



A Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of *Bauhinia purpurea*

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Abstract

In this study, we conducted a comparative investigation into the phytochemical screening and antioxidant activity of aqueous extracts from various parts of the *Bauhinia purpurea* plant, including leaf, flower, stem bark, and root. The qualitative analysis was performed to screen the phytochemical content of each extract, followed by quantitative analysis to determine the total phenolic and total flavonoid contents. Our findings revealed that different parts of the *B. purpurea* plant yielded distinct natural products upon extraction. Both the leaf and flower extracts contained alkaloids, flavonoids, saponins, carbohydrates, polyphenols, and phenolics. On the other hand, the aqueous extracts of the stem barks and root parts of *B. purpurea* only contained alkaloids, flavonoids, and phenolics. Consistent with the phytochemical assay, the flower extract exhibited the highest total phenolic content ($40.14 \pm 0.65 \mu\text{g}/\text{mL GAE}$) and the highest flavonoid content ($387.57 \pm 0.63 \mu\text{g}/\text{mL CE}$) compared to the other parts. Consequently, the flower extract displayed the highest antioxidant activity ($51.76 \pm 0.32\%$) with DPPH radical assay, closely approaching the antioxidant activity of ascorbic acid ($70.54 \pm 0.51\%$), which served as the positive control. This significant finding highlights the potential of the *B. purpurea* flower as a potent source of antioxidant agents for future applications.

Keywords: *Bauhinia purpurea*, antioxidant, phytochemical, phenolic content, flavonoid content

1. INTRODUCTION

Studies on medicinal plants and traditional medicines have long fascinated herbalists worldwide. Traditional medicine, with its holistic approach, places emphasis on enhancing overall health, disease prevention, immune regulation, and personalized care. It is noteworthy that over 60% of pharmaceuticals have been derived from plants, owing to their gentle impact on the human body, minimal or negligible adverse effects, cost-effective production, and convenient accessibility. Consequently, medicinal plants provide considerable advantages for human well-being [1].

Bauhinia purpurea is a flowering plant commonly known as the Purple Orchid Tree or Butterfly Tree. It belongs to the family Fabaceae and is native to Southeast Asia. *B. purpurea* is renowned for its vibrant and showy flowers, which

range in color from pale pink to deep purple. The unique shape of its leaves, which resemble a pair of butterfly wings, gives the plant its distinctive appeal. *B. purpurea* is widely cultivated as an ornamental tree due to its beautiful flowers and attractive foliage [2]. It is often used in gardens, parks, and landscapes to add a touch of color and elegance. The plant thrives in warm, tropical climates and prefers well-drained soil and full sunlight. Apart from its aesthetic value, *B. purpurea* also holds significance in traditional medicine systems. Various parts of the plant, including the flowers, leaves, and bark, are believed to possess medicinal properties [3]. It has been used in herbal remedies for its potential anti-inflammatory [4]-[6], antioxidant [7]-[9], and antimicrobial [10] effects.

B. purpurea contains a diverse array of bioactive compounds, as demonstrated by various researchers. Phytoconstituents found in *B. purpurea* encompass glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepine, fatty acids, and phytosterols [11]. Negi et al. [12], not only identified ergosterol, beta-tocopherol, stigmasterol, and lanosterol, but also discovered additional bioactive compounds such as lupeol, phytol, hexadecanoic acids, methyl esters of hexadecanoic acids, octadecadienoic acids, and octadecatrienoic acid in the leaf extract. Zakaria et al. [13] demonstrated the presence of flavonoids, saponins, condensed tannins, and steroids. Pettit et

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Table 1. Qualitative phytochemical screening of *B. purpurea* aqueous extract.

Phytochemical	<i>B. purpurea</i> aqueous extract			
	Leaf	Flower	Stem Bark	Root
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	-	-
Carbohydrates	+	+	-	+
Polyphenols	+	+	-	-
Proteins	+	-	-	-
Amino Acids	+	-	-	-
Phenolics	+	+	+	+
Triterpenes	-	-	-	-
Anthraquinones	-	-	-	-

+ = presence; - = absence

al. [14] discovered the existence of bauhinia statins 1 and 2. Furthermore, numerous other investigators have identified various bioactive compounds, including flavonoids [15], xylopyranoside [16], and triperpine [17].

Khrisnaveni examine the secondary metabolites and antioxidant properties of an aqueous extract obtained from shade-dried *B. purpurea* leaves. The total phenolic content was determined using the Folin-Ciocalteu method, while the total flavonoid content was measured using the aluminum chloride method. Additionally, various standard methods were employed to evaluate antioxidant activities. The analysis revealed a higher concentration of flavonoids (160.0±6.9 mg/g) compared to phenolics (126.66±6.11 mg/g). Moreover, the extract exhibited higher levels of nitric oxide scavenging activity (258.66±4.61 mg/g) and reducing power activity (141.33±2.30 mg/g) when compared to total antioxidant activity (81.33±6.11 mg/g) and metal chelating activity (30.66±2.30 mg/g) [18]. Aye (2021) use *B. purpurea* for the screening of various bioactivities. Crude extracts were prepared through solvent extraction. The total phenolic content in the ethanol and aqueous extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent. The ethanol extract exhibited a higher phenolic content (32.58 µg GAE/mg of extract) compared to the aqueous extract (21.73 µg GAE/mg of extract). To evaluate antioxidant activity, both ethanol and aqueous extracts were subjected to DPPH radical scavenging assay. The ethanol extract demonstrated greater potency (IC₅₀ = 5.75 µg/mL)

in antioxidant activity compared to the aqueous extract (IC₅₀ = 8.51 µg/mL). The antimicrobial activity of both extracts was tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, and *Escherichia coli*. The results indicated the susceptibility of all tested microorganisms to both the ethanol and aqueous extracts of *B. purpurea* leaf. Furthermore, the acute toxicity of the ethanol extract was investigated using doses of 300, 2000, and 5000 mg/kg body weight on albino mice, and no lethality was observed up to fourteen days after administration [19]. The purpose of this work was to enrich the research on the *B. purpurea* variety from Yangon, Myanmar by performing phytochemical screening and describing the effect of different parts (leaf, flower, stem bark, and root) on the antioxidant activity.

2. MATERIALS AND METHODS

2.1. Materials

B. purpurea samples (leaf, flower, stem bark, and root) were collected from Thanlyin, Yangon, Myanmar during October-December 2021. Laboratory-grade Dragendroff's reagent, Anthrone reagent, sulphuric acid, Ninhydrin reagent, Millon's reagent, iron(III) chloride (FeCl₃), 3-indoleacetic acid, Folin-Ciocalteu reagents, sodium carbonate, gallic acid, catechin, aluminium(III) chloride (AlCl₃), sodium nitrite, and sodium hydroxide were purchased from Merck Sigma-Aldrich Reagent Pte, Singapore.

2.2. Methods

2.2.1. Preparation of Aqueous Extract

The extraction method refers to the research by previous literature with some modifications. Fresh *B. purpurea* (leaf, flower, stem bark, and root) samples were washed using flowing water, dried under direct sunlight, and finally ground into a powder and stored at room temperature. Powder samples of 4 g were mixed with 100 mL of distilled water. The mixture was then heated to 60 °C for 20 min. After heating, the solution was allowed to cool and filtered using filter paper (Whatman filter paper), and then the filtrate was collected.

2.2.2. Qualitative Phytochemical Screening

The qualitative phytochemical screening of the extracts was performed to identify the main groups of chemical constituents present in the extracts using the color reactions. The extract of *B. purpurea* was subjected to detect the presence of alkaloids (Dragendroff's test) [20], flavonoids (colorimetric AlCl₃ test) [21], saponins (Foam test) [21], carbohydrate (Anthrone test) [22], polyphenols (Puncal-D test) [23], proteins (Ninhydrin test) [23], amino acid (Millon's test) [24], phenolics (Follin-Ciocalteu test) [25], triterpene (Salkowski test) [26], and anthraquinones (Borntrager's test) [26]. The extract solution was further analyzed to determine its total phenolic content with Follin-Ciocalteu reagent [27] in which the results were shown as the equivalent of a $\mu\text{g mg}^{-1}$ gallic acid (GAE). Catechin as a standard was employed in a colorimetric AlCl₃ determination of the total flavonoid content of the extract. The total flavonoid content of the extract was expressed in $\mu\text{g mg}^{-1}$ equivalent of catechin (CE) [28].

2.2.3. FTIR Analysis

Fourier transform infrared (FTIR) spectroscopic technique was used to identify the presence of functional groups in various parts of *B. purpurea* aqueous extract. FTIR spectrum of samples was recorded on a spectrophotometer (Shimadzu IR Prestige 21). The spectrum was recorded in the wavelength range of 4000–500 cm⁻¹.

2.2.4. Antioxidant Activity

The extract sample's antioxidant activity was evaluated by DPPH radical testing following the procedure described by the previous report, using ascorbic acid as the positive control. DPPH 0.1 mmol L⁻¹ solution was prepared by dissolving in ethanol. As much as 1 mg of ascorbic acid was dissolved in 1 mL of methanol. Dilution was performed to produce a standard solution of ascorbic acid with varying concentrations (50–500 $\mu\text{g mL}^{-1}$). For each tube containing a stock solution of ascorbic acid (200 μL), 1 mL of 0.1 mmol L⁻¹ DPPH solution was added, and a further 800 μL 50 mmol L⁻¹ Tris-HCl buffer was added (pH 7.4). The final volume is adjusted to 4 mL with ethanol. Stock solutions for extract prepared by dissolving 1 mg of each sample in 1 mL of methanol. Different aliquots of stock solution (50–500 μg) were added to the separate tube, and the final volume was adjusted to 2 mL using ethanol. A total of 1 mL of 0.1 mmol L⁻¹ DPPH solution and 800 μL 50 mmol L⁻¹ Tris-HCl buffer (pH 7.4) were added to each tube. The control was prepared by mixing 1 mL DPPH 0.1 mmol L⁻¹, 800 μL 50 mmol L⁻¹ Tris-HCl buffer (pH 7.4), and 2 mL of ethanol. Absorbance was recorded at room temperature after incubation for 30 min, measured at 517 nm by UV-Vis spectrophotometer (Analytic Jena Specord 200 Plus). The percentage of antioxidant activity (inhibition percentage) was calculated using the

Table 2. Total phenolic and flavonoid contents as well as antioxidant activity.

Sample	Total Phenolic Content ($\mu\text{g/mg}$ GAE)	Total Flavonoid Content ($\mu\text{g/mg}$ CE)	DPPH Scavenging Activity (%)
Leaf	34.84 ± 0.37	345.71 ± 0.25	48.87 ± 0.78
Flower	40.14 ± 0.65	387.57 ± 0.63	51.76 ± 0.32
Stem Bark	23.76 ± 0.55	227.14 ± 0.87	34.65 ± 0.67
Root	13.76 ± 0.54	119.54 ± 0.79	21.26 ± 0.55
Ascorbic Acid	-	-	70.54 ± 0.51

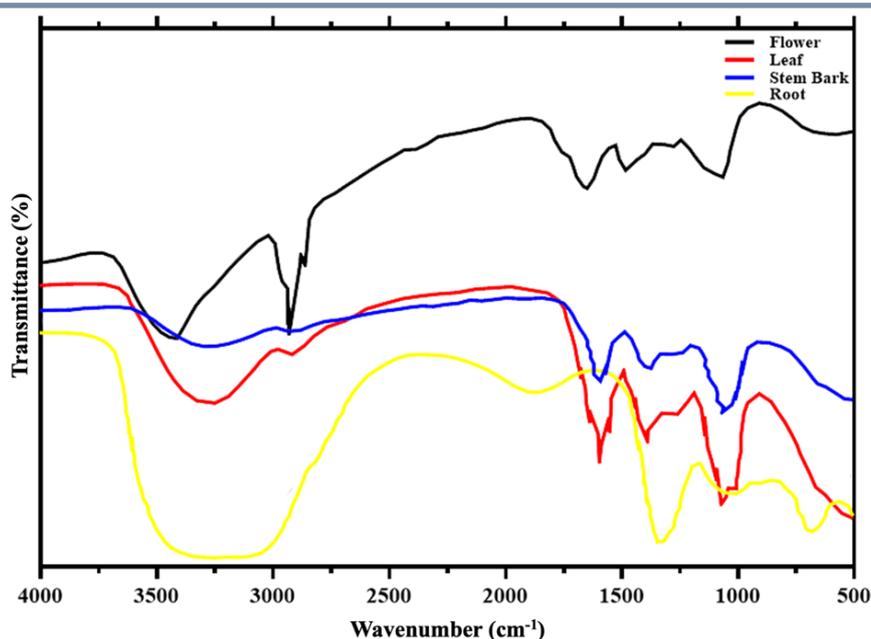


Figure 1. FTIR spectrum of different parts of *Bauhinia purpurea* aqueous extract.

following equation 1 [28];

$$\% \text{ DPPH Scavenging Activity} = (A_c - A_s) / A_c \times 100\% \quad (1)$$

whereas A_c is the absorbance value of the control solution while A_s is the absorbance value of the sample. The mean and standard deviation were calculated based on triplicate measurement.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical screening

In this study, the aqueous extracts of *B. purpurea* (leaf, flower, stem bark, and root) were screened for the presence of bioactive compounds. The results of the study revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids, and phenolics. However, triterpenes and anthraquinones were not found in any of the aqueous extracts (Table 1). The presence of alkaloids can be detected using Dragendroff's reagent. Dragendroff's reagent contains potassium iodide and bismuth(III) nitrate under acidic conditions. Since alkaloids typically contain tertiary amine groups, they can form a complex with tetraiodobismuth, resulting in an orange-colored precipitate [20].

The presence of flavonoids was observed through the $AlCl_3$ test based on color change. If the sample contains flavonoids, the addition of $AlCl_3$ will result in a yellow color [25]. The detection of

saponins was conducted using the Foam test, where the presence of saponins can be observed from the formation of stable foam on the surface of the sample solution [21]. The presence of carbohydrates was detected using the Anthrone test. Anthrone reagent will form a blue-green color when it reacts with the furfural form of carbohydrates under acidic conditions [22]. The presence of polyphenols was observed through the Puncal-D test. Positive results were indicated by the formation of a blue-colored complex. Protein detection was carried out using the Ninhydrin test. Free amino groups in proteins will form a blue-purple complex with ninhydrin reagent [23]. The presence of amino acids was evaluated with the Millon's test. Millon's reagent was prepared by dissolving mercury in nitric acid. Hydroxyl aromatic groups of amino acids will form a complex with mercury ions, resulting in a red precipitate [24].

The presence of phenolics was examined with the Folin-Ciocalteu test. Folin-Ciocalteu reagent contains a mixture of phosphomolybdate and phosphotungstate acids. Oxidation-reduction reactions with phenolic compounds will generate a blue-colored solution [28]. On the other hand, the detection of triterpenes was performed with the Salkowski test. Extracts containing triterpenes will form a golden yellow color solution with the addition of concentrated sulfuric acid in chloroform medium. Meanwhile, the presence of

anthraquinones was identified with the Borntrager's test, where positive results are indicated by the cherry-red color in the aqueous phase [26].

Qualitative phytochemical analysis showed that all extracts contained flavonoid and phenolic compounds. Therefore, further experiments were conducted to measure the total phenolic content and total flavonoid content in each extract from different parts of the *B. purpurea* plant. The extract samples were estimated for total phenolic content using Folin-Ciocalteu reagent, and the results were expressed in $\mu\text{g}/\text{mg}$ GAE. Catechin was used as a standard in the colorimetric determination of the total flavonoid content of the extract using AlCl_3 . The total flavonoid content of the extract was expressed in $\mu\text{g}/\text{mg}$ CE [27].

The results in total phenolic content and total flavonoid content in each extract of different parts of *B. purpurea* are listed in Table 2. The total phenolic content of the aqueous extracts of leaf, flower, stem bark, and root part of *B. purpurea* plant was 34.84 ± 0.37 ; 40.14 ± 0.65 ; 23.76 ± 0.55 ; and 13.76 ± 0.54 $\mu\text{g}/\text{mL}$ GAE, respectively. It means that the aqueous extract of flower part of *B. purpurea* plant contains a slightly higher total phenolic content than leaf, stem bark, and root parts. On the other hand, the total flavonoid content of the aqueous extracts of leaf, flower, stem bark, and root parts of *B. purpurea* plant was 345.71 ± 0.25 ; 387.57 ± 0.63 ; 227.14 ± 0.87 ; and 119.54 ± 0.79 $\mu\text{g}/\text{mL}$ CE, respectively. In agreement with the results of total phenolic content, the aqueous extract of flower part of *B. purpurea* plant contains the highest total flavonoid content than other parts whereas the root part contains the lowest total flavonoid content.

3.2. FTIR Analysis

The qualitative analysis of phytochemicals revealed that the aqueous extracts obtained from different parts of the *B. purpurea* plant contained diverse phytochemical compounds. Therefore, to further investigate these extracts, FTIR analysis was conducted to identify the functional groups present in the natural compounds extracted from various parts of the *B. purpurea* plant. Figure 1 displays the FTIR spectra of the *B. purpurea* plant extracts obtained from different parts, indicating that the extracts from distinct plant parts exhibited varying FTIR spectra due to the presence of different natural compounds.

The FTIR spectrum of the leaf and flower

extracts, as depicted in Figure 1, exhibited a broad signal around ~ 3400 cm^{-1} , accompanied by sharp signals at $1650\text{-}1750$ cm^{-1} , $1400\text{-}1500$ cm^{-1} , and ~ 1100 cm^{-1} . These findings align with the results obtained from the phytochemical analysis, indicating that the leaf and flower extracts share similar natural components. In contrast, the FTIR spectrum of the stem bark extract showed a simpler pattern with a broad signal around ~ 3400 cm^{-1} , along with sharp signals at $1650\text{-}1750$ cm^{-1} and ~ 1100 cm^{-1} . The root extract displayed the simplest FTIR spectrum, characterized by a broad signal at ~ 3400 cm^{-1} and a sharp signal at $1400\text{-}1500$ cm^{-1} , reflecting its lower phytochemical content compared to the other extracts.

The FTIR analysis results indicated that all extracts contained the O–H functional group (broad peak at ~ 3400 cm^{-1}) due to the use of distilled water for extraction. Moreover, the C=O functional group was present in all extracts, possibly originating from polar natural compounds, as evidenced by the sharp peak at $1650\text{-}1750$ cm^{-1} . The leaf, flower, and stem bark extracts exhibited the C–O functional groups, which were detected as sharp peaks at 1100 cm^{-1} . Additionally, sharp peaks at $1400\text{-}1500$ cm^{-1} and ~ 1250 cm^{-1} indicated the presence of aliphatic and aromatic carbons, respectively.

The qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids, and phenolics as the identified natural products in the leaf and flower extracts. These compounds displayed functional groups such as O–H, C=O, C–O, aliphatic, and aromatic carbons, as observed in the FTIR spectra of the leaf and flower extracts. In contrast, the stem bark and root extracts contained flavonoids, carbohydrates, and phenolics as the observed natural products, leading to the detection of only the O–H, C=O, and C–O functional groups in their respective FTIR spectra.

3.3. Antioxidant Activity

To assess the potential use of various parts of the *B. purpurea* extract as an antioxidant agent, an additional experiment was conducted using the *in vitro* DPPH assay. The antioxidant activity test results are presented in Table 2. The leaf, flower, stem bark, and root aqueous extracts from the *B. purpurea* plant exhibited DPPH scavenging activities of $48.87 \pm 0.78\%$, $51.76 \pm 0.32\%$, $34.65 \pm$

0.67%, and $21.26 \pm 0.55\%$, respectively. These findings align with the reported total phenolic content and total flavonoid content in this study and previous research. It is evident that higher total phenolic and flavonoid contents correspond to increased antioxidant activity. As natural phenolic and flavonoid compounds contain free O–H groups thus all extracts showed antioxidant activity. The aqueous extract of flower part of *B. purpurea* plant exhibited the highest antioxidant activity. Furthermore, it is worthy to note that the antioxidant activity of flower extract is close to the antioxidant activity of ascorbic acid, which is remarkable.

4. CONCLUSIONS

Natural compounds were extracted from various parts of *B. purpurea*, including leaf, flower, stem bark, and root parts. The phytochemical analysis revealed the presence of flavonoids, carbohydrates, and phenolic compounds in all extracts, while triterpenes and anthraquinones were absent. Quantitative analysis showed that the aqueous extract from the flower part of *B. purpurea* exhibited the highest total phenolic content ($40.14 \pm 0.65 \mu\text{g/mL GAE}$) and the highest flavonoid content ($387.57 \pm 0.63 \mu\text{g/mL CE}$) compared to other parts. The flower aqueous extract contained natural compounds with functional groups such as O–H, C–O, C=C, and C=O, as observed in the FTIR analysis. Consequently, the aqueous extract of the flower part of *B. purpurea* plant demonstrated remarkable antioxidant activity ($51.76 \pm 0.32\%$), which was close to the antioxidant activity of ascorbic acid ($70.54 \pm 0.51\%$), the positive control. Therefore, the flower of the *B. purpurea* plant holds great potential as a source of antioxidant agents for future applications.

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Author Contributions

T. M. H. contribution to investigation, data curation, and writing the original draft; K. K. S. contribution to investigation (antioxidant assays); H. H. contribution to conceptualization, data curation, supervision, and Writing—Review and editing.

Conflicts of Interest

The authors declare no conflict of interest.

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