ISSN: 0975-5772

S.S.Sawheney et al / Journal of Pharmaceutical Science and Technology Vol. 3 (8), 2011,645-650

# Phytochemical Screening and Antioxidant properties of *Bauhinia Variegata (bark).*

Dr. S. S. Sawhney, M. Amin Mir*, Sandeep Kumar
Uttaranchal College of Science and Technology, Dehradun
Uttarakhand Technical University, Dehradun -248001 (Uttarakhand) India.

**Abstract:** This paper describes simple method for performing qualitative analysis of phytochemicals and antioxidant properties of the concerned plant. The distribution of the main active principles (Alkaloids, carbohydrates, proteins/ amino acids, fixed oils and fats, phenolic compounds, tannins and saponins) were assessed along with the antioxidant potential of plant in various solvents. The plant extracts were found to behave differently in different solvents as per their phytochemicals and antioxidant potential is taken into consideration. The plant extracts were found to contain different constituents differently in various solvents (like petroleum ether, methanol, and water), as performed by one or other particular method for every constituent as given below. Antioxidant property were carried out only in two solvents (methanol and water) by FRAP method (ferric reducing ability of plasma or plant) and was found more in methanolic extract than in water extract. The importance of the distribution of the above mentioned phytochemicals were discussed. The study provides scientific method for the investigation of the active components of concerned plant along with its antioxidant potential.

Key words: Antioxidant activity, Active constituents, Phytochemicals, *Bauhinia variegata.*

## Introduction:

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Approximately 80% of the world population depends exclusively on plants for their health and healing. People in recent years are complementing their treatment with natural supplements [1]. Further more motivation of people towards herbs is increasing due to their concern about the side effect of drugs, those prepared from synthetic materials. Many botanical and some common dietary supplements are good sources of antioxidants and anti-inflammatory compounds [2,3]. The importance of the antioxidant constituents of plant material is to maintain the health and protection from heart diseases and cancer [4]. They are vital substances that possess the ability to protect the body from damage caused by free radicals induced oxidative stress [5].

Medicinal and aromatic plants a gift of nature are being used against various infections and diseases in the world since past history. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhabiting bacterial or fungal growth. The substances that can inhabit pathogens and have little toxicity to host cells are considered for developing new antimicrobial drugs. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These could be nutritive/ non-nutritive chemicals but have a protective or disease preventive property. The important of these phytochemicals are alkaloids, flavonoids, tannins and phenolic compounds.

*Bauhinia variegata* commonly known as Kachnar or orchid tree belongs to the family fabaceae grows about 3-8 m tall. The plant bark possesses the property to overcome hypertension, elimination of cholesterol helping the body to develop elasticity to the arterial walls, is always used as a folk medicine. It had been found that the plant possesses the ability to increase calcium and potassium channels.
These properties of Bauhinia Variegata, prompt us to carry out the phytochemical screening and antioxidant properties of the plant bark.

## Materials and methods

### Chemicals and equipments
All the chemicals used in the present investigation were of analytical grade (AR) and were purchased from the sigma Merck etc. All the glass wares and equipments used were sterilized prior to use. Deionised water was used in the complete study.

### Plant materials
The plant was collected from Forest Research Institute Dehradun, and the collection process was preferably done in the dry condition. The plant samples were air dried and grounded into uniform powder with a grinder.

### Experimental

### Extraction
The extraction procedure was carried out with three solvents, petroleum ether, methanol and water based upon their polarity index. The extraction was done by Soxhlet extraction method. A thimble was used in order to get the purest form of extract. The same thimble along with plant material was used in all the three extractions only changed the solvents in accordance with their polarity index, (petroleum ether, methanol and then water).

### Extraction by Organic solvents

### Extraction (A)
75g of plant powdered bark was weighted accurately and extracted for 14 hours, with petroleum ether in a Soxhlet apparatus. The extract was evaporated to remove the volatile solvent and get the plant extract in solid form after being kept in a dessicator for complete dryness.

### Extraction (B)
The same procedure was done in extraction (B) as in extraction (A), with the replacement of only solvent, i.e solvent used was methanol.

### Aqueous Extraction (C)
Water extraction was done by decoction technique, in which the plant material residue after extracted from petroleum ether and methanol was dissolved in near about 500ml of water and heated over a heating element to evaporate the water and then again putted the same quantity of water into it. The same procedure was repeated for two times. Finally filter the solution to get a filtrate which was dried to get the dried form of plant extract.

### Chemical tests for the phytochemicals
Chemical tests were carried on the all extracts using known procedures to identify the plant phytochemicals namely alkaloids, carbohydrates, proteins, phenolic compounds and gums.

### (A) Test for alkaloids.
Solvent free 50 mg extract was stirred with few ml of dilute HCl and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

**(1) Wagner's test.** To a few ml of filtrate, few drops of Wagner's reagent were added along the sides of the test tube. A reddish – brown precipitate confirmed the test.

**(2) Hager's test.** To a few ml of filtrate 1 or 2 ml of Hager's reagent were added. A prominent yellow precipitate indicated the test as a positive.

**(B) Test for Carbohydrates and Glycosides.**

**(1) Molish test.** To 2ml of filtrate two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

**(2) Fehling test.** One ml of filtrate was boiled on water bath with 1ml each of Fehling solution A and B. A red precipitate indicates the presence of sugar.

**(3) Barfoed test.** To 1ml filtrate, 1ml of Barfoed reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicates the presence of sugar.

**(4) Benedict's test.** To a 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicates the presence of sugar.

**(C) Test for proteins and Amino Acids.**

**(1) Biuret test.** An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1 ml of ethanol (90%) was added, fallowed by excess of potassium hydroxide pellets. Pink colour in ethanol layer indicated the presence of proteins.

**(2) Ninhydrin test.** Two drops of ninhydrin solution were added to 1 ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

**(D) Test for Fixed Oils and Fats.**

**(1) Spot test.** A small quantity of extract was pressed between two filter papers, oil stain on the filter paper indicated the presence of fixed oil.

**(E) Test for Phenolic compounds and Tannins.**

**(1) Ferric chloride test.** The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of Phenolic compounds.

**(2) Gelatin test.** The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% gelatin solution containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compound.

**(F) Test for flavonoids.**

**(1) Alkaline reagent test**. An aqueous solution of extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

**Antioxidant activity determination**

The antioxidant activity was measured by FRAP method (Ferric reducing ability of plasma or plant) Benzie and Strain, (1996-1990).

**FRAP - Working solution**. 25 ml acetate buffer, 2.5 ml TPTZ solution (2,4,6-Tripyridyl-S-triazine) and 2.5 ml $FeCl_3.6H_2O$ solution was freshly prepared. Aqueous solution of known $FeCl_4.7H_2O$ was used for calibration.

## Observation

### Table -1 Phytochemical Screening

The data sheet for various types of phytochemicals which were found in various extracts of *Bauhinia variegata* bark are as follows.

| S/No. | ***Phytochemical tests*** | ***Petroleum Ether*** | ***Methanol*** | ***Water*** |
|---|---|---|---|---|
| **A.** | **Alkaloid Test** | | | |
| 1. | Hager's Test | -ve | +ve | +ve |
| 2. | Wagner's test | -ve | -ve | -ve |
| **B.** | **Carbohydrate Test** | | | |
| 1. | Molish Test | +ve | +ve | +ve |
| 2. | Fehling Test | +ve | -ve | -ve |
| 3. | Barfoed Test | -ve | -ve | -ve |
| 4. | Benedict Test | -ve | +ve | -ve |
| **C.** | **Proteins and Amino Acids** | | | |
| 1. | Biuret Test | -ve | +ve | -ve |
| 2. | Ninhydrin Test | +ve | +ve | +ve |
| **D.** | **Fixed oils and Fats** | | | |
| 1. | Spot Test | +ve | -ve | -ve |
| **E.** | **Phenolic compounds** | | | |
| 1. | Ferric chloride test | +ve | +ve | +ve |
| 2. | Gelatin Test | -ve | +ve | -ve |
| **F.** | **Flavonoid Test** | | | |
| 1. | Alkaline reagent Test | -ve | +ve | -ve |

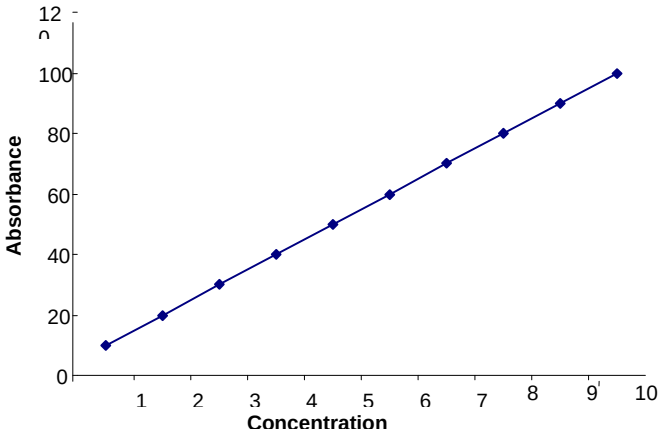
### Antioxidant property

Preparation of standard solution; 0.01ml of $FeSO_4$, solution was mixed with 1.5 ml of FRAP reagent and volume was made up to 5 ml with distilled water, rest of dilutions were prepared by varying the volume of ferrous sulphate solution and distilled water and the absorbance was recorded at 593mμ.

**Table (2)**

| Concentration | Absorbance (μM) |
|---|---|
| 10 | 0.038 |
| 20 | 0.066 |
| 30 | 0.096 |
| 40 | 0.121 |
| 50 | 0.152 |
| 60 | 0.184 |
| 70 | 0.216 |
| 80 | 0.252 |
| 90 | 0.277 |
| 100 | 0.304 |

**Standard curve for FRAP reagent solution.**
**Fig (1)**




From the standard curve the value of Extinction Coefficient (□) comes out to be.

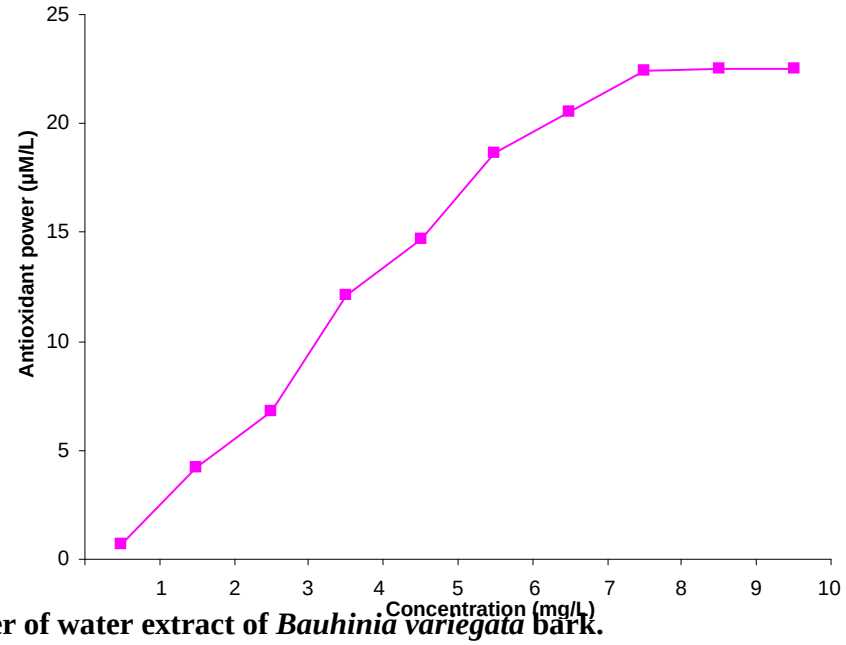
$$\square = 1.5 \times 10^{-4}\ Lmol^{-1}cm^{-1}.$$

**Antioxidant power of methanolic extract of *Bauhinia variegata* bark;**

**Table (3)**

| S/No. | Concentration (mg) | Antioxidant Power (µm/L) |
|---|---|---|
| 1 | 0.006 | 0.7 |
| 2 | 0.012 | 4.2 |
| 3 | 0.018 | 6.8 |
| 4 | 0.024 | 12.1 |
| 5 | 0.030 | 14.7 |
| 6 | 0.036 | 18.6 |
| 7 | 0.042 | 20.5 |
| 8 | 0.048 | 22.4 |
| 9 | 0.054 | 22.5 |
| 10 | 0.060 | 22.5 |

**Line graph showing the antioxidant power verses concentration. (Fig 2)**

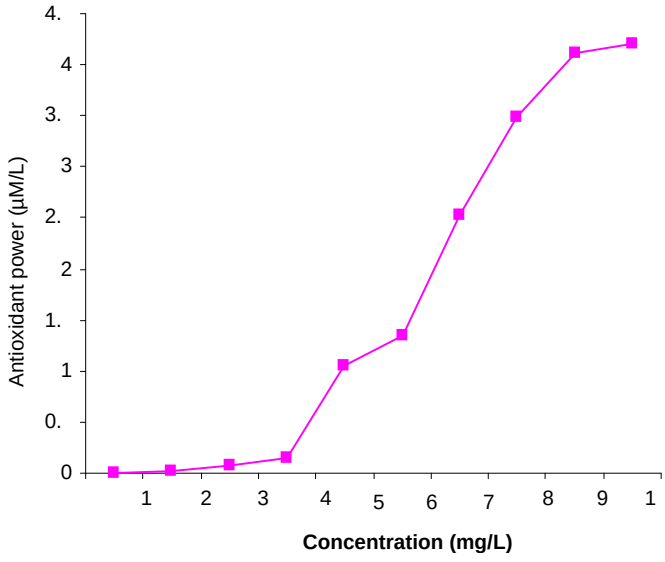



**Antioxidant power of water extract of *Bauhinia variegata* bark.**

**Table (4)**

| S/No. | Concentration (mg) | Antioxidant Power (µm/L) |
|---|---|---|
| 1 | 0.006 | 0.00 |
| 2 | 0.012 | 0.01 |
| 3 | 0.018 | 0.15 |
| 4 | 0.024 | 0.23 |
| 5 | 0.030 | 1.20 |
| 6 | 0.036 | 1.42 |
| 7 | 0.042 | 2.53 |
| 8 | 0.048 | 3.55 |
| 9 | 0.054 | 4.12 |
| 10 | 0.060 | 4.34 |

**Line graph showing the antioxidant power verses concentration Fig (3)**




## Result and Discussion

The present study carried out on the plant sample revealed the presence of medicinally active constituents, alkaloids, flavonoids, carbohydrates, proteins, fixed oils and phenolic compounds as are summarized in table (1). The alkaloids were found in the methanolic and water extract, but were found absent in petroleum ether.

Carbohydrates and phenolic compounds were found to be present in all the extracts. Proteins were found present only in methanollic extract, and fixed oils only in petroleum ether. Flavonoids, phenolic compounds, alkaloids etc have been linked or suggested to be involved with antibacterial and anti-viral activity [6]. Investigations on the mode of action indicates that flavonoids increase colonic water and electrolyte reabsorption and other phytochemicals act by inhabiting intestinal mobility, while some components have been shown to inhabit particular enteropathogens [7]. Carbohydrates are the main components of the cell wall, protoplasm and cell-sap while other accumulates are insoluble storage products. Nitrogen is one of the "major" nutrients needed to support good plant growth. Plants use nitrogen to form amino acids needed in the formation of protein [8]. The fixed oils have been widely used as a fragrance component, a flavoring agent and a herbal medicine to terminate pregnancy [9].

Further investigation was carried on the antioxidant activity of the plant. In extracts (B) and (C) the antioxidant power showed a gradual increase with the increase in concentration, upto a certain limit, after which the antioxidant power graph showed no increase but fallowed a linear path although with increase in concentration. The results also showed that the methanollic extract showed a greater increase in antioxidant potential than the water extract. The diseases caused by the free radicals production in human system can be treated with the *bauhinia variegata* bark, because of its high antioxidant potential. So the bark of this concerned plant can be taken in a good quantity in order to reduce the risk of various types of diseases caused due to free radicals.

**References:**

[1] Dursum, E., S. Otles and E. Akcicek, 2004. Herds as Food source in Turkey. Asian Pacific J. Cancer Prev., 5: 334-339.

[2] Nakarni, N., 1976. Antioxidants from spices and herbs in Natural Antioxidants: Chemistry, Health effects NS Applications. Shahidi, F. Ed; AOCS Press; Champaign I L., PP: 64-75.

[3] Leung, A.Y and S. Foster, 1996. Encyclopedia of common natural ingredients used in food, Drugs and Cosmetics (Ed.). Rube John Wiley, New York.

[4] Lolinger, J., 1991. The use of antioxidants in food, free Radicals and Food Additives. O.I. halliwel, O.I.; B. Eds.; Taylor and Francis, London, pp: 129 0 150.

[5] Souri, E., G. Amin, A.D. Sharifabadi, A. Nazifi and H. Farsam, 2004. Antioxidative activity of sixty plants from Iran. Ir. J. Pharmac. Res. 3; 55-59.

[6] Enzo AP (2007). Traditional plants and herbal remedies used in the treatment of diarrheal disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F, Owais M, editors. Modern Phytomedicine Turning Medicinal Plants in to Drugs. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 248-260.

[7] Enzo AP (2007). Traditional plants and herbal remedies used in the treatment of diarrheal disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F, Owais M, editors. Modern Phytomedicine Turning Medicinal Plants in to Drugs. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 248-260.

[8] Paine TD, Stephen FM, Cates RG (1988). Moisture stress, tree suitability and southern pine beetle population dynamics. In: Payne TL, Saarenmaa H, editors. Integrated control of Scolytid Bark Beetles. Virginia Polytechnique Institute and State University, Blacksburg, pp. 85-103.

[9] Frohlichl. O and Shibamoto T (1990). Stability of pulegone and thujone in ethanolic solution. Journal of Agricultural and Food Chemistry, 38: 2057-2060.