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Original Article

ANTIOXIDANT POTENTIAL OF BAUHINIA PURPUREA (L) LEAF

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ABSTRACT

Objective: *Bauhinia purpurea* is called as Mandarai in Tamil and in English as butterfly tree. The aim of the present study was to determine the secondary metabolites and antioxidant activities of shade dried *Bauhinia purpurea* leaf aqueous extract.

Methods: Quantitative analysis for total phenolics was done by Folin-ciocalteau method and total flavonoids by aluminium chloride method. Likewise, various antioxidant activities were assessed by following standard methods.

Results: The flavonoid content was higher (160.0±6.9mg/g) compared to phenolics (126.66± 6.11mg/g). Similarly, the nitric oxide scavenging activity (258.66±4.61mg/g) and reducing power activity (141.33±2.30mg/g) was found to be higher compared to total antioxidant (81.33±6.11mg/g) and metal chelating activity (30.66±2.30mg/g).

Conclusion: The results obtained reveal that *Bauhinia purpurea* leaf extract proves to be a good antioxidant and needs further characterization to confirm its diversified therapeutic applications.

Keywords: Antioxidant, *Bauhinia purpurea,* Dried leaves, Phenolics, Flavonoids.

INTRODUCTION

The genus *Bauhinia* L is called as 'Orchid Tree' of ornamental value [1] *Bauhinia purpurea* (Linn.) is a medium sized deciduous flowerings tree, bark ashy to dark brown belonging to the family Leguminosae and subfamily Caesalpinioidae [2, 3, 4] sparingly grown in India. *Bauhinia purpurea* is widely used in Ayurvedic and Yunani medical system. Leaves of *Bauhinia purpurea* (Linn) were extensively used in the treatment of wounds [5], *In vitro* study has demonstrated that *B. purpurea* possesses antiproliferative [6] antioxidant [6, 7] and antimicrobial activities [7, 8] as well as potential hepatocellular carcinoma inhibitor [9].

Interestingly, other studies have proved that *B. purpurea* leaf possesses antiulcer activity [10, 11]. According to World Health Organization, medicinal plants are the greatest source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [12]. Hence, the present study was undertaken to evaluate the secondary metabolites and antioxidant activities of aqueous extract of *Bauhinia purpurea* leaf.

MATERIALS AND METHODS

Sample collection

The *Bauhinia purpurea* leaves were collected from Navodaya academy senior secondary school located at Namakkal, Namakkal District, Tamil Nadu, India, during the month of November, 2013. The collected leaves were cleaned thoroughly and dried under the shade. Once the drying process is complete, the dried leaves were ground to powder using blender for further use.

Preparation of extract

Aqueous extract was prepared by taking different concentrations of dry *Bauhinia purpurea* leaf powder (25, 75, 100mg). Each concentration was dissolved in 10ml water, mix using 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. 0.1ml was used for each experiments. Each experiment was repeated thrice.

Determination of secondary metabolites

The phenol and flavonoid content of aqueous extract was analysed.

Determination of total phenol content

Total phenolic content were determined by Folil-ciocalteau method. The extract (0.1ml) was mixed with folinciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5min and added aqueous $NaCo_3$ (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 [13, 14].

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 $AlCl_3$ (10%), 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm 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 [15].

Determination of antioxidant activities

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

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 $FeCl_3$ (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 [16].

Total antioxidant capacity

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (V1) 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 [17].

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 [18].

Metal chelating activity

Add extract (0.1ml) to a solution of 2mM $FeCl_2$ (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 [19].

For all estimations, readings were taken using UV- Visible spectrophotometer- Shimadzu, Japan make. Model UV 1800. Standard graph were plotted for all 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 experiments were 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), $s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$.

RESULTS

Secondary metabolites

The results of secondary metabolites are shown in Table.1. The total phenolics was lower when compared to total flavonoids. The results of three different concentrations studied are as follows: The phenolic content observed was 41.66±1.52mg/g for 25mg *Bauhinia* leaf powder, while it was 74.66±1.15mg/g for 75mg, whereas, with 100mg, the phenolic activity was found to be 126.66±6.11mg/g. The total flavonoids analysed quantitatively shows, that *Bauhinia* leaf aqueous extract contains 43.0±2.0mg/g, 57.33±1.15, 160.0±6.9mg/g when studied at 25, 75, 100mg. At higher concentration, the levels of secondary metabolites was found to be higher (Table.1).

Table 1: Phenolic and Flavonoid content of *Bauhinia purpurea* leaf aqueous extract

S. No.	Leaf (mg)	Total phenolics GAE mg/g	Total flavonoids QEmg/g
1.	25	41.66±1.52	43.0±2.0
2.	75	74.66±1.15	57.33±1.15
3.	100	126.66±6.11	160.0±6.9

Values are Mean ± SD for three experiments

Antioxidant ctivities

The results of antioxidant activities are illustrated in Table.2. Among the antioxidant activities assessed, nitric oxide scavenging and reducing power activity was higher compared to total antioxidant, metal chelating activity. Nitric oxide activity assessed shows that, *Bauhinia purpurea* leaves contain 258.66±4.61mg/g when experimented with 100mg, and it was 88.66±1.15mg/g with 75mg, and 41.0±2.64mg/g with 25mg. Reducing power activity results are as follows: 141.33±2.30mg/g (100mg), 90.66±1.15mg (75mg), 35.33±3.05mg/g (25mg). Likewise, the results of total antioxidant activity obtained are: 21.33±1.52mg/g (25mg), 35.33±2.30mg/g (75mg), 81.33±6.11mg/g (100mg). And for metal chelating activity, 100mg of *Bauhinia* leaf powder showed 30.66±2.30mg/g when assessed with 100mg, and with 75mg it was 29.0±3.46mg/g, whereas, it was 16.16±2.51mg/g with 25mg. (Table.2).

Table 2: Antioxidant activities of *Bauhinia purpurea* leaf aqueous extract.

S. No.	Leaf (mg)	Reducing power	Total antioxidant Ascorbic acid equivalent mg/g	Nitric oxide	Metal chelating
1.	25	35.33±3.05	21.33±1.52	41.0±2.64	16.16±2.51
2.	75	90.66±1.15	35.33±2.30	88.66±1.15	29.0±3.46
3.	100	141.33±2.30	81.33±6.11	258.66±4.61	30.66±2.30

Values are Mean ± SD for three experiments

DISCUSSION

In plants, flavonoids play a role in flower and seed pigmentation, in plant fertility and reproduction, and in various defence reactions to protect against abiotic stresses like UV light or biotic stresses such as predator and pathogen attacks [20, 21, 22]. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens.

They show antiallergic, antiinflammatory, antimicrobial and anticancer activity [23]. Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages [24]. The literature reports showed that there is high correlation between antioxidant activity and phenolics content [25]. Antioxidants have been established to be the most effective way to eliminate adverse effects caused by free radicals as antioxidants can scavenge them or endorse their decomposition [26]. The use of plant extracts and phytochemicals with antioxidant activity can be of great significance in the treatment of many diseases [27, 28]. One of most the important role played by natural products as therapeutic agents are through their antioxidant activity.

CONCLUSION

The results of this study showed that the antioxidant activity, total phenolic, total flavonoid content were exhibited by the aqueous extracts of shade dried *Bauhinia purpurea* leaf, which might find its use in therapeutic applications. Since, the extract used is aqueous extract, only water soluble phenolic compounds might have induced antioxidant activities. The screening of leaf extract for antioxidant activities reveal that it can be a potential source of natural antioxidant.

CONFLICT OF INTERESTS

Declared None

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