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In-Vitro Antioxidant and Free Radical Scavenging Activity of *Bauhinia Variegata* Linn

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Abstract- In this present study our aim was to evaluate *In vitro* antioxidant and free radical scavenging potential of methanolic extracts of *Bauhinia variegata* Linn. Different parts of *Bauhinia variegata* like leaves, bark and flowers have free radical scavenging activity by hydroxyl radical scavenging method. All extracts have different level of antioxidant activity. Methanol extracts was found to be good solvent for extraction and having good antioxidant activity. IC_{50} value of *Bauhinia variegata* leaf, stem bark and floral buds are 17.9, 19.5 and 17.2 ug/ml. The Reducing power of extracts was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer (UV -1601 SHIMADZU). In this plant (*Bauhinia variegata)* leaf, stem bark, floral buds extracts there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. These findings demonstrated that *Bauhinia variegata* possess free radical and hydroxyl radical scavenging activity as well as antioxidant activity *in vitro*. In conclusion the present study indicates that *Bauhinia variegata* may be a potential source of natural antioxidant.

Index Terms- Antioxidant activity, Hydroxyl radical, ascorbic acid, *Bauhinia variegata,* TBARS

I. INTRODUCTION

Bauhinia variegata is a medium sized deciduous tree found on the rocky hills of Circars, Deccan, and Carnatic regions of South India (Farnsworth, *et. al*., 1985). An infusion from its bark is used as an astringent, tonic and useful in scrofula, skin diseases, and ulcers. The decoction of the roots is used in dyspepsia and as an antidote to snake poison (Gupta, *et. al*., 1979). Previous phytochemical studies on the stems (Gupta, et. al., 1980; Gupta and Chauhan, 1984; Gordon and David, 2001) flowers (Hirano, 1989; Jose, et. al., 2007) and seeds (Ontbriand, 2000b) of this species have led to the isolation of several flavonoids. There are various types of the fatty acid compound found in *Bauhinia variegata* such as linolinic acid, oleic, steric, palmitic and myristic acid (Preston, *et. al*., 1987) A new lectin from seeds of the *Bauhinia variegata* was purified and biochemically characterized (Rajkapoor, *et. al*., 2006). The Anti-inflammatory and antibacterial activity of all the extracts of *Bauhinia variegata* was reported (Rajkapoor, *et. al*., 2004; 2003). There are few reports on the antitumour activity of *Bauhinia variegata* ethanolic extract against Dalton's ascetic lymphoma (DAL) in *Swiss albino* mice (Rajkapoor, *et. al*., 2003). The increasing awareness among consumers about the relation between diet and health is a sign for food producers to pay more attention to the possibilities of health protecting properties in new product development. Product characteristics such as sensory properties (taste, color and texture), microbiological safety, nutritive value, and keep ability have always been regarded as the only important quality attributes in food product development. Nowadays interest is growing for compounds that have been considered as nonnutritive, but which may play a physiological role in the human body. These compounds might be important in maintaining human health and are referred to as "bioactive compounds". Examples are flavonoids, glucosinolates, carotenoids, organosulfides, sterols, and peptides (Steinmetz and Potter, 1996). Since ancient times, many official herbs have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants. A distinction must be made between water-soluble and fat-soluble antioxidants (Hirasa and Takemasa, 1998). Examples of water-soluble antioxidants are ascorbic acid (vitamin C) and B vitamins (e.g., thiamin and riboflavin), while tocopherols (such as vitamin E) and carotenoids (e.g., *beta*-carotene and Lycopene) are fat-soluble antioxidants. Antioxidants in the polyphenol and flavonoids groups vary in their hydrophilic-lipophilic properties (Steinmetz and Potter, 1996).

The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents (Hirasa and Takemasa, 1998). A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts (Nakatani, 1997). The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi, *et. al*., 1992), which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Many of these phytochemicals possess significant antioxidant capacity that may be associated with lower incidence and lower mortality rates of cancer in several human populations (Velioglu, *et. al*., 1998). The purpose of this study was to evaluate *Bauhinia variegata* (kachnar) Linn as new potential sources of natural antioxidants. The antioxidant activities were determined by *in vitro* assays: inhibition of hydroxyl radicals by TBRAS system.

II. MATERIALS AND METHODS

Plant material – Aerial parts of *Bauhinia variegata* (Kachnar) like leaves, stem bark and floral bud were collected in the early

stages of vegetation from the Bhopal, and District Mandla (M.P.) ,India during the October month of 2006. The identification of the plant *Bauhinia verigata L.* (Kachnar) (family*: Leguminose*) was done by botanist Dr. S.S. Khan (Voucher Specimen No: SP/101/LGOB/2006), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh (India).

Reagent and authentic samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany). Sample absorbances were read using a Lambda 532 nm, UV - 1601 Spectrometer Schimadzu (Japan).

Preparation of *B. variegata* extract - Dried powdered plant material such as leaves, stem bark and floral buds (10 g) were extracted by continuous mixing in 100 ml 50% methanol, and stem bark in 95% methanol, 24 h at room temperature. After filtration, methanol was evaporated until only water remained through evaporation on water bath at 60-70 ^{0}c temperature. The dired powder were kept in air tied box.

Deoxyribose assay to assess OH^- radical scavenging activity
The OH^- radical scavenging activity of *Bauhinia variegata* leaves, stem bark, floral buds extract (10–100 ug/ml) were determined according to the deoxyribose method of Halliwell, *et. al*., (1987) in the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H_2O_2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Follow in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture which was then heated in a boiling water bath for 15 min. Once samples were cooled, the absorbances were read at 532 nm. The IC_{50} value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent inhibition of hydroxyl radical was calculated as follows:

$$\%\text{Inhibition} = \frac{\text{Abs: 532 nm Control Abs. - 532 nm sample Abs.}}{\text{532 nm Control Abs}} \times 100$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

III. RESULT AND DISCUSSION

The effect of *Bauhinia variegata* extracts on inhibition of hydroxyl radical production was assessed by the iron (II)–dependent deoxyribose damage assay. The Fenton reaction generates hydroxyl radicals (OH^-) that degrade deoxyribose using Fe^{++} salts as an important catalytic component (11). Oxygen radicals may attack the sugar, which leads to sugar fragmentation. Addition of transition metal ions such as iron at low concentrations to deoxyribose causes degradation of the sugar into malondialdehyde and other related compounds which form a chromogen with thiobarbituric acid (TBA). Antioxidant activity of the extracts was compared with the standard drug ascorbic acid (Table 1) .The results of the effects of the examined *Bauhinia variegata* leaf, stem bark and floral buds, extracts as well as control solutions on OH^- radical production. They show that all extracts of *Bauhinia variegata* and control solutions inhibited the production of OH^- radicals. The % of free racial scavenging activity of methanolic extract of *Bauhinia variegata* leaf, stem bark and floral bud presented in Table 1. have reducing power, the free radial OH^- scavenging activity of the extract increases with increasing the concentration.

The IC_{50} value of *Bauhinia variegata* leaf, stem bark and floral buds were shown the highest inhibitory activity with IC_{50} of 17.9, 19.5 and 17.2 ug/ml, respectively. When compared to the reference substances, the all aerial parts of *Bauhinia variegata* extracts were found to be less efficient in radical scavenging. The scavenging effects of the examined extracts could be due to the flavonoids, but could also be a result of the activity of other secondary bimolecular present in the extracts. This indicates that the concentration of flavonoids is not the only factor related to the antioxidant activity. The possible synergism of flavonoids with other components present in the extracts may be responsible for this observation. Plant extract exhibited antioxidative potential and increased concentration of plant extract has shown increased antioxidative potential.

Table 1: Antioxidant activity of methanolic extracts of *B. variegata*

Concentration (µg/ml)	**% of inhibition (TBARS)**			
	Ascorbic acid	***B. variegata* leaf extract**	***B. variegata* stem bark extract**	***B. variegata* floral buds extract**
10	25.89 ± 2.36	20.86 ± 1.94	19.42 ± 2.31	21.58 ± 1.92
20	60.43 ± 2.59	55.39 ± 1.73	51.43 ± 1.46	56.83 ± 1.93
30	70.50 ± 3.20	62.58 ± 2.53	60.07 ± 2..58	65.46 ± 1.87

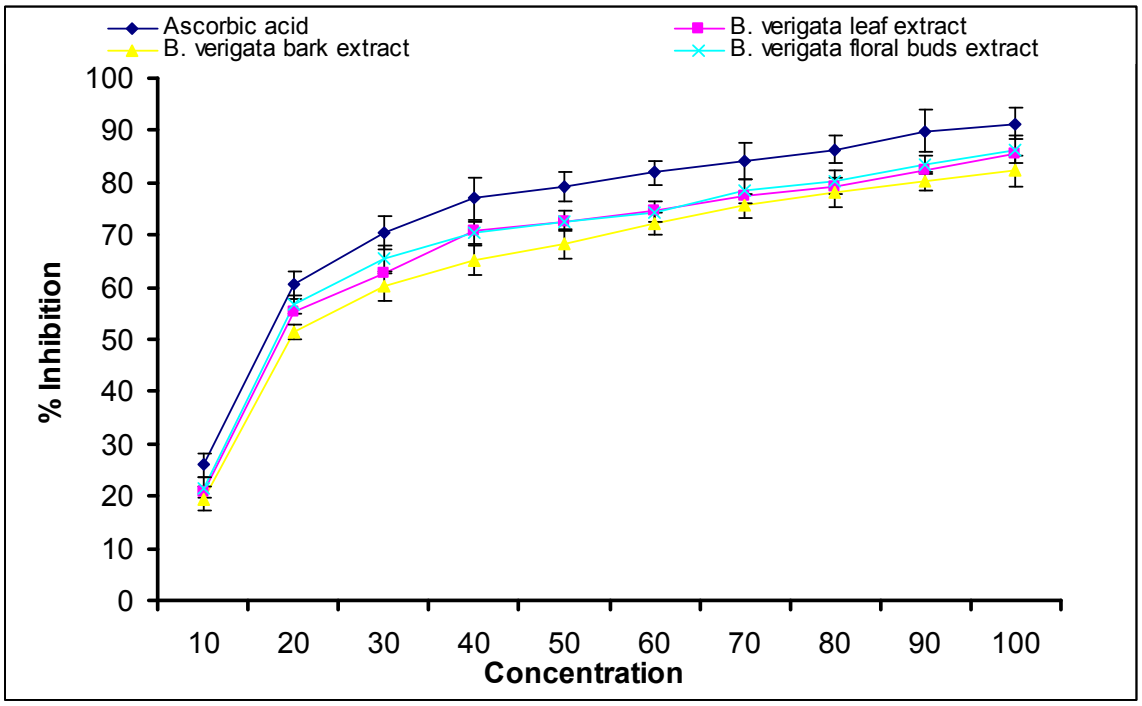
40	76.97 ± 4.03	70.86 ± 2.19	65.10 ± 2.80	70.50 ± 3.10
50	79.13 ± 2.85	72.66 ± 1.84	68.34 ± 2.79	72.66 ± 2.34
60	82.01 ± 2.31	74.82 ± 1.80	72.30 ± 2.10	74.46 ± 1.97
70	84.17 ± 3.44	77.33 ± 2.30	75.53 ± 2.34	78.41 ± 2.04
80	86.33 ± 2.90	79.13 ± 2.34	78.05 ± 2.81	80.21 ± 2.07
90	89.92 ± 4.16	82.37 ± 1.67	80.21 ± 1.81	83.45 ± 1.78
100	91.36 ± 2.87	85.61 ± 2.68	82.37 ± 2.98	86.33 ± 1.92
Control OD at 532 nm – 0. 280				

IC_{50} values:

S. No.	**Group**	**IC_{50} Values**
1.	Ascorbic acid	16 µg/ml
2.	*B. variegata* leaf extract	17.9 µg/ml
3.	*B. variegata* leaf extract	19.5 µg/ml
4.	*B. variegata floral* buds extract	17.2 µg/ml

a absorbance at 532 nm, **b** Scavenging effect (%) = [(Abs. Blank − Abs. sample)/ Abs. Blank] × 100

Group 1: Calibration curve for the total TBARS content of the *B. variegate* Leaf, stem bark and floral buds extracts.



IV. Discussions

In these experiments antioxidant activity was determined by measuring the inhibition of TBARS by a range of ten different concentrations of a certain antioxidant. For each concentration the percent inhibition was measured after 60 min of incubation. The concentrations were chosen in such a way that the lowest concentration provided minimum inhibition of TBARS and the highest concentration gave maximum inhibition of TBARS. The reproducibility of this method is good, the variation in IC_{50} values that are obtained when standard antioxidant compounds are applied is 10-100ug/ml. Methanolic extracts of *Bauhinia variegata* species possess significant free radical scavenging, hydroxyl radical scavenging and antioxidant activity *in vitro*, which offer the possibility of using these extracts as natural antioxidants. In conclusion the results of this study demonstrated that using in *vitro* model *Bauhinia variegata* was found to have antioxidant activity. This activity was found due to presence of polar phenolic compounds flavonoid, tannin etc. Overall *Bauhinia variegata* can be considered as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetes, atherosclerosis etc. Further studies are required to establish its *in vivo* antioxidant activity using different animal models.

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