

Potential Antibacterial and Antioxidant Activity of Methanolic Extract of *Vigna unguiculata* (L.) Walp Leaves

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Abstract: This study was performed to determine the bioactivity of methanolic extract of *Vigna unguiculata* (L.) Walp leaves as potential antibacterial and antioxidant. Based on the phytochemical screening, the methanolic extract of methanolic extract of *V. unguiculata* (L.) Walp leaves showed the presence of phenolic, terpenoid and saponin. The 15 wt.% of methanolic extract of *V. unguiculata* (L.) Walp leaves exhibited a moderate antibacterial activity against Gram positive bacterium (*S. aureus*) with 15.5 cm of inhibition zone diameter. But negative result was obtained against Gram negative (*E. coli*). The antioxidant activity of methanolic extract of *V. unguiculata* (L.) Walp leaves against DPPH that determined as IC50 was 66.71 mg/L.

1 INTRODUCTION

Vigna unguiculata (L.) Walp is the original plant from Africa to cure epilepsy, chest pain, and constipation (Kritzinger, 2005). *Vigna unguiculata* (L.) Walp also can be used as the source of protein and other nutrients (Siddhuraju, 2007).

Insect pest is still an issue for the *Vigna unguiculata* (L.) plantation in Africa. There is a correlation between the concentration of flavonoid/anthocyanine that presence in *V. unguiculata* (L.) and the ability to inhibit the attack of insect pest (Makoi, 2010).

The HPLC measurement of *V. unguiculata* showed the presence of three aglycon flavonoid, i.e. quercetin, kaempferol, and isorhamnetin. Also, the resistency correlation between insect pest and flavonoid content. This result showed that the presence of quercetin and isorhamnetin play a role in the resistency mechanism (Latanzio, 2000).

Other than flavonoid compound, phenolic and tannin can be found in the seed of *V. unguiculata* and the processed *V. unguiculata* is claimed as the strong antioxidant sources. This is as the impact of the formation of complex between tannin and protein, and the presence of phenolic compound in the dietary based on *V. unguiculata*. The presence of the potential antioxidant compound in the *V. unguiculata* can protect the human body from oxidative process (Siddhuraju, 2007).

The objective of the current research was to determine the antibacterial and antioxidant activity of *V. unguiculata* (L.) Walp leaves.

2 EXPERIMENTAL

2.1 Materials

Equipment : glassware, rotary evaporator, petri dish, spectrophotometry UV-Vis.

Materials : leaves of *V. unguiculata* (L.) Walp., 5 wt.% of FeCl₃, ethyl acetate, Lieberman-Burchard's reagent, NA medium, MHA medium, DMSO, distillate water, DPPH, methanol, culture of *S. aureus* and *E. coli*.

2.2 Extraction of *V. unguiculata* (L.) Walp Leaves

About 600 g of simplicia of *V. unguiculata* (L.) Walp leaves was macerated in 4 L of methanol. The filtrate was separated and concentrated using rotary evaporator. The obtained extract was named as concentrated methanolic extract.

2.3 Antibacterial Activity of *V. unguiculata* (L.) Walp Leaves

The concentration of methanolic extract of *V. unguiculata* (L.) Walp leaves was varied to be 5, 15 and 25 wt.% in DMSO. Mueller Hinton Agar (MHA) medium was poured into petri dish in the temperature of 45-50°C and the bacterium suspension was spread in the medium. The antibacterial was determined using dish paper method. The incubation of treated medium was kept at 35°C for 18-24 h. *S. aureus* and *E. coli* were chosen as tested bacterial.

2.4 Antioxidant Activity of *V. unguiculata* (L.) Walp Leaves

The antioxidant activity of methanolic extract of *V. unguiculata* (L.) Walp leaves was determined using spectrophotometer UV-Vis as an impact of colour changed due to the reaction between of DPPH and the extract. w milligram of DPPH was dissolved in methanol until its volume 450 mL with the final concentration of DPPH was 50 µM. The concentration was varied to be 5, 10, 15 dan 20 ppm.

One millilitre of DPPH 50 µM was mixed with 2 mL of methanolic extract of methanolic extract of *V. unguiculata* (L.) Walp leaves. The mixture was homogenized, and it was left dark condition for 30 minutes. The absorbance of extract was measured at 516 nm. The inhibition percentage was determined from the equation below:

$$\text{inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{\text{Absorban Control}} \times 100\% \quad (1)$$

3 RESULT AND DISCUSSION

3.1 Antibacterial Activity

The antibacterial properties of methanolic extract of *V. unguiculata* (L.) Walp leaves was evaluated against *S. aureus* and *E. coli*. The diameter of inhibition zone was appeared as a clear zone around the paper dish that used in antibacterial evaluation. The inhibition zone result was showed in Table 1.

Table 1: The antibacterial activity of methanolic extract of *V. unguiculata* (L.) Walp leaves

No	Concentration (%)	Diameter of inhibition zone (mm)	
		<i>S. aureus</i>	<i>E. coli</i>
1	5	14	-
2	15	15,5	-
3	25	15	-

Based on that result, the methanolic extract of methanolic extract of *V. unguiculata* (L.) Walp leaves has a moderate potency to inhibit *S. aureus* but diameter of inhibition was not found in *E. coli*. The moderate potency of the extract had an inhibition zone diameter was 15.5 mm.

The different result between the both bacterial can be caused there was no active compound that can be found presence of active compound that can inhibit *E. coli*. Also, this assumption can be caused by the different of cell walls structure in the Gram and Positive negative. Bacterium Gram positive has single laye with low lipid content (1-4%) and the Gram negative bacterium has higher lipid content (11-12%) and the outer membrane has three component that constructed by liposaccharide, lipoprotein, and phospholipid (Fardiaz, 1992).

3.2 Antioxidant Activity

The antioxidant activity determination of methanolic extract of *V. unguiculata* (L.) Walp leaves was carried out using DPPH method and the absorbance was measured using spectrophotometer UV-VIS at 516 nm of wavelength. The following result in Table 2 was the absorbance of methanolic extract at various concentration.

Table 2: Absorbance of methanolic extract of *V. unguiculata* (L.) Walp leaves

No	Concentration (ppm)	Absorbance
1	Blank	0,963
2	5	0,864
3	10	0,843
4	15	0,832
5	20	0,810

The radical scavenging activity of this methanolic extract were determined as %inhibition. Minami *et al* (1998) The %inhibition of antioxidant was categorized according to IC50 value. The antioxidant molecules were categorized as very strong antioxidant activity when the IC50 ≤ 10, strong antioxidant activity when the IC50 between 10 and 100, and the poor antioxidant activity when

the $IC_{50} > 100$. The IC_{50} of methanolic extract of *V. unguiculata* (L.) Walp leaves was obtained using regression linear equation with value of 66.71 mg/L. Based on the Minami classification, this methanolic extract has strong antioxidant activity.

4 CONCLUSIONS

The evaluation of the 15 wt.% of methanolic extract of *V. unguiculata* (L.) Walp leaves as potential antibacterial showed a moderate diameter inhibition zone against *S. aureus* with value 15.5 mm. The antioxidant activity of the methanolic extract has strong antioxidant activity with IC_{50} of 66.71 mg/L.

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