

## Phytochemical Screening, Antioxidant Activity and Phenolic Content of Different Plant Parts of *Brucea javanica* (L.)

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### ABSTRACT

*In this study, phytochemical screening, antioxidant activity as well as phenolic content in the leaves, twigs and barks of Brucea javanica (L.) were determined using standard phytochemical screening method, DPPH radical scavenging assay and Folin-Ciocalteu reagent method, respectively. Methanolic extracts of these different plant parts were prepared by maceration method. Phytochemical screening revealed the presence of flavonoids, terpenoids and tannins in all plant parts. This test also revealed the presence of alkaloids and saponins in all plant parts except they were absent in the twigs and barks parts, respectively. On the other hand, steroids was absent in all plant parts. DPPH method was used to evaluate the antioxidant properties of the plant parts by measuring the absorbance at 517 nm. The antioxidant activity was then compared with standard ascorbic acid. Among all parts, methanolic extract of leaves of Brucea javanica (L.) exhibited the highest antioxidant activity at the concentration of 100 ppm with  $86.19 \pm 0.20\%$  inhibition which was comparable with standard ascorbic acid with  $97.62 \pm 0.13\%$  inhibition at the same concentration. The  $IC_{50}$  value of the methanolic extract of leaves was  $54.52 \pm 0.16$  ppm while the other parts were more than 100 ppm. All plant parts showed  $IC_{50}$  value higher than the standard ascorbic acid which recorded  $IC_{50}$  of  $9.04 \pm 0.09$  ppm. The methanolic extract of the leaves also exhibited the highest total phenolic content which was  $105.58 \pm 0.21$  mg GAE/g extract compared to the other parts. This result correlated well with the higher antioxidant activity exhibited by the methanolic extract of the leaves. Thus, the leaves part of methanolic extract of Brucea javanica (L.) exhibited the highest antioxidant activity compared to the other plant parts.*

**Keywords:** *Brucea javanica* (L.), simaroubaceae, phytochemical screening, antioxidant activity, phenolic content

### INTRODUCTION

*Brucea javanica* (L.) belongs to the family of Simaroubaceae and is known as “Melada pahit or Lada pahit”. It is widely distributed in the lowland and bush of Malaysia especially in the northern part of the Malay Peninsula. In terms of morphology, this evergreen shrub can be up to 10m in height and their leaves are compound, 12-62 cm in length with 5-15 leaflets, alternate, ovate or lanceolate (Department of Forestry, n.d.). This plant can be used to treat fever, hemorrhoids, ulcers and dysentery (Samy, Sugumaran & Lee, 2005). Previous study revealed the presence of triterpenoids and quassinoids from the plant (Dong et al., 2013; Ye et al., 2015). This plant is known to have important biological activities such

as antiplasmodial, antitrypanosomal, antioxidant and antiproliferative activities (Bagheri, Hajiaghaalipour, Nyamathulla, & Salehen, 2018; Bawm et al., 2008; Cai, Luo, Sun, & Corke, 2004; Hout et al., 2006). However, most of the reports focused only on the roots and fruits of the plant. Data on the preliminary screening and antioxidant activity in different plant parts of *Brucea javanica* (L.) such as leaves, twigs and barks has not yet been extensively recorded so far. Thus, this research is carried out to investigate the presence of the secondary metabolites, antioxidant activity and the phenolic content in different plant parts of *Brucea javanica* (L.).



Figure 1: A picture of *Brucea javanica* (L.)

## METHODOLOGY

### Preparation of the Methanolic Crude Extracts

Fresh leaves, twigs and barks of *Brucea javanica* (L.) were collected from Kampung Ulu Inas, Negeri Sembilan Darul Khusus, Malaysia. The methanolic extracts of different plant parts of *Brucea javanica* (L.) were prepared using maceration method. Firstly, the collected plant parts were washed and allowed to dry at room temperature for two weeks. The samples were then further dried in an oven at 40 °C for two hours. After this period, they were ground to fine powder. Then, they were soaked in methanol at room temperature for 48 hours. The extracts were then filtered after 48 hours using Whatmann filter paper number 1 into three different Erlenmeyer flasks. The soaking process was repeated for two more times using fresh methanol. After that, all filtrates were combined according to their plant parts. Then, they were concentrated using rotary evaporator at 40 °C. Lastly, the crude extracts were stored in the refrigerator before further use.

## Phytochemical Screening

Phytochemical screening was carried out on the methanolic extracts of the leaves, twigs and barks of *Brucea javanica* (L.) to determine the presence of alkaloids, flavonoids, terpenoids, steroids, saponins and tannins using standard method (Ayoola et al., 2008; Kumar, Jha, Kumar, Agrawal, & Gupta, 2012; Mojab, Kamalinejad, Ghaderi, & Vahidipour, 2003; Uddin, Rauf, Siddiqui, & Shah, 2011; Vennila et al., 2012).

## Antioxidant Activity

The free radical scavenging activity of the methanolic extracts of different plant parts of *Brucea javanica* (L.) against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by Spectronic 20 at 517 nm. This activity was measured according to the method previously described with slight modification (Boxi, Rajesh, Kumar, Praveen, & Mangamma, 2010). Firstly, 0.1 mM of DPPH solution was prepared in methanol and the solution was placed in the dark throughout the test. Then, a stock solution of standard ascorbic acid in methanol was prepared by dissolving 0.05 g of ascorbic acid in 50 mL of volumetric flask to make a concentration of 1000 ppm. A series of standard solutions of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm were then prepared in 25 mL of volumetric flask. The same procedure was used in preparing the stock solution and a series of dilutions of the plant extracts. After that, 3.0 mL of different concentrations (20-100 ppm) of ascorbic acid was mixed with 1.0 mL of DPPH and shaken vigorously. They were placed in the dark for 30 minutes and the absorbance was measured at 517 nm. The same proportion was used to prepare the plant extracts (leaves, twigs and barks) and control where each extract and methanol was used respectively instead of the ascorbic acid. The measurement was made on the control, followed by ascorbic acid and plant extracts. Samples were measured in triplicates.

## Total Phenolic Content

Total phenolic content of methanolic extracts of different plant parts of *Brucea javanica* (L.) using Folin-Ciocalteu reagent was determined by Spectronic 20 at 765 nm. This activity was measured according to the previous method with slight modification (Maizura, Aminah, & Wan Aida, 2011). Firstly, Folin-Ciocalteu reagent was prepared with 10 times of dilution with distilled water. Then, 7.5% of sodium carbonate was prepared, followed by a preparation of stock solution of standard gallic acid and a series of standard solution of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm which were obtained from dilution of the stock solution. After that, 0.5 mL of different concentrations (20-100 ppm) of gallic acid was mixed with 2 mL of Folin-Ciocalteu reagent. After 5 minutes, 4 mL of sodium carbonate solution was added and allowed to react at room temperature for 30 minutes (Alhakmani, Kumar, & Khan, 2013). The same procedure was used to prepare the reaction mixture for plant extracts (leaves, twigs and barks) and blank where 1000 ppm of plant extracts and methanol were used instead of the gallic acid, respectively (Stankovic, 2011). The measurement was made on the blank, followed by gallic acid and plant extracts. Samples were measured in triplicates.

## RESULTS AND DISCUSSION

### The Extractive Yield of Methanolic Extracts of Different Plant Parts of *Brucea javanica* (L.)

The extraction method used was maceration where the powdered samples were soaked in methanol at room temperature. The samples were soaked three times in order to extract more compounds as possible in the plant. They were soaked in the dark glass bottle to prevent the active constituents in the sample to react with the surroundings such as light and form other compounds. The percentage extractive yield of the methanolic crude extracts was calculated using the formula as mentioned below (Kumar et al., 2012).

$$\text{Extractive yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried sample}} \times 100 \quad (1)$$

**Table 1: The extractive yield of methanolic extracts of different plant parts of *Brucea javanica* (L.)**

Plant Part	Weight of Dried Sample (g)	Weight of Dried Extract (g)	Extractive Yield (%)
Leaves	123.8744	3.9136	3.16
Twigs	66.3806	1.8051	2.72
Barks	161.8451	7.8049	4.82

Based on Table 1, the barks part of *Brucea javanica* (L.) showed the highest extractive yield compared to the other plant parts with 4.82%, followed by 3.16% from the leaves part and 2.72% from the twigs part. Methanol is a solvent that can be used to extract all non-polar, intermediate and polar compounds from the plant extracts.

### Phytochemical Screening of Different Plant Parts of *Brucea javanica* (L.)

The phytochemical screening of methanolic crude extracts of different plant parts of *Brucea javanica* (L.) were carried out in order to determine their phytochemical contents such as alkaloids, flavonoids, terpenoids, steroids, saponins and tannins.

**Table 2: The extractive yield of methanol extracts of different plant parts of *Brucea javanica* (L.)**

Secondary Metabolites/Plant Part	Leaves	Twigs	Barks
Alkaloids	+	-	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Steroids	-	-	-
Saponins	+	+	-
Tannins	+	+	+

(+) present, (-) absent

Alkaloids were tested using Mayer's reagent. The presence of alkaloids was based on the presence of precipitation or cloudy appearance in the samples. Based on the observation, leaves and barks parts revealed the presence of alkaloids whereas there was no alkaloids present in the twigs part. On the other hand, flavonoids were tested using Shinoda test. Based on the observation, all plant parts exhibited the formation of brown coloration. This showed that flavonoids were present in all parts of the plant. Terpenoids were tested by using Salkowski test. Based on the observation, all plant parts exhibited the

formation of reddish brown coloration at the interface. This showed that terpenoids were present in all parts of the plant. Meanwhile, steroids were tested using Liebermann-Burchard test. Initially, all plant parts successfully changed their color to violet. As the reaction progress, theoretically, the color should change to blue or green but the solution turned to dark brown. This showed that steroids were absent in all parts of the plant. Saponins were tested using Froth test. Based on the observation, the formation of stable persistent froth for 20 minutes was observed in the leaves and twigs parts of the sample with the length of froth was less than 1 cm each. There was no froth present in the barks part. The result revealed that saponins were present in the leaves and twigs part while there was no saponins present in the barks part. Lastly, tannins were tested using ferric chloride solution. Based on the observation, all plant parts revealed the formation of brown coloration. The result showed that tannins were present in all plant parts.

### Antioxidant Activity of Different Plant Parts of *Brucea javanica* (L.)

In this study, a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to estimate the antioxidant activity of different plant parts of *Brucea javanica* (L.). Initially, the color of DPPH itself was purple. When this radical had reacted with antioxidant such as ascorbic acid, the color of DPPH radical changed to yellow. This means that, a stable free radical, 2,2-diphenyl-1-picrylhydrazyl had already changed into a stable, non-radical compound which was 2,2-diphenyl-1-picrylhydrazine. The effect of antioxidants on DPPH radical is based on the hydrogen-donating ability or the ability of DPPH to accept electron from antioxidant compound. The degree of decolorization from purple to yellow was determined by the decrease in its absorbance at 517 nm. Lower absorbance of the reaction mixture indicated higher antioxidant activity. This absorbance was evaluated by Spectronic 20 at 517 nm. Based on the absorbance readings, the radical scavenging activity (% inhibition of radicals) of each plant extract can be calculated by using Equation 2. Then, a graph of % inhibition versus concentration was plotted in order to display equation which will then be used to calculate  $IC_{50}$ .  $IC_{50}$  is a parameter widely used to evaluate the antioxidant activity of the plant.  $IC_{50}$  is a concentration of the sample or standard needed to scavenge or inhibit the formation of DPPH radicals by 50%. Lower  $IC_{50}$  value showed a strong antioxidant activity. Free radicals scavenging activity (% inhibition) was calculated by using formula as shown below (Boxi et al., 2010).

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100 \quad (2)$$

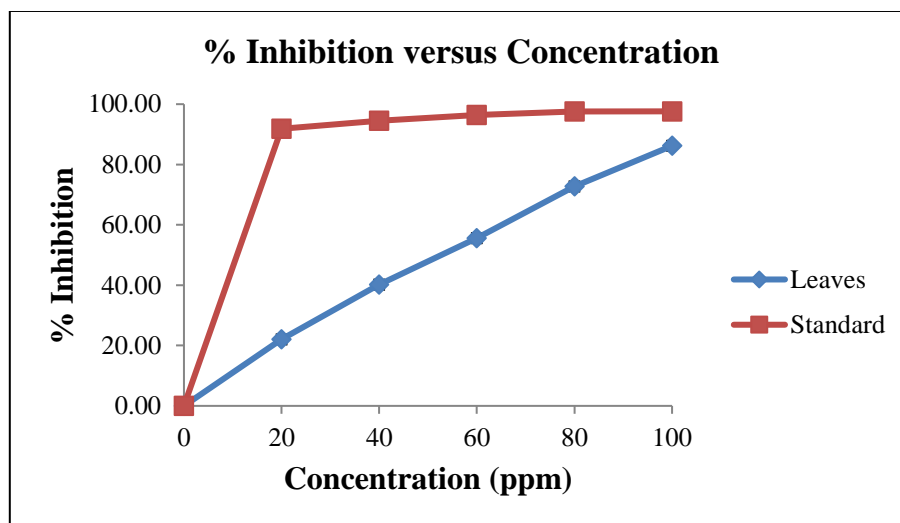


Figure 2: A graph of % inhibition versus concentration of methanolic extract of leaves of *Brucea javanica* (L.)

Table 3: Percentage of inhibition in methanolic extracts of leaves of *Brucea javanica* (L.)

Concentration (ppm)	Absorbance Readings (A)			% Inhibition
	1	2	3	
0	0.000	0.000	0.000	0.00 ± 0.00
20	0.228	0.227	0.228	22.03 ± 0.20
40	0.174	0.175	0.175	40.18 ± 0.20
60	0.130	0.129	0.130	55.59 ± 0.20
80	0.080	0.079	0.080	72.72 ± 0.20
100	0.041	0.040	0.040	86.19 ± 0.20

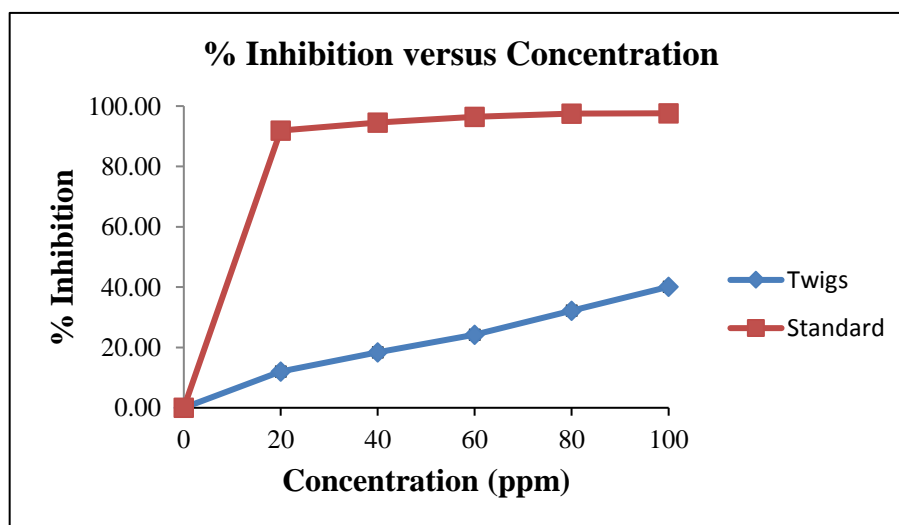


Figure 3: A graph of % inhibition versus concentration of methanolic extract of twigs of *Brucea javanica* (L.)

Table 4: Percentage of inhibition in methanolic extracts of twigs of *Brucea javanica* (L.)

Concentration (ppm)	Absorbance Readings (A)			% Inhibition
	1	2	3	
0	0.000	0.000	0.000	0.00 ± 0.00
20	0.286	0.286	0.286	12.00 ± 0.00
40	0.265	0.266	0.265	18.36 ± 0.18
60	0.246	0.246	0.247	24.21 ± 0.18
80	0.221	0.220	0.220	32.21 ± 0.18
100	0.194	0.195	0.195	40.10 ± 0.18

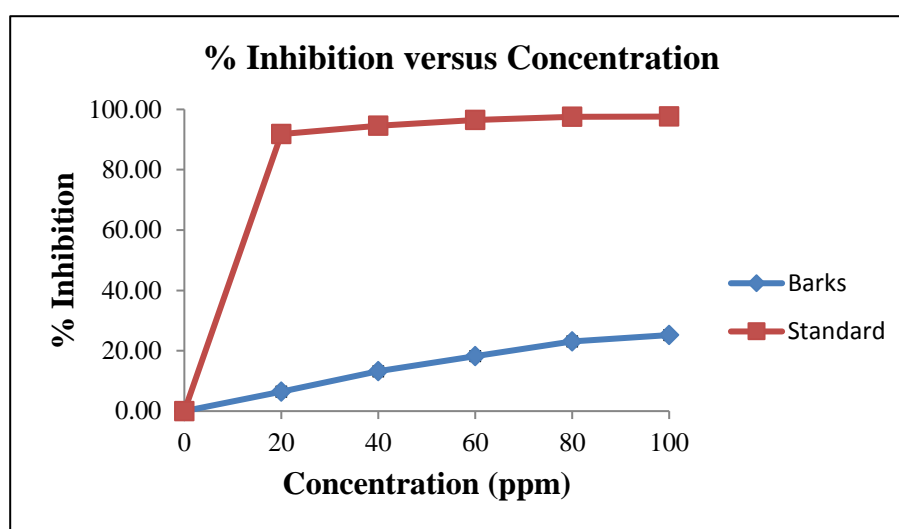


Figure 4: A graph of % inhibition versus concentration of methanolic extract of barks of *Brucea javanica* (L.)

Table 5: Percentage of inhibition in methanolic extracts of barks of *Brucea javanica* (L.)

Concentration (ppm)	Absorbance Readings (A)			% Inhibition
	1	2	3	
0	0.000	0.000	0.000	0.00 ± 0.00
20	0.296	0.295	0.296	6.44 ± 0.18
40	0.274	0.274	0.275	13.18 ± 0.18
60	0.258	0.259	0.258	18.25 ± 0.18
80	0.243	0.243	0.243	23.10 ± 0.00
100	0.237	0.236	0.236	25.21 ± 0.18

Table 6: Results of IC<sub>50</sub> values of standard and extract of different plant parts of *Brucea javanica* (L.)

Extract/Standard	Plant Part	IC <sub>50</sub> (ppm)
Methanol	Leaves	54.52 ± 0.16
	Twigs	125.64 ± 0.19
	Barks	187.74 ± 0.73
Ascorbic acid	-	9.04 ± 0.09

Based on Table 6, standard ascorbic acid exhibited strong antioxidant activity with the  $IC_{50}$  value of  $9.04 \pm 0.09$  ppm. The result achieved from this study is acceptable in comparison to the previous study. According to Karagozler et al. (2008), the  $IC_{50}$  value of standard ascorbic acid revealed an almost similar value to the conducted study which was  $9.00 \pm 2.26$  ppm. This might prove the validity of the result obtained as it was published in Journal of Food Chemistry. Based on the results obtained, the leaves were extracted using methanol showed the lowest  $IC_{50}$  value of  $54.52 \pm 0.16$  ppm compared to other plant parts which showed more than 100 ppm. Lower  $IC_{50}$  value indicated a stronger antioxidant activity. By referring to the Table 3, 100 ppm of this extract gave  $86.19 \pm 0.20\%$  inhibition meanwhile standard ascorbic acid gave  $97.62 \pm 0.13\%$  inhibition at the same concentration. This showed that the methanolic extract of leaves part possessed quite high antioxidant activity. However, the  $IC_{50}$  values of all plant parts of *Brucea javanica* (L.) were larger than ascorbic acid which was  $9.04 \pm 0.09$  ppm. Overall, all plant parts showed increase in the % inhibition when their concentrations increased and showed antioxidant property.

### Total Phenolic Contents of Different Plant Parts of *Brucea javanica* (L.)

Total phenolic content of different plant parts of *Brucea javanica* (L.) were determined by Spectronic 20 at 765 nm. They were calculated by using Equation 3 and expressed in terms of gallic acid equivalent (Nickavar & Esbati, 2012).

$$\text{Gallic acid equivalent} = \frac{C \times V}{M} \quad (3)$$

where C = Concentration of gallic acid established from the calibration curve (ppm)

V = Volume of extract (L)

M = Weight of the extract (g)

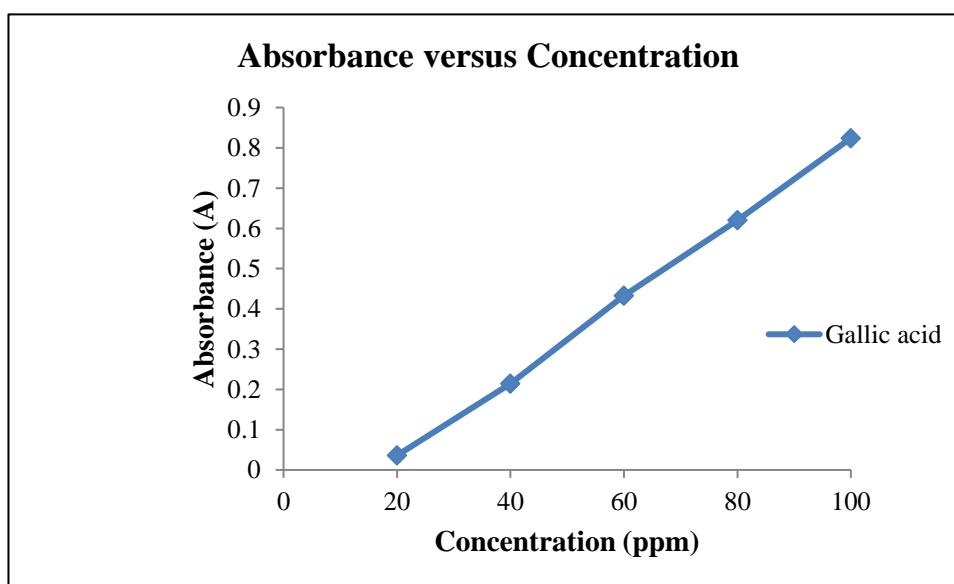


Figure 5: Calibration curve of standard gallic acid



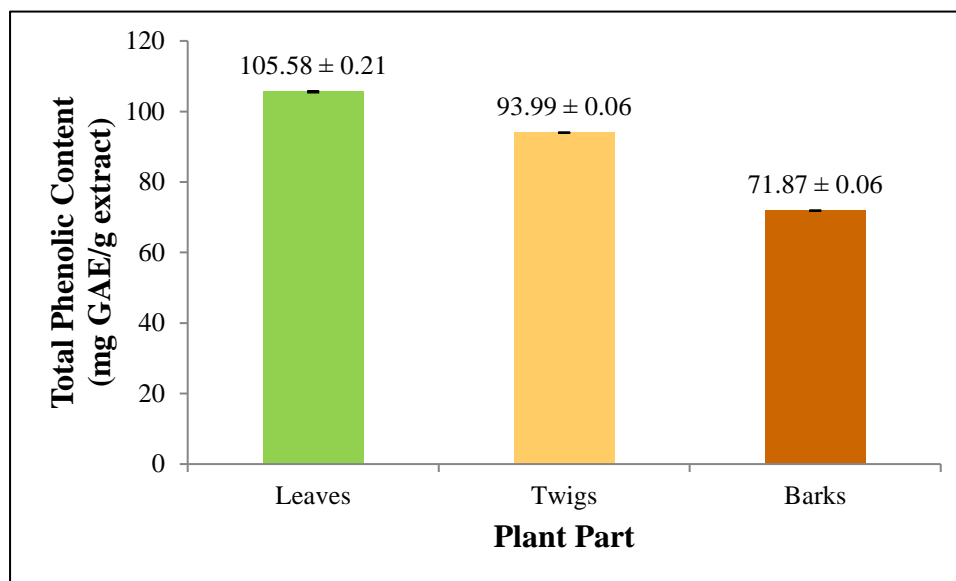


Figure 6: Results of total phenolic content of different plant parts of *Brucea javanica* (L.)

The amounts of total phenolic in different plant parts of *Brucea javanica* (L.) were determined by Spectronic 20 at 765 nm. 765 nm is the maximum wavelength ( $\lambda_{\max}$ ) of standard gallic acid.  $\lambda_{\max}$  is the highest sensitivity for the measurement to occur. In this study, gallic acid was used as a standard to represent all phenolic compounds in the extract. Based on Figure 6, leaves extracted by using methanol showed the highest quantity of phenolic content which was  $105.58 \pm 0.21$  mg GAE/g extract compared to the other plant parts. This might be the indicator of the higher antioxidant activity in this plant part. The total phenolic content increases from  $71.87 \pm 0.06$  mg GAE/g extract of the barks part to  $93.99 \pm 0.06$  mg GAE/g extract for twigs part. This showed why different plant parts of *Brucea javanica* (L.) vary in their antioxidant activity. In short, the higher the total phenolic content, the higher the antioxidant activities of the plant (Karagozler et al., 2008). The presence of phenolic compounds such as flavonoids and tannins contributed to the observed results on the total phenolic and antioxidant activity of the different plant parts of *Brucea javanica* (L.).

## CONCLUSION

In conclusion, phytochemical screening of methanolic extracts revealed the presence of flavonoids, terpenoids and tannins in all plant parts such as leaves, twigs and barks. Alkaloids and saponins were also present in all plant parts except in the twigs and barks parts, respectively. On the other hand, steroids was absent in all plant parts of *Brucea javanica* (L.). Methanolic extract of the leaves of *Brucea javanica* (L.) exhibited the highest antioxidant activity as well as the total phenolic content compared to other plant parts. Thus, the leaves part of *Brucea javanica* (L.) showed the highest antioxidant activity followed by twigs and barks parts, respectively.

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