x+0.033 (r^2 = 0.991) (Rohadi et al., 2016).

Antioxidant activity

Several concentrations (50–400 µg/mL) of 50% methanolic extract was obtained from *Syzygium cumini* L. seed and tested for their in vitro antioxidant activity. It was observed that the test compounds scavenged free radicals in a concentration-dependent manner in all model (**Table 1**). The highest DPPH inhibition percentage was 93.51% at 400 µg/mL, while the highest FRAP was –4.05% (the negative value was caused by higher sample absorbance than reference absorbance). Inhibitory activity against lipid peroxidation was 60.37% at 96 h during initial incubation.

Oxidative degradation of lipid especially that induced by reactive oxygen species (ROS) lead to quality deterioration of food and could have harmful effects on health (Laguerre et al., 2007; Vasi and Austin, 2009). The natural antioxidant may offer resistance against the lipid autoxidation occurs by a free-radical chain reaction (Vasi and Austin, 2009).

The DPPH (1,1-diphenyl-2-picryl hydrazyl radical) assay have been widely used to determine the free radical-scavenging activity of various plants and pure compounds. DPPH is stable free radicals which dissolve in methanol and its color show characteristic absorption at 517 nm.

When an antioxidant scavenges the free radicals by hydrogen donation, the colors in the DPPH assay mixture to become lighter. As presented in **Table 1**, DPPH inhibition percentage values were dose dependent, whereby they increased in the range on the tested concentration for the and the positive control (BHA). At the same concentrations (400 µg/mL) certainly, the DPPH inhibition of MEJS (93.05%) was stronger than BHA (73.71%).

The reducing power of a MEJS may serve as a significant indicator of its potential antioxidant activity. It can be seen that the reducing power percentage values of MEJS and the positive control (Vitamin C) were concentration-related and increase with the increase in sample concentration in the range 50-200 µg/mL. Meanwhile, the

Table 1. Antioxidant properties of MEJS as measured by lipid peroxidation inhibition activity, DPPH scavenging, and FRAP

Conc. (µg/mL)	Scavenging of free radicals (%)		
	PoV (96 h)	DPPH	FRAP
50	49.63	83.513	52.53
100	50.13	91.89	54.88
150	51.09	92.21	52.34
200	51.93	92.87	-1.46
400	60.37	93.045	-4.05

Results are mean of triplicate

absorbance value of Vitamin C in the range 250-1000 µg/mL showed weaker than MEJS absorbance value so that the percentage reduction of the sample as compare to standard was negative. The high tannin content of MEJS allegedly contributes on strong RSA-DPPH and FRAP assay. Zhang and Lin (2009) note that tannins extracted from Java plum fruits showed a very good DPPH radical scavenging activity and ferric reduction antioxidant power.

In $FeCl_2/H_2O_2$ stimulated linoleic acid peroxidation system showed the MEJS in moderate inhibitory activity against peroxidation (60.4%) and significantly lower ($p<0.05$) than BHA, however, stronger than grape seed extract (the picture not showed).

Identification of phenolic compounds using TLC

Aqueous MEJS was fractioned using separating funnel using ethyl acetate as solvent and one of the fractions (ethyl acetate fraction) was hydrolyzed using sulfuric acid (7% H_2SO_4) based on method previously applied by Meena and Patni (2008), whereby 3 fractions were obtained, water, ethyl acetate, and hydrolyzed ethyl acetate fraction of sample. The results of phenolic compounds qualitative measurements by TLC are presented in **Table 2**.

Table 2. Phenolic compounds of MEJS fraction measured by TLC

No	Fraction	Compound	Rf	Results
1	Water	Flavonoid (Rutin)	0.47	(+)
		Phenolic (gallic acid)	0.76	(+)
		Tanin (tannic acid)	0.74	(+)
2	Ethyl acetate	Flavonoid (Rutin)	0.47	(+)
		Phenolic (gallic acid)	0.76	(+)
		Tanin (tannic acid)	0.74	(+)
3	Hydrolyzed-Ethyl acetate	Flavonoid (Rutin)	0.47	(+)
		Phenolic (gallic acid)	0.76	(+)
		Tanin (tannic acid)	0.74	(+)

Gallic acid determination. Silica gel plate 60 F254 (Al-sheet) previously spotted by the sample was put in a chamber filled with 200 mL mobile phase made from methanol:10% formic acid (97:3, v/v). Visualization under UV ray using TLC scanner was conducted at λ 254 nm and 365 nm. Fluorescence was found in all 3 samples similar to reference (gallic acid) at retention factor (R_f) of 0.76, it was suspected that gallic acid was suspected to be present in all fractions.

Flavonoid rutin determination. Silica gel plate 60 F254 (Al-sheet) previously spotted by the sample was put in a chamber filled with 200 mL mobile phase made from butanol:acetic acid:water (3:1:1, v/v/v). Visualization under UV ray using TLC scanner was conducted at λ 254 nm and 365 nm. Fluorescence was found in all 3 samples similar to reference (rutin) at R_f of 0.47. It indicated that water, ethyl acetate, and hydrolyzed ethyl acetate contained compound suspected as rutin.

Tannic acid determination. Silica gel plate 60 F254 (Al-sheet) previously spotted by the sample was put in a chamber filled with 200 mL mobile phase made from ethyl acetate-formic acid-acetic acid-water (100-11-11-27, v/v/v/v). Visualization under UV ray using TLC scanner was conducted at λ 254 nm and 365 nm. Fluorescence was found in all 3 samples similar to reference (tannic acid) at R_f of 0.74 indicated that water, ethyl acetate, and hydrolyzed ethyl acetate contained compound suspected as tannin.

Identification of Phenolic Compounds using HPLC-DAD

To measure the quantity and the type of phenolic compounds of MEJS, the sample was analyzed using HPLC referred to retention time (tR) and the peak of phenolic compounds standard, which was phenolic's gallic acid, flavonol's rutin and quercetin, and flavanol's (+/–) catechin. Analysis of gallic acid, catechin, and rutin was done based on the method applied by Santagati et al., (2008), quercetin by those of Phani et al., (2010), while quantitative analysis of catechin was done according to Zu et al. (2006). The results are presented in **Table 3.**

Identification of flavonoid's rutin. Analysis of phenolic compound using HPLC showed that gallic acid, catechin, and rutin were not found in water fraction. In ethyl acetate fraction, gallic acid and catechin were also not found, but 54.1 ppm (mg/L) rutin was found. Similarly, in hydrolyzed ethyl acetate fraction, there was only rutin found of 404 ppm (mg/L), as seen in **Fig. 1**.

Table 3. Phenolic compound of MEJS fractions

Fraction	Concentration (mg/L)			
	Gallic acid	Rutin	Quercetin	(+)-Catechin
Water	n.d	n.d	25	55181
Ethyl acetate	n.d	54.1	n.d	258
Hydrolyzed Ethyl acetate	n.d	404	n.d	28692

n.d = not detected

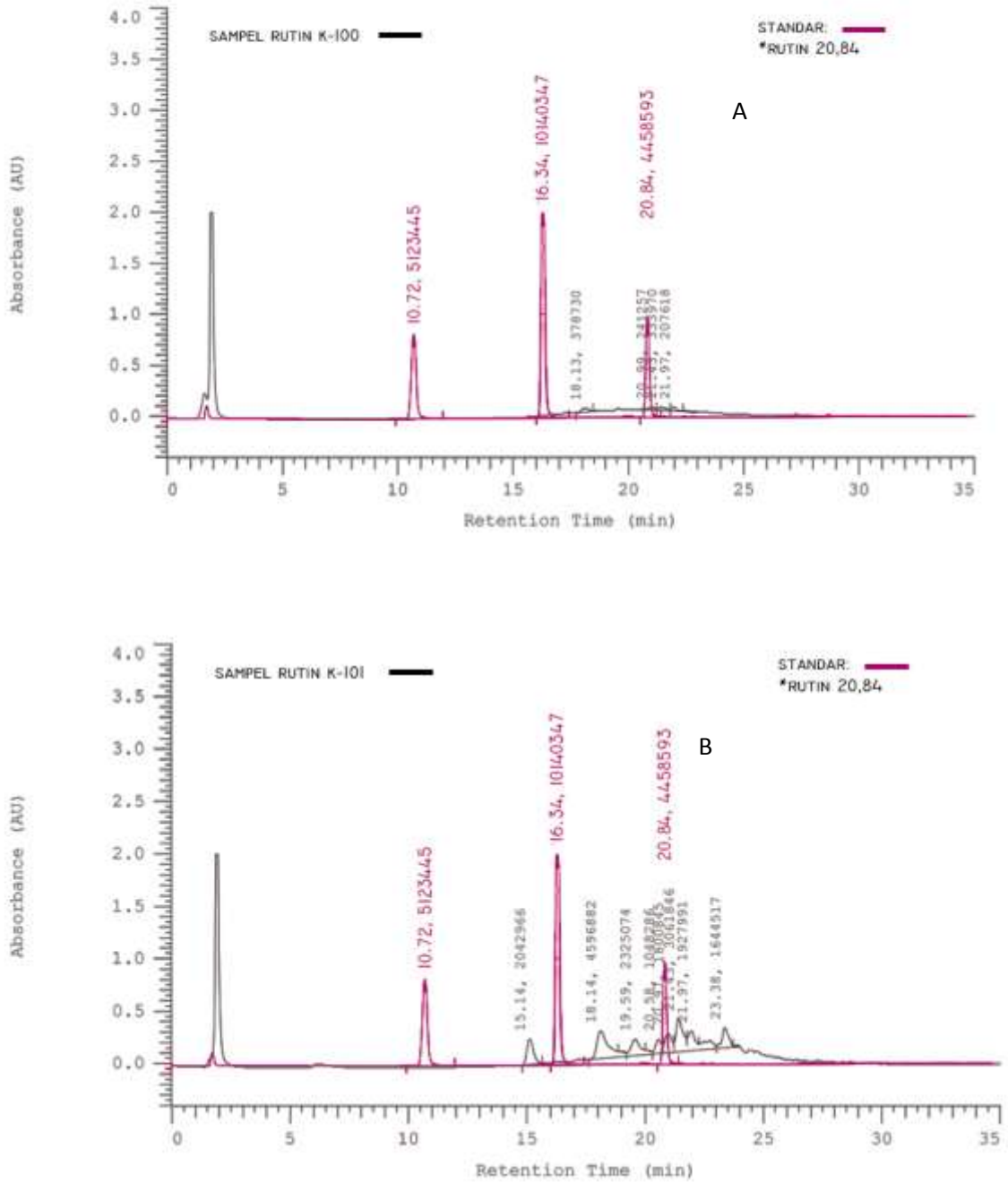

Fig 1. Chromatogram confirmed gallic acid (tR=10.72), catechin (tR=16.34) and rutin (tR=20.84) were not detected (n.d) in water fraction (A), but 54.1 ppm and 404 ppm of flavonoid's rutin was found in ethyl acetate fraction and hydrolyzed ethyl acetate fraction, respectively (B).

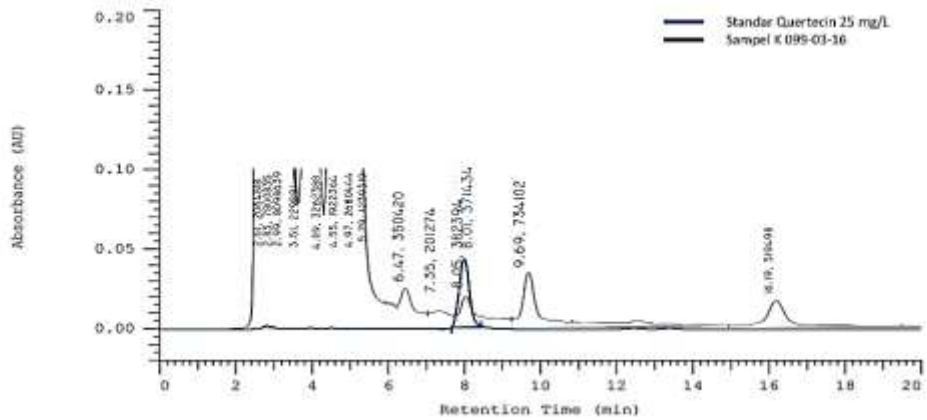

Fig. 2 Quercetin (insert) was detected in water fraction of MEJS (25 ppm), but not detected in two other fractions (ethyl acetate and hydrolyzed ethyl acetate fraction), tR = 8.05

Identification flavonoid's quercetin. Quantitative analysis of flavonoid's quercetin was done according to the method applied by Phani et al. (2010) on all three fractions, compare to chromatogram of quercetin (tR= 8.05) as standard. Water fraction chromatogram showed that 25 ppm (mg/L) quercetin was found, but not in other two fractions (**Fig. 2**).

The presence of quercetin in Java plum seed was confirmation of previous researches (Baliga et al*.,* 2011; Swami et al*.,* 2012). Flavonoid tends to dissolve in water and other polar solvents such as methanol, ethanol, ethyl acetate and their mixture, which classified them as a suitable solvent to extract plant flavonoid (Heim et al. 2002; Harborne, 2006; Durackova, 2008). Quercetine was not found, but another flavonol family such as kaempferol of 236.98±5.63 mg/100 g with strong absorbance at λ 243 nm (Rydlewsky et al*.,* 2013).

Identification of (+)-catechin. Quantification of tannin (+)-catechin was done according to the method applied by Zu et al., (2006) on 3 fractions. Chromatogram of water fraction (**Fig**. **3a**) showed that catechin was found for 55181 ppm (mg/L). Catechin was found in ethyl acetate fraction (not presented in the figure) and hydrolyzed ethyl acetate fraction (**Fig. 3b**) of 258 ppm (mg/L) and 28692 ppm (mg/L), respectively, with standard catechin as a reference (tR= 2.79).

The presence of tannin in *duwet* with its antioxidative property was previously reported (Mura et al., 2000; Zhang and Lin, 2009). Rydlewsky et al., (2013) were the first study reported epicatechin content in Java plum seed of 48.14±0.57 (mg/100 g), which did not found in other parts of the fruit (pulp and peel). Catechin is commonly found in tea leaves (Heim et al., 2002; Brewer, 2011), grape seed, onion and its family (Brewer, 2011).

There was significant increasing of catechin found in ethyl acetate and its hydrolyzed fraction, from 258 ppm (mg/L) to 28692 ppm (mg/L). That higher content of (+)-catechin was caused by hydrolysis of flavan-3-ols, either in monomer form (gallocatechin) or its oligomer and polymer (proanthocyanidins). Tavares et al., (2016) noted 8 monomers of flavan-3-ols were found in edible part of duwet (peel and pulp), which were catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin-3-O gallate, catechin-3-O-gallate, epigallocatechin-3-O-gallate, and gallocatechine-3-O-gallate.

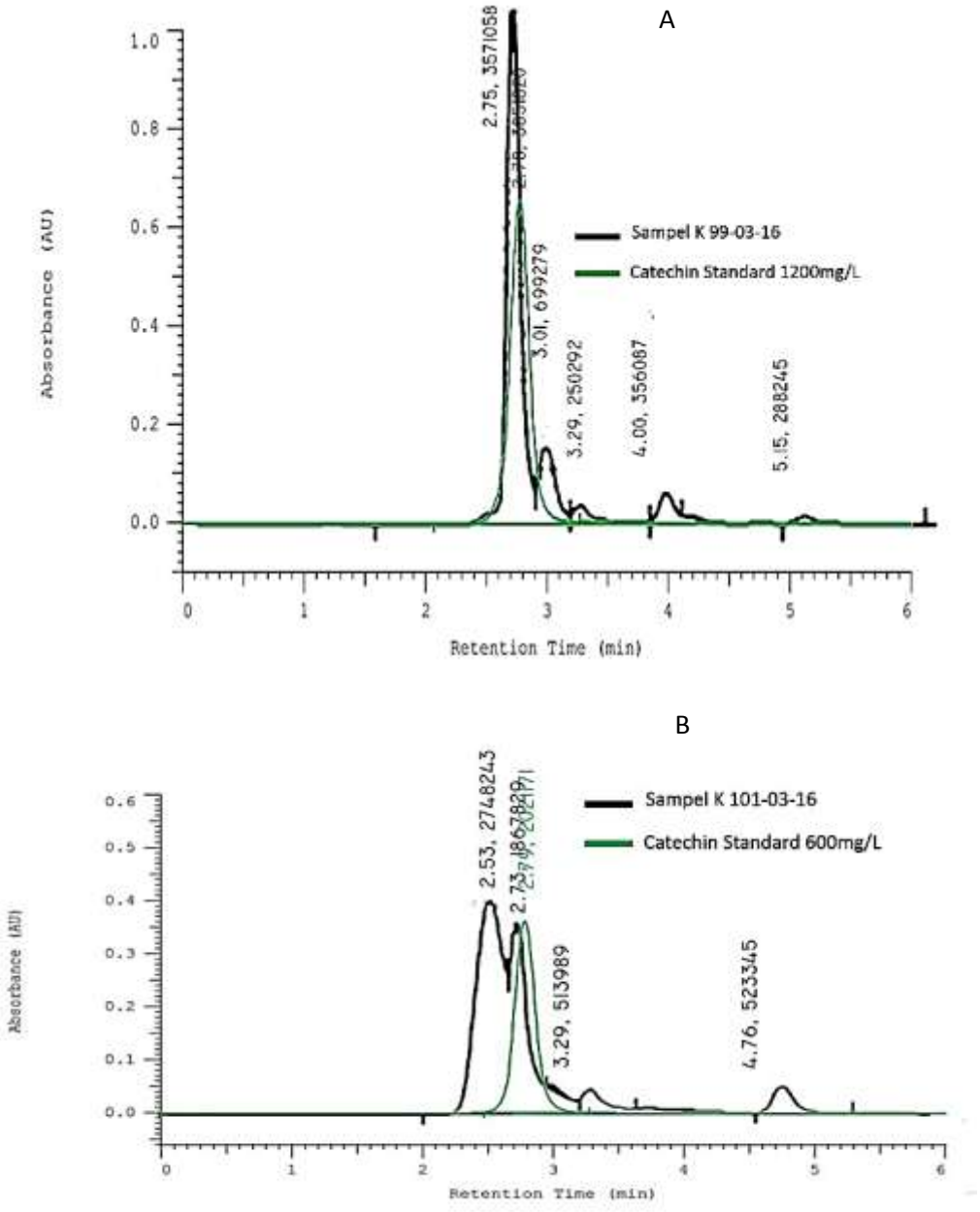

Fig. 3 The high amount of flavanoids (+)-catechin was found in MEJS in water fraction (A) as well as ethyl acetate and hydrolyzed ethyl acetate fraction (B).

Conclusion

MEJS exhibited strong antioxidant activity as DPPH radical scavenger and ferric reducing agent, with moderate lipid peroxidation inhibition activity, with flavonoid's rutin and quercetin and abundant tannin (+)-catechin responsible for the action. It can be concluded that Java plum seed is the potential natural antioxidant source.

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