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Research paper

Phytochemical analysis and cytotoxicity evaluation of flowering buds of *Bauhinia variegata*

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Abstract: The current investigation was focused to access the phytochemical studies of the crude aqueous extract and methanol extract of flowering buds of *Bauhinia variegata* contains secondary metabolites like saponins, flavonoids, carbohydrates, proteins, amino acids, and terpenoids. The quantity of these secondary metabolites varied in crude aqueous extract and methanol extract of the buds. The highest levels of phenolics, flavonoids, and terpenoids were observed in methanol extract with the respective standard drugs. The amino acid content was highest followed by phenol and flavonoids.The quantitative determination of secondary metabolites proteins, amino acids, flavonoids, saponins, etc. were also conducted and found that crude aqueous extract of the drug contains the maximum amount of secondary metabolites. In cytotoxic studies (brine shrimp lethality assay) the use of the extract of methanol was reported as potent with LD50 of 241.778 µg/ml as compared to aqueous extract with LD_{50} of 489.706 1µg/ml.

Keywords*: Bauhinia variegata,* phytochemical analysis, cytotoxic activity, Secondary metabolites

INTRODUCTION

The ethnobotanical importance of plants is as old as mankind. In pre-historic societies, plant medicines were found everywhere, whereas 7,000 conventional medicines such as aspirin, digoxin, morphine, quinine, etc. have been obtained from plants and have been included in the modern pharmacopeia. A review from 1981 to 2007, showed that since 1994 the majority of the drugs (35%) approved by the FDA are derived from natural sources. Researchers have made many attempts to explore many secondary metabolites (flavonoids, alkaloids, saponins, tannins, phenols, carbohydrates, phenolic glycosides, cyanogen glycosides) with pharmacological and biological activities against cancer, diabetes, ulcers, and for many other diseases. Keeping in view the importance of natural drugs pharmaceutical industries are anxious about the knowledge of indigenous people regarding the uses of herbs to search for new active secondary metabolites. (Talukdar *et al*., 2011; Farais *et al*.,2013).

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The family Fabaceae covers the genus Bauhinia. This family comprised of 630 genera and 18,000 species. The plants included in this family are deciduous trees, shrubs, vines, and are well known for ornamental purpose. Bauhinia is a famous plant called by various names throughout the world like Kachnar in Hindi linguistic, Raktakanchan in Marathi language, English users named it as an orchid tree as well as Mountain ebony. The word Kachnar means "A glowing beautiful lady" in transcripts of Sanskrit language (Patil *et al*., 2012). It is widely planted in many native countries like India, South China, Pakistan, Burma, Vietnam, Cambodia, Laos, and Thailand (Sharma *et al*., 2013). In native countries, flowers, buds, pods, and seeds of kachnar are cooked and used to make pickles. (Gupta *et al*., 2009)The different plant parts are used for medicinal purposes in South Asia in Unani, Ayurveda, and Homeopathic health belief models. The plant parts that are most frequently used are flowers, stem, buds, stem bark, leaves, roots, and seeds (Koti *et al.*, 2009).

The antidiabetic activity of stem bark and leaves of *Bauhinia variegata* was discovered in rats by using extracts of hydro-alcohol, ethanol, and aqueous extract. The metabolism of glucose was increased in the rats on oral extract administration of various strengths that resulted in the reduction of raised glucose levels in the blood. Later experimental work established that the leaves of *Bauhinia variegata* contained insulin similar proteins that stimulate the metabolism of glucose(Kapoor *et al.*, 2003) (Azevedo *et al.*, 2006). The preparation of leaves in an ethanolic extract with a dose of 300mg/kg was related to decrease blood glucose levels and enhanced lipid profile. The results of in-vitro study emphasized that *Bauhinia variegata* and roesoside leaves extracts in ethanol could upsurge the insulin release in INS-1 beta-cell line. The decrease in blood glucose levels in alloxan-induced rats was also proposed on the regeneration of β-cells with the use of ethanolic bark extract ranging from 250-500 mg/kg(Baraiya *et al.*, 2012) (Bodak *et al.*, 2012). The presence of flavonoid rhamnocitrin in *Bauhinia variegata* stem bark confirmed the anti-cataract potential of the plant.

The anti-cancerous effect of *Bauhinia variegata* ethanolic stem extract was experimentally determined on Swiss Albino mice. The cell line growth of cancer against Dalton's ascetic lymphoma was inhibited by stem. The effect was also estimated by using the same extract against Ehrlich ascites carcinoma. The stem extract showed antimutagenic activity. The orally given ethanol extract successfully decreases N-nitrosodiethylamine enhanced enzymes (Kumar et al., 2012). *Bauhinia variegata* flowering buds contain amino acids of four types in the early stages. They are glutamic acid, aspartic acid, glycine, and α alanine. The mature stages of buds contain α keto glutaric acid, Oxaloacetic acid, and Phospho phenol pyruvic acid. In the folkloric medicinal system, the flowering buds of *Bauhinia variegata* were used to treat hemorrhoids, dysentery, and diarrhea. The flowers of *Bauhinia variegata* were used to treat edema and dysentery and as a laxative and anthelmintic activity. The floral bud and flowers were ingested frequently for mumps and leucorrhoea treatment. *Bauhinia variegata* flowering buds in the Unani system of medicine were used for cough treatment, ophthalmic infections, liver complications, and hematuria menorrhagia(Rajkapoor *et al*., 2003a) (Rajkapoor *et al*., 2006). The presence of *Bauhinia variegata* in Pakistani flora with traditional usage and established pharmacological activities from investigations evoked to explore the phytochemical analysis and cytotoxicity evaluation of flowering buds of *Bauhinia variegata* (Mukherjee *et al*., 1977). This investigation was planned to estimate the preliminary phytochemical analysis, with the purpose to determine the secondary metabolites that may be associated with the cytotoxicity anti-diabetic activity of flowering buds of *Bauhinia variegata* (Nadkarni *et al.*, 1954) (Bhatnagar *et al*., 1973) (Malhotra *et al.*, 1973) (Badhe *et al*.,1990).

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MATERIAL AND METHODS

Plant collection: Bauhinia variegata was procured from Faislabad for experimental purposes.The expert Botanist Prof Dr. Qazi Najam us Saqib from the pharmacy department, Comsats University Abbottabad identified and authenticated the plant. One portion of the sample was placed in a local herbarium that served as a reference. The flowering buds were used as plant parts. The plant part was shade dried at room temperature. After adequate drying, the plant material was then chopped to a fine powder. The powdered material was then weighed properly and stored in a tight polythene bag for further study(Seidel et al., 2006) (Handa et al., 2008).

Extract preparation: The extraction flask was used for soaking of 200g of dried powder in 1 liter of menthol. The herbal mixture was kept in the darkroom for 14 days. Daily shaking of the mixture was done twice during this period. After 14 days filtration was carried out with Whatman filter paper No. 1. The methanolic extract and residues were separated.The residues were added in 300 ml of methanol and the same process was adopted thrice. The rotary evaporator was used for drying of filtrate at 40C. The same procedure was used for the aqueous extract to get 2.5g and 8.6g of methanol extract respectively(Sonam *et al.*,2015).

Chemicals and reagent solutions: Analytical grade pure chemicals and reagent solutions procured from E.Merck Germany were used in the investigation(E.Merck, Germany).

Phytochemical analysis: Qualitative phytochemical analysis of both extracts *Bauhinia variegata* flowering buds was performed by following standard procedures for screening of secondary metabolites such as saponins, flavonoids, carbohydrates, proteins, phenols, terpenoids, glycosides, alkaloids, tannins in the respective plant.

The total content of amino acid was assayed by following the Ninhydrin method (Gayathri *et al.*, 2013). In 0.5ml of both extracts add 4ml of distilled water then add 1ml of ninhydrin reagent and mixed well. After that boil, the reaction mixture for 15 minutes in a hot water bath, allowed cooling.Now add ethanol of 1 ml quantity to the reaction mixture and mixed well and absorbance was measured at 570nm with the help of a calorimeter. The Glycine-100 µg/ml as a standard was assessed in the same way. The amounts of amino acid in flowering buds were expressed as mg/g of flowering buds.

Plant material was investigated for total protein contents following the method described by (Gayathri et al., 2013). To 0.4ml of both extracts, add 9ml distilled water in order to increase the volume and permitted to stand for some time, before the addition of 1.0ml of Folins Ciocalteu. The resultant mixture was shaken well before allowed to incubate at room temperature. After five minutes 10ml of 7% sodium carbonates solution was added to the resultant mixture. The mixture was again incubated at 23°C for 90 minutes. After incubation, the calorimeter was used to measure absorbance at 750nm. The Gallic acid-100 µg/ml as a standard was assessed in the same way. The amounts of phenol in flowering buds were expressed as milligram of GAE/g of flowering buds.

The total flavonoid contents were accessed with the same procedure as adopted by Gayathri *et al,* in 2013(Gayathri et al., 2013).The 0.5 ml volume of extracts were added to aluminum chloride(10%) and sodium acetate(0.1 M).The distilled water of 5.3ml was added to the resultant mixture and then incubated for 30 minutes at room temperature. A calorimeter was used to measure absorbance at 420nm. The Quercitin-100 µg/ml as a standard was assessed in the same way. The amounts of flavonoids in flowering buds were expressed as milligram of QE/g of flowering buds.

ASIAN JOURNAL OF PHARMACOGNOSY *Research paper*
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Cytotoxic evaluation: The cytotoxic evaluation utilized Brine shrimp lethality assay as standard to determine the anticancerous prospect of *Bauhinia variegata* (Atta-ur-Rehman *et al.*, 2001). The 20mg of *Bauhinia variegata* flowering buds crude aqueous and methanol extracts were dissolved in 2ml of distilled water and Dimethyl Sulfoxide (DMSO) which served as a stock solution. Concentrations of five diverse strengths of 500, 250,125,50, and 25 µg/ml ppm was transported into the vials (5 vials/concentration) from the stock solution. Evaporation of vials solvents was carried out and the residue was resolubilized in 2ml of seawater. The Pasteur pipette was used to place 10 larvae/vial. Seawater was used to makeup final volume up to 5 ml with 100ppm, 125ppm, 225ppm, 500ppm,and 1000 ppm of final concentrations and incubated at 250-270 C for 24 hours under the light. The negative control vials have Dimethyl Sulfoxide and the positive control vial have to reference cytotoxic drug. The magnifying glass was used to count the survivors after 24 hours.The perforated partition assisted in the unequal division of the hatching tray. A sprinkling of eggs was done in minor quantity over the solution (Sea salt (3.8g) + 1000ml Distilled water). Aluminum foil was used to cover the solution for darkness. The tray was positioned under the lamp, when the eggs hatched out the larvae swam energetically and traveled to the illuminated portion of the tray.

Biostat software was used to calculate LD_{50} values by probit analysis(Barkatullah *et al.*, 2011).

RESULTS AND DISCUSSION

Phytochemical analysis: The phytochemical analysis of the crude aqueous extract and methanol extract of flowering buds of *Bauhinia variegata* showed the existence of a variety of significant secondary metabolites like saponins, flavonoids, proteins, amino acids, terpenoids, etc as presented in table 1. The highest levels of phenolics, flavonoids, and terpenoids were observed in methanol extract with the respective standard drugs. This can be attributable to the higher solubility of these compounds in methanol than the aqueous medium.

Secondary metabolites	Extracts
Crude aqueous	Methanol
Alkaloids - -	- -
Flavonoids ++	++
Phenols ++	++
Carbohydrates ++	++
Saponins ++	++
Triterpenoids ++	++
Tannins ++	++
Glycosides ++	++
Proteins ++	++
Phytosterol ++	++

Table1: Phytochemical analysis of crude aqueous and methanol extract of flowering buds of

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Analysis of Phytochemicals: The quantitative analysis of crude aqueous extract and methanol extract of flowering buds of *Bauhinia variegata* in Table 2.The aminoacid content was highest followed by phenol and flavonoids.

Phytochemical	BVM	BVA
Total Flavonoids mg/QE/g	1.26±0.06	4.26±0.26
Total Phenols mg/GAE/g	3.96±0.09	4.74±0.97
Total Amino acid mg/Gly/g	7.71±9.8	9.47±23.74

Table 2: Quantitaive Analysis of Phytochemicals

Analysis of flavonoids: The results of the quantitative analysis indicated that the content of flavonoids was higher in the aqueous medium of extract when compared with the methanol medium of extract. The antioxidant action and metal chelation potential of flavonoids are well recognized from an herbal medicine prospective(Okwu *et al.*, 2004). *Bauhinia variegata* is used in herbal therapy for anti-inflammatory actions and hypoglycemic effects.

Analysis of phenols: The results of the quantitative analysis of phenols presented that the content of phenol was higher in the aqueous medium of extract when compared with methanol medium of extract. The rich phenolic content used in food products minimizes heart disease due to antioxidant potential as well as effective in chemotherapy and osteoporosis treatment(Frankel *et al.*, 1993) (Kanner *et al*., 1994).

Analysis of aminoacids: The results showed that aqueous extract (9.74mg/g ±23.74) contains a higher amount of amino acids as compared to methanol extract(7.71mg/g ±9.8). The human body needed protein in adequate amounts for development and as a structural constituent of body cells.Proteins act as precursors for vital molecules for lifespan. The analyzation of flowering buds of *Bauhinia variegata* resulted in glutamic acid, aspartic acid, glycine, and α alanine at the early stages. The mature stages of buds contain α keto glutaric acid, Oxaloacetic acid, and Phospho phenol pyruvic acid. *Bauhinia variegata* has enriched nutrition value with amino acids and protein content and also served as a source of energy during malnourishment. The probit analysis of amino acids is presented in the graph 3.

Cytotoxic evaluation: The crude aqueous extract and methanol extract of *Bauhinia variegata* flowering buds were subjected to cytotoxicity assay using Brine shrimp assay to calculate LD_{50}. This assay is considered a convenient method for the detection of plant compounds that are responsible for the toxicity of the freshly hatched brine shrimp larvae. The probit analysis of aqueous crude drug and methanol crude drug is presented in graph 1.

The results of this activity showed that methanol extract is more toxic than aqueous crude extract with LD_{50} = 241.778 µg/ml with the upper and lower confidence interval of 315.78 µg/ml and 174.98 µg/ml and 489.7061 µg/ml with the upper and lower confidence interval of 653.5212 µg/ml and 352.890 µg/ml respectively. The results are presented in Table 2.

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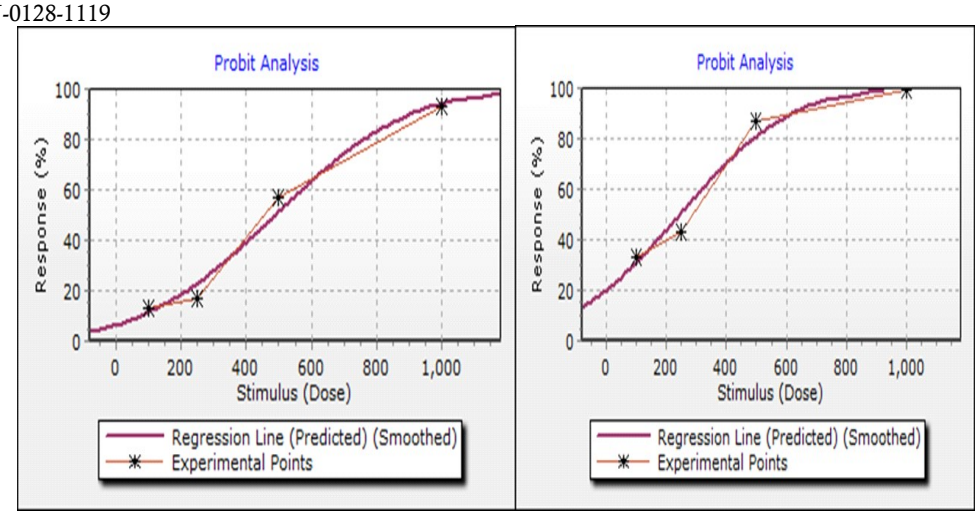

Graph 1:Graphical presentation of aqueous crude drug and methanol drug

Cytotoxicity results of aqueous crude drug				
Dose (µg/ ml)	Log dose	Total No. shrimps	No. of dead shrimps	Probit
1000	3.000	30	28	7.9394
500	2.699	30	17	6.1108
250	2.3979	30	5	4.8324
100	2.097	30	4	4.5697
Cytotoxicity results of methanol drug				
1000	3.000	30	30	7.9394
500	2.699	30	26	6.1108
250	2.3979	30	13	4.8324
100	2.097	30	10	4.5697

Table 3: Cytotoxicity results of aqueous crude drug and methanol drug

CONCLUSION

All over the world, the success of any health care system depends on the availability of suitable drugs. The medicinal plants have always contributed a vital role in the "world health". 80% of the population throughout the world depends on the herbal remedy according to the survey of the World health organization. The above investigation concluded that plant *Bauhinia variegata* flowering buds possess many advantages /uses and can be incorporated for therapeutic efficacy against various ailments as folklore medication. The antioxidant action and metal chelation potential of flavonoids, chemotherapeutic and osteoporosis treatment probability of phenols and enriched protein content should be investigated further for herbal and synthetic molecules isolation and development. The cytotoxic studies of crude aqueous extract and methanol extract of *Bauhinia variegata* flowering buds showed that methanol extract possesses cytotoxic activity and thus can be used for the treatment of tumors.

CONFLICTS OF INTERESTS

Authors have declared that no competing interests exist.

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eISSN-0128-1119

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