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Investigation of biochemical characters and antioxidant properties of different winged bean (*Psophocarpus tetragonolobus*) genotypes grown in Indonesia

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Abstract. *Calvindi J, Syukur M, Nurcholis W. 2020. Investigation of biochemical characters and antioxidant properties of different winged bean (*Psophocarpus tetragonolobus*) genotypes grown in Indonesia. Biodiversitas 21: 2420-2424.* Winged bean, *Psophocarpus tetragonolobus* (L) DC, is described as having antioxidant properties. This work evaluated the biochemical and antioxidant characteristics of the *P. tetragonolobus* genotypes. Twelve-winged bean genotypes were calculated for total phenolic content (TPC) and total flavonoid content (TFC) of biochemical characters and antioxidant properties by using four methods: 2,2-diphenyl picrylhydrazyl (DPPH), cupric reducing antioxidant power (CUPRAC), ferric reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC). The plant material was grown in the same location conditions in Indonesia. The total phenolic content varied from 154.6 to 161.5 mg GAE/ 100 g, and flavonoid ranged from 105.2 to 112.4 mg QE/ 100 g fresh weight. The antioxidant capacities were 30.6 - 47.0, 140.4 - 167.6, 66.9 - 170.8, and 28.0 - 52.4 µmol TE/ 100 g fresh weight as calculated by the DPPH, TEAC, FRAP, and CUPRAC assays, respectively. The antioxidant activities were significantly correlated with the polyphenol content of winged bean genotypes fruits. The genotypes TU, L3, H3U, H1P, and TH were recognized higher based on their TPC, TFC, and antioxidant activities, indicating that these genotypes to be promising for further breeding program and commercial purposes.

Keywords: Agricultural biochemistry, antioxidant, flavonoid, polyphenol, winged bean

INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus*; Family-Fabaceae) is a diploid (2n=2X=18) underutilized legume crop and is widely cultivated in Asia and Africa (Ng, 2013; Mohanty et al. 2020). Winged bean is called as "soybean of tropics" with excessive potentiality the nutritional contents (Amoo et al. 2011). The flowers, leaves, tubers, pods, and seeds of plant parts are eatable (Mohanty et al. 2020). The essential amino acids, proteins, vitamins, minerals, and oil contents in winged bean seed are similar to soybean (Amoo et al. 2011). Moreover, the winged bean was found for containing anti-nutrients such as polyphenol (Singh et al. 2019), tannin (Singh et al. 2017), phytic acid, trypsin inhibitor, chymotrypsin inhibitor (Mohanty et al. 2020), protease inhibitor (Kaur and Sohal 2019), and oxalate (Alalade et al. 2016). Traditionally, the winged bean is used to treat diabetes, cancer, infection, eye and migraine diseases, muscle weakness, and asthma (Singh et al. 2019). Previously, several studies have been reported pharmacological activities of winged beans, such as an antioxidant (Koley et al. 2019; Singh et al. 2019), antihypertensive (Chay et al. 2018) and antifungal (Zakuan et al. 2018).

Reactive oxygen species, a free radical, is destructive to persons, leading to digestive, cardiovascular, cancer, and respiratory diseases (Liu et al. 2018). The vegetables and fruits are rich in antioxidant compounds. Consuming them will help to protect against several diseases caused by free radicals (Li et al. 2014; Ishihara et al. 2018). The function of plant antioxidants is to defend the cell from free radicals and regulate reactive oxygen species-related enzymes (Djordjevic et al. 2011). The synthetic antioxidant of butylated hydroxyanisole and butylated hydroxytoluene are promoted carcinogenesis (Devi et al. 2019). Their consumption has now been a decline. Thus, the finding of natural antioxidants with non-toxic compounds is important to be done. Winged bean is rich in metabolite with antioxidant properties, including peptide (Wan Mohtar et al. 2014), phenolic acid, and flavonoid (Gan et al. 2016; Singh et al. 2019). Olaiya et al. (2018) reported that different winged bean accessions have variate antioxidant properties.

The work aimed to determine the biochemical characters and antioxidant properties of twelve-winged bean genotypes, including total phenolic content (TPC) and total flavonoid content (TFC) for biochemical characteristics, and 2,2-diphenyl picrylhydrazyl (DPPH), cupric reducing antioxidant power (CUPRAC), ferric reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC) for antioxidant activities. Besides, we predicted the variation and association between

the biochemicals characters and antioxidant properties in winged bean genotypes. So, this work could be provided scientific information knowledge in the selection, improvement, and breeding of different winged bean genotypes.

MATERIALS AND METHODS

Plant material and sample preparation

Twelve-winged bean genotypes germplasm were selected in the F6 population, which derived from crossing (F1) of Thailand-introduced purple winged bean x Cilacap-accessed local green-winged bean (Table 1). The genotypes were cultivated in the experimental fields of Bogor Agricultural University, Bogor, West Java, Indonesia. The cultivation was performed in a randomized block design in three replications. For biochemical determination components (total phenolic and flavonoid) and antioxidant capacities, the sample fruits of studied genotypes were harvested at 10^{th}-day pod after anthesis. The sample preparation was extracted using ethanol according to the method recommended by Singh et al. (2019) with slight modification. Briefly, after the pod sample was cut into minor pieces, then the seeds were removed. After the sample was homogenized for 2 min using the blender, 2 g sample each genotype was extracted with 80% ethanol (10 ml). The sample was sonicated for 30 min, then macerated with stirring at 125 rpm for 24 h in the dark. Then, the sample homogenate was centrifuged (at 10000xg and 4°C) for 15 min. Finally, the sample extract (supernatant) concentration of 0.2 g/ml was used for the determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity.

Biochemical and antioxidant analyses

Extract fruit samples were determined total phenolic and total flavonoid contents for biochemical traits evaluation. TPC was assessed spectrophotometrically using Folin-Ciocalteu reagent with the method recommended by Khumaida et al. (2019) with modification. TPC was measured based on the standard curve and expressed as mg gallic acid equivalents (GAE) / 100 g fresh weight (FW). Supernatant extracts each genotype (20 µL) were mixed with 50% Folin-Ciocalteu reagent (100 µL) in 96-well microplate and incubated for 5 min. Then, the mixture was added with 7.5% Na_2CO_3 (80 µL) and incubated in the dark for 2 h. Finally, the absorbance at 750 nm was measured with a microplate reader (Epoch BioTek, USA). Whereas TFC was determined using the colorimetric method (Khumaida et al. 2019) with quercetin as standard. TFC expressed as mg QE/ 100 g FW. The sample or diluted standard (10 µL) was mixed with methanol (60 µL), 10% AlCl3 (10 µL), 1 M CH3COOK (10 µL), and distilled water (110 µL). The mixtures were incubated for 30 min at room temperature and then the absorbance of mixture was measured at 415 nm using microplate reader (Epoch BioTek, USA).

Table 1. Information of winged bean genotypes used in this work

Genotype codes	Origin	Pod color
H1U	Selected (F6)	Green
H2	Selected (F6)	Green
H3P	Selected (F6)	Green
H1P	Selected (F6)	Green
H3U	Selected (F6)	Green
L4	Selected (F6)	Purple
L2	Selected (F6)	Purple
H4P	Selected (F6)	Green
L1	Selected (F6)	Purple
L3	Selected (F6)	Purple
TU	Parent (Thailand-introduced purple winged bean)	Purple
TH	Parent (Cilacap-accessed local green winged bean)	Green

Four in-vitro methods were used to evaluate the antioxidant capacities of extract fruit samples of winged bean genotypes. The assays are 22-diphenyl picrylhydrazyl (DPPH) and Trolox equivalent antioxidant capacity (TEAC) for evaluating the free radical scavenging activities, and cupric reducing antioxidant power (CUPRAC) and ferric reducing antioxidant power (FRAP) for assessing the reducing power. DPPH, TEAC, FRAP, and CUPRAC were determined.

The DPPH radical scavenging activity was measured as explained by Nurcholis et al. (2017) with modification. DPPH (125 µM) in ethanol was used in the experiment. Briefly, 100 µL aliquot extract was mixed with DPPH (100 µL) and then incubated at room temperature (dark) for 30 min. Final absorbance was measured at 517 nm using a microplate reader (Epoch BioTek, USA). The value was expressed as µmol Trolox equivalent per 100 g FW.

TEAC of the sample was determined using 2, 2-azino-di-(3-ethylbenzothialozinesulphonic acid (ABTS) radical according Re et al. (1999) with modification. Briefly, 20 µL aliquot extract was mixed with seven mM ABTS reagent (280 µL). The mixture incubated in the dark for 6 min. Absorbance was measured using a microplate reader (Epoch BioTek, USA) at 734 nm. The result was expressed as µmol Trolox equivalent per 100 g FW.

Ferric reducing antioxidant power (FRAP) was estimated as described by Benzie and Strain (1996) with modification. FRAP reagent consisted of 10 mM TOTZ in 40 mM HCl, 20 mM FeCl3.6H2O in distilled water, and acetate buffer at pH 3.6, in comparison to 1: 1: 10, respectively. Before used, the FRAP reagent was stored at 37°C for 30 min. Briefly, 10 µL aliquot extract was mixed with 300 µL FRAP reagent and then incubated at 37°C for 30 min in the dark. The absorbance at 593 nm was measured using a microplate reader (Epoch BioTek, USA) at 734 nm. Antioxidant activity was expressed as µmol Trolox equivalent per 100 g FW.

CUPRAC method was determined as described by Öztürk et al. (2011) with modification. Briefly, the sample (50 µL) was mixed with 50 µL each of neocuproine (7.5 x

10^{-3} M), copper (II) chloride (10^{-2} M), and ammonium acetate buffer (pH 7) solutions. After the mixture incubated in the dark for 30 min, the absorbance was recorded at 450 nm, and value was expressed as µmol Trolox equivalent per 100 g FW.

Data analysis

The means value ± SD of three replicates were determined. ANOVA was performed using ExpDes packages in R, followed by the Scott-Knott test (Ferreira et al. 2014). The PerformanceAnalytics packages in R was used to create the Pearson correlation coefficients between biochemical and antioxidant variables (Peterson et al. 2014).

RESULTS AND DISCUSSION

The biochemical components and antioxidant capacities of different winged bean genotypes, including TPC, TFC, FRAP, CUPRAC, DPPH, and TEAC are presented in Table 2.

Polyphenol is a group of biochemistry compounds that have antioxidant properties (Khumaida et al. 2019; Kalisz et al. 2020). Flavonoid is one of the polyphenol compounds and is widely reported as antioxidant activity (Nurcholis et al. 2016; Tanleque-Alberto et al. 2020). Significant variances of TPC and TFC of studied winged bean genotypes were observed in the present work (Table 1). The highest TPC was recorded as 161.5 mg GAE/ 100 g FW in TU genotype, and the lowest was noted in H3P genotype as 154.6 mg GAE/ 100 g FW. In this work, TFC in the twelve-winged bean genotypes was significantly different, with the maximum value recorded in L3 (112.4 mg QE/ 100 g FW) and the minimum recorded in H3P (105.2 mg QE/ 100 g FW). TPC and TFC in this study are satisfactorily higher compared to the phenol and flavonoid content recorded in the works performed by Singh et al. (2019) for winged bean genotypes from India with value ranged from 48.4 to 143.5 mg GAE/100 g FW and 9.1 to 37.0 mg CE/ 100 g FW, respectively. In date, plant phenol and flavonoid metabolites have found significant interest, because of the usefulness in overcoming several human diseases (Vauzour et al. 2010; Ma and Chen 2020). Thus, it is recommended that the winged bean genotypes having high TPC and TFC should be nominated for bred in further development.

The antioxidant activities among 12 selected genotypes were observed, which is seen in Table 1. Antioxidant activity depends on the mechanism of antioxidant metabolites in extract (Mercado-Mercado et al. 2020). Thus, different assays are needed to evaluate natural antioxidant capacity. In this study, FRAP, CUPRAC, DPPH, and TEACH methods are applied to assess the antioxidant capacity of studied winged bean genotypes. Free radical scavenging activity of the winged bean genotypes was evaluated using TEAC and DPPH assays (Mareček et al. 2017), whereas reducing power was performed using CUPRAC and FRAP assays (Suktham et al. 2019). The antioxidant activities among the winged bean genotypes were varied from 66.9 to 170.8 µmol TE/ 100 g FW, as measured by the FRAP method. In the CUPRAC method, the antioxidant activity of studied genotypes ranged from 28.0 to 52.4 µmol TE/ 100 g FW. The genotype H3U was the highest antioxidant capacity, whereas lowest noted in genotype TH based on FRAP and CUPRAC assays. In TEAC assay, the antioxidant capacity of the selected genotypes varied from 140.4 (H1U) to 167.6 (TH) µmol TE/ 100 g FW, whereas DPPH method values ranged between 32.8 (TU) to 47.0 (H1P) µmol TE/ 100 g FW. Genotype H3U, H1P, and TH showed the highest performance for their antioxidant capacities. Thus, these genotypes are recommended in future plant breeding of winged bean based on antioxidant capacities. Antioxidant activity with FRAP and CUPRAC assays showed highest compared with DPPH and TEAC assays in studied winged bean genotypes. These results were linear with formerly reported findings by (Singh et al. (2019) with studied genotypes from India. Therefore, antioxidant activity in winged bean extract dominant showed reducing power than free radical scavenging activity.

Table 2. Biochemical component and antioxidant properties of different winged bean genotypes

Genotypes	TPC	TFC	FRAP	CUPRAC	DPPH	TEAC
	(mg GAE/100 g FW)	(mg QE/100 g FW)	(µmol TE/100 g FW)	(µmol TE/100 g FW)	(µmol TE/100 g FW)	(µmol TE/100 g FW)
H1U	159.2 ± 0.3a	107.5 ± 2.0c	119.4 ± 38.0b	46.3 ± 2.2b	35.5 ± 1.7c	140.4 ± 2.2d
H2	157.2 ± 0.8a	105.6 ± 0.7d	89.9 ± 15.9c	37.7 ± 1.2d	38.4 ± 1.4c	161.6 ± 4.0b
H3P	154.6 ± 0.8b	105.2 ± 0.3d	76.0 ± 15.7c	31.8 ± 1.6e	34.7 ± 0.3c	166.3 ± 0.5a
H1P	154.8 ± 0.0b	105.3 ± 0.7d	75.5 ± 15.5c	34.5 ± 3.7d	**47.0 ± 6.8a**	164.0 ± 1.9b
H3U	158.1 ± 1.8a	107.1 ± 2.1c	**170.8 ± 26.0a**	**52.4 ± 4.0a**	30.6 ± 2.6d	146.5 ± 3.5d
L4	157.9 ± 1.9a	109.5 ± 0.2b	106.0 ± 23.5b	38.5 ± 0.7d	36.3 ± 0.6c	160.5 ± 2.0b
L2	157.9 ± 1.9a	108.6 ± 12.1b	117.4 ± 23.8b	46.5 ± 3.6b	37.2 ± 3.1c	155.0 ± 3.5c
H4P	157.5 ± 0.7a	108.6 ± 0.3b	93.0 ± 10.2c	36.6 ± 4.1d	42.5 ± 1.3b	162.2 ± 1.6b
L1	159.7 ± 2.0a	107.7 ± 1.5c	118.0 ± 18.7b	43.0 ± 2.7c	37.0 ± 1.4c	143.7 ± 0.4d
L3	157.0 ± 0.8a	**112.4 ± 1.1a**	98.8 ± 24.0c	41.3 ± 4.9c	35.8 ± 0.6c	156.4 ± 1.7c
TU	**161.5 ± 4.4a**	110.1 ± 1.2b	113.3 ± 7.1b	48.1 ± 1.9b	32.8 ± 1.2d	143.2 ± 5.3d
TH	155.2 ± 0.8b	105.9 ± 0.5d	66.9 ± 5.4c	28.0 ± 1.2e	37.6 ± 2.2c	**167.6 ± 0.7a**

Note: DPPH, 22-diphenyl picrylhydrazyl; CUPRAC, cupric reducing antioxidant power; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; and TFC, total flavonoid content. The value ± SD of three replicates. Mean value in each column marked with different letters differ significant at $p < 0.05$ by the Scott-Knott test

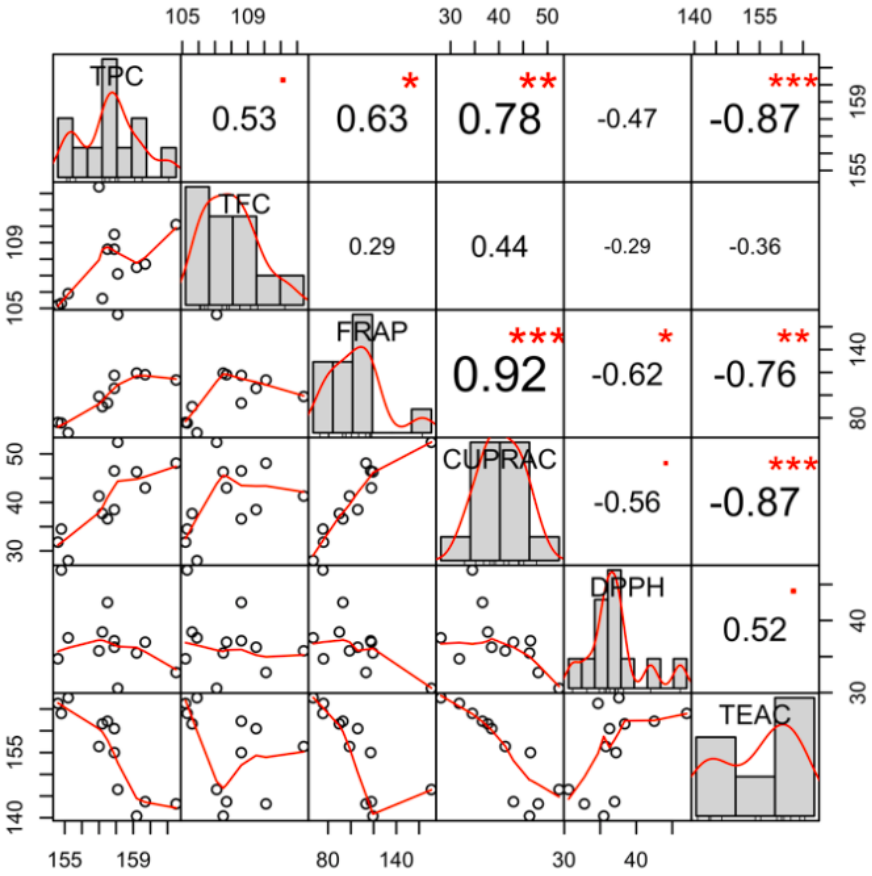

Figure 1. The correlation for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties (DPPH, 22-diphenyl picrylhydrazyl; CUPRAC, cupric reducing antioxidant power; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity). ***, **, *, · showed significant level with *p*-values of 0.001, 0.01, 0.05, and 0.1, respectively. The figure showed the variable on the diagonal, the bivariate scatter plots with a fitted line on the bottom of the diagonal, and the value of the correlation with the significance level on the top of the diagonal

Polyphenol, such as flavonoid and phenolic content, is a metabolite that often associated with the antioxidant capacities in the plant extract (ZHANG et al. 2018; Lim et al. 2019). Moreover, the flavonoid and phenolic acid metabolites have been shown to be the primary compounds responsible for fruit antioxidant capacities (Wang et al. 2018). As shown in Figure 1, for all the studied genotypes, there were correlations between biochemical variables (TPC and TFC) and antioxidant capacities (DPPH, CUPRAC, FRAP, and TEAC). Positive correlation ($p < 0.1$, r2 value of 0.53) were noted between TPC and TFC. In this work, TPC was positive and significantly correlated with the antioxidant capacity of FRAP and CUPRAC assays, whereas negative correlation detected with DPPH and TEAC assays. The result indicated that the antioxidant capacity in winged bean fruit extract might be strongly affected by TPC. This suggests that the phenolic compounds in winged bean were antioxidants of reducing power mechanisms. The lowest positive and negative correlation was detected between TFC and FRAP and CUPRAC assays, and among TFC and DPPH and TEAC assays, respectively. Based on all assays used to evaluate the antioxidant activity, the coefficients of correlation among FRAP/CUPRAC, FRAP/DPPH, and FRAP/TEAC were 0.92, -0.62, and -0.76, respectively. The associations between CUPRAC/DPPH and CUPRAC/TEAC were -0.56 and -0.87, respectively. The correlation between DPPH/TEAC was 0.52. These reports suggest that the antioxidant activity in winged bean fruit can be reliably measured using FRAP and CUPRAC assays than DPPH and TEAC methods.

In summary, the evaluation of the data recorded from this work showed that the antioxidant activities and biochemical characters depended on different winged bean genotypes. The TU, L3, H3U, H1P, and TH genotypes have significantly superior biochemical and antioxidant capacities, which acknowledges us to suggest them for plant breeding and commercial consumption.

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