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Research Article

Correlation of Total Phenolic, Flavonoid and Carotenoid Content of *Sesbania sesban* (L. Merr) Leaves Extract with DPPH Scavenging Activties

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ABSTRACT

Antioxidants are found in many plants and can neutralize free radicals. *Sesbania sesban* (L.)Merr is a plant that has been used empirically by Indonesian people and its proven have many pharmacologycal activity. The objectives of this research were to study antioxidant activity *Sesbania sesban* leaves extract using DPPH (2,2-diphenyl-1-picrylhydrazyl) method and correlation with its total phenolic, flavonoid and carotenoid content. Extraction was performed by reflux using different polarity solvent. The extracts were vaporated using rotary evaporator. Antioxidant activity was tested using DPPH assay. Determination of total phenolic, flavonoid and carotenoid content were performed by UV-visible spectrophotometry and its correlation with inhibitory concentration 50 (IC_{50}) DPPH scavenging activity were analyzed by Pearson's method. N-hexane, ethyl acetate and ethanol extracts of *Sesbania sesban* leaves had IC_{50} DPPH < 50 µg/ml and it can be classified as very strong antioxidant. Ethanol extract of *Sesbania sesban* had the highest of total phenolic content (5.18 g GAE/100 g) and highest total flavonoid content (4.56 g QE/100 g), while the highest total carotenoid content (4.56 g BE/100 g) was given by n-hexane extract. Total phenolic content in *Sesbania sesban* leaves extracts had significant and negative correlation with their IC_{50} DPPH scavenging activities. Phenolic compounds in *Sesbania sesban* leaves extracts were contributor major in its antioxidant activities by DPPH method.

Keywords: Antioxidant, *Sesbania sesban*, flavonoid, phenolic, carotenoid.

INTRODUCTION

Antioxidants are found in many plants and can neutralize free radicals[1]. Antioxidants are compounds that are able to inhibit the negative impact of free radicals in the body[2] that is preventing tissue damage as well as on cellular components in the body as a result of chemical reactions involving free radicals[3].

Sesbania sesban (L.) Merr is a plant that has been used empirically by the people of Indonesia. *Sesbania sesban* is a wild plant, can grow up to 2-6 meters, is widespread in tropical countries, especially in Indonesia. Based on the classification of Cronquist[4], *Sesbania sesban* belong to Magnoliophyta division, Magnoliopsida class, Fabales nation, and Fabaceae family. Pharmacological activity of *Sesbania sesban* leaves were anthelmintic for children, cough medicine[5] and anti - inflammation in rheumatic[6]. *Sesbania sesban* seeds were used to reduce pain during menstruation and inflammation in skin disease[6]. Saponin from *Sesbania sesban* leaves indicated a topical anti-inflammatory activity[7]. *Sesbania sesban* leaves water extract showed potential antidiabetic activity[8]. Phytochemical study of *Sesbania sesban* exposed the presence of polyphenols, saponins and flavonoids[5], cyanidin, delphinidin glucoside[6]. Some researches suggested that total phenolic contnet in plant have a correlation with its antioxidant activity. Plants contain phenolic and polyphenols groups may have antioxidant activity[9,10]. Previous study reported antioxidant of methanol extracts *Sesbania sesban* flowers[11]. Research regarding antioxidant activity of *Sesbania sesban* leaves using different polarity solvents and correlation of total phenolic, flavonoids and carotenoids with their antioxidant activity have not been reported yet.

This study aimed to determine the antioxidant activity of *Sesbania sesban* leaves extract with various polarity solvents using 2,2- diphenyl - 1 - picrylhydrazyl (DPPH) and analyze the correlation of total phenolic, flavonoids and carotenoids content with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH (2,2- diphenyl - 1 picrylhydrazyl), gallic acid, quercetin, beta carotene, ascorbic acid were purchased from Sigma-Aldrich (MO, USA), *Sesbania sesban* leaves, methanol, ethanol, ethyl acetate, n-hexane, and all other analytical materials used in the study.

Sample preparation

Sesbania sesban leaves freshly collected from Ujung Berung- Bandung, West Java - Indonesia on August 2013, determined in the Herbarium Bandungense, School of Life

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Sciences and Technology, Bandung Institute of Technology. Leaves are sorted, washed, dried at 40°C - 45°C and grinded into powder.

Extraction

Sesbania sesban leaves crude drug was extracted by reflux, using increasing polarity solvents which were n-hexane, ethyl acetate and ethanol. Crude drug was extracted using n-hexane triplicate, then the residue extracted using ethyl acetate triplicate, and the end the residue extracted using ethanol triplicate. Each extract was concentrated by rotary evaporator and resulted thick n-hexane extract (E1), ethyl acetate extract (E2) and ethanol extract (E3).

Antioxidant activity using DPPH method

Antioxidant activity was adopted from Blois's method[12]. Sample was prepared in various concentration, then added with DPPH solution 50 ug/ml (volume 1:1). After incubation for 30 minutes, absorbance was measured at λ 517 nm using a UV-visible spectrophotometry. Methanol is used as a blank, DPPH solution 50 µg/ml as control and ascorbic acid as standard. Antioxidant activity was determined triplicate for standard and each extract. Antioxidant activity was measured as percentage of decreasing in DPPH absorbance of sample, which presented by changing in color of DPPH. The color change is caused the presence of antioxidant in each extract by donating hydrogen atom to DPPH[13]. IC_{50} of DPPH was calculated using a calibration curve obtained from the antioxidant activity of each extract at various concentration.

Determination of total phenolic content

Determination of total phenolic content was conducted using Folin - Ciocalteu reagent[2]. Each extract was dissolved in methanol. The presence of phenolic groups are indicated by changing to blue color, and absorbance was measured at λ 765 nm. Analysis was performed tripicate for each extract. Gallic acid solution was used as standard and prepared in various concentration 60-150 µg/ml to be a curve standard[2]. Total phenolic content was calculated using the linear regression equation of the calibration curve and expressed as gallic acid equivalent per 100 grams of extract (g GAE/100 g).

Determination of total flavonoid content

Determination of total flavonoid content was carried out by UV - Vis spectrophotometer using adopted method from Chang[14]. Each extract was dissolved in methanol. Changing in color solution was measured at λ 415 nm. Analysis was conducted triplicate for each extract. Quercetin solution was used as standard and various concentration (40-160 µg/ml) of quercetin prepared for making a standard curve. Total flavonoid content was calculated using the linear regression equation of the calibration curve and presented as quercetin equivalent per 100 grams extract (g QE/100 g).

Determination of total carotenoid content

Determination of total carotenoid was adopted from Thaipong[15]. Each extract diluted in n-hexane. The absorbance was measured at wavelength of 470 nm. Analysis was carried out triplicate for each extract. Beta carotene is used as standard in various concentration of 10-40 mg/ml to obtain standard curve. Total carotenoid content was evaluated using the linear regression equation of the calibration curve and exposed as beta-carotene equivalent per 100 grams of extract (g BE/100 g).

Statistical analysis

Each sample analysis was performed in triplicate. All of presented results are means (± standard deviation) of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc LSD procedure was done with SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, carotenoid content and antioxidant activities were conducted using the Pearson's method[10].

RESULTS

The percentage of DPPH scavenging activity of ethanol extract of *Sesbania seban* leaves (E3) at a concentration of 50 µg/ml was 52.87%, ethyl acetate extract (E2) 52.58% and n-hexane extract (E1) 51.31%. IC_{50} of DPPH scavenging activities of E1, E2 and E3 could be seen in Fig 1.

Total phenolic content

Total phenolic content in E1, E2 and E3 varied from 1.20 to 5.18 g GAE/100 g and could be seen in Fig 2. The total phenolic content in different polarity extracts from *Sesbania seban* leaves was obtained by using standard curve equation of gallic acid, $y = 0.0027x + 0.138$, $R^2 = 0.9913$.

Total flavonoid content

Total flavonoid content in E1, E2 and E3 in the range of 1.61 - 3.22 g QE/100 g (Fig 3). The total flavonoid content in different polarity extracts from *Sesbania seban* leaves was calculated using standard curve equation of quercetin, $y = 0.007x-0.0311$, $R^2 = 0.9984$.

Total carotenoids content

Total carotenoid content in E1, E2 and E3 ranged from 0.75 to 4.51 g BE/100 g (Fig 4). The total carotenoid content in different polarity extracts from *Sesbania seban* leaves was determined using standard curve equation of beta-carotene, $y=0.0217+0.0049$, $R^2 = 0.9873$.

Pearson correlation coefficient of total phenolic, flavonoid, carotenoid content of Sesbania sesban leaves extracts with their antioxidant activities

Correlation of total phenolic, flavonoid and carotenoid with their IC_{50} of DPPH scavenging activities was represented by Pearson's correlation coefficient. The Pearson's correlation coefficient of total phenolic, flavonoid and carotenoid content with their IC_{50} of DPPH scavenging activities were $r = -0.943$, $p < 0.01$; $r = -0.396$, $p< 0.291$; $r = 0.871$, $p < 0.01$), respectively.

DISCUSSION

The antioxidant potential of the genus Sesbania had been reported from some of the earlier researches[11,16,17]. The antioxidant activity of ethanol leaves extract of *Sesbania seban* had been reported by Mani[18], while the antioxidant properties from different polarity extracts (n-hexane, ethyl acetate and ethanol) of *Sesbania seban* leaves have not been reported yet. DPPH is stable free radical and sensitive to determine the antioxidant activity of plants[19]. DPPH scavenging activities of compound that act as antioxidant

in the extract was shown by changing of DPPH solution color [20,21].

The percentage of DPPH scavenging activities of E1, E2 and E3 50 µg/ml ranged from 52.87% to 52.31%. This result suggested that antioxidant compound in E1, E2 and E3 was able to scavenge the free radical DPPH. The previous research by Katiresh[1] showed that DPPH of scavenging activities of methanol extract of *Sesbania sesban* flower was 37.09% and the ethanol extract of *Sesbania sesban* leaves had antioxidant activity by scavenging free radical[18]. In the same genus, Ouattara[16] denoted that the methanol extracts of leaves and steam bark of *Sesbania grandiflora* had very strong antioxidant activity with an IC_{50} of DPPH scavenging activities 40 and 24 µg/ml, respectively. Previous study[17] stated that various concentrations (1.43-4.29 mg/ml) of 50% of ethanol extract of *Sesbania grandiflora* leaves had DPPH scavenging activities 18.03%-53.12% and various concentrations (2.85-0.95 mg/ml) of 70% acetone extract of *Sesbania grandiflora* leaves had DPPH scavenging activities 23.5% – 65.0%. The potential of antioxidant can be shown with IC_{50} values. IC_{50} is the concentration of samples that can scavenge 50% of free radical DPPH activity. The highest antioxidant activity is indicated by the lowest value of IC_{50}. IC_{50} of DPPH scavenging capacities of E1, E2 and E3 were compared to IC_{50} of DPPH of ascorbic acid standard.

E3 had the highest antioxidant activity compared to E1 and E2, while E1 had the lowest antioxidant activity. According to Blois[12], sample which had IC_{50} lower than 50 µg/ml was a very strong antioxidant. The IC_{50} DPPH values of E1, E2 and E3 ranged from 4.12 to 8.36 µg/ml, while IC_{50} DPPH of ascorbic acid was 1.39 µg/ml. It showed that E1, E2 and E3 can be categorized as very strong antioxidant.

The extract has antioxidant activity may be suspected of containing the compound capable of donating hydrogen on free radicals. Hydrogen donors may be groups of phenolic[22] compounds and flavonoids[23]. Acidic compound contribute to the high antioxidant activity of cinnamic acid and benzoic acid[10,24]. Cinnamic acid had higher antioxidant activity than benzoic acid[10,24]. Determination of total phenolic content in E1, E2 and E3 varied from 1.20 to 5.18 g GAE/100 g. It showed that E1, E2 and E3 consisted of phenolic compounds. Total phenolic content was calculated using a standard curve equation of gallic acid $y = 0.0027x + 0.138$, $R^2 = 0.9913$. Gallic acid standard curve was obtained from various concentrations of gallic acid solution. The highest total phenolic content 5.18 % was given by E3. Several studies demonstrated that genus Sesbania has different result in total phenolic content. Quattara[16] reported that the total phenolic content in methanol extract of leaves and steam of *Sesbania grandiflora* were very high when compared to its root and fruit. Previous research presented that methanol extract of *Sesbania rostrata* leaves had total phenolic content 46.33 mg GAE/100 mg[25]. Study by Shymala[17], total phenolic content in ethanol and acetone extracts of *Sesbania grandiflora* leaves were 3.01% and 3.06%, respectively, while Kalpana[26] found that total phenolic content in 70 % acetone extract of *Sesbania grandiflora* flower was 49.1 µg/mg.

Total flavonoid content in E1, E2 and E3 represented as quercetin equivalent per 100 grams extract (QE g/100 g). The results revealed that the highest total flavonoid content (3.22%) was shown by E3. Total flavonoid content was measured by the presence of quercetin after adding $AlCl_3$ reagent. $AlCl_3$ will form a complex with the OH of flavonoid compounds at C3'-C4', and or OH at C-3 and 4 oxo, and or OH at C-5 and 4 oxo. Testing method using $AlCl_3$ is still limited, and it will show that the antioxidant activity of flavonoids only derived by certain groups[26]. Determination of total flavonoid content in Sesbania genus showed that 70 % acetone extract of *Sesbania grandiflora* flower had total flavonoid content 12.86 µg/mg[26]. Methanol extract of *Sesbania grandiflora* fruit had the highest total flavonoid content compared to the leaves, stems and roots of *Sesbania grandiflora*[16], as well as in methanol extract of *Sesbania rostrata* fruit had higher total flavonoid content than the methanol extract of leaves, stems and root of *Sesbania rostrata*[25]. *Sesbania grandiflora* can be identified to have high levels of quercetin, kaemferol and myricetin[27].

The value of total phenolic content, total flavonoids content and carotenoids content can be correlated with the total antioxidant activity which was represented by the IC_{50} of DPPH, and analyze by Pearson's correlation coefficient (R). If r value of $0.61 \leq r \leq 0.97$[15] its mean the high positive correlation and if the value of $-0.61 \leq r \leq -0.97$[23] showed high negative correlation. Total phenolic content in *Sesbania sesban* leaves extract had significant and negative correlation with IC_{50} of DPPH ($r = -0.943$, $p < 0.01$). Increasing in total phenolic content will give the higher antioxidant activity which show by the lower IC_{50} of DPPH value. It might be supposed that phenolic compounds in *Sesbania sesban* leaves extract were the major contributor in its antioxidant activity by DPPH method.

The Pearson's correlation coefficient between total flavonoid content of *Sesbania sesban* leaves extract with IC_{50} scavenging of DPPH gave no significant correlation ($r = -0.396$, $p < 0.291$). It means total flavonoid content of *Sesbania sesban* leaves extract have no correlation with IC_{50} of DPPH. It showed that flavonoid compounds in *Sesbania seban* leaves extract had no influence in antioxidant activity by DPPH method. Flavonoid had a role in antioxidant activity of extract. Position OH and double bonds in flavonoid were determined to give improving in antioxidant activity. OH at C-3 and double bonds between C-2 and C-3 will increase the antioxidant activity in extract[28,29]. OH orthoposition at C-3' and C-4' and oxo functional group in position C-4 will provide the highest impact in improving the antioxidant activity[24]. Correlation of total phenolic content and total flavonoid content in *Sesbania sesban* leaves extract with their antioxidant activities by Pearson's method had not been widely reported yet. However, research which was conducted by Katiresh[1] stated that anthocyanins (belong to phenolic compounds) in methanol extract of *Sebania sesban* flower had very strong antioxidant activity and

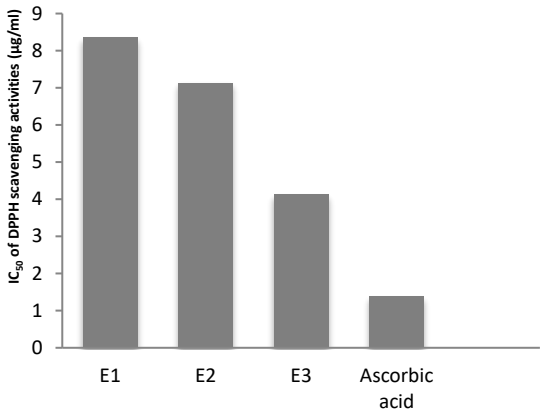

Figure 1: IC_{50} of DPPH scavenging activities in various *Sesbania sesban* leaves extract.

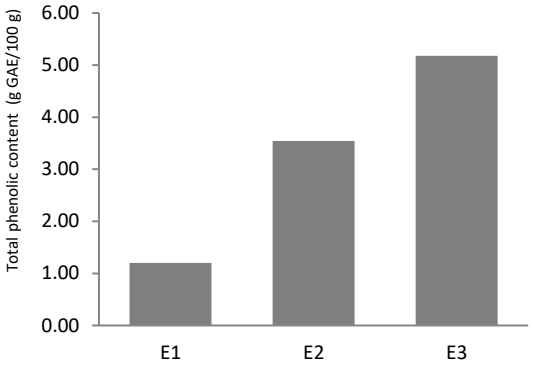

Figure 2: Total phenolic content in various extracts of *Sesbania sesban* leaves.

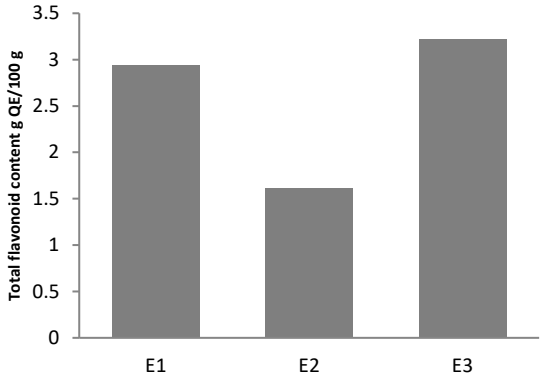

Figure 3: Total flavonoid in various extracts of *Sesbania sesban* leaves.

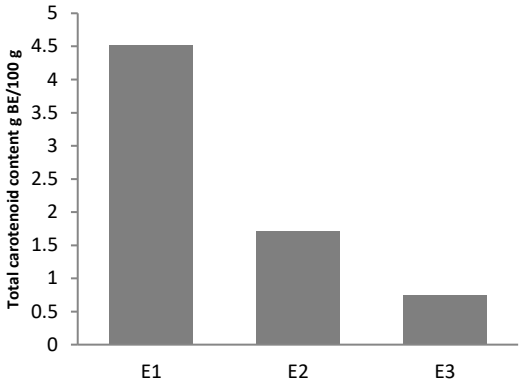

Figure 4: Total carotenoid content in various extracts of *Sesbania sesban* leaves.

highly efficient to scavenge free radical DPPH. Quattara[16] denoted that *Sesbania grandiflora* flower stalks and leaves extracts showed antioxidant activity by DPPH and FRAP methods which related to high value of phenolic content and flavonoid content. Methanol extract of leaves *Sesbania rostrata* had polyphenolic content 46.33 mg GAE/100 mg and tannin 25.89 mg TAE/100 mg which contributed in providing antioxidant activity using DPPH and reduction of Fe^{3+} methods[25]. Total flavonoid and total phenolic content in the same family (Legumes/Fabaceae) have been reported by Fidrianny[23], which stated that ethyl acetate extract of *Phaseolus radiatus* leaves had high total flavonoid content and ethanol extract of *Phaseolus radiatus* leaves have high phenolic content.

The highest total carotenoid content was indicated by E1 (4.512 g BE/100 g), while the smallest value given by E3 (0.75 g BE/100 g). The specific of carotenoid is strong yellow-orange color. The strong color is exposed by conjugated double bonds in carotenoid compounds. E1 gave stronger orange-yellow color than E2 and E3. Astaxanthin and beta-carotene are often used as a standard in total carotenoid content[30,31]. Beta-carotene can be an effective antioxidant in human health by interfering the chain reaction of free radicals[32,33]. Conjugated double bonds in the beta-carotene make it very effective as an antioxidant[32,34,35] therefore beta-carotene was used as a standard. Total carotenoid content in *Sesbania sesban* leaves extracts had significant and positive correlation with their IC_{50} DPPH ($r = 0.871$, $p < 0.01$), which showed the higher total carotenoid content will give the higher IC_{50} of DPPH, that means the lower antioxidant activity. Carotenoid compounds are potentially powerful of antioxidant activity that are like zeaxanthin, astaxanthin and astaxanthin-β-glucoside, beta-carotene and α-tocopherol. It can be suggested that carotenoid compounds in *Sebania sesban* leaves extract had low antioxidant activity. Previous research[23] revealed that total carotenoid content of n-hexane, ethyl acetate and ethanol extract of three species belong to Legumes *Phaseolus radiatus*, *Glycine max*, and *Arachis hypogaea* had high total carotenoid content in the range of 0.14 - 18.42 g BE/100 g. The highest total carotenoid content was shown in n-hexane extract of *Glycine max* 18.42 g BE/100 g. It similar to n-hexane, ethyl acetate and ethanol peel extracts of four species of Legumes that where *Glycine max, Phaseolus. vulgaris, Vigna subterranea and Arachis hypogaea* had high total carotenoid content varied from 0.026 to 0.33 g BE/100 g. The highest total carotenoid content was found in n-hexane peel extract of *Glycine max* 0.33 g BE/100 g[28].

CONCLUSION

All of *Sesbania sesban* leaves extracts with different polarity solvents (n-hexane extract, ethyl acetate extract

and ethanol extract) can be categorized as very strong antioxidant. The highest total phenolic and total flavonoid content was showed by ethanol extract whereas the highest total carotenoid content was given by n-hexane extract. Total phenolic content in *Sesbania sesban* leaves extracts had significant and negative correlation with their IC_{50} of DPPH scavenging activities. *Sesbania sesban* leaves are potentially to develop as source natural antioxidant.

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