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FTIR-Based Metabolomics for Characterization of Antioxidant Activity of Different Parts of *Sesbania grandiflora* Plant

(Metabolomik Berasaskan FTIR untuk Pencirian Aktiviti Antioksidan Bahagian Berbeza Tumbuhan *Sesbania grandiflora*)

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ABSTRACT

Sesbania grandiflora, one of the flowering plants with great potential as a source of natural antioxidants because it contains chemicals such as tannin, phenolics, and flavonoids. However, there has been no extensive investigation on the antioxidant activity of isolated from different parts of this plant. This study aims to investigate the correlation between antioxidant activity and secondary metabolites extracted from three different parts (leaves, stem barks, and roots) of *S. grandiflora* plant using Fourier-transform infrared spectroscopy (FTIR) based metabolomics approach. The FTIR is a very useful technique for identifying the functional groups present in the mixtures, while antioxidant assay provides the base to select the part of the plant as the most potential source of antioxidant. The antioxidant properties were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), and potassium ferricyanide reduction method. The multivariate data - analyses using Principal Component Analysis (PCA) and Partial Least Square (PLS) were conducted to compare the distribution of metabolites extracted from different parts of the *S. grandiflora* plant investigated. The PLS was performed to evaluate the relationship between the components of the extracts obtained from different parts of the plant and their antioxidant activities. The results exhibited that antioxidant activities of the extract of the stem barks, and roots are higher than that of the extract of the leaves. Also, the PLS model indicated that the functional group absorption data were significantly correlated with the IC_{50} values of antioxidant activity. Subsequently, based on the results of PLS analysis displayed that C=C, C=O, and along with C-O functional groups are proposed as the main contributors to the antioxidant activity of the extracts tested. The extracts of different parts were grouped using PCA analysis with a total of principal components (PC) of 94%.

Keywords: Antioxidant property; metabolomics approach; secondary metabolites; *Sesbania grandiflora*

ABSTRAK

Sesbania grandiflora, salah satu tumbuhan berbunga yang berpotensi besar sebagai sumber antioksidan semula jadi kerana mengandungi bahan kimia seperti tanin, fenol dan flavonoid. Walau bagaimanapun, tiada kajian meluas mengenai aktiviti antioksidan terpencil daripada bahagian berlainan tumbuhan ini. Penyelidikan ini bertujuan untuk mengkaji korelasi antara aktiviti antioksidan dan metabolit sekunder yang diekstrak daripada tiga bahagian berbeza

(daun, kulit batang dan akar) tumbuhan *S. grandiflora* menggunakan pendekatan metabolomik berasaskan spektroskopi transformasi Fourier inframerah (FTIR). FTIR ialah teknik yang sangat berguna untuk mengenal pasti kumpulan berfungsi yang terdapat dalam campuran, manakala ujian antioksidan menyediakan asas untuk memilih bahagian tumbuhan sebagai sumber antioksidan yang paling berpotensi. Sifat antioksidan telah ditentukan menggunakan 2,2-difenil-1-picrilhidrazil (DPPH), asid 2,2'-azino-bis-3-etilbenztiazolina-6-sulfonik (ABTS) dan kaedah pengurangan kalium feriksianida. Data multivariat - Analisis menggunakan Analisis Komponen Utama (PCA) dan Kuasa Dua Terkecil Separa (PLS) telah dijalankan untuk membandingkan taburan metabolit yang diekstrak daripada bahagian berlainan tumbuhan *S. grandiflora* yang dikaji. PLS dijalankan untuk menilai hubungan antara komponen ekstrak yang diperoleh daripada bahagian tumbuhan yang berlainan dan aktiviti antioksidannya. Keputusan menunjukkan bahawa aktiviti antioksidan ekstrak kulit batang dan akar adalah lebih tinggi daripada ekstrak daun. Juga, model PLS menunjukkan bahawa data penyerapan kumpulan berfungsi secara signifikan berkorelasi dengan nilai IC_{50} aktiviti antioksidan. Selepas itu, berdasarkan keputusan analisis PLS menunjukkan bahawa C=C, C=O dan bersama-sama dengan kumpulan berfungsi C-O dicadangkan sebagai penyumbang utama kepada aktiviti antioksidan ekstrak yang diuji. Ekstrak bahagian yang berbeza dikumpulkan menggunakan analisis PCA dengan jumlah komponen utama (PC) sebanyak 94%.

Kata kunci: Metabolit sekunder; pendekatan metabolomik; *Sesbania grandiflora*; sifat antioksidan

Introduction

Medicinal plants are still the primary alternative sources in discovering and developing new medicines to prevent or cure various diseases. More recent studies showed that some diseases, particularly degenerative diseases, including neurodegenerative disorders, inflammatory, diabetes, cancer, cardiovascular, Alzheimer's, and Parkinson's disease exhibited a significant correlation with antioxidant agents (Gülçin 2020; Huang, Zhang & Chen 2016; Manna & Jain 2015; Nani et al. 2021). Antioxidants are substances that can neutralize the harmful effects of free radical species (reactive and unstable) in the body. Free radicals can be produced through biochemical processes in the body or due to external cause, such as ultraviolet radiation, thereby increasing unsaturated lipid peroxidation associated with degenerative diseases. Therefore, antioxidant compounds are essential to inhibit the redox reactions of free radicals and unsaturated lipids (Coulibaly et al. 2014).

The utilization of natural antioxidants from medicinal plants for reducing the risk of oxidative stress-induced neurological diseases continues to gain growing interest since many secondary metabolites such as terpenoids, coumarins, quinones, lignans, alkaloids, and phenolics, are known to possess antioxidant activity (Greenwell & Rahman 2015; He & Yan 2013; Lister et al. 2020; Rodríguez-García et al. 2019). Another study showed that phenolic compounds, including simple phenols, tannin, flavonoids, phenolic acids, and anthocyanins, have encouraging activities as antioxidant, anti-inflammatory, antibacterial, and antidiabetic (Petlevski et al. 2013).

One of the plants with promising potential as a source of antioxidants is *S. grandiflora* (local name: *turi*). The phytopharmacological study on *S. grandiflora* has been published in previous study (Noviany et al. 2021). Previous workers also reported antituberculosis activity of nine flavonoids and new natural phenolic compounds isolated from roots of *S. grandiflora* (Hasan et al. 2012; Noviany et al. 2020a, 2012). Moreover, biological properties of some 2-arylbenzofurans and its derivatives isolated from the stem bark of *S. grandiflora* have been reported in previous investigations (Noviany et al. 2021, 2020b, 2018). As we know, certain biological activities are associated with bioactive compounds in medicinal plants. The composition and amount of these bioactive compounds depend on several factors such as the geographic location, the parts of the plant, and extraction method (Skrovankova et al. 2015). Taking into account the possible diversity of bioactive compounds in different parts of the plant, in this study, bioactive compounds were extracted from three parts (leaves, stem barks, and roots) of the *S. grandiflora* plant, followed by antioxidant activity test of the extracts. This study was conducted since to the best of our knowledged based on the literature search, there has been no extensive research on the antioxidant potential of *S. grandiflora*. Therefore, in the present study, we investigated whether different parts of *S. grandiflora* plant possessed the potential as a valuable source of antioxidants as an effective free-radical inhibitor. Furthermore, the relationship between

antioxidant activity and secondary metabolites from different parts of the plant was also examined using multivariate data analyses.

Metabolomics is a comprehensive approach used to analyze metabolites in a sample at a certain point in time both qualitatively and quantitatively through targeting fingerprinting, and profiling analysis (Re et al. 1999). Metabolomic approaches such as fingerprinting and profiling can be used to find a correlation between the composition and concentration of the metabolites and their biological activity (Cambiaghi, Ferrario & Masseroli 2017; Umar et al. 2021). Several workers have reported application of FTIR-based fingerprinting with chemometric analysis to study the correlation between metabolite profiles and antioxidant activity of several plant samples, such as *Orthoshiphon aristatus* (Adámez et al. 2012), *Phaleria macrocarpa* (Easmin et al. 2017), and *Syzygium polyanthum* (Rohaeti, Karunina & Rafi 2021).

Materials and Methods

Potassium bromide (KBr)-type IR spectra were performed using FTIR SHIMADZU spectrophotometer (Japan), and UV spectra were displayed using Agilent Cary 100.

PLANT MATERIALS

Sample collections

Samples of *S. grandiflora* plant were collected in March 2019 from Gisting, 5th under block, Kec. Gisting Village, Kab. Tanggamus. Lampung Province, Indonesia. The leaves, stem barks, and roots of the plant were kept separately. The plant specimens (NV6/NRGD/2019) were identified at the Herbarium Bogoriense, LIPI Bogor, Indonesia.

Extraction process

Fresh samples (leaves, stem barks and roots) were chopped and cleaned by rinsing under running tap water. The samples were dried in an open space for three weeks and then ground into powder. The extraction was done according to the methods described in our previous studies with minor modification (Noviany et al. 2018; Nurmaida et al. 2018). Each sample was macerated with methanol as a solvent in the ratio of 1: 5 for 24 h. The extract was filtered, and then the solvent was removed under reduced pressure using a rotary evaporator at 40 °C. In this study, the masses of leaves, stem barks, and roots used for extraction were 100, 200, and 50 g, respectively, and the masses of the crude extract from each of the samples were 80, 70, and 10 g, respectively. The crude extracts were analyzed by FTIR spectroscopy and assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays.

FTIR analysis

Secondary metabolites fingerprinting was performed with an FTIR spectrophotometer (SHIMADZU-Japan). The measurement of FTIR spectroscopy was referred to our previous paper (Umar et al. 2021). The sample was provided by mixing the crude extract with potassium bromide and made into a pellet using a hydraulic press. FTIR spectrum was recorded by scanning the sample in the regions from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. The spectra were processed by OPUS ver. 4.2 software (Bruker, Germany) and Spectragryph v1.2.11. Five replicate measurement was carried out for all samples. All analyses were measured using five biological replicates.

DPPH Radical Scavenging Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of the extracts was carried out to study the radical scavenging effect of plant extracts following the method described by Blois (1958) and Braca et al. (2001). Briefly, the DPPH stock solution was prepared by carefully weighing 0.0039 g DPPH then dissolved in 25 mL methanol, and the stock solutions of the extracts were made at a concentration of 1000 µg/mL. Ascorbic acid and methanol were used as a positive and negative control, respectively. DPPH solution was dispensed into a test tube (1 mL/tube), and 1 mL of the tested extracts with the concentrations of 10, 25, 50, 125, 250 µg/mL were immediately introduced. The reaction mixture was shaken well and incubated at ambient temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. DPPH radical scavenging activity of the extracts was expressed as IC_{50} (the sample concentration required to inhibit 50% of the DPPH concentration). The values of IC_{50} were calculated using linear regression of plots.

ABTS Method

2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) assay of the extracts was performed to evaluate the radical scavenging activity according to Re et al. (1999) with minor modification. The ABTS stock solution was prepared and stored in the dark at room temperature

for 16 h. Before assay, this solution was diluted with in methanol (about 1:1 v/v) and homogenized. The ABTS radical scavenging activity was measured by adding 1 mL of different extracts in concentrations series (10, 25, 50, 125, 250 µg/mL), and after 15 min, the absorbance of the mixtures was read at 750 nm. Ascorbic acid was used as a positive control.

FRAP Method

The determination of reducing power of the extracts was conducted based on antioxidant ability to form a colored complex with potassium ferricyanide, trichloroacetic acid (TCA), and ferric chloride ($FeCl_3$). It was measured by the method as described by Jayaprakasha, Singh and Sakariah (2001) with minor modification. The tested extracts were added at different concentrations (10, 25, 50, 125, 250) µg/mL to 1 mL of phosphate buffer (0.2 N, pH 6.6) and 1 mL of potassium ferricyanide (1%), then the mixtures were incubated at 50 °C for 20 min. After 20 min of incubation, 1 mL of TCA (10%) was added, and the mixture was shaken well. The upper layer of the solution (1 mL) was added with distilled water (1 mL) and 0.1% $FeCl_3$ (0.4 mL), followed by measuring the absorbance at 681 nm.

Data Analysis

The multivariate data analyses, including principal component analysis (PCA) and partial least square discriminant, were performed with *The Unscrambler X* version 10.1 (CAMO, Norwegia). The formation of a prediction model of antioxidant activity was carried out by involving the abscissa, which represented the FTIR measurement results and the ordinate of the analysis data, which are the average percentages of scavenging capacity from five replicates (Rohaeti, Karunina & Rafi 2021).

Results and Discussion

FTIR Fingerprinting of *S. grandiflora* Samples

Evaluation of metabolite composition extracted from different parts of *S. grandiflora*, plant (leaves, stem barks, and roots extracts) was carried out using FTIR spectroscopy in the range 4000 to 400 cm^{-1} with the main purpose to the overall functional groups of metabolites found in the samples.

Five FTIR spectra for each sample were recorded, and from the average FTIR absorption spectra of all extracts (Figure 1), we could see a similar pattern among

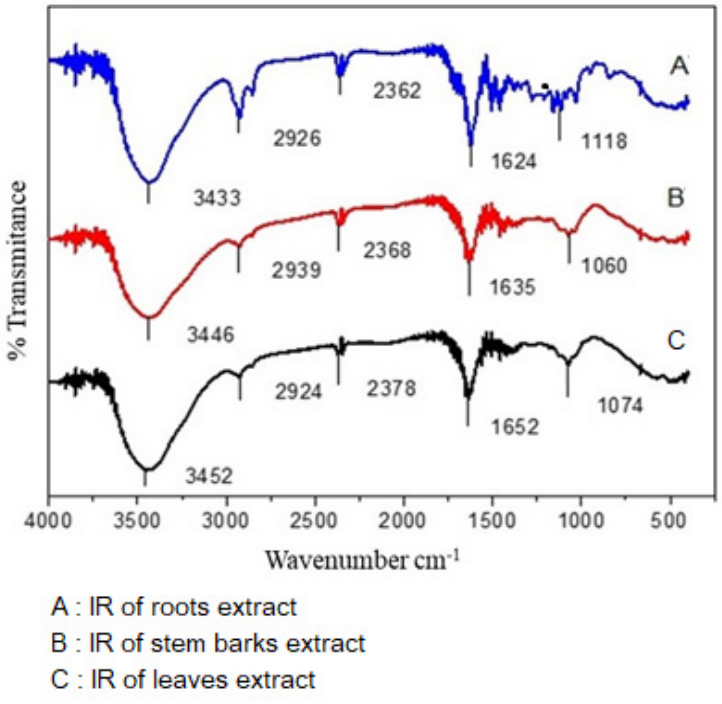


FIGURE 1. FTIR spectrum of three different extracts of *S. grandiflora*

the three different extracts. The results indicated that generally, the profile of metabolites containing in the leaves, stem barks, and roots is quite similar, with no significant differences in the absorption profile, all spectra are characterized by the presence of absorption bands at 3650-3200; 3000-2850; 1680-1600; and 1680-1630; as well as 1027-1070 cm^{-1} that represented for hydroxyl (O-H), saturated carbon (C-H), olefin (C=C), carbonyl (C=O) and ether (C-O) groups, respectively (Pavia, Lampman & Kriz 2010)(Table 1).

TABLE 1. List of wavenumbers of various bond types

Wavenumbers (ν, cm^{-1})	Types of bond	Types of vibration
3650-3000	O-H	bend
3000-2850	C-H	strecth
1680-1600	C=C	strecth
1680-1630	C=O (amide)	strecth
1027-1070	C-O	strecth

Despite the similarity in the FTIR spectra patterns, it was also observed a slight difference in the % transmittance values among the three different extracts indicating the slightly differences in the level of secondary metabolites concentration. The difference in the concentration of secondary metabolites contained in each parts may affect the plant's antioxidant property.

A series of multivariate analyses were applied to cluster the leaves, stem barks, and roots extract based on the FTIR spectra. *The Unscrambler X* version 10.1 (CAMO, Norwegia) was applied to assess the overall variation of the metabolite levels. Principal component analysis (PCA) was employed to cluster the samples. This statistical method is useful to explain the data set variance and grouping the samples based on their similarity. The PCA model was generated using absorbance value in the range of 400-1800 cm^{-1}. We used two principal components (PC1 and PC2) from the PCA, with the total variant obtained was 97% for grouping the parts of the plant. PC1 and PC2 values exhibited 92% and 5% of the variances, respectively. In other words, from the PCA analysis, there is 97% of the data variance can be explained by the model (Figure 2).

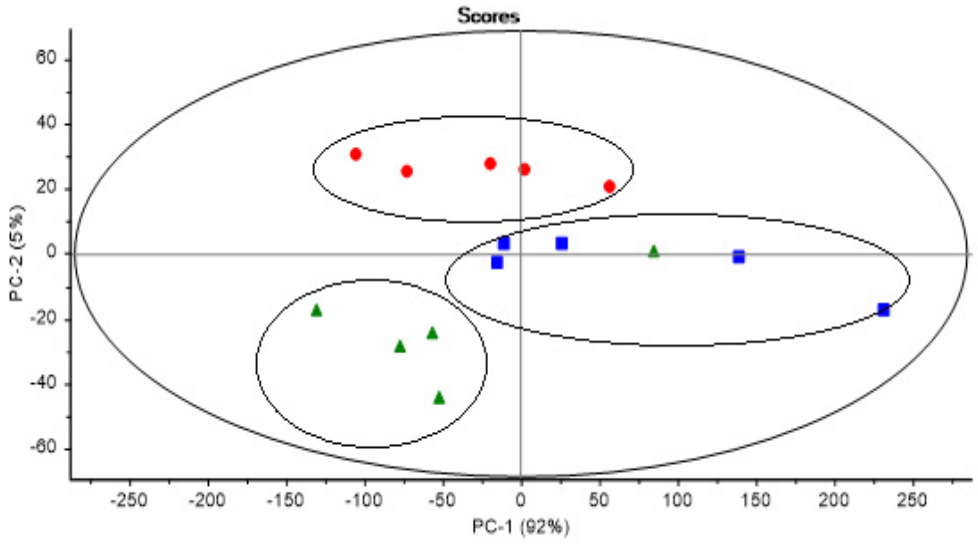


FIGURE 2. PCA analysis of three different extracts of. *S. grandiflora* (leaves: **blue**; bark: **red**; roots: **green**)

The PCA score plot displayed that each part of the leaves, bark, and roots of *S. grandiflora* can be distinguished and divided into three groups. The closer the distance between the plots on each part *S. grandiflora*, the more similar the secondary metabolite content of the fraction. Meanwhile, the other parts of *S. grandiflora* root were close together, which indicated the similarities in secondary metabolites. Furthermore, the results of the plot of the leaves and stem bark scores were clustered but still not so close, and it was indicated that there were slight differences in secondary metabolites. However, there was still an unclassified fraction (Code: AT3 fraction). It could be due to differences between parts from the same plant regarding their amount and type of secondary metabolites (Figure 2).

THE CORRELATION OF FTIR SPECTRUM WITH ANTIOXIDANT ACTIVITY

The correlation between FTIR data and antioxidants can explain the metabolite content and antioxidant activity by evaluating the data using a partial least square (PLS) chemometric model. PLS can predict the response variable from a large number of predictor variables. The formation of the PLS model analysis was carried out by inputting two sets of data. In contrast, the absorbance value data variable was used as the independent variable, while the antioxidant IC_{50} value (Y) and the FTIR absorbance data (X) as the response variables. PLS analysis results generated several plots that contain the X-Y relation score plot and the regression coefficient plot (Figure 3). The results of this analysis indicated a plot showing the existence of a grouping between the extracts of plant parts that play an active and less active role in antioxidant bioactivity. The plot showing the absorption of functional groups active on antioxidant activity is negative, while the less active functional groups are in the positive area. This occurs due to the high antioxidant activity had a low IC_{50} value.

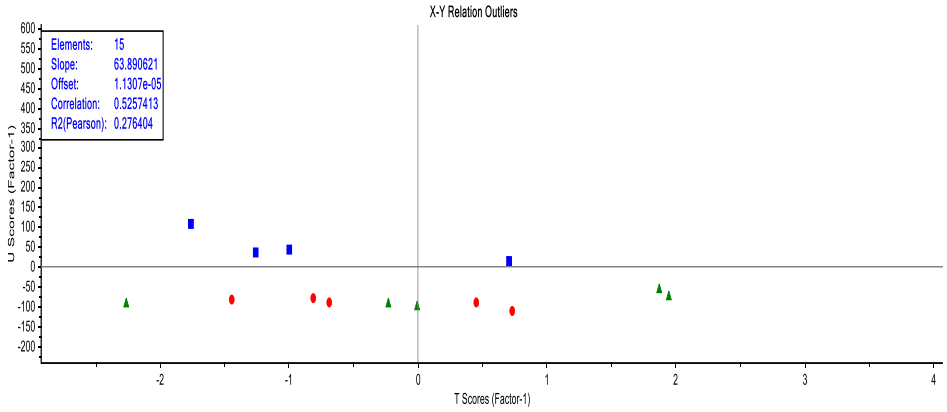
In the PLS X-Y relation analysis carried out on the DPPH antioxidant test and the IR spectrum, it can be seen that the correlation value is 0.52 (Figure 3(A)). Because the calculation uses IC_{50}, the negative value on the graph can be interpreted that the parts of *S. grandiflora* has strong antioxidant. In the PLS X-Y relation graph, it can be concluded that the stem barks and roots extracts of *S. grandiflora* showed strong antioxidant activity in which three plots of each part of the plant are in the negative area of the U and T scores. The PLS X-Y relation analysis observed on the ABTS antioxidant test and its IR spectrum displayed that the plot is still random and not clustered with a correlation value of 0.41 (Figure 3(B)). Even the PLS analysis on the ABTS antioxidant assay is different with the DPPH method, but the stem barks and roots are still showing antioxidant activity. While the PLS X-Y relation analysis of the FRAP method is similar with the DPPH method with the correlation value of 0.52 (Figure 3(C)). Therefore, the stem barks and roots of *S. grandiflora* have active antioxidant property. Overall, the results of this analysis indicated that the stem barks and roots of *S. grandiflora* displaying the existence of a grouping an active role in antioxidant bioactivity, in contrast the leaves part of the plant showing the less active.

Metabolites that play a role in antioxidant activity can be identified from their functional groups by observing the plot of regression coefficient in the PLS analysis. The regression coefficient plot shows the value of the regression coefficient, which is the magnitude of the influence of each independent variable on the response variable. The regression coefficients of the methanol extract of *S. grandiflora* leaves can be observed in Figure 1. According to Guo et al. (2017), the absorption of functional groups that play an active role in antioxidant activity is expressed by the IC_{50} value and has a negative regression coefficient value.

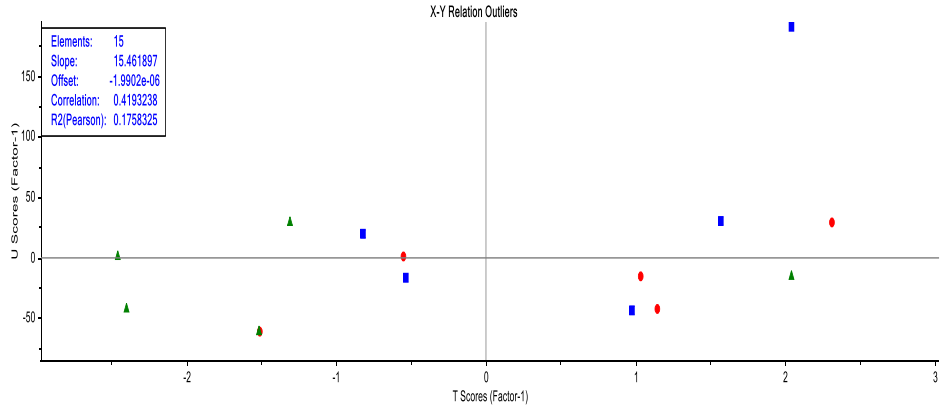
From the PLS X-Y relation analysis on three antioxidant test methods (Figure 3), it was observed that the stem bark and roots were located in the negative area on the U and T scores. This observation indicated that roots and stem bark might play an active role in their antioxidant activity. Besides that, among the three antioxidant methods applied, the best correlation between FTIR data and the antioxidant activity was obtained from DPPH and FRAP evaluation, which showed the same correlation value of 0.52.

The PLS analysis results can also be used to predict the functional groups that contributed most to the antioxidant activity. A negative coefficient regression value was indicated as the most responsible functional group that may play an active role in the antioxidant assay from the regression plot obtained. The PLS analysis of the important variables between FTIR data and DPPH assay can be seen in Figure 4.

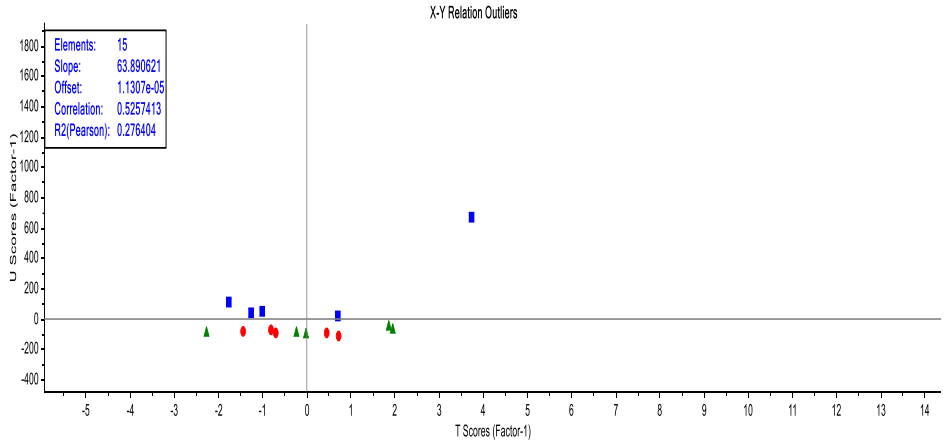
The functional groups that play an active role in antioxidant activity generated the negative regression. In the DPPH assay, these types of functional groups were observed in the range of wavenumber at 1650-



(A)



(B)



(C)

FIGURE 3. Plot of X-Y score correlation FTIR and DPPH (A), ABTS (B), and FRAP (C) on leaves (**blue**), stem bark (**red**), root extract (**green**)

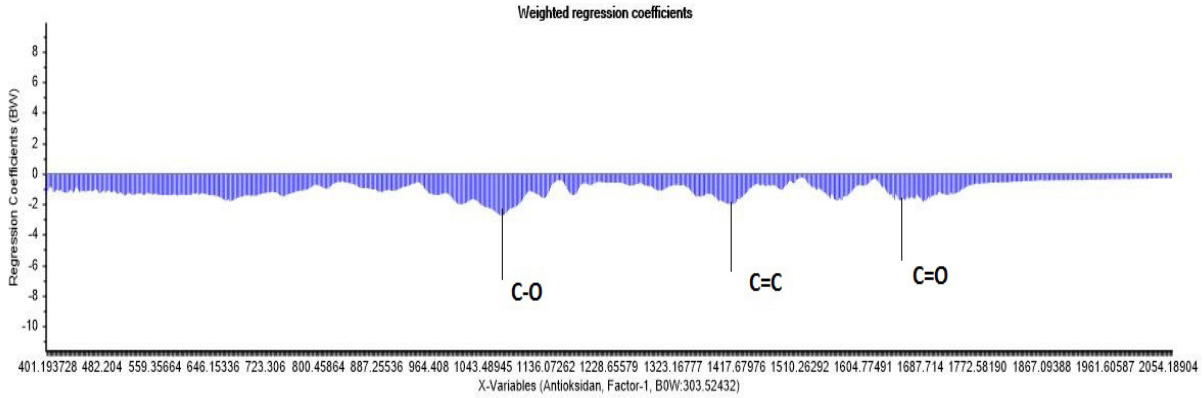


FIGURE 4. PLS analysis of the important variables between FTIR data and DPPH assay

1700, 1400-1450 and 1040-1140 $cm^{-1,}$ which indicated the absorption of C = O (carbonyl group), C = C (aromatic), and C-O, respectively. Among these functional groups, the carbonyl group displayed the lowest regression coefficient. Therefore, the C = O functional group was predicted to be the most significant contributing functional group to the antioxidant activity of *S. grandiflora* extract. The same observation also was afforded from the PLS analysis between FTIR data and FRAP antioxidant assay (Figure 5). The differences only at the regression coefficient value of the data. It can be seen in Figure 5 that the lowest regression coefficient was found in the aromatic C = C functional group. Hence, the C = C functional group was assumed to be the most affecting functional group for the antioxidant activity of *S. grandiflora* extract using the FRAP method. However, from the correlation between FTIR data and antioxidants with the ABTS method (Figure 6), there is no finding of functional groups that act as antioxidants.

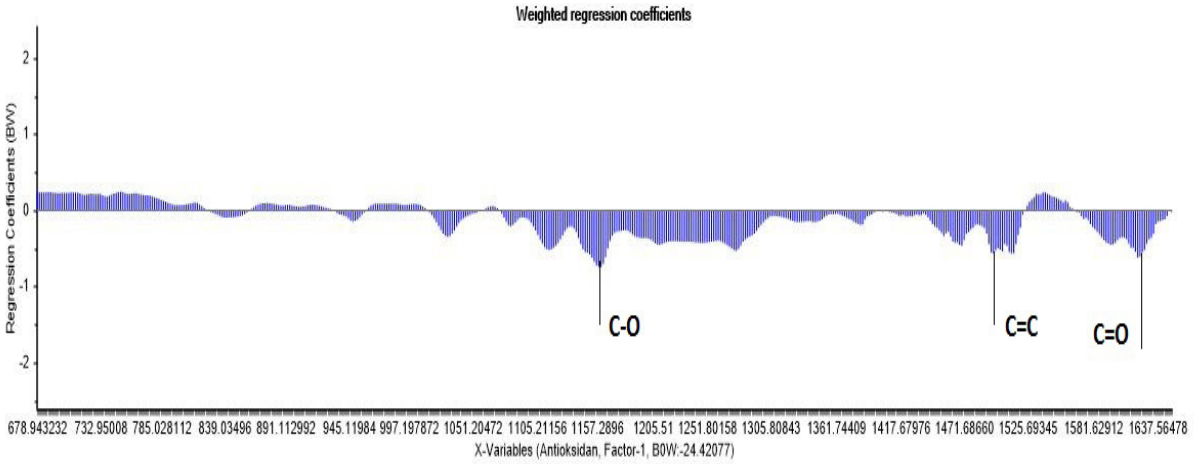


FIGURE 5. PLS analysis of the important variables between FTIR data and FRAP assay

Based on the PLS analysis, it can be concluded that there is a consistency of functional groups that are predicted to play an active role as an antioxidant, i.e., the C – O, C = C, and C = O. Even though the results exhibited the exact prediction of functional groups using the FRAP and DPPH methods, no predicted functional groups were shown using the ABTS method. From the results of the important variable analysis, the O-H group was not considered to have contribution to the antioxidant activity of the samples. Instead, the C = C

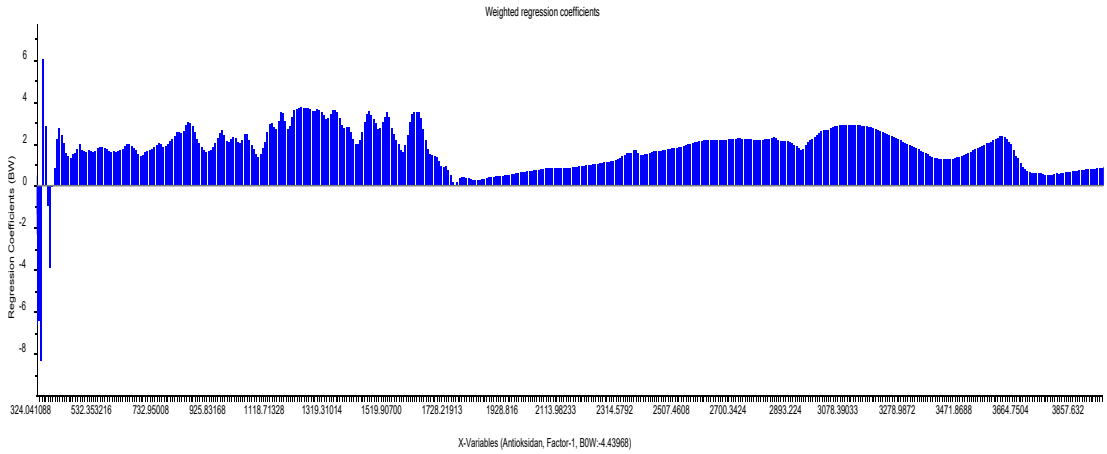


FIGURE 6. PLS analysis of the important variables between FTIR data and ABTS assay

and C-O functional groups were observed, which were predicted to be derived from tannin, phenolic acid, anthocyanins and flavonoid type compounds (Petlevski et al. 2013). Previous research was done by Coulibaly et al. (2014) on bioprospecting some medicinal plants for their antioxidant components. They reported that flavonoid compounds such as quercetin, myricetin, kampferol, rutin, and isoquercetin were the major constituents that may justify their potent antioxidant activities. Additionally, Bittencourt et al. (2015) reported that antioxidant capacity is usually closely associated with the content of phenolic compounds which may exert a synergetic effect between themselves and with some lipophilic compounds such as vitamins C and E. Phenolic compounds are presumed to be good antioxidant molecules due to their extensive conjugated π-electron systems that facilitates the donation of electrons from the hydroxyl moieties to oxidizing radical species. These results are in agreement with our previous findings on phytochemical study of *S. grandiflora* roots and stem barks, in which flavonoids and phenolics compounds have been successfully separated (Noviany et al. 2021, 2020b, 2018, 2012). However, the continuous investigation on the isolation and purification of all parts of *S. grandiflora* extracts based on the metabolomics approach was required to clarify the most important functional groups that play an active role as an antioxidant.

CONCLUSIONS

In this research, the correlation between antioxidant activity and its secondary metabolites from different parts of *S. grandiflora* plant was successfully achieved through the metabolomic approach. This is the first study on the plant belongs to the genus of *Sesbania* and other members of the family Fabaceae. However, further purification the plant extracts is still required in order to gain more scientific information regarding the chemical constituents contributing to their antioxidant activity.

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