Antioxidant Capacity of Various Extracts from Whole Plant of *Bauhinia Purpurea* (Linn) Evaluated by Three *In Vitro* Methods

C.D.Shajiselvin[1], G.Somasundaram[2], A. Kottai Muthu[*]

[1]Karpagam University, Coimbatore, India
[2]Mahatma Gandhi Medical College and Research Institute, Pondicherry- 607 402, India
[*]Department of Pharmacy, Annamalai University, Annamalai Nagar-608 002, India

*Corresponding author

Dr. A. Kottai Muthu, M.Pharm, Ph.D,

Assistant Professor of Pharmacy,

Annamalai University.

Mobile .No: 91-9443171712

E-mail: arthik03@yahoo.com

Summary

The antioxidant capacity of the whole plant of *Bauhinia purpurea* extracts, obtained by sequential extraction with various polarities of solvents, was evaluated by three different *in vitro* methods: hydroxyl, nitric oxide and total phenol activity. The ethyl acetate extract showed best free radical scavenging activity than that of other two extracts. The nitric oxide radical scavenging activity of ethyl acetate extract (IC_{50} = 180 µg/ml) was better than that of standard ascorbate (IC_{50} = 410 µg/ml). It showed good reducing capacity assessment. The ethyl acetate extract of *Bauhinia purpurea* contains high amount of phenolic content than that of other two extracts. The high antioxidant capacity observed for ethyl acetate extract suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

Key words: Whole plant of *Bauhinia purpurea*, *Invitro* antioxidant, Hydroxyl radical scavenging activity, Nitric oxide radical activity, total phenolic content.

Introduction

Oxidative stress resulting from the toxic effects of free radicals on the tissue plays an important role in the pathogenesis of various pathological conditions such as ageing process, anemia, arthritis, asthma, inflammation, ischemia, mongolism, neurodegeneration, Parkinson's disease, and perhaps dementia. Antioxidants are radical scavengers, which protect the human body against free radicals[1, 2]. Free radical also induces liver damage. Likewise, metabolism of certain drugs like paracetamol, produce free radicals, which cause liver damage. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thereby help in preventing the free radical induced diseases[3].

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 septicaemia[4]. 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[5]. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycaemic activity, etc [6].Even through, traditionally, leaves of *Bauhinia purpurea* (Linn) were extensively used for the treatment of variety of wounds[7], and no scientific data in its support is available.

However, information pertaining to the systematic studies on the antioxidant properties of whole plant of *Bauhinia purpurea* (Linn) is lacking. In view of the above fact, in the present study the possible antioxidant capacity of various extracts from whole plant of *Bauhinia purpurea* (Linn) were evaluated by three in -vitro free radical scavenging models.

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[8] 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

Determination of Hydroxyl radical scavenging activity[9]

The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA – H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Determination of Nitric oxide radical scavenging activity[10]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964)[10].The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization.

Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Determination of total phenol[11]

The measurement of total phenol is based on Mallick and Singh (1980)[11]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^oC for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Results and Discussion

Inhibition of Hydroxyl radical

The percentage of Hydroxyl radical scavenging activity of petroleum ether extract of *Bauhinia purpurea* was summarized in Table 1. The maximum scavenging activity of petroleum ether extract of *Bauhinia purpurea* is 37.65 % at 1000 µg/ml and ascorbate were found to be 55.23 % at 1000 µg/ml. The IC_{50} values of petroleum ether extract of *Bauhinia purpurea* and ascorbate were found to be 1050µg/ml and 410µg/ml respectively.

Table 1: Hydroxyl radical scavenging activity of Petroleum ether extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.27 ± 0.06	26.87 ± 0.07
2	250	28.58 ± 0.01	30.30 ± 0.05
3	500	36.42 ± 0.02	60.64 ± 0.02
4	1000	37.65 ± 0.06	55.23 ± 0.01
		IC_{50} = 1050 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of ethyl acetate extract of *Bauhinia purpurea* was summarized in Table 2. The maximum scavenging activity ethyl acetate extract of *Bauhinia purpurea* is 73.82 % at 1000 µg/ml and ascorbate were found to be 55.23 % at 1000 µg/ml. The IC_{50} values of ethyl acetate extract of *Bauhinia purpurea* and ascorbate were found to be 230µg/ml and 410µg/ml respectively.

Table 2: Hydroxyl radical scavenging activity of Ethyl acetate extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	44.23 ± 0.08	26.87 ± 0.07
2	250	52.81 ± 0.06	30.30 ± 0.05
3	500	69.21 ± 0.04	60.64 ± 0.02
4	1000	73.82 ± 0.03	55.23 ± 0.01
		IC_{50} = 230 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of methanolic extract of *Bauhinia purpurea* was presented in Table 3. The methanolic extract of *Bauhinia purpurea* was exhibited a maximum degradation of deoxy-ribose mediated by hydroxyl radical scavenging activity of 64.17 % at 1000 µg/ml whereas for ascorbate (standard) were found to be 55.23 % at 1000 µg/ml. The IC_{50} values of methanolic extract of *Bauhinia purpurea* and ascorbate were found to be 420µg/ml and 410µg/ml respectively.

Table 3: Hydroxyl radical scavenging activity of Methanolic extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	22.18 ± 0.01	26.87 ± 0.07
2	250	46.79 ± 0.05	30.30 ± 0.05
3	500	52.18 ± 0.03	60.64 ± 0.02
4	1000	64.17 ± 0.02	55.23 ± 0.01
		IC_{50} = 420 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the results, it can be concluded that the ethyl acetate extract of *Bauhinia purpurea* have potent antioxidant activity when compared with other two extracts. When comparison of all the three extracts with ascorbate the ethyl acetate extract of the *Bauhinia purpurea* were showed similar result.

Inhibition of Nitric oxide radical

Table 4 was shows the scavenging of nitric oxide radical by the petroleum ether extract of *Bauhinia purpurea* and ascorbate. The maximum scavenging activity 38.77% and 55.23% respectively at 1000 µg/ml petroleum ether extract and ascorbate. The concentration required for 50% inhibition of petroleum ether extract and ascorbate was recorded as 1290µg/ml and 410µg/ml respectively.

Table 4: Nitric oxide radical scavenging activity of Petroleum ether extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	12.46 ±0.44	26.87 ± 0.07
2	250	20.87 ± 0.41	30.30 ± 0.05
3	500	31.91 ± 0.22	60.64 ± 0.02
4	1000	38.77 ± 0.36	55.23 ± 0.01
		IC_{50} = 1290 µg/ml	**IC_{50} = 410 µg/ml**

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

Table 5 was shows the scavenging of nitric oxide radical by the ethyl acetate extract of *Bauhinia purpurea* and ascorbate. The maximum scavenging activity 71.01 % and 55.23% respectively at 1000 µg/ml ethyl acetate extract and ascorbate. The concentration required for 50% inhibition of ethyl acetate extract and ascorbate was recorded as 180µg/ml and 410µg/ml respectively.

Table 5: Nitric oxide radical 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	38.78 ± 0.29	26.87 ± 0.07
2	250	58.73 ± 0.46	30.30 ± 0.05
3	500	64.83 ± 0.44	60.64 ± 0.02
4	1000	71.01 ± 0.47	55.23 ± 0.01
		IC_{50} = 180 µg/ml	**IC_{50} = 410 µg/ml**

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

Table 6 was shows the scavenging of nitric oxide radical by the methanolic extract of *Bauhinia purpurea* and ascorbate. The maximum scavenging activity is being 64.86 % and 55.23% respectively at 1000 µg/ml methanolic extract and ascorbate. The concentration required for 50% inhibition of methanolic extract and ascorbate was recorded as 250µg/ml and 410µg/ml respectively.

Table 6: Nitric oxide radical scavenging activity of Methanolic extract of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	41.75 ± 0.35	26.87 ± 0.07
2	250	50.01 ± 0.21	30.30 ± 0.05
3	500	58.59 ± 0.19	60.64 ± 0.02
4	1000	64.86 ± 0.46	55.23 ± 0.01
		IC_{50} = 250 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the above results clearly indicated that the ethyl acetate and methanolic extract of *Bauhinia purpurea* were found more effective nitric oxide scavenging activity than that of petroleum ether extract. But when compare to the all the three extracts with ascorbate (standard), the ethyl acetate extract of the *Bauhinia purpurea* was showed the strong nitric oxide radical scavenging activity than that of other two extracts.

Total phenol

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[12]. The phenolic compounds may contribute directly to antioxidative action[13]. The total phenolic content of various extract of whole plant of *Bauhinia purpurea* was depicted in Table 7.

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

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Bauhinia purpurea*	1.23 ± 0.05
2	Ethyl acetate extract of *Bauhinia purpurea*	2.46 ± 0.05
3	Methanolic extract of *Bauhinia purpurea*	1.35 ± 0.04

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

Based on the result the ethyl acetate extract of *Bauhinia purpurea* was found higher content of phenolic components than that of petroleum ether and methanolic extracts of *Bauhinia purpurea.*

Conclusion

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. In the present work, the high antioxidant capacity observed for ethyl acetate extract of whole plant of *Bauhinia purpurea* suggest that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

Acknowledgments

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

References

1. Mahakunakorn P, Tohda M, Murakami Y, Matsumoto K and Watanabe H. Antioxidant and Free radical-scavenging activity of Choto-san and its related constituents. *Biol. Pharm. Bul* 2004; **27**: 38-46.
2. Polterait O . Antioxidants and free-radical scavengers of natural origin. *Current Org. Chem* 1997; **1**: 415-440.
3. Miller NJ and Rice-Evans C. Factors influencing the antioxidant activity determined by the ABTS radical cation assay. *Free Rad. Res* 1997; **26**: 195-199.
4. Asolker LV, Kakkar KK, Chakre OJ. Supplement to glossary of Indian medicinal plants. Part-I (A-K). National Institute of Science Communication, New Delhi, 2000; pp 116-7.
5. Kirthikar KR, Basu DB. Indian Medicinal Plants. Vol. 4.2n ed. Dehradun: Oriental Enterprises, 2000; pp 1255-7.
6. Rajanarayana K, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J Pharmacol* 2001; **33**:2-16.
7. Chopda MZ, Mahajan RT. Wound Healing Plants of Jalgaon District of Maharashtra state, India. *Ethnobotanical Leaflets* 2009; **13**:1-32.
8. Harborne JB. Phytochemical methods 11 Edn In Chapman &,Hall. New York,1984; pp 4-5.
9. Elizabeth K and Rao MNA. Oxygen radical scavenging activity of curcumin. *Int.J.Pharm* 1990; **58**: 237-240.
10. Garrat DC.The quantitative analysis of drugs, Champman and Hall, Japan 1964; **3**: 456-458.
11. Mallick CP and Singh MB . Plant enzymology and Histoenzymology (eds), Kalyani publishers, New Delhi, 1980; pp 286.
12. Hatano T, Edamatsu R, Mori A, et al. Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical and Pharmaceutical Bulletin 1989; **37**: 2016–2021.
13. Duh PD, Tu YY, Yen GC.Antioxidant activity of water extract of Harng Jyur (Chrysanthemum morifolium Ramat). *Lebnesmittel-Wissenschaft und Technologie* 1999; **32**: 269–277.