

Determination of Antioxidant Activity and Total Phenol Content of Crude Extract from the Bark of *Salix alba* L. (Moe-ma-kha)

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Abstract : Over the years, research on antioxidants, total phenol content and total flavonoid content as potential therapeutic agents to prevent free radical generated damage in the human body has gained popularity. The study was carried out to determine the antioxidant activity and total phenol content of crude extracts from the bark of *Salix alba* L., in order to validate the medicinal potential of the plant. In the present work, the sample was collected from Yankin Township, Yangon Region in Myanmar and identified at Botany Department, Dagon University in Myanmar. *Salix alba* L., (Moe ma kha) belonging to the genus *Salix* and *Salicaceae* family, has a very long history of medicinal usage and it is known as one of the medicinal plants in Myanmar. The antioxidant activity of bark extract was determined by DPPH assay method and total phenol content of bark extract was determined by Folin-Ciocalteu reagent method. From the results of free radical scavenging assay, IC₅₀ values of ethanol and water extracts were found to be 0.72 µg/mL and 1.09 µg/mL while compared with that is standard vitamin C (IC₅₀= 1.17 µg/mL). The total phenol contents of ethanol and water extracts were found to be (163.80 ± 0.018 mg GAE/g) and (173.58 ± 0.047 mg GAE/g) respectively.

Key Words: *Salix alba* L., Antioxidant activity, DPPH, Total phenol content, Folin-Ciocalteu reagent

1. INTRODUCTION :

Countries in Asia, including Myanmar, have rich traditional medicinal plants for the treatment of diseases. Medicinal values of the plant extracts can be determined and application of these chemical constituents can have effective action on undesirable microbes and insects. Plants are rich sources of secondary metabolites with interesting biological constituents. *Salix alba* L., commonly known as white willow in English, Moe-ma-kha in Myanmar and considered medicinal. In recent years, it has been used for herbal drugs due to its bioactive constituents with pharmacological properties. The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. The Department of Chemistry has undertaken research works of identifying anticancer, antibacterial, antimalarial and antioxidant new compounds in which the compounds have been isolated and identified from natural sources for research works. As an important category of phytochemicals, phenolic compounds widely exist in plants and have been considered to be a major contributor to the antioxidant activity. And so, it makes possible to screen the presence of antioxidant activity and total phenol content of the extracts from the bark of *Salix alba* L. (Moe-ma-kha).

2. MATERIALS AND METHODS :

Antioxidant activity of crude extract of *Salix alba* L. was determined by DPPH (2, 2-diphenyl--picrylhydrazyl) assay. Crude extract (2 mg) and 10 mL of EtOH were thoroughly mixed by shaker, the mixture solution was filtered and the stock solution was obtained. The sample solution (20, 10, 5, 2.5, 1.25 and 0.625 µg/mL concentration) was prepared from this stock solution by dilution with appropriate amount of EtOH. The effect on DPPH radical was determined using the method by Marinova and Batch varov (2011). The control solution was prepared by mixing 1.5 mL of 60 M DPPH solution and 1.5 mL of EtOH using shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 60 M DPPH solution and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance of these solutions was measured at 517 nm by using UV-7504 spectrophotometer. Absorbance measurements were done in triplicate for each concentration and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation.

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} = absorbance of control solution

A_{sample} = absorbance of tested sample solution

IC₅₀ value was calculated by linear regressive excel program.

The total phenol content of crude extracts was determined by Folin-Ciocalteu reagent method. A volume of 1 mL of crude extract (0.5 mg/mL) was mixed with 5 mL of Folin-Ciocalteu reagent (diluted 1 :10 with distilled water) and were neutralized with 4 mL of 1 M sodium carbonate solution. The reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking for color developed. The absorbance of the resulting blue color was measured at 765 nm using UV-visible spectrophotometer. The total phenol content was calculated with the help of the graph shown in Figure 3 and standard curve equation was $y = 0.0304 x + 0.0445$, where $R^2 = 0.9976$.

3. RESULTS AND DISCUSSION :

From the observation of DPPH free radical scavenging assay, both ethanol and water extracts from the bark of *Salix alba* L. have shown that the effectiveness of antioxidant activities of extracts showed more potency than that of standard vitamin C. The radical scavenging activities of extracts were expressed in terms of % RSA and IC₅₀ (50 % inhibitory concentration). Ethanol extract (IC₅₀ = 0.72 µg/mL) possessed the higher radical scavenging property than the water extract (IC₅₀ = 1.09µg/mL). However, both extracts showed more potency than that of standard vitamin C (IC₅₀ = 1.17 µg/mL). These results are shown in Table 1, Figures 1 and 2 respectively. Since the lower the IC₅₀ value, the higher the antioxidant activity of the extract. High antioxidant values are valuable for human health because antioxidant have potential beneficial effects in protecting against diseases. According to the results, the total phenol contents of ethanol and water extracts from the bark of *Salix alba* L. were found to be (163.80 ± 0.018 mg GAE/g) and (173.58 ± 0.047 mg GAE/g) respectively. The results are described in Table 2 and 3. From the result, there was lack of correlation between TPC and DPPH radical scavenging activity of plant because the plant had higher TPC value was not necessary better in DPPH inhibition. Thus, the bark extracts of *Salix alba* L. are better sources of natural antioxidants, which may be useful in preventing the progress of various oxidative stresses. The remedial potency of such plant extracts for antimicrobial, anti-inflammatory, anticancer and pain killer etc. may be employed to justify their potencies.

Table 1. %RSA (Radical Scavenging Activity) and IC₅₀ Values of Tested Samples and Standard Vitamin C

Extract	% Inhibition (mean ± SD) in different concentration (µg/ml)						IC ₅₀ (µg/mL)
	0.625	1.25	2.5	5	10	20	
<i>Salix alba</i> (water)	28.94 ± 3.56	57.41 ± 4.29	85.88 ± 0.54	85.53 ± 0.54	87.43 ± 1.14	84.94 ± 1.25	1.09
<i>Salix alba</i> (EtOH)	47.81 ± 5.05	62.16 ± 1.09	82.44 ± 0.74	85.41 ± 0.94	85.65 ± 0.90	84.46 ± 0.54	0.72
Standard Vitamin C	25.20 ± 1.40	53.58 ± 0.88	65.53 ± 1.13	74.82 ± 0.59	83.32 ± 0.73	90.15 ± 0.48	1.17

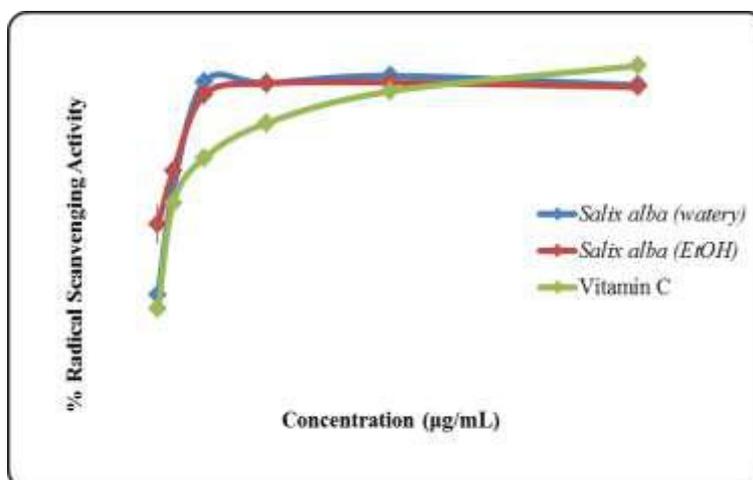


Figure 1. Radical scavenging activity of different concentrations of crude extracts from *Salix alba* L. (Moe-ma-kha) bark and vitamin C

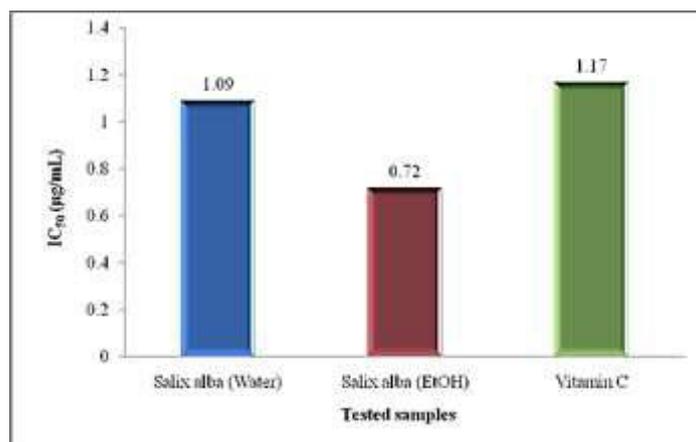


Figure 2. A bar graph of IC₅₀ values of crude extracts from *Salix alba* L. (Moe-ma-kha) bark and vitamin C

Table 2. The Absorbance of Gallic Acid Standard Solution at 765 nm

Sr No.	Concentration (µg/mL)	Absorbance
1	3.125	0.0918
2	6.250	0.1997
3	12.500	0.4244
4	25.000	0.8705
5	50.000	1.6284
6	100.00	3.0449

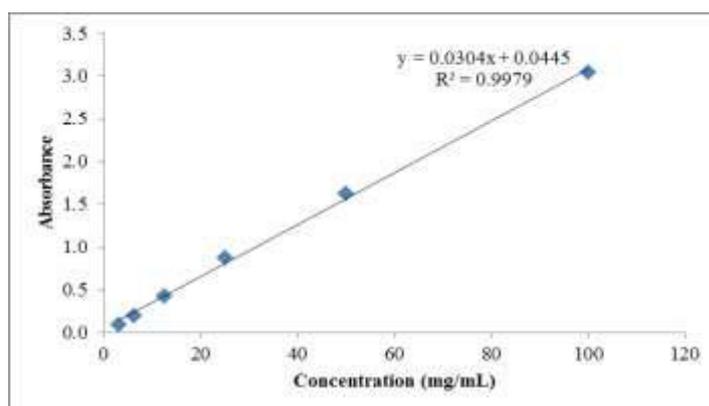


Figure 3. Construction of gallic acid standard calibration curve

Table 3. Total Phenol Content (TPC) of Extracts of *Salix alba* L. by Folin-Ciocalteu Reagent Method

Extracts	TPC (mg GAE ± SD)/ g of extract
H ₂ O	173.58 ± 0.047
EtOH	163.80 ± 0.018

4. CONCLUSION:

It may be concluded that from the study of the extracts from bark of *Salix alba* L. indicated a potency for medicinal used since *Salix alba* L. has been known to have antioxidant potential. It had been proved that extracts from the bark showed antioxidant active potency.

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