

# Total Phenolic, Mineral Contents, Antioxidant and Antimicrobial Activities of *Crotalaria pallida* Aiton.

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## Abstract

*Crotalaria pallida* Aiton. (family –Fabaceae) is used for treating urinary problems and fever. The phytochemical constituents, the total phenolic and mineral contents, antioxidant and antimicrobial activity of the stem of *Crotalaria pallida* Aiton. were reported. The phytochemical analysis by standard methods showed the presence of all tested phytochemical constituents in *Crotalaria pallida*. The mineral contents by EDXRF displayed that potassium, calcium, iron, silicon, sulfur, manganese, phosphorus, zinc, titanium, copper, rubidium were detected in *Crotalaria pallida*. The result of total phenolic content of *Crotalaria pallida* using Folin Ciocalteu's reagent was found to be 47.6 mg GAE/g. The evaluation of antimicrobial potential by agar well diffusion method revealed that the methanolic extract of *Crotalaria pallida* showed high activity against *Staphylococcus aureus* (25 mm), medium sensitivity against *Bacillus cereus* (17 mm), *Shigella boydii* (14 mm) and *Salmonella typhi* (19 mm). The antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging assay exhibited that *Crotalaria pallida* extract showed moderate activity with  $EC_{50}$  62.77  $\mu$ g/ml.

**Keywords:** Antioxidant, antimicrobial, phytochemical, mineral, scavenging

## 1. Introduction

Plants have been used as traditional medicine for the treatment of different diseases. Plants are one of the important sources of bioactive secondary metabolites used in the development of antidiabetic, anti-inflammatory, anticancerous, and antimicrobial drugs. The application of antimicrobial agents derived from plant sources are safe and might be helpful in reducing the dependence on antibiotics [1]. Many studies have investigated the antioxidant properties of plants based natural products. Plants involving phenolic compounds are valuable to human health to their anti-cancer, antimicrobial and antioxidant activities [2]. Many researchers reported that plant phenolic compounds have been found to possess powerful antioxidants.

Kachin State is rich in natural resources including the forests with valuable medicinal plants. Most people who stay in this region rely on medicinal plants for their health care. In Kachin State, there are so many

medicinal plants that haven't studied scientifically. Thus, these medicinal plants are valuable to be chosen for chemical and biological studies. *Crotalaria pallida* Aiton. of the family Fabaceae, collected from Kachin State, was selected for the present study. The plant is used in this region to treat urinary diseases, fever and skin infections [4]. The present study was aimed at evaluating the phytochemical constituents, total phenolic and mineral contents, antimicrobial and antioxidant activity of the stem of *Crotalaria pallida* Aiton.

## 2. Materials and Methods

### 2.1. Plant Material

The medicinal plant of *Crotalaria pallida* Aiton. was collected from Wyne Maw Township, Kachin State, Myanmar and identified by Dr Soe Myint Aye. The collected samples were allowed to dry for one week in well ventilated shade. Then, the air dried samples were grounded into powder by grinding machine.

### 2.2. Phytochemical Analysis

The preliminary phytochemical analysis of alkaloids, flavonoids, terpenes, sterols, glycosides, reducing sugars, lipophilics, polyphenols, saponins, phenolic compounds and tannins in the stem extracts was carried out using standard methods and each of the tests was quantitatively expressed as negative (-) or positive (+) [3].

### 2.3. Determination of Mineral Contents by EDXRF

The mineral contents of the stem of *Crotalaria pallida* were recorded on spectro XEPOS Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometa, Germany. flush right.

### 2.4. Determination of Total Phenolic Contents in Plant Extract

Total phenolic content of methanolic extract of stem extract was determined with Folin-Ciocalteu's method [6]. The concentration, 1 mg/mL of stem extract (dissolved in ethanol) was used in the analysis. The

reaction mixture was prepared by mixing 0.5 mL of ethanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% NaHCO<sub>3</sub>. Blank was concomitantly prepared, containing 0.5 mL of ethanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of NaHCO<sub>3</sub>. The sample was thereafter incubated at 45°C for 45 mins. The absorbance was determined using spectrophotometer at  $\lambda_{\max} = 765$  nm. The sample was prepared in triplicate for each analysis and the mean value of absorbance was obtained. To obtain a calibration curve, various concentrations of gallic acid solutions (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL) were prepared. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extract was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Total Phenolic Content =  $c \ V/m$

$c$  = concentration from calibration curve

$m$  = mass of the extract used

$v$  = volume of the extract

## 2.5. Antimicrobial Activities of the Plant Extract

The antimicrobial activities of the methanolic extract of the stem of *Crotalaria pallida* were tested by using agar-well diffusion method on five selected organisms such as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Shigella boydii*, *Salmonella typhi* at Department of Biotechnology, Mandalay Technological University, Mandalay, Myanmar.

## 2.6. Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activity of the methanolic extract of stem of *Crotalaria pallida* was determined by DPPH scavenging activity assay [5]. DPPH assay is applied to analyze free radical scavenging activity of plant extract by hydrogen transfer mechanism. 500 µL of test solutions in three different concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL) and 500 µL of 0.2 M acetate buffer pH 5.5 solutions are mixed in a test tube, followed by addition of 250 µL of  $5 \times 10^{-4}$  M DPPH solution to the mixture in dark. The mixture was homogenized applying a vortex mixer in a dark room (resistant to UV light) and stand for 30 minutes at room temperature. After that, the mixture was measured by a spectrophotometer UV absorbance at  $\lambda_{\max}$  517 nm. Ascorbic acid was used as a reference compound in the same concentration range as the test sample. A control solution was prepared by mixing 500 µL of buffer (pH 5.5) solution, 500 µL of ethanol and 250 µL of  $5 \times 10^{-4}$  M DPPH solution in the test tube. Blank solution was prepared by mixing 500 µL of buffer (pH 5.5) solution with 750 µL of ethanol in the test tube. The mean values were obtained from triplicate experiments. The capability of scavenging DPPH radicals as a percentage of DPPH remaining in the

resulting solution was determined using the following equation:

$$\text{DPPH (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}$$

Where Abs control is absorbance of control and Abs sample is absorbance of sample. The antioxidant power (EC<sub>50</sub>) is expressed as the test substance concentration (µg/mL) that result in a 50% reduction of initial absorbance of DPPH solution and that allows to determine the concentration. EC<sub>50</sub> (50% effective concentration) values were calculated by linear regressive excel program.

## 3. Results and Discussion

### 3.1. Botany Description of *Crotalaria pallida* Aiton.

Family name	: Fabaceae
Botanical name	: <i>Crotalaria pallida</i> Aiton.
Myanmar name	: Kwe ka laut
English name	: Smooth Rattlebox
Part used	: Stem



Figure 1. *Crotalaria pallida* Aiton. plant

### 3.2. Preliminary Phytochemical Analysis

The preliminary phytochemical study of the stem extract of *Crotalaria pallida* showed the presence of alkaloids, flavonoids, terpenes, sterols, glycosides, reducing sugars, lipophilics, polyphenols, saponins, phenolic compounds and tannins.

### 3.3. Mineral Contents Analysis by EDXRF

As shown in Table (1), the elements present in the stem powder of *Crotalaria pallida* were potassium, calcium, iron, silicon, sulfur, manganese, phosphorus, zinc, titanium, copper, rubidium. The powder of *Crotalaria pallida* contains potassium with the highest amount of 0.63193%.

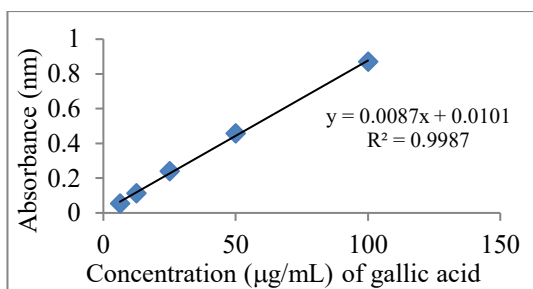
### 3.4 Determination of Total Phenolic Contents in Stem of *Crotalaria pallida*

The total phenolic content of stem extract of *Crotalaria pallida* was studied with Folin-Ciocalteu's reagent according to the Singleton et al [6] method using gallic acid as a standard. The standard calibration curve of gallic acid was shown in Figure (2). The total phenolic content of extract was investigated from

regression equation for the calibration curve of gallic acid ( $y = 0.0087x + 0.0101$ ,  $R^2 = 0.9987$ ). The total phenolic content of the plant extract expressed as gallic acid equivalents (GAE). The result of total phenolic content of *Crotalaria pallida* was found to be 47.6 mg GAE/g.

**Table 1. Mineral contents of stem of *Crotalaria pallida***

No	Elements	Results (mass %)
1	Potassium	0.63193
2	Calcium	0.21382
3	Iron	0.07911
4	Silicon	0.01689
5	Sulfur	0.01495
6	Manganese	0.01399
7	Phosphorus	0.01056
8	Zinc	0.00987
9	Titanium	0.00557
10	Copper	0.00195
11	Rubidium	0.00132



**Figure 2. Plot of standard calibration curve of gallic acid (GA) for the determination of total phenolic content**

### 3.5. Antimicrobial Activities of the Methanolic Extract of *Crotalaria pallida*

According to the antimicrobial experiment, (Table 2), the methanolic extract of stem of *Crotalaria pallida* showed high activity against *Staphylococcus aureus*, medium sensitivity on *Bacillus cereus*, *Shigella boydii* and *Salmonella typhii* but it did not inhibit the growth of *Pseudomonas aeruginosa*.

**Table 2. Antimicrobial activities of methanolic extract of *Crotalaria pallida***

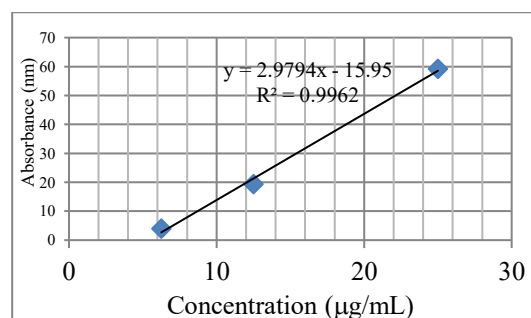
Test Organisms	Inhibition zone (mm)
<i>Pseudomonas aeruginosa</i>	-
<i>Bacillus cereus</i>	17 (++)
<i>Staphylococcus aureus</i>	25 (+++)
<i>Shigella boydii</i>	14 (++)
<i>Salmonella typhii</i>	19 (++)

Agar well – 10 mm  
 10 mm~14 mm (+)  
 15 mm~19 mm (++)

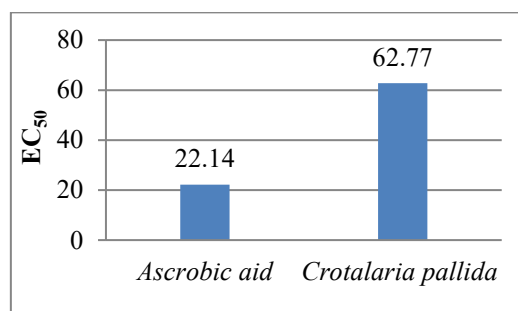
20 mm above (+++)

### 3.6. Antioxidant Activity by DPPH Scavenging Activity Assay

The antioxidant activity of methanolic extract of stem of *Crotalaria pallida* was tested by DPPH free radical scavenging assay using ascorbic acid as a reference standard. The result of antioxidant activity of standard ascorbic acid was shown in Figure (3). As shown in figure (4), DPPH radical scavenging activity of the methanolic extract of *Crotalaria pallida* showed moderate free radical scavenging activity with  $EC_{50}$  62.77  $\mu$ g/mL in comparison to that of standard ascorbic acid was 22.14  $\mu$ g/mL.



**Figure 3. Standard calibration curve of ascorbic acid for the determination of DPPH scavenging activity**



**Figure 4. DPPH radical scavenging activity of ascorbic acid and methanolic extract of *Crotalaria pallida***

**Table 3. DPPH scavenging activity and total phenolic content of *Crotalaria pallida* (n = 3)**

Plant Sample	TPC (mg GAE/g dry extract wt)	DPPH scavenging $EC_{50}$ ( $\mu$ g/ml)
<i>Crotalaria pallida</i>	47.6	62.77

In the present study, the phytochemical investigation of the extracts of *Crotalaria pallida* revealed that alkaloids, flavonoids, terpenes, sterols, glycosides, reducing sugars, lipophilics, polyphenols, saponins, phenolic compounds and tannins that help for treating various diseases were detected. The high content of potassium in stem powder of *Crotalaria pallida* aids to reduce blood pressure and water retention, and protect

against stroke. The antimicrobial experiment of the stem extract of *Crotalaria pallida* showed significant antimicrobial effect against *Staphylococcus aureus*, and moderate activity against *Bacillus cereus*, *Shigella boydii* and *Salmonella typhi*. Hence, this plant can be applicable to cure some infection diseases. In recent years, explore for plant-based phytochemicals possessing antioxidant effects has been increased due to their potential use in the treatment of various diseases. Total phenolic compounds in plants are important natural antioxidants for neutralizing free radicals. The present investigation showed that the extract of *Crotalaria pallida* contains moderate amount of phenols (47.6 mg GAE/g). According to antioxidant experimental data, the methanolic extract of stem of *Crotalaria pallida* was found to be moderate antioxidant effect with  $EC_{50} = 62.77 \mu\text{g/mL}$ .

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#### 4. Conclusion

The antimicrobial activity exhibited high activity against *Staphylococcus aureus*, and moderate activity against *Bacillus cereus*, *Shigella boydii* and *Salmonella typhi* due to the presence of various phytochemicals in the extract of *Crotalaria pallida*. There was a direct correlation between antioxidant activity and the content of phenols in the extract of *Crotalaria pallida* in this study because it displayed moderate phenolic contents (47.6 mg GAE/g) and moderate antioxidant activity ( $EC_{50} = 62.77 \mu\text{g/mL}$ ). Hence, this plant will be useful for the development of antimicrobial and antioxidant agents.

#### Acknowledgement

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