Total Phenolic Content and Antioxidant Activity of Mung bean Seed Cultivars from Optimized Extraction Treatment

Fenolik Total dan Aktivitas Antioksidan Ekstrak Biji Kacang Hijau dari Perlakuan Ekstraksi yang Dioptimasi

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ABSTRACT

Secondary metabolites are produced by plants both during normal growth and under biotic and abiotic stresses. Apart from genetic and environmental factors, the secondary metabolite contents are also influenced by analytical methods. The aims of this study were to obtain suitable solvents and extraction treatments which produced high total flavonoid and phenolic contents as well as antioxidant activity in shiny and dull green mung beans. An extraction of 0.5 g sample with grade ≤80 mesh in 70% acetone was selected to estimate the contents of total flavonoids and phenolics. A treatment of shaking the sample in 70% acetone (1:10 w/v) for 2 h and followed by 18 h of maceration with twice extractions showed the highest amounts of flavonoid as well as phenolic contents. Total flavonoid and phenolic contents of 14 mung bean cultivars ranged from 1.28 to 2.35 mg CE/g and 3.74 to 6.58 mg GAE/g, respectively. Antioxidant activity represented by percentage of DPPH inhibition varied from 66.8 to 91.5%. A dull green mung bean cultivar Vima 1 had the highest total flavonoid and phenolic contents (2.35 mg CE/g and 6.58 mg GAE/g). Antioxidant activity of Vima 1 cultivar (91.5%) was not different from those of Perkutut (91.5%), Murai (90.1%) and Sriti (88.9%). The small quantity of sample (0.5 g) combined with the simple extraction treatment was effective in quantifying the different contents of total flavonoids and phenolics in mung bean seeds.

Keywords: antioxidant activity, extraction, phenolic, flavonoid, mung bean

ABSTRAK

Metabolit sekunder merupakan senyawa yang dihasilkan tanaman selama pertumbuhannya, baik dalam kondisi normal maupun tercekam. Kandungan metabolit sekunder, selain dipengaruhi oleh faktor genetik dan lingkungan, juga dipengaruhi oleh metode analisis yang digunakan. Penelitian bertujuan untuk mendapatkan jenis pelarut dan perlakuan ekstraksi yang menghasilkan kandungan flavonoid dan fenolik total tinggi serta aktivitas antioksidan biji kacang hijau. Perlakuan ekstraksi untuk analisis kandungan flavonoid dan fenolik total menggunakan bobot sampel 0,5 g, ukuran partikel ≤80 mesh dan pelarut 70% aseton. Kandungan flavonoid dan fenolik total tertinggi diperoleh pada ekstraksi dengan pengocokan selama 2 jam dalam pelarut 70% aseton (1:10 b/v) diikuti maserasi selama 18 jam dan dua kali ekstraksi. Kandungan flavonoid dan fenolik total dari 14 varietas kacang hijau berkisar antara 1,28-2,35 mg CE/g dan 3,74-6,58 mg GAE/g dengan aktivitas antioksidan antara 66,8% hingga 91,5%. Kacang hijau dengan warna kulit biji hijau kusam, yaitu Vima 1, memiliki kandungan flavonoid dan fenolik total tertinggi, masing-masing 2,35 mg CE/g dan 6,58 mg GAE/g. Aktivitas antioksidan dari varietas Vima 1 (91,5%) tidak berbeda dengan Perkutut (91,5%), Murai (90,1%) dan Sriti (88,9%). Penggunaan sampel 0,5 g dan diekstrak dengan cara sederhana efektif untuk mengukur perbedaan kandungan flavonoid dan fenolik total pada biji kacang hijau.

Kata kunci: aktivitas antioksidan, ekstraksi, fenolik, flavonoid, kacang hijau

INTRODUCTION

Mung beans are rich in starch, oligosaccharides, proteins, amino acids, fibre, polyphenols, and natural antioxidants (Kim *et al*. 2012; Tang *et al*. 2014). Regarding to their good nutrients and high bioactive compounds as well as good taste and flavor, mung bean seeds are considerably utilized for both traditional and industrial foods (Tang *et al*. 2014). Sprouts, porridge, weaning food, snacks, cookies, and transparent noodle are some foods prepared from mung bean.

During the growth phase, plants normally produce secondary metabolites such as phenolic compounds including phenolic acids (benzoic and cinnamic acid derivatives), simple phenols, flavonoids, stilbenes, tannins, lignans, and lignins (Chon 2013). The production of these compounds significantly increase when the plants are triggered by both biotic and abiotic stresses as well as symbiotic micro-organisms such as infection by plant pathogens, wounding caused by insect pests and herbivores, excess amount of UV radiation, and nodulation (Boue *et al*. 2009; Couto *et al*. 2011; Dardanelli *et*

al. 2012; Silva *et al*. 2013). The roles of secondary metabolites, particularly phenolic compounds are to protect the seedling from infection of soil borne pathogens after germination (Swamy *et al*. 2016). These compounds are also beneficial to protect leaves, fruits, and seeds against plant pathogens at the later growth of the crops (Lygin *et al*. 2009; Mikulic-Petkovsek *et al*. 2011; Ruiz-Garcia and Gomez-Plaza 2013).

A number of phenolic content analyses in seeds of leguminous crops has been studied (Nithiyanantham *et al*. 2012; Siger *et al*. 2012; Boudjou *et al*. 2013) for either determination of total phenolic contents or an individual phenolic or a group of phenolic compounds (Chon 2013). Prior to be quantified, the targeted compounds must be extracted from the samples using suitable solvents and extraction treatments. Therefore, extraction and sample preparation techniques as the first step of any secondary metabolite studies are essential as they would influence the final results and outcome (Azmir *et al*. 2013).

Various extraction methods of plant phenolic compounds can be conducted either using traditional or non-traditional methods for specific goals. Traditional techniques include solid liquid extraction, soxhlet extraction, sonication and blending, while microwave heating, extrusion, enzyme digestion, electric field, ohmic heating, supercritical fluids, and accelerated solvents belong to non-traditional methods (Ghafoor *et al*. 2012; Azmir *et al*. 2013). Even though traditional methods are considered time consuming and need large amount of samples, sorbent, and organic solvents (Mustafa and Turner, 2011), these approaches are still applied in many laboratories where access to modern equipments is limited.

In particular for mung bean, extraction of defatted mung bean was conducted through stirring and sonicating in acidified 80% ethanol for 1 h (Lee *et al*. 2011). Ethyl acetate, methanol, *n*-hexane, and *n*-butanol were also used to extract dry seeds and sprout of mung beans by boiling the samples under reflux (Kim *et al*. 2012). Repeated extraction up to three times was done for mung bean seeds and sprouts using methanol (99.8%) and shaking for 20 minutes (Pajak *et al*. 2014).

Studies on the extraction efficiency of mung bean secondary metabolites, particularly employing different concentration levels of organic solvents as well as extraction methods are still limited. Yusnawan (2018) extracted soybean flour using methanol, ethanol, and acetone in combination with several traditional extraction methods. The similar treatments for extraction of mung bean flour have not been carried out yet. Therefore, this current study aimed to investigate effects of different concentration levels of organic solvent, particle sizes, and traditional extraction methods on total flavonoid and phenolic contents as well as antioxidant activity in mung bean seeds. The optimized extraction method was then used to determine the contents of the secondary metabolites in selected mung bean cultivars.

MATERIALS AND METHODS

Preparation of Mung Bean Sample

Intact seeds of 14 mung bean cultivars, *i.e.* Betet, Walet, Gelatik, Parkit, Merpati, Sriti, Kenari, Murai, Perkutut, Sampeong, Kutilang, Vima 1, Vima 2, and Vima 3 were crushed into fine powder using a sample mill. The powder was gradually sieved to obtain different particle sizes (>70 – ≤60 mesh, >80 – ≤ 70 mesh, and ≤ 80 mesh). All seeds were obtained from mung bean germplasm collection of Indonesian Legumes and Tuber Crops Research Institute.

Trial 1: Extraction of Mung Bean Flour using Different Solvents

Organic solvents of acetone, methanol and ethanol with different concentration levels (50%, 70% and 80%) were used to extract mung bean secondary metabolites (Yusnawan 2018). Acetic acid at a final concentration of 0.5% was added into solvents to acidify the solvents. Mung bean flour of Vima 1 cultivar and the extraction solvents (1:10 w/v) were shaked for 2 h in capped amber glass prior to 18 h of maceration. Twice extractions were conducted. The supernatant obtained from the first and the second extractions were pooled and stored at 4 °C. Total flavonoid and phenolic contents as well as antioxidant activity were determined based on the wet basis of the samples, and the optimized concentration was used for trial 2.

Trial 2: Effect of Different Weights of Mung Bean Flour in Combination with Particle Sizes on Secondary Metabolites

Three weight samples of mung bean flour (0.5 g, 1.0 g, and 2.5 g) in combination with three particle sizes (>70 – ≤ 60 mesh, >80 – ≤70 mesh, and ≤ 80 mesh) were extracted with the optimized solvent obtained from trial 1. Total flavonoid and phenolic contents as well as antioxidant capacity were determined, and the optimized treatment was used for trial 3.

Trial 3: Effect of Different Extraction Treatments on Mung Bean Secondary Metabolites

Treatments of shaking, maceration and ultrasonic assisted extraction (UAE) were employed to extract the secondary metabolites. There were 12 combinations derived from these treatments:

1) the sample in extraction solvent (1:20 w/v) were shaked for 2 h and macerated for 18 h, and the supernatant was collected after centrifugation,
2) the sample in extraction solvent (1:10 w/v) was shaked for 2 h and macerated for 18 h and then centrifuged (this procedure repeated twice), then the supernatant obtained from the first and the second extraction was combined,
3) the sample in extraction solvent (1:20 w/v) was macerated for 20 h, and the supernatant was collected,
4) the sample in extraction solvent (1:10 w/v) was macerated for 20 h, and then centrifuged (this prosedure repeated twice), then the first and the second supernatant were pooled,
5) UAE for 5 min followed by maceration for 20 h was conducted to the sample in extraction solvent (1:20 w/v), and the supernatant was collected,
6) the same procedure as treatment 5 was performed with prolong UAE for 10 min,
7) twice extractions of UAE for 5 min followed by maceration for 20 h of the sample in extraction solvent (1:10 w/v), and then the two supernatants were pooled,
8) the same treatment as that of 7 was performed with prolong UEA for 10 min,
9) UAE for 5 min was carried out to the sample in extraction solvent (1:20 w/v), and then the supernatant was collected,
10) the same treatment as that of 9 was conducted with prolong UAE for 10 min,
11) twice extractions of UEA for 5 min of the sample in extraction solvent (1:10 w/v) was performed, and the supernatant obtained from the first and the second extractions was pooled,
12) the same treatment as that of 11 was carried out with prolong UAE for 10 min.

Total flavonoid (Lee *et al*. 2011) and phenolic contents (Kim *et al*. 2012) as well as antioxidant capacity (Yusnawan 2018) were determined.

Total Flavonoid Content Determination

Determination of total flavonoid content in samples using following procedure developed by Lee *et al*. (2011) with a minor modification. The sample extracts and distilled water at ratio of 1:5 v/v were thoroughly mixed in a tube. Sodium nitrite solution (1:20 v/v) was added to the sample and mixed. After incubation for 6 min, aluminum chloride solution was added and incubated for 5 min. Prior to addition of distilled water, sodium hydroxide (1000 μL) was added to the mixed solution. After being thoroughly mixed, absorbance values were measured at 510 nm using a spectrophotometer. Total flavonoid contents in the sample were expressed as catechin equivalents per gram of the sample (mg CE/g).

Total Phenolic Content Determination

An assay to determine total phenolic content was conducted according to Kim *et al*. (2012) with a slight modification. The mung bean extracts were diluted in distilled water (1:60 v/v) and Folin Ciocalteu's reagent was added (250 μL). Incubation for 8 min was carried out after the solution was added with sodium carbonate (750 μL) and distilled water. After incubation for 2 h, the absorbance value of the samples was spectrophotometrically determined at 765 nm. Total phenolic content was expressed as gallic acid equivalent per gram of sample (mg GAE/g).

Antioxidant Activity Determination

Mung bean extracts (1:19 v/v) were reacted in 1 mM ethanolic DPPH solution, and then it incubated in the dark room for 30 min to allow complete reaction. Absorbance values (A) of the control and sample were measured using a spectrophotometer at 515 nm (Yusnawan 2018). Discoloration percentage which represents antioxidant activity of the mung bean extracts was calculated as follows:

$$\text{percent discoloration} = [1-(A_{sample}/A_{control})] \times 100\%$$

Contents of Flavonoids, Phenolics and Antioxidant Activity

Dry seeds of 14 mung bean cultivars which had dull green *testa*/seed coat color (Vima 3, Vima 1, Murai, Sriti, Gelatik, Betet) and shiny seed coat color (Vima 2, Kutilang, Sampeong, Perkutut, Kenari, Merpati, Parkit, Walet) were finely crushed as described in the sample preparation. The samples were extracted using the optimized method obtained from trial 3. The data were analyzed in a completely randomized design with triplicate. Least significant difference was calculated to determine the differences among the treatments.

RESULTS AND DISCUSSION

Effect of Solvents on Secondary Metabolite Contents

Solvents used for extraction significantly influenced the contents of total flavonoids and phenolics in mung bean seeds (Table 1). Acetone gave higher values than both methanol and ethanol. Acetone at a concentration of 70% yielded the highest total flavonoid and phenolic contents as well as antioxidant activity, and therefore it was selected for further optimization. The result was in accordance with Zhang *et al*. (2013) which showed that extraction using acetone-based solvent resulted in higher total phenolic and flavonoid contents as well as DPPH scavenging capacity in mung beans than those of extracted in methanol. Kumar *et al*. (2010) also used 70% aqueous acetone to extract antioxidant components from yellow, green, and black soybeans.

Effect of Quantity and Particle Sizes of Mung Bean Flour Sample on Secondary Metabolites

The sample quantities in combination with the particle sizes influenced contents of total flavonoid and phenolic compounds extracted with 70% acetone (Table 2). Extraction using the smallest particle size showed the highest contents of total flavonoid and phenolic contents. Quantity of 0.5 g mung bean flour sample with particle size ≤80 mesh was not different from those of 1.0 and 2.5 g at the same particle sizes, and therefore it was chosen for practical extraction.

Small particle size of soybean sample (100 mesh) was also used by Kumar *et al*. (2010) to extract total phenolic contents from different seed coat colors. Yusnawan (2018) also used 0.5 g of sample flour with particle size ≤ 177 μm (or equal to≤ 80 mesh) to extract phenolic and flavonoid contents in soybean seeds. Smaller particle size has larger surface contact between target analyte and extraction solvent, and therefore enhancing extraction efficiency (Azwanida 2015).

Effect of Different Extraction Treatments on Mung Bean Secondary Metabolites

The highest amounts of total flavonoid and phenolic contents (2.45 mg CE/g and 5.63 mg GAE/g) were obtained from treatment of shaking sample for 2 h followed by maceration for 18 h in 70% acetone (1:10 w/v) with twice extractions. Lesser amounts of these compounds (1.96 mg CE/g and 4.64 mg GAE/g) were obtained using the same treatment in a single extraction. Antioxidant activity measured using the combination of shaking and maceration treatments with single or twice extractions, on the other hand, showed the same values with 5-10 min of UAE treatment combined with maceration (Table 3).

Twice extractions in combination with overnight maceration to extract phenolic contents in soybeans was also used by Kumar *et al*. (2010). The repeated extraction allowed more time for analytes to contact with the solvents, and therefore extracted more phenolic contents. However, Yusnawan (2018) found the same amounts of extract soybean flavonoid and phenolic contents, either extracted with UAE followed

Table 1. Total flavonoid and phenolic contents, and antioxidant activity of Vima 1 mung bean cultivar extracted using different solvents

No.	Solvent	Total flavonoid content (mg CE/g)	Total phenolic content (mg GAE/g)	Inhibition of DPPH (%)
1	50% acetone	0.88 d	4.18 b	62.59 c
2	70% acetone	2.29 a	5.96 a	90.55 a
3	80% acetone	1.39 c	4.29 b	66.84 c
4	70% acidified acetone	1.60 b	5.89 a	82.67 b
5	50% methanol	0.30 gh	2.60 cd	22.96 d
6	70% methanol	0.33 g	2.18 e	18.64 de
7	80% methanol	0.47 f	1.68 f	13.26 f
8	70% acidified methanol	0.21 h	2.35 cde	22.16 d
9	50% ethanol	0.45 f	2.70 c	23.93 d
10	70% ethanol	0.62 e	2.19 e	16.27 ef
11	80% ethanol	0.54 ef	1.37 f	12.36 f
12	70% acidified ethanol	0.50 f	2.27 de	19.03 de

The numbers followed by the same letters in the same column are not significantly different, LSD (α = 0.05).

Table 2. Total flavonoid and phenolic contents, and antioxidant activity of Vima 1 mung bean cultivar extracted using various sample quantity and particle sizes

No.	Combination of sample weight and particle size	Total flavonoid content (mg CE/g)	Total phenolic content (mg GAE/g)	Inhibition of DPPH (%)
1	0.5 g, >70 – ≤60 mesh	1.81 bc	4.51 cd	84.07 a
2	0.5 g, >80 – ≤70 mesh	1.78 c	4.65 bc	83.65 a
3	0.5 g, ≤80 mesh	2.06 a	5.16 a	82.96 a
4	1.0 g, >70 – ≤60 mesh	1.74 c	4.72 abc	82.11 ab
5	1.0 g, >80 – ≤70 mesh	1.76 c	4.77 abc	83.19 a
6	1.0 g, ≤80 mesh	2.07 a	5.08 ab	85.38 a
7	2.5 g, >70 – ≤60 mesh	1.38 d	4.04 de	69.19 c
8	2.5 g, >80 – ≤70 mesh	1.35 d	3.98 e	68.29 c
9	2.5 g, ≤80 mesh	2.02 ab	4.91 abc	76.58 b

The numbers followed by the same letters in the same column are not significantly different, LSD ($\alpha = 0.05$).

Table 3. Total flavonoid and phenolic contents, and antioxidant activity of Vima 1 mung bean cultivar extracted using different extraction methods

No.	Extraction method	Total flavonoid content (mg CE/g)	Total phenolic content (mg GAE/g)	Inhibition of DPPH (%)
1	Shaking-maceration-1 x 10 mL extraction	1.96 c	4.64 c	86.95 abc
2	Shaking-maceration-2 x 5 mL extraction	2.45 a	5.63 a	90.51 a
3	Maceration-1 x 10 mL extraction	1.87 cd	4.65 c	83.61 c
4	Maceration-2 x 5 mL extraction	2.21 b	5.19 b	89.24 ab
5	UAE 5 min-maceration-1 x 10 mL extraction	1.82 d	4.85 bc	85.61 bc
6	UAE 10 min-maceration-1 x 10 mL extraction	1.49 e	4.16 d	74.63 d
7	UAE 5 min-maceration-2 x 5 mL extraction	2.11 b	5.07 b	90.22 ab
8	UAE 10 min-maceration-2 x 5 mL extraction	2.17 b	5.21 b	88.62 ab
9	UAE 5 min-1 x 10 mL extraction	0.63 g	1.75 f	13.09 g
10	UAE 10 min-1 x 10 mL extraction	0.65 g	1.87 f	12.90 g
11	UAE 5 min-2 x 5 mL extraction	0.92 f	2.93 e	36.02 f
12	UAE 10 min-2 x 5 mL extraction	0.96 f	3.07 e	42.91 e

The numbers followed by the same letters in the same column are not significantly different, LSD (= 0.05).
UAE = ultrasound-assisted extraction

by maceration and repeated twice or with shaking followed by maceration. This suggests that UEA gives the same effect as shaking because it enhances plant cell disruption that facilitate solvent penetration and mass transfers (Yusnawan 2018).

Total Flavonoid Content in Mung Bean Cultivars

Several flavonoid compounds are found in mung beans (Wang *et al*. 2008; Prokudina *et al*. 2012). Polyhydroxy substitutions in most flavonoids are classified as polyphenols and they have significant DPPH radical scavenging activity (Tang *et al*. 2014). Total flavonoid content of 14 mung bean samples varied significantly (Figure 1). Total flavonoid content of the six dull green mung bean cultivars varied from 1.51 to 2.35 mg CE/g, and the highest content was found in Vima 1 (2.35 mg CE/g). Eight shiny green mung bean cultivars had lower values of flavonoid content ranging from 1.28 to 2.06 mg CE/g, and the highest content was observed in Perkutut cultivar. The difference of total flavonoid contents between dull and shiny mung bean cultivars needs to be further studied.

Genotypic variations of total flavonoid contents in mung beans were also investigated by Zhang *et al*. (2013). The highest value obtained from a particular cultivar was about 3.3 folds higher than the lowest one. The difference of the highest to the lowest flavonoid contents in the current study was much lower than found by Zhang *et al*. (2013) that was about 1.84 times, and it because of different flavonoid standard used. Lee *et al*. (2011) investigated a defatted sample of mung bean extracted with ethanol, and found the total flavonoid content of 1.49 mg CE/g, which was within the range of total flavonoid contents (1.28 to 2.35 mg CE/g) observed in the present study.

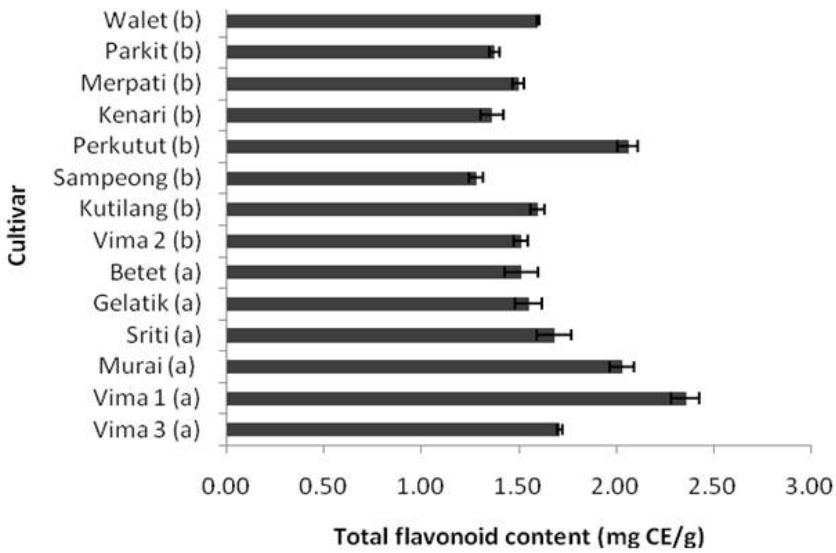

Figure 1. Total flavonoid content of fourteen mung bean cultivars. Bars represent standard deviation. (a) dull green and (b) shiny green seed coat

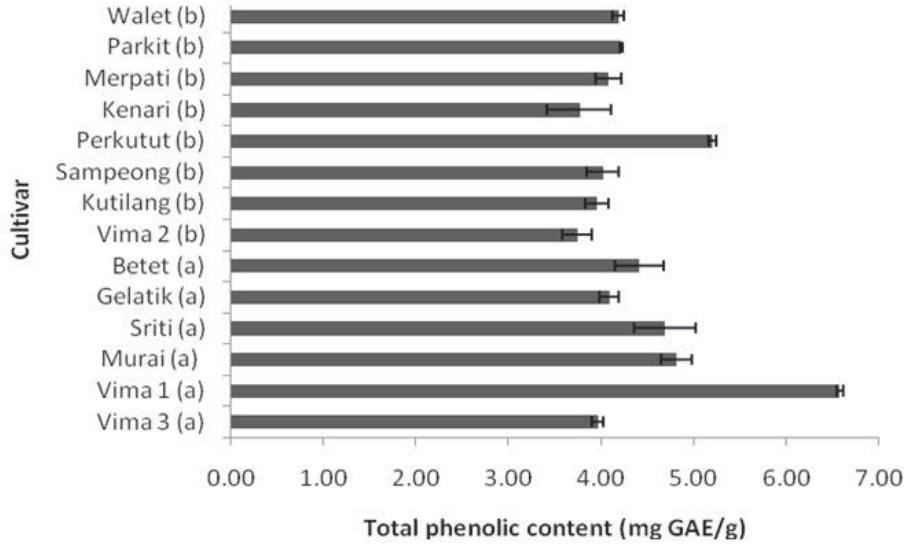

Figure 2. Total phenolic content of fourteen mung bean cultivars. Bars represent standard deviation. (a) dull green and (b) shiny green seed coat.

Total Phenolic Content in Mung Bean Cultivars

Mung beans are rich in phenolic acids which contribute to antioxidant activity. These phenolic acids are synthesized through phenylpropanoid, shikimate, and pentose phosphate pathways (Tang *et al*. 2014). The dull green mung bean cultivar of Vima 1 having the highest value of total flavonoid content also exhibited the highest total phenolic content (6.58 mg GAE/g) (Figure 2). The total phenolic contents of the dull group varied from 3.97 to 6.58 mg GAE/g, slightly higher than the contents in shiny green group (3.74 to 5.20 mg GAE/g). Among the shiny group, Perkutut cultivar showed the highest value (5.20 mg GAE/g). Among the 14 cultivars studied, Vima 1 has the highest phenolic content followed by Perkutut. Again, the genotypes of mung bean could be the reason in the difference of total phenolic contents and the compounds which are responsible for the high phenolic contents, but still needs to be investigated further.

Variations in total phenolic contents of mung bean cultivars have also been reported by Zhang *et al*. (2013). In their study, the phenolic contents of ten mung bean samples extracted with acetone-water (1:1 v/v) varied from 1.86 to 5.07 mg GAE/g, whereas those extracted in pure methanol ranged from 0.08 to 0.28 mg GAE/g. The lowest and the highest contents of total phenolics were still less than in the current study (3.74 to 6.58 mg GAE/g).

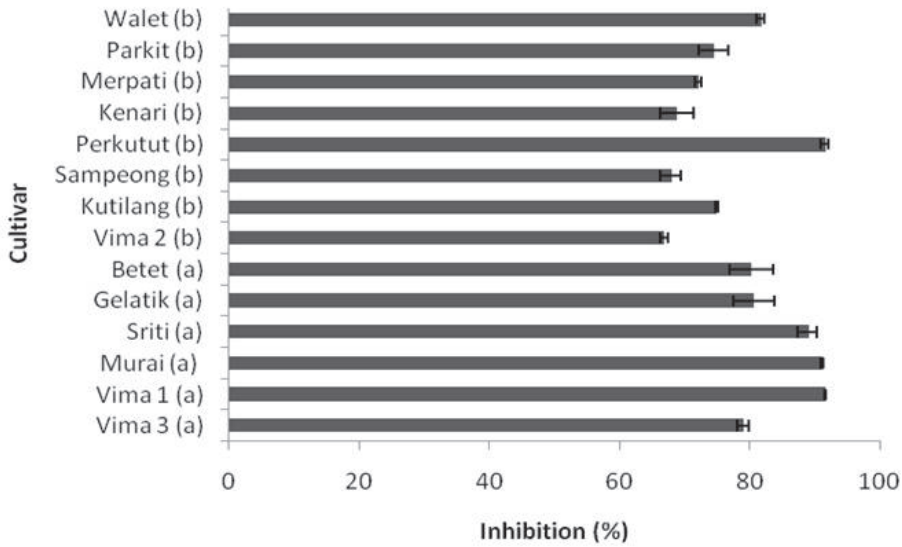

Figure 3. Antioxidant activity of fourteen mung bean cultivars. Bars represent standard deviation. (a) dull green and (b) shiny green seed coat.

Different secondary metabolite contents which were influenced by the extraction solvents were also investigated in this present study. This indicates that differences in total phenolic contents are determined by genotype, extraction solvent and extraction treatment.

Antioxidant Activity in Mung Bean Cultivars

Antioxidant activity as indicated by DPPH radical scavenging activity in 14 mung bean cultivars varied significantly (Figure 3). Antioxidant activities of three dull green mung bean cultivars, *i.e.* Vima 1 (91.5%), Murai (91.1%) and Sriti (88.9%) were similar to that of a shiny green mung bean, Perkutut (91.5%). Wider range of antioxidant activities from 66.8% to 91.5% were found in the shiny green group as compared to those in dull green mung bean cultivars, which ranged from 79.0% to 91.5%.

Antioxidant activity positively correlated to total flavonoid contents ($r = 0.85$, $p < 0.05$) and total phenolic contents ($r = 0.78$, $p < 0.05$). This finding was in agreement with Zhang *et al.* (2013) that found a strong positive correlation of water-acetone extract of mung beans between DPPH scavenging capacity and total phenolic contents ($r = 0.95$, $p < 0.01$) as well as between the antioxidant activity and total flavonoid contents ($r = 0.87$, $p < 0.01$). Flavones, flavonoids, isoflavone, and isoflavonoids are major flavonoid compounds in mung beans having high antioxidant activity. At least 44 individual compound of flavonoids have been identified in the mung bean seeds and sprouts (Wang *et al*. 2008; Prokudina *et al*. 2012). Two major antioxidant components of flavonoids, *i.e.* vitexin and isovitexin have been confirmed with HPLC, NMR and MS techniques (Li *et al*. 2012; Zhang *et al*. 2013). Total phenolic contents of these two compounds varied depending on the cultivars, ranging from 4.41 to 5.01 mg/100 mL and 4.74 to 5.25 mg/100 mL for vitexin and isovitexin, respectively (Li *et al*. 2012).

Phenolic acids also contribute to antioxidant activity in legumes. Compounds of *p*-hydroxybenzoic, protocatechuic, syringic, gallic acid, vanillic acid, gentisic acid, shikimic acid, *p*-coumaric, cinnamic acid, caffeic acid, ferulic acid, chlorogenic acid, salycilic acid, and 3-hydroxycinnamic acid are phenolic acids identified in mung beans (Sosulski and Dabrowski 1984; Sawa *et al*. 1999; Zhang *et al*. 2013). Instead of phenolics compounds, tochoperols, carotenoids, phytic acid, saponins or *L*-ascorbic acid that are normally present in legumes may also collectively contribute to antioxidant activity (Lee *et al*. 2011; Pająk *et al*. 2014). Mung bean seeds contain high tocopherols (α-tocopherol ranged from 1.1 to 10.1 mg/kg, γ-tochoperol ranged from 60.7 to 80.9 mg/kg, and δ-tochoperol ranged from 4.6 to 11.2 mg/kg) and these contents depend on the cultivars (Anwar *et al*. 2007).

CONCLUSION

Extraction of secondary metabolites in mung beans using finer particle (≤80 mesh) produced higher amounts of total flavonoids and phenolics, and antioxidant activity. Shaking the sample in 70% acetone (1:10 w/v) for 2 h followed by maceration for 18 h with twice extractions is an effective and simple method for analyzing total flavonoid and phenolic contents. Small quantity of sample (0.5 g) is as effective as 1.0 g and 2.5 g for assessing total flavonoid and phenolic contents in mung beans,

and this is more practical and efficient particularly during assessing a large number of samples.

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