

Research Article



A Preliminary Study on Phytochemical Screening and Antioxidant Potential of *Bauhinia purpurea* Linn Flower

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ABSTRACT

Antioxidants are central substance, which have the ability to guard the body from damages caused by free radical-induced oxidative stress. A mixture of free radical scavenging antioxidants is found in a number of dietary sources. The main idea of the study is to assess the antioxidant activity of *Bauhinia purpurea* flower. The phytochemical screening showed the presence of terpenoid, flavonoid, tannins, and also contain total flavonoid in highest level. The decreasing order of various antioxidant activities studied is shown as follows: Nitric oxide scavenging activity < Reducing power activity < Total antioxidant activity < Metal chelating activity. From the results, it is known that *Bauhinia purpurea* flower have potential antioxidant activity and thereby scavenges free radicals in vitro.

Keywords: *Bauhinia purpurea*, Flower, Free radical, Phytochemicals, Scavenger.

INTRODUCTION

Natural products obtained from the plant resources have been the major supplements to combat many serious diseases in the developing countries.¹ The plant *Bauhinia purpurea* Linn. Is commonly known as Hong Kong orchid tree, belonging to the family leguminosae (sub family- Cesalpinioideae) native to South China and Southeastern Asia and it is found throughout India, ascending to an altitude of 1300m in the Himalayan region. It is a moderate evergreen tree and is used in abundance for medicinal and cattle feed purposes by the tribes of India² and also being used in various indigenous systems of medicine like Ayurveda and Unani. The attention paid by health authorities to the use of herbal medicines has increased considerably, both because they are often only medicine available in less developed areas and because they are becoming a popular alternative medicine in more developed areas.³ *Bauhinia purpurea* reported to exhibit various pharmacological activities such as anti-oxidant, hepatoprotective, hypoglycaemic, antiproliferative activity.^{4,5} Earlier reports are there for studies in ornamental flower⁶ and in flowers of medicinal plants.⁷ The bark or root, flower mixture with boiled rice water is used as maturant for boils and abscesses.⁸ Considering the importance of flower, the present study was aimed to analyze the phytochemicals, secondary metabolites, antioxidant activities.

MATERIALS AND METHODS

Sample collection

The *Bauhinia purpurea* 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

flowers were cleaned thoroughly and dried under the shade. Once the drying process is complete, the dried flowers were ground to powder using blender for further use.

Aqueous extract preparation

Aqueous extract was prepared by taking 50, 75 and 100mg of powdered *Bauhinia purpurea* flowers. Each concentration was dissolved in 10ml water mixing with a magnetic stirrer at 4°C for 4h. The mixture was filtered through nylon cloth and centrifuged at 20,000g for 30min. 0.1ml of supernatant was used for the analysis.

All quantitative experiment was performed three times and 0.1ml was used for each experiments.

Phytochemical analysis

Phytochemical screening was carried out for the aqueous extract as per the standard methods.

Phlobatannins

Extract was boiled with 1% hydrochloric acid. Deposition of red precipitate is an evidence for the presence of Phlobatannins.

Saponin

5 ml extract was mixed with 20ml of distilled water then agitated in a graduated cylinder for 15 min. Formation of foam indicates saponin.

Steroid

1ml extract was dissolved in 10ml of chloroform; equal volume of concentrated sulphuric acid was added from the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence indicating the presence of steroid.



Tannin

4ml extract was treated with 4ml FeCl₃. Formation of green colour indicates the presence of tannin.

Terpenoids

2ml of the extract was mixed with 2ml of chloroform, 3ml concentrated Sulphuric acid was carefully added to form a layer. A reddish brown colour formation indicates the presence of terpenoids.

Flavonoids

Extract was treated with a drop of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Cardial Glycosides

Extract was treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

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Each experiment was performed three times and 0.1ml was used for each experiments.

Determination of Secondary Metabolites

The phenol and flavonoid content of aqueous extract was analyzed.

Determination of total phenol content

Total phenolic content were determined by Folin-ciocalteau method. The extract (0.1ml) was mixed with folin-ciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5min and added aqueous sodium carbonate (4ml, 1M). The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765nm. The standard curve was prepared. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.⁹⁻¹¹

Estimation of flavonoids

The aluminium chloride method was used for the determination of the total flavonoid content. Extract solution were taken and to this 0.1ml of 1M potassium acetate, 0.1ml of Aluminium chloride (10%), 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415nm was recorded after 30min of incubation. A standard calibration plot was generated using known concentration of quercetin. The concentration of flavonoid in the test samples were calculated from the

calibration plot and expressed as mg quercetin equivalent/g of sample.⁹⁻¹¹

Determination of Antioxidant Activities

Nitric oxide scavenging assay, Reducing power assay, Total antioxidant assay, Metal chelating activities were performed.

Nitric oxide scavenging activity

This procedure is based on the principle that, sodium nitroprusside in aqueous solution, at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM), in phosphate buffered saline, was mixed with extract and incubated at room temperature for 150min. After the incubation period, 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. Ascorbic acid was used as a positive control.¹²

Reducing power assay

Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20min. 1.0 ml of Trichloro acetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (1.5ml) was mixed with distilled water (1.5ml) and FeCl₃ (0.1ml, 0.1%) after mixing, the contents were incubated for 10min and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control.¹³

Total antioxidant capacity

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH by adding 4ml reagent solution containing 0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM Ammonium molybdate. The tubes were incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to RT, the absorbance of mixture was measured at 695nm against blank. The phosphomolybdenum method is quantitative, since, the total antioxidant activity is expressed as the number of equivalents of ascorbic acid.¹⁴

Metal chelating activity

Add extract (0.1ml) to a solution of 2mM FeCl₂ (0.05ml). The reaction was initiated by the addition of 5mM Ferrozine (160µl), the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was then measured spectrophotometrically at 562nm. Standard curve was plotted using ascorbic acid. Distilled water (1.6ml) instead of sample solution was used as a control. Distilled water



(160µl) instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution.¹⁰

For all estimations, readings were taken using UV- Visible spectrophotometer- Shimadzu, Japan make. Model UV 1800. Standard graph were plotted for quantitative experiments using their respective standards and samples were plotted against standard by taking concentration in X axis and OD in Y axis.

Statistical Tool

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

Table 2: Secondary metabolites and Antioxidant activities of *Bauhinia purpurea* flower

Orchid flower (mg)	Total Phenolics (mg/g)	Total flavonoids (mg/g)	Reducing power activity (mg/g)	Total antioxidant assay (mg/g)	Nitric oxide scavenging activity (mg/g)	Metal chelating activity (mg/g)
50	11.96±3.35	39.96±1.35	58.63±2.65	35.06±3.32	51.53±4.08	22.4±1.01
75	34.0±2.0	54.66±1.15	64.66±1.15	35.33±2.30	91.33±1.15	30.33±0.57
100	138.66±2.30	156.0±6.9	129.33±2.30	82.66±6.11	232.0±6.92	65.33±3.05

Table 1 show that aqueous extract contains phytochemicals such as terpenoid, flavonoid, tannins, saponin, steroid, phlobatannins, cardiac glycosides. (Table 1)

Table 2 shows the result of secondary metabolites and antioxidant activities of *Bauhinia purpurea* flower. The total phenolics and flavonoids was found to be higher at 100mg concentration. Among the various antioxidant activities tested, nitric oxide scavenging activity was higher. The reducing power activity was high compared to total antioxidant and metal chelating activity. The results of three different concentrations studied shows that secondary metabolites as well as antioxidant activities were found to be high at higher concentration. (Table 2)

The flowers having significant compounds with antioxidant activity can be favored as one among the consumables in human diet or for therapy. The carotenoid, anthocyanin, flavonoid, and vitamin rich flowers can be potentially dominated nutraceutical industries for the production of value added foods. Flowers rich in antioxidants can be used as an alternate for butylated hydroxyanisole and butylated hydroxyl toluene used in food industry for food storage as these chemicals are carcinogenic.¹⁵

CONCLUSION

From the results, it is concluded that *Bauhinia purpurea* flower contain phytochemicals like terpenoid, flavonoid, tannin, phlobatannins, cardiac glycosides and might be considered as a potential source of flavonoids, thus find

RESULTS AND DISCUSSION

Table 1 Shows the results of phytochemical constituents present in the aqueous extract of *Bauhinia purpurea* flower.

Table 1: Phytochemicals in aqueous extract of *Bauhinia purpurea* flower

Phytochemicals	Present(+) /Absent (-)
Terpenoid	+
Flavonoid	+
Tannins	+
Saponins	+
Steroid	+
Phlobatannins	+
Cardiac glycosides	+

suitable for application in pharmaceutical, nutraceutical industries.

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