Total phenolic content and antioxidant activity in eight cowpea (Vigna unguiculata) genotypes

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Abstract. Cowpea is considered as one of the underutilized legumes which is rich in secondary metabolites. These metabolites can be detected during the plant growth and the contents increase especially after the plants are stressed both abiotic and biotic. This study aimed to determine total phenolic and flavonoid contents as well as antioxidant activity in seeds of eight cowpea genotypes. Two extraction solvents (70% acetone and 70% acidified acetone) were used to extract the secondary metabolites in those cowpea genotypes. Total flavonoid contents of those genotypes extracted in 70% acetone and 70% acidified acetone were in the range from 7.28 to 10.90 mg CE/g and 7.84 to 11.58 mg CE/g, whereas total phenolic contents were from 15.20 to 19.99 mg GAE/g and 15.03 to 21.15 mg GAE/g. Inhibition percentages of antioxidant activity were in the range from 46.90 % to 59.46 % and 50.71 % to 63.81 % for 70% acetone and 70% acidified acetone, respectively. Both solvents were effective to extract those secondary metabolites. Flavonoid and phenolic contents of MLGU 0239 were the highest among other genotypes, which were 11.58 mg CE/g and 21.15 GAE/g and antioxidant activity was 63.81 % inhibition after being extracted with 70% acidified acetone. This study observed the variation of the secondary metabolites among eight cowpea genotypes with different seed coat colours.

1. Introduction

Cowpea (Vigna unguiculata) is one of the legume protein sources and also rich in carbohydrates with relatively low-fat content [1]. This legume contains high polyphenols, including phenolic acid derivatives and flavonol glycosides. Anthocyanins and flavan-3-ols are also found in some cultivars [2]. Several seed coat colours were observed among cowpea genotypes, and some secondary metabolites such as delphinidin, catechin and epicatechin were higher in the dark seeded cowpeas [3].

Higher polyphenolic compounds in leguminous plants are challenged by various abiotic stresses including heat, salt, drought and heavy metals. Other compounds such as terpenes and alkaloids are also accumulated during these stresses [4]. Accumulation of these compounds is also observed in plants during fungal, viral, bacterial infections and other pathogens. These plant phenolics act as antimicrobial agents to protect the plants from further cell damage [5].

Phenolic acid derivatives, flavonol glycosides, flavonols, anthocyanins, tannins, sphingolipids and fatty acids are observed in cowpea extracts [2-3]. Catechin and epicatechin, catechin glucoside, and delphinidin were also found in trace amounts of some genotypes which showed variation among the genotypes [3]. Extraction of phenolic contents of leguminous plants either using traditional or non-traditional techniques has been conducted to determine total phenolics or an individual compound [6-8].
A previous study showed that acetone solvents were effective to extract secondary metabolites in cowpea [9]. Similar amount of cowpea secondary metabolite contents was observed when the cowpea flour was extracted with acetone and acidified acetone. The addition of weak acid in the organic solvent was to enhance the detectability of the targeted compounds by hydrolysing the sample [16]. However, the use of several cowpea genotypes has not been conducted in this previous study to determine variations of cowpea secondary metabolites. Therefore, this current study aimed to determine total phenolic and flavonoid contents as well as antioxidant activity in seeds of eight cowpea genotypes using extraction solvents of 70% acetone and 70% acidified acetone.

2. Material and methods

2.1. Cowpea sample preparation
Eight cowpea genotypes, i.e., MLGU 0239, MLGU 0233, MLGU 0240, MLGU 0234, MLGU 0235, MLGU 0241, MLGU 0113, and MLGU 0242 were obtained from cowpea germplasm collection of Indonesian Legumes and Tuber Crops Research Institute. The eight cowpea genotypes had different seed coat colours of dark brown (MLGU 0239), reddish brown (MLGU 0233), brown (MLGU 0240, MLGU 0235) and red (MLGU 0234, MLGU 0241, MLGU 0113, MLGU 0242). The cowpea genotypes were powdered using a sample grinder. The powders were stored at 4 °C in sealed sample plastic bags.

2.2. Cowpea flour extraction with organic solvents
Two extraction solvents of 70% acetone and 70% acidified acetone were used to extract the cowpea flour according to Yusnawan et al. [10]. The cowpea powders in the extraction solvents (1:10 w/v) were shaked for 2 h and macerated for 18 h. Twice extractions were performed and the first supernatant obtained from the first extraction was combined with the second supernatant from the second extraction. This combined supernatant was stored in amber vials at 4 °C and used for estimation of total phenolic and flavonoid contents as well as antioxidant activity.

2.3. Flavonoid content determination
A slight modification of total flavonoid determination conducted by Lee et al. [11] was carried out to estimate the content in the cowpea extract. Diluted samples in distilled water were reacted with sodium nitrite (1:20 v/v) and followed by the addition of 10% aluminium chloride. After incubation for a few minutes, sodium hydroxide and distilled water were poured in the solution. Absorbance values were measured using a spectrophotometer. Total flavonoid content was expressed as catechin equivalent/g of the sample (CE/g).

2.4. Phenolic content determination
A reagent of Folin Ciocalteu was reacted with the sample to estimate total phenolic content [12]. The cowpea extract was diluted in distilled water prior to the addition of 250 µL of the Folin Ciocalteu’s reagent. Blue color was developed after being added with 750 µL of sodium carbonate and incubated for 90 min. The mixture was read using a spectrophotometer. Gallic acid equivalent per gram of the sample (GAE/g) was used to estimate total phenolic content in the cowpea extract.

2.5. Antioxidant activity determination
Antioxidant activity as represented by percentage of inhibition was determined by reacting the cowpea extracts with methanolic DPPH solution [13]. The cowpea extract was reacted with the methanolic DPPH solution (1:19 v/v) and the reaction was incubated for 30 min in the dark. Absorbance values were recorded using a spectrophotometer. Inhibition percentage (% inhibition) was calculated as [1- (Asample/Acontrol)] x 100%, where Asample was absorbance value of the sample and Acontrol was absorbance value of the control.
3. Results and discussion

3.1. Effect of extraction solvents on total flavonoid, phenolic contents and antioxidant activity

The use of both 70% acetone and 70% acidified acetone was effective to extract phenolic, flavonoid contents as well as antioxidant activity in all cowpea samples. The secondary metabolite contents extracted using these two solvents were quite similar (Figure 1, 2, 3). This finding was in line with a previous study that solvents-based acetone has high efficiency in extracting the secondary metabolites of cowpea [9]. In addition, acetone was also effective to extract secondary metabolites in mung beans [10].

3.2. Total flavonoid, phenolic contents and antioxidant capacity of cowpea affected by different preparation treatments

Total flavonoid contents extracted with 70% acetone were from 7.28 to 10.89 mg CE/mg (Figure 1). The highest flavonoid content was MLGU 0239 among other seven cowpea genotypes. Extraction using 70% acidified acetone which was from 7.84 to 11.57 mg CE/mg was comparable with that of 70% acetone. Flavonoid contents of MLGU 0233 and MLGU 0235 were around two-thirds of that of MLGU 0239, which were 7.28 and 7.29 mg CE/g for 70% acetone and 7.84 and 8.65 mg CE/g for 70% acidified acetone, respectively. The MLGU 0239 had dark seed coat colour which may correlate with anthocyanins apart from flavanol and tannins. Particularly in legumes, major flavonoids accumulated in seeds were anthocyanins, proanthocyanidins, and isoflavones [14]. In dark or black seed coat colour for instance, anthocyanins and proanthocyanidins were more concentrated than isoflavones [15].

![Figure 1. Total flavonoid contents (TFC) of eight cowpea genotypes extracted with 70% acetone (A) and 70% acidified acetone (Ac-A). Bars represent standard deviation.](image)

Total phenolic contents of eight cowpea genotypes were from 15.05 to 19.99 mg GAE/g and from 15.03 to 21.15 mg GAE/g after being extracted with 70% acetone and 70% acidified acetone (Figure 2). Similar results with total flavonoid contents, among eight cowpea genotypes, MLGU 0239 was the highest of total phenolic content, whereas two genotypes of MLGU 0233 and MLGU 0235 were consistently having the lowest phenolic contents, which were three quarters of MLGU 0239. Higher phenolic content was observed in seed coats than that in cotyledons and embryonic axis which contributed to the total phenolic acid of the whole seeds [15].

Antioxidant activity as represented by the inhibition percentage of DPPH slightly varied among cowpea genotypes (Figure 3). Inhibition values were in the ranges from 46.90 to 59.46% and from 50.71 to 63.81% in 70% acetone and 70% acidified acetone extracts. Extraction of antioxidant components using 70% acidified acetone was slightly higher activity than those of using 70% acetone. Again, MLGU
0239 had the highest antioxidant activity which was similar to that of MLGU 0240 extracted using 70% acetone (59.47% and 58.39%) and 70% acidified acetone (63.81% and 59.85%). According to Tsamo et al. [3], cowpea with darker seed coat colour contained higher secondary metabolites such as delphinidin, catechin and epicatechin which may contribute to high antioxidant activity. Higher antioxidant activity in cowpea with dark seed coat colour was also observed in the previous study [9], in which brown seed coat colour of cowpea genotype had higher antioxidant activity than that of red seed coat colour. Higher antioxidant activity in seeds of darker seed coat colour was also observed in soybean [17], which could possibly be correlated with high polymerized procyanidin and anthocyanin [18].

![Figure 2](image2.png)

**Figure 2.** Total phenolic contents (TPC) of eight cowpea genotypes extracted with 70% acetone (A) and 70% acidified acetone (Ac-A). Bars represent standard deviation.

![Figure 3](image3.png)

**Figure 3.** Antioxidant activity of eight cowpea genotypes extracted with 70% acetone (A) and 70% acidified acetone (Ac-A). Bars represent standard deviation.

There are correlations between total flavonoid and phenolic contents (r = 0.88), total flavonoid and antioxidant activity (r = 0.77), as well as total phenolic and antioxidant activity (r = 0.731). These three linear correlations were also observed in darker seed coat colours of soybeans than those of yellow ones as reported in the previous studies [15, 19]. Since flavonoids contributed to the majority of phenolic
compounds [15], the strong correlation between total phenolic and flavonoid contents was observed in this current study. Major anthocyanins in cowpea seeds as a part of flavonoids were most likely responsible for antioxidant activity as shown in that high correlation value \((r = 0.77)\). This finding was also in agreement with antioxidant activity studies in soybeans [20].

A study of a variation in secondary metabolites among cowpea genotypes was valuable to determine a potential variation of the genotypes in facing biotic stress particularly seed and soil borne pathogens. Phenolics are important compounds to combat such stress. High secondary metabolite contents of MLGU 0239 cowpea genotypes are needed to be further studied in conjunction with biotic stress.

4. Conclusions
Contents of total flavonoids and phenolics as well as antioxidant activity in eight cowpea genotypes extracted with 70% acetone and 70% acidified acetone were comparable. The MLGU 0239 cowpea genotype had high in flavonoid, phenolic contents as well as antioxidant activity. The potency of this genotype to alleviate both biotic and abiotic stresses is needed to be further evaluated.

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