

Evaluation of antioxidant potential of alcoholic stem bark extracts of *Bauhinia variegata* Linn.

Ashwani Kumar*

Institute of Pharmacy, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu - 333001 (Rajasthan), India

Vikas Anand

School of Pharmaceutical Sciences and Applied Chemistry, Sardar Bhagwan Singh University, Balawala, Dehradun-248161 (Uttarakhand), India

R. C. Dubey

Department of Botany and Microbiology, Gurukul Kangri Vishwavidyalaya, Haridwar-249407 (Uttarakhand), India

Kapil K. Goel

Department of Pharmaceutical Sciences, Gurukul Kangri Vishwavidyalaya, Haridwar-249407 (Uttarakhand), India

*Corresponding author. E-mail: ashwanipharma03@gmail.com

Abstract

The present study was carried out for the evaluation of *in-vitro* antioxidant potential of alcoholic stem bark (ALSB) extract of *Bauhinia variegata* Linn. Dried stem bark extract of *B. variegata* Linn. was screened to evaluate its free radical scavenging effect. Various methods like DPPH assay, reducing power assay, percentage scavenging activity of hydrogen peroxide and superoxide radical scavenging activity were used for screening *in-vitro* antioxidant potential. Antioxidant potentials were concentration dependent which were compared with standard antioxidants such as butylated hydroxyanisole (BHA) and ascorbic acid. The maximum scavenging effect of *B. variegata* Linn. alcoholic stem bark extract on DPPH free radical, superoxide radical and hydrogen peroxide was 72.19 ± 0.20 , 81.60 ± 0.22 and 76.06 ± 0.16 respectively at a concentration of 2500 $\mu\text{g/mL}$. It was clearly indicated that the alcoholic extract of the stem bark has significant *in vitro* antioxidant activity. Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), Ascorbic acid and gallic acid appear to be associated with hepatotoxicity and many others negative health effects. Therefore, natural antioxidants may be preferred over the synthetic antioxidants.

Keywords: Alcoholic extract, Antioxidant activity, *Bauhinia variegata* Linn., Scavenging potential, Stem bark

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INTRODUCTION

From the ancient time, herbal plants are widely used for the treatment of various human disorders all over the world because of the presence of active constituents of therapeutic value. According to World Health Organization (WHO) more than 80% of the world's population still relies on traditional medicine for their primary health care needs (Chidi, 2014).

Among the hundreds of medicinal plant, *Bauhinia variegata* Linn. (family: leguminosae), medium sized tree with hairy branches is one of them, and its value in medicine is known since ancient age (Bansal *et al.*, 2014). The various parts of the tree like flowers, flowers bud, stem bark, stem, leaves, seeds and roots are popular in various system of medicines like ayurveda, unani and homeopathy

in India for the cure of variety of disease (Sahu and Gupta, 2012).

Phytochemical screening of the stem bark and leaves of *B. variegata* Linn. showed that the plant contained various active constituents like carbohydrates, resins, saponins, terpenoids, alkaloids, steroids, flavonoids, tannins, proteins and cardiac glycosides (Dhale, 2011). These constituents make this plant as a folk medicines for treatment of various pathological disease. Various pharmacological studies showed that this plant exerted antidiabetic, anti-inflammatory, anti-tumor, hepatoprotective, antibacterial, haemagglutinating, haematinic, antimicrobial, immunomodulatory and antiulcer activities (Rubaiyat *et al.*, 2016).

In the treatment of diseases, antioxidant therapy has gained an immense importance. Antioxidants have been reported to prevent oxidative damage

caused by free radical and may prevent the occurrence of aging and various diseases even the cancer (Johora *et al.*, 2013).

The aim of the present investigation was to evaluate *in vitro* antioxidant, free radical scavenging activity of the *B. variegata* Linn. stem bark extract.

MATERIALS AND METHODS

Collection of plant samples: Sample of *B. variegata* Linn. (family leguminosae) stem were collected from the plant grown in Botanical Garden, Department of Botany and Microbiology, Gurukula Kangri University, Haridwar (India) in the month of December, 2015 and were positively identified by the experts of the department (specimen identification No: 291/Bot and Micro/16-01-16). The plant samples were washed with running tap water to remove the adhered dust, dirt and other foreign material and dried in shade at room temperature. The dried samples were powdered which were stored in air tight container at room temperature for further studies.

Preparation of crude extracts: The plant samples were powdered separately in shade and subjected to hot extraction in Soxhlet continuous extraction apparatus with alcohol solvents for 48 - 72 h. The extracts were collected in a beaker, filtered separately and considered as the 100 % concentrated stock extract. These extracts were evaporated by vacuum at reduced pressure (Kew *et al.*, 2018). The extracts were then dried and weighed, and yield was calculated using the formula: yield % = $X/Y \times 100$, where X = weight of the beaker with dried drug-weight of the beaker, and Y = total amount of the dried drug.

Phytochemical analysis of plant extracts: Alcoholic stem bark extract (ALSB) of *B. variegata* Linn. was subjected for qualitative analysis of phytoconstituents viz., alkaloids (Dragendorff's test), steroids (Salkowski test), saponins (Foam formation test), cardiac glycosides (Raymond test), tannins (Ferric Chloride test), flavonoids (Lead Acetate test), Proteins (Biuret test), carbohydrates (Molish's test), amino acids (Ninhydrin test), fats and oils (Solubility test), phenolic compounds (Iodine test) as cited in Treatse and Evans (1983) and Kumari *et al.* (2017).

Methods for antioxidant activity

DPPH free radical scavenging activity: The free radical scavenging activity of alcoholic stem bark extract was measured by decrease in the absorbance (Nuraniye *et al.*, 2017). A stock solution of DPPH (33 mg in 1 L) was prepared in alcohol. To the 5mL of the stock solution, added 1 ml of extract solution at different concentrations (250–2500 mg/mL). After 30 min. of the reaction mixture, absorbance was measured at 517 nm and compared with standards i.e. butylated hydroxyanisole (BHA) and ascorbic acid. The following equation was used for calculating the percentage

inhibition of DPPH free radical generation:

$$\% \text{ Scavenging activity} = [(Ab_0 - Ab_1) / Ab_0] \times 100 \quad (\text{Eq. 1})$$

Where, Ab_0 was the absorbance of the control and Ab_1 was the absorbance of extract or standard compounds.

Superoxide radical scavenging assay: The reaction mixture was prepared by adding 1ml of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer, having pH 7.4) and 1 ml NADH solution (468 mM NADH in phosphate buffer, having pH 7.4) to 1mL of sample solution of alcoholic stem bark extract. To the above reaction mixture, added 100 ml of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, having pH 7.4). The mixture was then incubated for 5 min. at 25°C and the absorbance was measured at 560 nm against blank sample and compared with standards i.e. butylated hydroxyanisole (BHA) and ascorbic acid (Naskar *et al.*, 2010).

The following equation was used for calculating the percentage inhibition of superoxide anion generation:

$$\% \text{ Inhibition} = [(Ab_0 - Ab_1) / Ab_0] \times 100 \quad (\text{Eq. 2})$$

Where, Ab_0 was the absorbance of the control and Ab_1 was the absorbance of extract or standard compounds.

Scavenging of hydrogen peroxide: A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (250-2500 µg/mL) of extracts were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. The phosphate buffer without hydrogen peroxide was used as blank (Najat *et al.*, 2015). The following equation was used for calculating the percentage scavenging of hydrogen peroxide of extract and standard compounds:

$$\% \text{ Scavenged } [H_2O_2] = [(Ab_0 - Ab_1) / Ab_0] \times 100 \quad \text{Eq. 3}$$

Where, Ab_0 was the absorbance of the control, and Ab_1 was the absorbance in the presence of the sample and standards i.e. butylated hydroxyanisole (BHA) and ascorbic acid.

Reducing power assay: Different concentrations of extract (250–2500 mg/mL) in 1 mL of alcohol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min (Bhattacharjee *et al.*, 2011). 2.5 mL of 10% trichloroacetic acid was added to the blends. After mixing of trichloroacetic acid, the blend was then centrifuged for 10 min. at 3000rpm. Then upper layer (2.5mL) of the blend was separated and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance of mixture

was measured at 700nm and compared with standards i.e. butylated hydroxyanisole (BHA) and ascorbic acid.

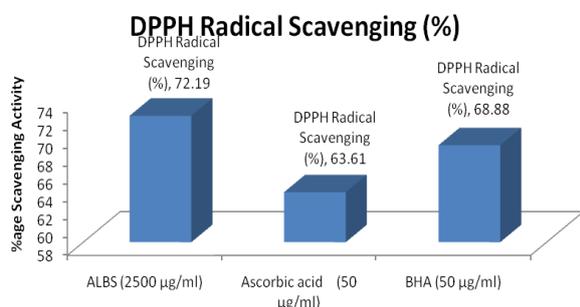


Fig. 1. Showing maximum DPPH radical scavenging percentage at 2500 µg/mL of alcoholic stem bark extract (ALBS) of *B. variegata* Linn.

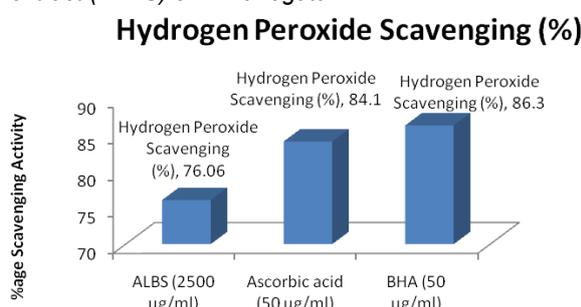


Fig. 2. Showing maximum hydrogen peroxide scavenging at 2500 µg/mL of alcoholic stem bark extract (ALBS) of *B. variegata* Linn.

Table 1. Phytochemical screening of alcoholic stem bark extract (ALSB) of *B. variegata* Linn.

S.N.	Phytochemical test	Alcoholic stem bark extract
1	Alkaloids	+ve
2	Steroids	+ve
3	Saponins	+ve
4	Cardiac Glycosides	+ve
5	Tannins	+ve
6	Flavonoids	+ve
7	Proteins	-ve
8	Carbohydrates	-ve
9	Amino Acids	-ve
10	Fats and Oils	+ve
11	Phenolic Compounds	+ve

Table 2. Antioxidant potential of alcoholic stem bark extract (ALSB) of *B. variegata* Linn.

Sample	Conc. (µg ml ⁻¹)	DPPH radicle scavenging (%)	Hydrogen peroxide scavenging (%)	Superoxide anion Scavenging (%)	Reducing power activity (Absorbance) ^a
ALSB *	(2500)	72.19 ± 0.20	76.06 ± 0.16	81.60 ± 0.22	1.633
Ascorbic acid (Standard)	(50)	63.61 ± 0.10	84.1 ± 0.1	79.62 ± 0.35	1.230
BHA (Standard)	(50)	68.88 ± 0.03	86.3 ± 2.1	72.44 ± 0.64	1.461

* ALSB: Alcoholic stem bark extract; ^aIncreased absorbance indicates increased reducing power [Values are mean ± SEM of three different concentrations (250 µg/mL, 1500 µg/mL and 2500 µg/mL) of alcoholic stem bark extract of *B. variegata* Linn., when compared with standards viz. BHA (10 µg/mL, 30 µg/mL and 50 µg/mL) and Ascorbic acid (10 µg/mL, 30 µg/mL and 50 µg/mL)].

RESULTS AND DISCUSSION

The present study clearly indicated that the alcoholic stem bark extract showed positive result for alkaloid, steroids, saponins, cardiac glycosides, tannins, Flavonoids, fats and oils and phenolic compounds, while the carbohydrates and amino acids were found absent in the give extract (Table 1). The evaluation of free radical scavenging activity of alcoholic stem bark extract of *B. variegata* Linn. was performed by numerous *in-vitro* assays. In case of DPPH radical scavenging assay, the scavenging ability of alcoholic stem bark extract on DPPH radical was found to be 72.19 ± 0.20% (Table 2) which was more significant as compared to the standards BHA and ascorbic acid i.e. 68.88 ± 0.03 and 63.61 ± 0.10 respectively. Fig.1 clearly

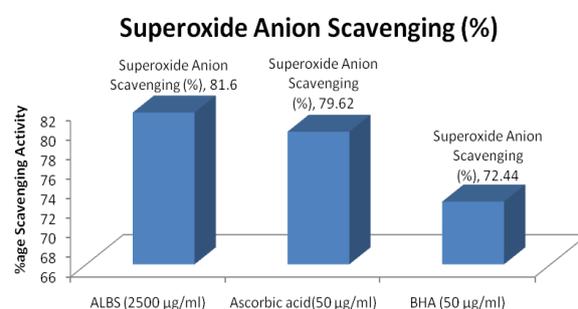


Fig. 3. Showing maximum superoxide anion scavenging at 2500 µg/mL of alcoholic stem bark extract (ALBS) of *B. variegata* Linn.

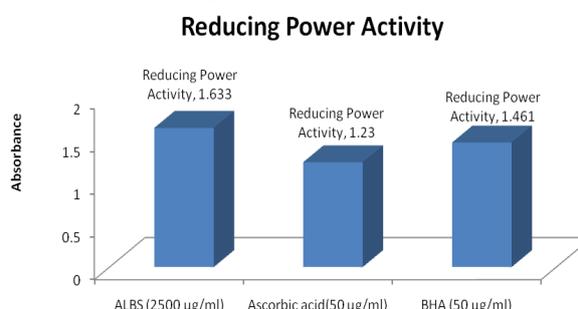


Fig. 4. Showing maximum reducing power activity at 2500 µg/mL of alcoholic stem bark extract (ALBS) of *Bauhinia variegata* Linn.

shows antioxidant potential of alcoholic stem bark extract of *B. variegata* Linn. when compared to BHA and ascorbic acid.

The hydrogen peroxide scavenging activity of alcoholic stem bark extract of *B. variegata* Linn. was found as $76.06 \pm 0.16\%$ which was comparable to standard antioxidants i.e. BHA and ascorbic acid $86.3 \pm 2.1\%$ and $84.1 \pm 0.1\%$ respectively. Fig. 2 clearly shows the comparative scavenging potentiality of alcoholic stem bark extract with standards.

The percentage inhibition of superoxide generation by alcoholic stem bark extract of *B. variegata* Linn. was found as $81.60 \pm 0.22\%$. On the other hand, ascorbic acid and BHA at concentration of $50\mu\text{g/ml}$ had $79.62 \pm 0.35\%$ and $72.44 \pm 0.64\%$ inhibition of superoxide radical (Table 2). Fig. 3 indicates that the alcoholic stem bark extract of *B. variegata* Linn. is more potent to inhibit the superoxide generation when compared to BHA and ascorbic acid.

The potential reducing power activity of a compound may be indicated by its increased absorbance. The reducing power of extract was found to be 1.633 as compared with BHA and Ascorbic acid 1.461 and 1.230 respectively (Table 2). Fig. 4 clearly shows the potential reducing power capacity of alcoholic stem bark extract when compared to standards i.e. BHA and ascorbic acid.

Indigenous systems of medicine have a strong repository of plants that have been used traditionally to offer some sort of oxidative stress. Less side effects, easy availability and highly economic factors makes the herbal drug better alternative of synthetic drugs. It is considered worthwhile to investigate some indigenous plants which have reputation in Ayurveda and folk medicines. Asokan et al. (2015) revealed comparative study of antioxidant potential of *Saraca indica* stem bark with ascorbic acid. Labiad et al. (2017) also performed comparative antioxidant activity of *M. thymus satureioides* extracts with ascorbic acid. In present study, antioxidant potential of alcoholic stem bark extract of *B. variegata* compared with BHA and ascorbic acid and the Observed data (Table 2) clearly indicated that stem bark extract showed significant antioxidant potential when compared with BHA and ascorbic acid.

Conclusion

The present study concluded that the alcoholic stem bark extract of *B. variegata* Linn. had significant *in vitro* antioxidant potentials. It showed maximum % scavenging activity i.e. 72.19 ± 0.20 , 76.06 ± 0.16 , 81.60 ± 0.22 for DPPH radical scavenging, hydrogen peroxide scavenging and superoxide anion scavenging respectively at $2500\mu\text{g/ml}$ when compared with BHA and ascorbic acid. It also showed maximum absorbance i.e. 1.633

which indicates more reducing power activity of alcoholic stem bark extract when compared with BHA and ascorbic acid i.e. 1.461 and 1.230 respectively. The extract of *B. variegata* stem bark showed the positive test of flavonoids. The antioxidant activity may be due to flavonoid and phenolic content present in the stem bark of this plant. Therefore, further studies may be performed for isolation and identification of more antioxidant components of *B. variegata* plant.

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REFERENCES

- Asokan, A., Thangavel, M. and Nisha, P. (2015). In vitro antioxidant activity of *Saraca indica* methanolic bark extract. *International Journal of Current Microbiology and Applied Sciences*, 4(3) : 515-520.
- Bansal, V., Malviya, R., Deeksha, Malviya, T. and Sharma, P.K. (2014). Phytochemical, pharmacological profile and commercial utility of tropically distributed plant *Bauhinia variegata*. *Global journal of pharmacology*, 8(2) : 196-205.
- Bhattacharjee, P., Hullatti, K.K., and Vijay Kumar, M.L. (2011). Anthelmintic and antioxidant activity of alcoholic extracts of different parts of *Coleus Amboinicus* Lour. *International Journal of Research in Ayurveda and Pharmacy*, 2(1):181-185.
- Chidi, N.I. (2014). Heavy metal contamination of herbal medicinal products and cosmetics: A course for concern. *Pelagia Research Library*, 5(2) : 9-22.
- Dhale, D.A. (2011). Phytochemical screening and antimicrobial activity of *Bauhinia variegata* Linn. *Journal of Ecobiotechnology*, 3(9) : 04-07.
- Johora, S., Kabir, A.S., Hakim, A., Abdullah, M., Islam, N. and Reza, A. (2013). Evaluation of the antioxidant activity of *Ficus racemosa* plant extracts from north-western district of Bangladesh. *Journal of Life and Earth science*, 8 : 93-99.
- Kew, K.S., Neivashini, M., Ooi, X.C., Nabila, P. and Khan, N.H. (2018). Qualitative study on the phytochemical constituents of the flower buds of *Bauhinia variegata*. *Madridge Journal of Pharmaceutical Research*, 2(1) : 47-51.
- Kumari, R., Kumar, S., Kumar, A., Kumar, K. and Dubey, R.C. (2017). Antibacterial, antioxidant and immunomodulatory properties in extracts of *Barleria lupulina* Lindl. *BMC Complementary and Alternative Medicine*, 17(484) : 1-11.
- Labiad, M.H., Harhar, H., Ghanimi, A., and Tabyaoui, M. (2017). Phytochemical screening and antioxidant activity of Moroccan *thymus satureioides* extracts. *Journal of Materials and Environmental Sciences*, 8 (6) : 2132-2139.
- Najat, A. B., Reem, A. A., and Mohammed M. I. (2015). Phytochemical and taxonomic evaluation of *Rhazya stricta* in Saudi Arabia. *Saudi journal of biological sciences*, 24(7): 1513-1521
- Naskar, S., Islam, A., Mazumder, U. K., Saha, P.,

- Haldar, P. K., and Gupta, M. (2010). In Vitro and In Vivo Antioxidant Potential of Hydromethanolic Extract of Phoenix dactylifera Fruits. *Journal of Scientific Research*, 2(1) :144-157.
12. Nuraniye, E., Mehmet, A., Ozge, Ç., and Mehmet, T. (2017). Investigating of Phytochemicals, Antioxidant, Antimicrobial and Proliferative Properties of Different Extracts of Thymus spathulifolius Hausskn. and Velen. Endemic Medicinal Plant from Sivas, Turkey . *International Journal of Secondary Metabolite*, 4(3) : 155-166.
13. Rubaiyat Hasan, M.d., Nasirul Islam, M.d. and Rokibul Islam, M.d. (2016). Phytochemistry, pharmacological activities and traditional uses of Emblica officinalis: A review . *International Current Pharmaceutical Journal*, 5(2), 14-21.
14. Sahu, G. and Gupta, P.K. (2012). A review on Bauhinia Variegata Linn. *International Research Journal of Pharmacy*, 3(1): 48-51.
15. Trease, G.E. and Evans, W.C. (1983). Textbook of pharmacognosy. 12th Edition, Tindall and Co., London.