International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp 919-924, April-June 2011

In-vitro antioxidant potential of various extracts from whole plant of *Bauhinia purpurea* (Linn)

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Abstract: The objective of the present investigation was to evaluate the *in-vitro* antioxidant potential various extracts of whole plant of *Bauhinia purpurea* with three *in-vitro* methods: DPPH scavenging activity, superoxide anion scavenging activity and estimation of total flavonoids. The ethyl acetate extract of *Bauhinia purpurea* was showed significant result in DPPH scavenging and superoxide anion scavenging activity methods. Similar result was not found in other two extracts. High amount of flavonoids were found in ethyl acetate extract of *Bauhinia purpurea* than that of other two extracts. The high antioxidant capacity observed for ethyl acetate extract of *Bauhinia purpurea* suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.
Key words: *Bauhinia purpurea,* DPPH scavenging activity, superoxide anion scavenging activity, total flavonoids.

Introduction

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions, or from exogenous factors[1]. *In vivo*, some of these ROS play positive roles such as energy production, phagocytosis, regulation of cell growth and intercellular signalling, or synthesis of biologically important compounds[2].However, ROS may also be very damaging as they can induce oxidation of lipids, causing membrane damage, decreasing membrane fluidity, and leading to cancer via DNA mutation[3]. A potent scavenger of these ROS may serve as a possible preventative against free radical mediated diseases[4].

Bauhinia purpurea Linn. (Leguminosae) is a medium sized deciduous tree, sparingly grown in India. This plant is used traditionally in dropsy, pain, rheumatism, convulsions, delirium, and septicemia[5]. The bark of the plant is used as an astringent in the treatment of diarrhea. Its decoctions are recommended for ulcers as a useful was solution[6]. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc[7].Even through, traditionally, leaves of *Bauhinia purpurea* (Linn) were extensively used for the treatment of variety of wounds[8], and no scientific data in its support is available.

Literature survey revealed that there is a lack of enough scientific reports regarding the antioxidant properties of whole plant of *Bauhinia purpurea* (Linn). Hence the objective of the present investigation was to evaluate the *in-vitro* antioxidant potential of various extracts from whole plant of *Bauhinia purpurea* (Linn) with various *in-vitro* antioxidant methods.

Material and Methods

Collection and Identification of Plant materials

The whole plant of *Bauhinia purpurea* (Linn), were collected from Nagercoil, Kanyakumari District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Bauhinia purpurea* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[9] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then marc was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Determination of Antioxidant activity

DPPH photometric assay[10]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[10]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity[11]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[11]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Total flavonoids[12]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

Results and Discussion

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[13]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[14].

DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Bauhinia purpurea* was presented in Table 1. The petroleum ether extract of *Bauhinia purpurea* and Rutin (standard) showed DPPH free radical scavenging activity in a concentration range of 125-1000 µg/ml and its inhibition ranged from 15-33% and 18-69% respectively. The IC_{50} values of the petroleum ether extract of *Bauhinia purpurea* and Rutin were found to be 1440µg/ml and 480µg/ml respectively.

Table 1: Effect of Petroleum ether extract of *Bauhinia purpurea* on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	15.97 ± 0.07	18.85 ± 0.07
2	250	19.78 ± 0.03	22.08 ± 0.05
3	500	25.18 ± 0.02	52.21 ± 0.02
4	1000	33.05 ± 0.11	69.83 ± 0.01
		IC_{50} = 1440 µg/ml	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Bauhinia purpurea* was presented in Table 2. The ethyl acetate extract of *Bauhinia purpurea* and Rutin (standard) showed DPPH free radical scavenging activity in a concentration range of 125-1000 µg/ml and its inhibition ranged from 24-69% and 18-69% respectively. The IC_{50} values of the ethyl acetate extract of *Bauhinia purpurea* and Rutin were found to be 290µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of methanolic extract of *Bauhinia purpurea* was presented in Table 3. The methanolic extract of *Bauhinia purpurea* and Rutin (standard) showed DPPH free radical scavenging activity in a concentration range of 125-1000 µg/ml and its inhibition ranged from 19-67% and 18-69% respectively. The IC_{50} values of the methanolic extract of *Bauhinia purpurea* and Rutin were found to be 570µg/ml and 480µg/ml respectively.

Table 2: Effect of Ethyl acetate extract of *Bauhinia purpurea* on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	24.04 ± 0.08	18.85 ± 0.07
2	250	47.67 ± 0.07	22.08 ± 0.05
3	500	63.68 ± 0.04	52.21 ± 0.02
4	1000	69.22 ± 0.03	69.83 ± 0.01
		IC_{50} = 290 µg/ml	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 3: Effect of Methanolic extract of *Bauhinia purpurea* on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	19.51 ± 0.01	18.85 ± 0.07
2	250	29.31 ± 0.04	22.08 ± 0.05
3	500	45.62 ± 0.03	52.21 ± 0.02
4	1000	67.23 ± 0.02	69.83 ± 0.01
		IC_{50} = 570 µg/ml	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations

From the above result clearly indicated that the ethyl acetate extract of *Bauhinia purpurea* exhibits significant DPPH scavenging activity when compared with standard Rutin. Similar result was not found in other two extracts. The IC_{50} values of ethyl acetate extract of *Bauhinia purpurea* and Rutin were observed at 290µg/ml and 480µg/ml respectively.

Superoxide anion scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical[15]. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[16]

The percentage of superoxide anion scavenging activity of petroleum ether extract of *Bauhinia purpurea* was presented in Table 4. Maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 44.35% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 1390µg/ml and 60µg/ml respectively.

The percentage of superoxide anion scavenging activity of ethyl acetate extract of *Bauhinia purpurea* was presented in Table 5. Maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 84.18% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 110µg/ml and 60µg/ml respectively.

The percentage of superoxide anion scavenging activity of methanolic extract of *Bauhinia purpurea* was presented in Table 6. Maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 59.87% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 490µg/ml and 60µg/ml respectively.

Table 4: Superoxide anion scavenging activity of Petroleum ether extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Pet. ether extract)	**Standard (Quercetin)**
1	125	15.20 ± 0.02	73.81 ± 0.006
2	250	29.80 ± 0.03	91.31 ± 0.011
3	500	36.35 ± 0.03	92.99 ± 0.024
4	1000	44.35 ± 0.02	98.01 ± 0.012
		IC_{50} = 1390 µg/ml	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 5: Superoxide anion scavenging activity of Ethyl acetate extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	**Standard (Quercetin)**
1	125	51.65 ± 0.04	73.81 ± 0.006
2	250	59.35 ± 0.02	91.31 ± 0.011
3	500	63.39 ± 0.03	92.99 ± 0.024
4	1000	84.18 ± 0.01	98.01 ± 0.012
		IC_{50} = 110 µg/ml	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 6: Superoxide anion scavenging activity of Methanolic extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	19.93 ± 0.02	73.81 ± 0.006
2	250	29.54 ± 0.01	91.31 ± 0.011
3	500	51.41 ± 0.03	92.99 ± 0.024
4	1000	59.87 ± 0.02	98.01 ± 0.012
		IC_{50} =490 µg/ml	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 7: The total flavonoids content of various extracts of whole plant of *Bauhinia purpurea*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Bauhinia purpurea*	2.17 ± 0.08
2	Ethyl acetate extract of *Bauhinia purpurea*	4.19 ± 0.96
3	Methanolic extract of *Bauhinia purpurea*	2.86 ± 0.05

*All values are expressed as mean ± SEM for three determinations

Based on the above results the IC_{50} values and percentage scavenging capacity, it was found that ethyl acetate extract of *Bauhinia purpurea* is more effective in scavenging superoxide radical than that of other two extracts.

Total phenol

The total amount of flavonoids content of various extract of whole plant of *Bauhinia purpurea* was summarized in Table 7. Flavonoids present in food of plant origin are also potential antioxidants[17, 18]. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities[19]. Recent studies showed that many flavonoids & related polyphenols contribute significantly to the total antioxidant activity of many plants[20].The higher content of flavonoids were found in ethyl acetate extract of *Bauhinia purpurea* than that of other two extracts.

Conclusion

The results of the present study was clearly indicated that the ethyl acetate extract of *Bauhinia purpurea* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. However, the ethyl acetate extract of *Bauhinia purpurea* was found high content of flavonoids. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

Acknowledgments

The authors are thankful to the Karpagam University, Coimbatore, India, for providing lab and technical support for the present investigation.

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