



## A Study on Phytochemicals in *Bauhinia purpurea* I. Leaf and Flower

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Accepted on: 21-09-2014; Finalized on: 30-11-2014.

### ABSTRACT

The present study was undertaken to evaluate the phytochemicals present in the *Bauhinia purpurea* L. leaf and flower. The samples *Bauhinia purpurea* leaf and flower were collected during the month of November-December 2013. The collected leaf and flower samples were allowed to shade dry and then powdered. Powdered *Bauhinia* leaf, flower samples were extracted with water and also hydrolyzed according to the standard prescribed protocol. Qualitative analysis showed positive result for carbohydrate, alkaloid, steroid, sterol, glycoside, saponin, flavonoid, tannin, phenolic compounds, flavonoid, protein, amino acid, fixed oil. The percent yield obtained with *Bauhinia purpurea* leaf was 29.57 whereas it was 20.20 with *Bauhinia purpurea* flower. Fluorescence study revealed that both the leaf and flower showed green fluorescence. The behavior of leaf and flower powder was negative for anthroquinone. The nutrients such as carbohydrate, protein and amino acids assessed quantitatively in leaf was 50.0±3.46mg/g, 05.19±0.23mg/g, 05.95±0.88mg/g, but in flower, the carbohydrate content was found to be 43.33±2.30mg/g. Similarly, the protein and amino acid content was 13.39±0.23mg/g, 00.59±0.17mg/g. The results showed that it is endowed with phytochemicals, nutrients which might find application in the treatment of diseases as curative agent.

**Keywords:** *Bauhinia purpurea*, Chemicals, Flower, Leaf, Nutrients.

### INTRODUCTION

**B**auhinia genus of family Cesalpinaceae consists of about 15 species that occur in India. Some of them are shrubs or trees, while a few are climbers.<sup>1</sup> India is a developing country which is valued for diversity of species, genetic, habitat. In developing countries like India, flowers are used in all cultural activities at all time.<sup>2</sup> Flowers are broadly known and used for their beauty as well as the color they radiate.<sup>3</sup> Medicinal plants find its application in the treatment of diseases since the dawn of world in the form of traditional medicine. The plant *Bauhinia purpurea* is a moderate evergreen tree used by tribes of India as cattle feed.<sup>4</sup> Various biological activities are ascribed to bauhinia species. *B.purpurea* a most important species used to treat several ailments in traditional system of medicine.<sup>5-8</sup> *B.purpurea* was reported for its antidiarrhoeal, anticancer, thyroid gland stimulating properties.<sup>9-11</sup> since, the flowers are rich in phytoconstituents, it was considered as a principle source in pharma and nutraceutical industries. Historically, all valuable medicinal preparations were derived from plants, whether in the simple form of plant parts or more complex form of crude extracts. Hence, the present study was aimed to experimentally analyze the phytochemicals present in the dry sample of *Bauhinia purpurea* leaf and flower.

### MATERIALS AND METHODS

#### Plant collection and identification

The samples *Bauhinia purpurea* leaf and flowers were collected from Navodaya academy senior secondary

school located at Namakkal, Namakkal District, Tamil Nadu, India, during the month of November-December, 2013. The collected leaves and flowers were cleaned thoroughly and dried under the shade. Once the drying process is complete, the dried leaves, flowers were ground to powder using blender for further use. The plant was identified by Dr. B. Sankar, Head and Assistant Professor, Department of Botany, Poompohar College, Tamil Nadu, India.



*Bauhinia purpurea*

#### Aqueous extract preparation

Aqueous extract was prepared by dissolving 15g of *Bauhinia purpurea* leaf and flower powder in 200ml of distilled water each separately. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage

yield, and the remaining marc left was extracted with water and used for qualitative analysis.

### **Phytochemical analysis**

The extract was tested for the presence of bioactive compounds by adopting standard procedures<sup>12,13</sup> fluorescence analysis,<sup>14</sup> behavior of drugs powder with different chemical reagents.<sup>15</sup>

#### **Test for carbohydrate**

##### ***Molisch's test***

To the extract added few drops of alcoholic alpha naphthol solution followed by few drops of concentrated sulphuric acid along the sides of the test tube. Purple or violet colored ring formed at the junction showed positive result.

##### ***Fehling's test***

To the extract added equal amount of Fehling's A and B solution, heat the tubes in a boiling water bath. Brick red precipitation of cuprous oxide formation confirmed the presence of reducing sugar.

##### ***Benedict's test***

To the extract added Benedict's reagent and the contents in the tubes were heated in a boiling water bath. Formation of red precipitate indicated positive result.

#### **Test for alkaloids**

##### ***Wagner's test***

To the extract added few drops of iodine solution in potassium iodide. Reddish brown precipitate showed positive result.

##### ***Hager's test***

To the extract added few drops of saturated solution of picric acid. Yellow color precipitation showed positive result.

#### **Test for steroids and sterols**

##### ***Libermann-Burchard test***

To the extract added 2ml chloroform, 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid. Positive result was confirmed by the formation of bluish red to cherry red color in chloroform layer.

##### ***Salwoski test***

To the extract, few drops of chloroform were added followed by concentrated sulphuric acid. Presence of bluish red to cherry red color denoted positive result.

#### **Test for Glycosides**

##### ***Legal test***

To the extract added pyridine and sodium nitroprusside. Positive result showed pink to red color formation.

##### ***Baljet test***

To the extract added picric acid. Appearance of orange color showed positive result.

##### ***Test for saponins***

##### ***Foaming test***

Foams produce when the extract was mixed with water.

##### ***Test for flavonoids***

##### ***Shinoda test***

To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

##### ***Zinc hydrochloride test***

To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

#### **Test for tannin and phenolic compounds**

##### ***Ferric chloride test***

To the extract added ferric chloride. Appearance of greenish black color showed positive result.

##### ***Potassium dichromate test***

To the extract added potassium dichromate solution. Positive result was confirmed by the formation of brown precipitate.

##### ***Gelatin test***

To the extract added 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate showed positive result.

#### **Test for protein and amino acids**

##### ***Biuret test***

To the extract added 4% sodium hydroxide followed by few drops of 15% copper sulphate. Appearance of purple color showed positive result.

##### ***Ninhydrin test***

Bluish violet color was formed when a solution of ninhydrin was added to the extract and heated.

##### ***Heat test***

The extract was heated by allowing it to boil in a water bath. Presence of coagulation showed positive result.

#### **Test for fixed oil**

##### ***Copper sulphate test***

Blue color was observed when the extract was mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

### Quantitative analysis of phytonutrients

Total carbohydrates,<sup>16</sup> proteins,<sup>17</sup> aminoacids<sup>18</sup> were performed according to the standard prescribed methods.

#### Estimation of carbohydrate

The total carbohydrate was estimated by Anthrone method. 1mg of *Bauhinia purpurea* leaf and flower powder was hydrolyzed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature. After neutralizing, the contents were centrifuged and 0.1ml of supernatant was used for the analysis. To the sample add 4ml of anthrone reagent and the contents were heated in a boiling water bath for 8minutes. The tubes were cooled and read at 630nm using spectrophotometer Shimadzu-Model-UV 1800. The standards were developed with glucose. Standard graph plotted was used to find the concentration of glucose present in the hydrolyzed extract.

#### Estimation of protein

The total protein was estimated by Lowry's method. To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of Folin ciocalteau reagent (diluted in the ratio of 1:2) was added and allowed for 30 minutes incubation, then read at 660nm using

spectrophotometer Shimadzu-Model UV 1800. The standards were developed with Bovine serum albumin. Standard graph plotted was used to calculate the concentration of protein present in the extract.

#### Estimation of aminoacid

The amino acid present was estimated by Ninhydrin method. To 0.1ml of extract added 1ml of ninhydrin solution dissolved in ethanol. The test tube was covered with a piece of paraffin film to avoid the loss of solvent due to evaporation. With gentle stirring, the contents were allowed to react at 80-100°C for 4-7minutes and then cooled. The color developed was read at 570nm. Tyrosine was used for developing standard. From the standard graph obtained the amino acid content in the extract was calculated.

#### Statistical Tool

Each experiment was carried out in triplicate and the results were given as Mean  $\pm$  Standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values),  $s = \frac{\sqrt{\sum(x-M)^2}}{n-1}$

### RESULTS AND DISCUSSION

The percentage recovery of the aqueous extract obtained was calculated and expressed in Table 1.

**Table 1:** Percentage yield of *Bauhinia purpurea* L. leaf, flower powder (Aqueous extract)

Name of the sample used	Weight taken for extraction	Initial weight of the beaker (gm)	Final weight of the beaker (gm)	Weight of the extract (gm)	% recovery
<i>Bauhinia purpurea</i> leaf powder	15g in 200ml	180.9209	185.2521	4.33	29.57
<i>Bauhinia purpurea</i> flower powder	14.6425g in 200ml	169.4589	172.4891	3.03	20.20

**Table 2:** Behavior of *Bauhinia purpurea* leaf, flower powder with different chemicals

Tests	Observation		Inference	
	leaf	flower	leaf	flower
Powder+Picric acid	Yellow color	Yellow color	Presence of alkaloid	Presence of alkaloid
Powder+Conc. H <sub>2</sub> SO <sub>4</sub>	Reddish brown color	Reddish brown color	Presence of steroids	Presence of steroids
Powder+ Aqueous FeCl <sub>3</sub>	Green inluoresence	Green inluoresence	Presence of flavonoids	Presence of flavonoids
Powder+Iodine solution	Brown color	Blue color	Absence of starch	Presence of starch
Powder+ Aqueous 5% KOH	Brown color	Brown color	Presence of anthroquinone	Presence of anthroquinone
Powder + NaOH	Yellow color	Yellow color	Presence of flavonoids	Presence of flavonoids
Powder+ Aqueous AgNO <sub>3</sub>	White precipitate	White precipitate	Presence of protein	Presence of protein

The yield in percentage was found to be 29.57 with *Bauhinia purpurea* leaf whereas, it was 20.20 with *Bauhinia purpurea* flower. (Table 1) The net weight used for the experiment was 14.6425gm. The weight of the dry extract obtained with respect to leaf was 4.33gm and 3.03gm for flower after the completion of drying process.

#### Analysis of *Bauhinia purpurea* leaf, flower powder for its behavior

The results of analysis of *Bauhinia purpurea* leaf, flower powder for its behavior is shown in Table 2.



The results of *Bauhinia purpurea* leaf, flower powder when tested with different chemicals showed positive results for alkaloid, steroid, flavonoid, starch, anthroquinone, protein. (Table 2)

### Fluorescence Analysis

**Table 3:** Fluorescence analysis of *Bauhinia purpurea* leaf, flower (Aqueous extract)

Name of the aqueous extract	Day light	UV light
leaf	Pale brown	Green
flower	Pale brown	Green

The results of fluorescence analysis showed green fluorescence for both the leaf and flower samples studied. (Table 3)

### Phytochemical analysis

The results of phytochemical analysis were shown in Table 4.

**Table 4:** Phytochemicals in *Bauhinia purpurea* leaf and flower (Aqueous extract)

Name of the test	Results	
	Leaf	Flower
Test for carbohydrate	+++	+++
Test for alkaloid	++	++
Test for steroid and sterol	+	+++
Test for Glycoside	+++	+++
Test for saponin	+	++
Test for flavonoid	+	+++
Test for tannin and phenolic compound	+++	+++
Test for protein and amino acid	+++	+
Test for fixed oil	+++	+++

+Slight changes, ++ Moderate, +++ Stronger reactions

The qualitative analysis showed strong positive reaction for most of the phytochemicals studied in both leaf and flower of *Bauhinia purpurea*. But only slight changes were observed with flavonoid, saponin, steroid, sterol for *Bauhinia purpurea* leaf. (Table 4)

**Table 5:** Phytonutrients in *Bauhinia purpurea* leaf and flower (Aqueous extract)

Phytonutrients assessed	Nutrient content (mg/g)
<b>Leaf</b>	
Total carbohydrate	50.0±3.46
Total protein	05.19±0.23
Amino acids	05.95±0.88
<b>Flower</b>	
Total carbohydrate	43.33±2.30
Total protein	13.39±0.23
Amino acids	00.59 ± 0.17

Values are Mean ± SD for three experiments

### Phytonutrients Analysis

The phytonutrients estimated were tabulated in Table 5.

Among the nutrients assessed, the carbohydrate content was higher for leaf (50.0±3.46mg/g) when compared to flower (43.33±2.30mg/g). But, the protein content was higher in flower (13.39±0.23mg/g) on comparison with leaf (05.19±0.23mg/g). Likewise, the amino acid content was higher for leaf (05.95±0.88mg/g) than flower (0.59 ± 0.17mg/g). The antioxidant activity of *Bauhinia purpurea* leaf and flower was reported by Krishnaveni et.al.<sup>19,20</sup>

### CONCLUSION

Developing country like India, herbal formulations forms a basis in primary health care for about 80% of the population as it gives fewer side effects due to its better compatibility with human body. From our results, it is understood that leaf and flower of *Bauhinia purpurea* contains chemical constituents, nutrients when tested qualitatively and quantitatively. Flower contains protein in higher concentration. While, the carbohydrate, amino acid content was higher in leaf. This shows that it might have a therapeutic potential which aids in the maintenance of good health.

**Acknowledgement:** The author wishes her thanks to Honorable Vice-chancellor Dr. C. Swaminathan Avl and Registrar Dr. K. Angamuthu Avl, Periyar University, Salem for their administrative support and excellent infrastructure facilities provided and also thank Dr. V. Raj, Professor and Head, Department of Chemistry, Periyar University, Salem for his help.

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Source of Support: Nil, Conflict of Interest: None.

