

Evaluation of Total Phenolic Content and Investigation of Potent Flavonoid Compound Isolated from *Butea frondosa* Roxb.

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Abstract

In this research work, the stem bark of *Buteafrondosa*Roxb., Myanmar name Pauk, was selected for chemical analysis. This sample was collected from Amarapura Township, Mandalay Region, Myanmar. The aim of this research is to get more interesting and bioactive compounds from this versatile medicinal plant. Firstly, the preliminary phytochemical test of this selected plant was carried out. Moreover, the total phenols of selected sample were extracted with methanol and distilled water. This extract was checked for qualitative test of phenols. In addition, total phenolic content of selected sample was evaluated by the Folin- Ciocalteu reagent using UV spectrophotometer at 765 nm. The total phenolic content of selected sample was found to be 15.4 ± 0.023 mg gallic acid equivalents (GAE) per g dry weight. Furthermore, the potent pure compound was isolated by Column Chromatography and HPLC methods at Meijo University, Nagoya, Japan. The structure of isolated compound was elucidated as flavonoid by advanced spectroscopic methods such as 1D, 2D NMR and EI Mass spectrometry. Based on the above findings, it can be suggested that this selected plant which is phenolic rich plant could be used as primary free radical scavengers.

Keywords: *Buteafrondosa*Roxb., phenols, spectrophotometric method, Folin-Ciocalteu reagent, HPLC method.

Introduction

It is evident that without nature human being life is impossible. There are three basic necessity of humans which are food, clothes and shelter and now the fourth one is good health and it is provided by plant kingdom. Nature stands a golden mark and provided the storehouse of remedies to cure all ailments of mankind. Plant kingdom represents a rich house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years (Sindhia and Bairwa, 2010).

Medicinal plants contain numerous active ingredients that may be potentially useful for the development of therapeutic agents. The identification and isolation of phytochemical groups and/or single chemical entities from them are, hence, crucial for drug discovery as these entities often work as individual agents or as a collective group of phytochemicals (purified extracts) to achieve the desired therapeutic effect. However, to assess their quality, standardisation of these plant parts needs to be carried out, which includes a series of tests to determine the quality, quantity and the purity of the phytochemicals or the extracts, along with the measure of contaminants or foreign matter present in them (Pradhan, et al., 2015).

Buteafrondosa Koenig ex Roxb./*Butea monosperma* (Lamk.) Taub. (Family: Fabaceae) is a long and deciduous tree, commonly distributed throughout India (Sharma, et al., 2001). It is also known as flame of the forest and found in greater parts of India, Myanmar (Burma) and Sri Lanka. It is a sacred deciduous tree, attaining a height of 12–15 m. It is reported to have excellent medicinal properties (Choedon, et al., 2010). The bark is reported to possess astringent, pungent, alliterative, aphrodisiac and antihelminthic properties. It is also useful in tumours, bleeding piles, gonorrhoea and ulcers (Burli and Khade, 2007). Even though a study on the aphrodisiac activity of *B. frondosa* (BF) bark extract is available, there is no existing

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evidence to elucidate the mechanism of action (Ramachandran, et al., 2004). *Butea monosperma* (BM), also known as Palas in the traditional system of medicine is a medicinal plant. As reported by the Indian Ayurvedic texts, its leaves, stem, flowers, seeds, gum (stem) and roots have been widely used as traditional medicine (Nadkarni, 2007) (Kirtikar and Basu, 1999).

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. Plant phenolics include phenolic acids, flavonoids, tannins and the less common stilbenes and lignans (D'Archivio, et al., 2007). Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized according to chemical structure into flavonols, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species (ROS). An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis and neurodegenerative diseases. Flavonoids may help to protection against these diseases (Hatano, et al., 1989).

The objective of present study was to evaluate the safe natural antioxidant products (Phenolic compounds) of *B. frondosa* Roxb. and to isolate and elucidate the secondary metabolite (Flavonoid compound) from this versatile medicinal plant.

Botanical Description

Family Name	:	Fabaceae
Botanical Name	:	<i>Butea frondosa</i> Koenig ex Roxb.
Synonym	:	<i>Butea monosperma</i> (Lamk.) Taub.
Genus	:	<i>Butea</i>
Species	:	<i>B. monosperma</i>
Myanmar Name	:	Pauk
English Name	:	Parrot tree



Figure 1. Flower and plant of *B. frondosa* Roxb.

Material and Methods

Sample Collection

The stem barks of *B.frondosa*Roxb. were collected from Amarapura Township, Mandalay Region, Myanmar (Figure 1). The collected samples were cut into small pieces and dried in air. It was stored in a well-stoppered bottle and used throughout the experiment.

Preliminary Phytochemical Test of *B.frondosa*Roxb.

The stem bark of *B.frondosa*Roxb.was tested by phytochemical screening according to the usual procedures.

Extraction of Phenol from *B.frondosa*Roxb.

2 g of dried plant sample was ground in a mortar and pestle. It was extracted with 20 mL of 0.3 % HCl in methanol. The mixture was centrifuged at 5000 rpm for 30 min. The supernatant was decanted to a small beaker. The extraction procedure was repeated for two times. The supernatant was poured to the same container.

The supernatant was evaporated to dryness and it was dissolved in distilled water. This solution was made up to 10 mLwith distilled water. This extract contains the phenols.

Qualitative Test for Phenols

Acid propertiestest:

The extract solutionselected sample was tested by blue litmus paper. This blue litmus paper turns red.

Colour with FeCl₃:

1mL of extract solutionwas taken and a few drops of very dilute solution of ferric chloride were added. The colour changes to brown and which indicates the presence of phenol. The reaction takes place as follows (Aparna, 2000).

Quantitative Determination of Total Phenolic Content

Principle

Phenols in alkaline medium react with phosphomolybdic acid of Folin- Ciocalteu reagent producing a blue coloured complex.

Estimation of λ_{\max} for gallicacid

To determine the absorption maximum, standard solution of gallic acid in concentration 7.5 $\mu\text{g}/\text{mL}$ was prepared. And then, 100 μL of Folin–Ciocalteu reagent and 300 μL of saturated Na₂CO₃ (20 %) solution were added. This standard solution was heated in the water bath at 40 °C for 30 min and then it was cooled at room temperature. The spectrum of this solution was measured in the wavelength interval 700 to 800 nm.

Preparation and determination of standard gallicacid

10 mg of the standard gallic acid was taken in a test tube. 10 mL of distilled water was added to the standard compound. 1 mL of this standard solution was taken in another test tube. The volume of this solution was made up to 10 mL with distilled water. The standard solution was taken by micro-pipette into a series of test tubes 20 μL , 40 μL , 60 μL , 80 μL and 100 μL respectively.

The volume was made up to 1.6 mL with distilled water in each test tube. And then, 100 µL of Folin-Ciocalteu reagent and 300 µL of saturated Na₂CO₃ (20%) solution were added. After the each standard solution was heated in the water bath at 40°C for 30 minutes, the values of absorbance of these prepared solutions were measured with a UV/ Visible spectrophotometer at 765 nm with respect to the blank solution. The calibration curve of standard gallic acid is shown in Figure3 (Slinkard and Singleton, 1977).

Determination of total phenolic content of stem bark of *B.froncosa*Roxb.

The total phenolic content of extract solution of stem bark of *B.froncosa*Roxb.was measured with the Folin-Ciocalteu reagent. Firstly, 20 µL of extract solution was taken in a test tube. It was made up to 1.6 mL with distilled water. 100 µL of Folin-Ciocalteu reagent was mixed, then 300 µL of saturated Na₂CO₃ (20%) was added.

The mixture was heated in a water bath at 40°C for 30 minutes and then cooled in an ice-bath. The absorbance of this prepared sample solution was measured at 765 nm by using UV/ Visible spectrophotometer. The assay was carried out in triplicate. The total phenolic content of extract solution of stem bark of *B.froncosa*Roxb.was expressed as mg gallic acid equivalent (GAE) /g dry weight.

Extraction and Isolation of Stem Barks of *B.froncosa*Roxb.

The air-dried stem barks of *B. frondosa*Roxb. (1.5 kg) were extracted with 4 L of MeOH at room temperature for one month. The MeOH extract was concentrated and the residue (25.8 g) was suspended in water. This suspension was successively extracted with ethyl acetate and *n*-butanol.

The ethyl acetate soluble extract was concentrated by rotary evaporator to produce a residue (5.4 g). The extract was fractionated on a reverse column using methanol and water gradient to afford 10 fractions (frs. I-X). Fraction V was rechromatographed over silica gel eluted with *n*-hexane and ethyl acetate to yield nine combined fractions. Then, the combined fraction (III) was found to be main fraction which showed only one spot on TLC and UV active. This compound was purified by HPLC (HighPerformance Liquid Chromatography). The pale yellow needle shape solid was obtained. The yield percent of this pure compound is 0.417 % (22.5 mg) based upon the ethyl acetate crude extract.

Results and Discussion

Determination of Phytochemical Test of *B.froncosa*Roxb.

According to the phytochemical investigation on the *B.froncosa*Roxb., the presence of chemical constituents such as alkaloids, flavonoids, glycosides, phenolic compounds, sugars, saponins, tannins, steroids and terpenes were detected in the extract of *B.froncosa*Roxb.

Evaluation of Total Phenolic Content in Stem Barks of *B.froncosa*Roxb.

Special test for phenol

In accordance with the special qualitative tests of phenol, it was found that the stem bark of *B.froncosa*Roxb. consists of phenolic compounds.

Absorption maximum wavelength of gallic acid

Scanning of the complex in a wavelength range from 700 nm to 800 nm showed a maximum absorbance (λ_{max}) at 765 nm as shown in Figure 2.

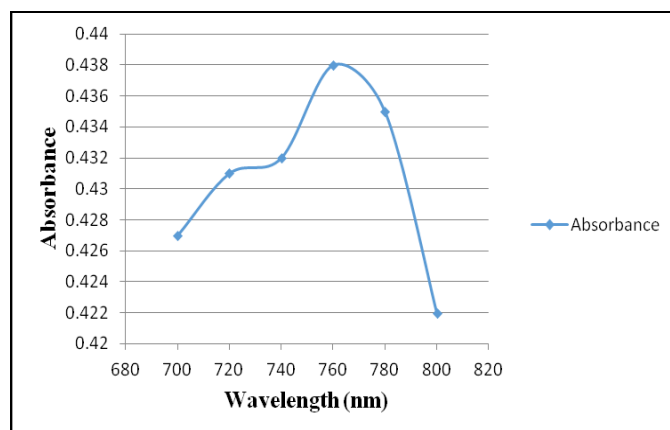


Figure 2. Maximum wavelength of standard gallic acid

Total phenolic content in stem barks of *B.froncosa* Roxb.

The calibration curve was plotted against by using the resulting data of standard gallic acid solution as shown in Figure3.

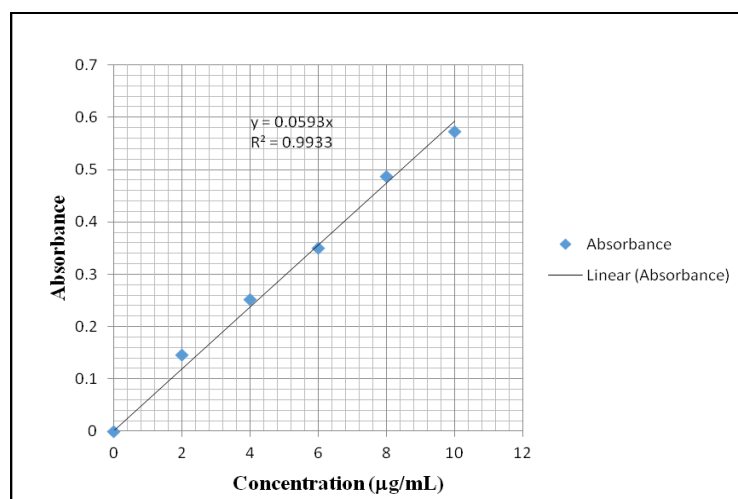


Figure 3. Absorbance concentration calibration curve for standard gallic acid

The total phenolic content of the extract solution was carried out by spectrophotometric method using the Folin-Ciocalteu reagent. The absorbance values of prepared sample solutions were measured with UV/ Visible spectrophotometer at 765 nm with respect to the blank solution. The amount of total phenolic content of analyzed sample was obtained by using the standard graph. The results are described in Table 1.

Table 1. The Results of Total Phenolic Content of *B.froncosa*Roxb.

Name of Sample	Phenol (mg/g)	Phenol (mg/g) Mean \pm Standard Deviation
Stem bark of <i>B.froncosa</i> Roxb.	15.41	
	15.37	15.4 \pm 0.023
	15.41	

From this result, the total phenolic content presented in the pomegranate juice was found to be 15.4 \pm 0.023 mg gallic acid equivalent (GAE) per g dry weight.

Structural Elucidation of Isolated Flavonoid Compound

According to the experimental section, one pure compound could be isolated from the EtOAc extract of stem bark of *B.froncosa*Roxb. Isolated compound is pale yellow solid. The structure of pure compound was assigned by FT IR, ¹H NMR, ¹³C NMR, DEPT, HSQC, EI-mass, DQF COSY and HMBC spectral data respectively (Figure 4-11).

According to FT-IR spectral data, compound consists of OH-group, sp² hydrocarbon, sp³ hydrocarbon, carbonyl group, aromatic benzene ring, C – C – O stretching vibration of alcohol group, C – O – C stretching vibration of ether functional group, trans or E and cis or Z alkenic groups respectively.

Its molecular formula is C₁₆H₁₂O₅ by applying FT IR, ¹H NMR, ¹³C NMR, HSQC, DEPT and EI Mass spectra. The molecular mass in EI mass spectrum is m/z-284 and degree of unsaturation in its structure is 11 which must be two benzene moieties and one pyran ring by using the DQF COSY and HMBC spectral data. The structure of this isolated compound could be assigned as flavonoid one. The ¹H- and ¹³C-NMR (CDCl₃), H-H, H-C correlation in DQF-COSY and HMBC spectra are shown in Table 2.

Flavonoid Compound :Pale yellow solid. ¹H-NMR (600 MHz, CDCl₃) δ : 8.4 (1H, s; H-2), 7.4(1H, d; *J* = 8.5 Hz; H-2'), 7.4(1H, d; *J* = 8.5 Hz; H-6'), 6.8 (1H, d; *J* = 8.5 Hz; H-3'), 6.8 (1H, d; *J* = 8.5 Hz; H-5'), 6.6 (1H, d; *J*=2.1 Hz; H-6) , 6.4 (1H, d; *J*=2.1 Hz; H-8), 3.9 (3H, s; C-7-OCH₃). ¹³C-NMR (125 MHz, CDCl₃) δ : 180.4 (C-4), 165.2 (C-7), 161.7 (C-5), 157.5 (C-4'), 157.4 (C-9), 154.3(C-2), 130.1 (C-2'), 130.1(C-6'), 122.5(C-3), 121.1(C-1'), 115.1 (C-3'), 115.1 (C-5'), 105.4 (C-10) , 97.9(C-6), 92.4(C-8), 56.1 (C-7-OCH₃), EI MS *m/z* (rel. int.): 284 [M⁺] (C₁₆H₁₂O₅).

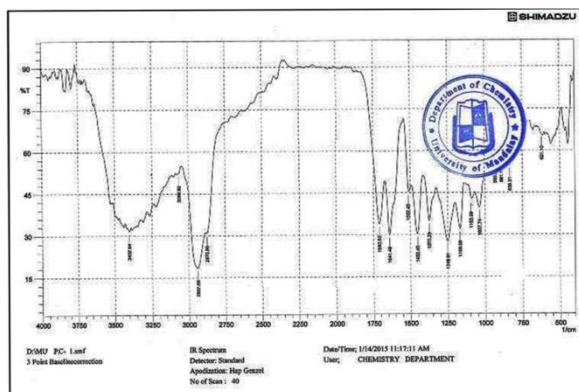
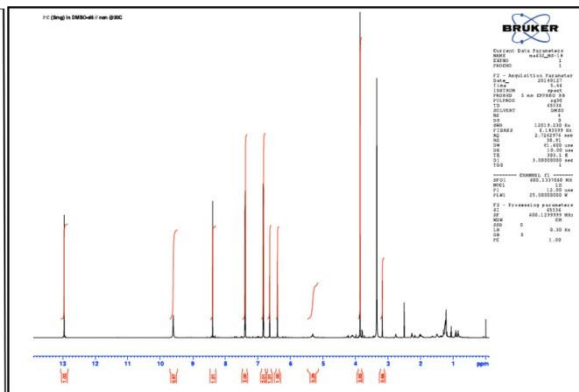


Figure 4. FT IR spectrum of isolated compound

Figure 5. ¹H NMR spectrum of isolated compound

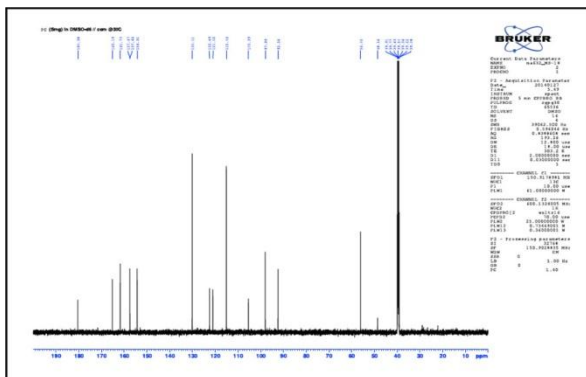


Figure 6. ¹³C NMR spectrum of isolated compound

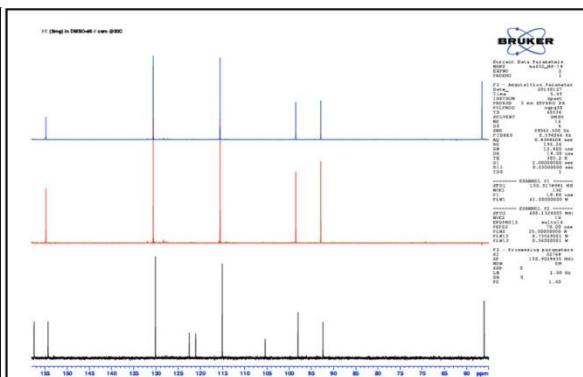


Figure 7. DEPT spectrum of isolated compound

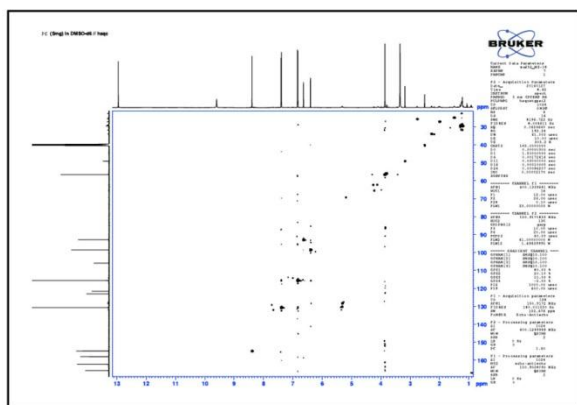


Figure 8. HSQC spectrum of isolated compound

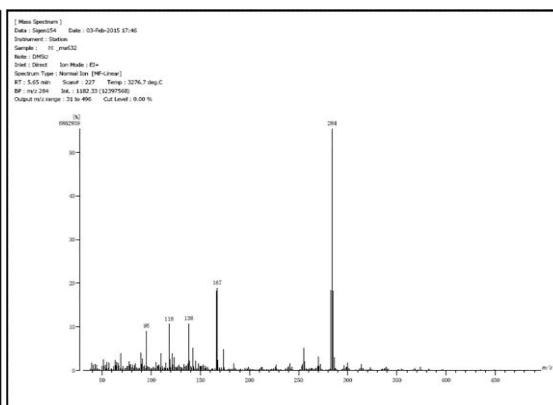


Figure 9. EI Mass spectrum of isolated compound

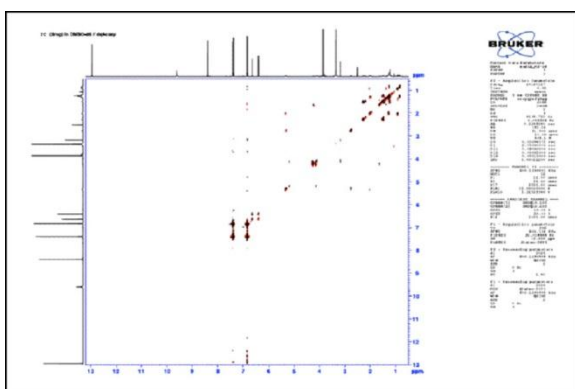


Figure 10. DQF-COSY spectrum of isolated compound

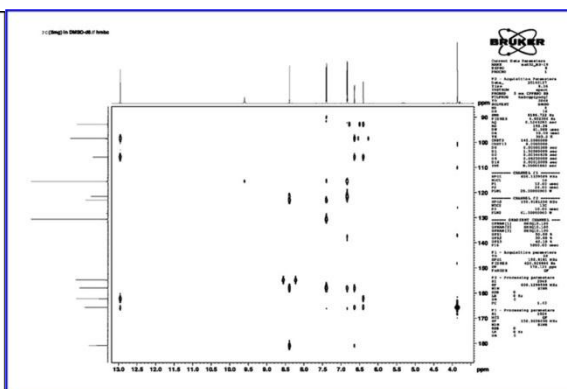


Figure 11. HMBC spectrum of isolated compound

TABLE 2. ¹³C, ¹H NMR DATA OF FLAVONOID COMPOUND AND ¹H-¹³C, ¹H-¹H CORRELATIONS EXHIBITED IN THE 2D NMR SPECTRA IN CDCl₃

No	Carbon	$\delta^{13}\text{C}$ (DEPT)	Proton	$\delta^1\text{H}$ (J Hz)	HMBC Correlation	COSY Correlation
1	OCH ₃	56.1 (OCH ₃)	OCH ₃	3.9 (s)	C-7	-
2	8	92.4(CH)	8	6.6(d, J=2.1)	C-4, C-7, C-9, C-10	H-6
3	6	97.9(CH)	6	6.4 (d, J=2.1)	C-7, C-5, C-10	H-8
4	10	105.4	-	-	-	-
5	3'	115.1 (CH)	3'	6.8 (d, J=8.5)	C-1', C-5'	H-2'
6	5'	115.1 (CH)	5'	6.8 (d, J = 8.5)	C-1', C-3'	H-6'
7	1'	121.1	-	-	-	-
8	3	122.5	-	-	-	-
9	2'	130.1(CH)	2'	7.4(d, J = 8.5)	C-4', C-3, C-6'	H-3'
10	6'	130.1 (CH)	6'	7.4(d, J = 8.5)	C-4', C-3, C-2'	H-5'
11	2	154.3(CH)	2	8.4 (s)	C-3, C-4, C-9	-
12	9	157.4	-	-	-	-
13	4'	157.5	-	-	-	-
14	5	161.7	-	-	-	-
15	7	165.2	-	-	-	-
16	4	180.4	-	-	-	-

The elucidated structure of isolated compound from the stem bark of *B.froncosa* Roxb. is described in Figure 12.

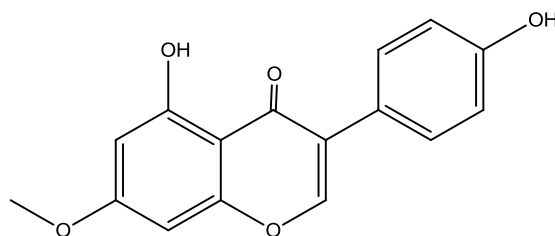


Figure 12. Structure of elucidated flavonoid compound

The IUPAC name of this flavonoid compound is 5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one.

Conclusion

The endeavour of present study is focused on phytochemical screening and determination of total phenolic content in the stem bark of *B. frondosa* Roxb. The total phenolic content was found to be significant amount in the selected sample. Further study is focused on the structural elucidation of isolated pure flavonoid compound from this selected plant. Flavonoids are phenolic compounds which are the secondary metabolites and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these natural antioxidant compounds (phenolic and flavonoid compounds) were found to be present in the stem bark of *B. frondosa* Roxb., it might be responsible for the potent antioxidant capacity of this plant. This will be most likely to improve the radical scavenging activity and other potential health benefits, promoting their use as natural antioxidant source.

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