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Preliminary Phytochemical, *In vitro* Pharmacological Study of *Bauhinia alba* and *Bauhinia variegata* flowers

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Abstract: The qualitative analysis of phytochemicals profile, antioxidant and antibacterial activities of *Bauhinia alba* and *Bauhinia variegata* flowers have been studied showing the presence of bioactive secondary metabolites. The phytochemical screening of *n*-hexane chloroform, ethyl acetate and methanolic fractions of *B. variegata* flowers revealed the presence of terpenoids, while steroids and cardiac glycosides were present in the entire fractions. *B. alba* flower showed the presence of terpenoids in *n*-hexane fraction while chloroform, ethyl acetate and methanol fractions contain steroids. The extracts/fractions of both plants were evaluated for antibacterial and antioxidant activity which indicated moderate activities against selected bacterial strains and scavenging property.

Key words: *Bauhinia alba* · *Bauhinia variegata* · Phytochemical screening · Antioxidant activity · Antibacterial activity

INTRODUCTION

Medicinal plants are an important source of producing valuable bioactive secondary metabolites which are great importance for the health of individuals and communities. The medicinal values of the plants are due to the chemical substances that produce a definite physiological action on human body [1-4]. *B. variegata* belongs to family Leguminosae and medium sized deciduous trees originate on the rocky hills of Circars and Carnatic regions of South India [5]. An infusion from *B. variegata* bark is used an astringent, tonic and useful in scrofula, ulcers and skin diseases. The plant possesses the property to overcome hypertension, elimination of cholesterol helping the body to develop elasticity to arterial walls and always used as a folk medicine. The phytochemical investigation of *B. variegata* showed that the stem, flowers and seeds of this species have led to the isolation of flavonides [6]. The previous studies on the roots bark revealed the presence (2S)-5,7-dimethoxy-3', 4'-methylenedioxyflavanone, dihydrodibenzoxepin, 5, 6-dihydro-1, 7-dihydroxy-3, 4-dimethoxy- 2-methyldibenzoxepin, flavonoids, quercetin 7-methylether, kaempferol 7,4'-dimethylether 3-O-β-D -glucopyranoside, and kaempferol 3-O-β-D-glucopyranoside [7]. *B. alba* belongs to family Caesalpiniaceae and also known white orchid semi-tropical tree with smooth or slightly ridged grey bark grows in moist rich soil in mild climates. The large fragrant blooms ranges in color from snowy to creamy white. The plant is ~15 ft in height, evergreen, deciduous, smooth-textured and veined. In current study, we have made an effort to identify potential bioactive secondary metabolites and their antioxidant and microbial activities.

MATERIALS AND METHODS

Plant Materials: The flowers *of B. alba* and *B. variegata* were collected from the University garden and identified by Prof. Dr. Abdur Rashid Department, of Botany, University of Peshawar, Peshawar, Pakistan.

Extraction and Fractionation: Shade dried flowers of *B. alba* and *B. variegata* were soaked in ethanol for 5 days. The extracts were concentrated under reduce pressure at 40°C using rotary evaporator and concentrated extracts were suspended in water and successively partitioned with *n*-hexane, chloroform, ethyl acetate and methanolic fraction followed by the standard protocol [8-11].

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Phytochemical Profiling: The chemical tests were performed on the *n*-hexane, chloroform, ethyl acetate and methanolic extracts of *B. alba* and *B. variegata* using reported procedures to identify the bioactive secondary metabolites.

Test for Alkaloids: 0.2 g of each fraction was warmed with 2% H_2SO_4 for 2 min. The reaction mixture was filtered and added a few drops of Dragendorff reagent to each filtrate. Orange red precipitate indicates presence of alkaloids [12].

Test for Tannins: A small quantity of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added. A dark green color indicates tannins [13].

Test for Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red color indicates presence of glycosides [14].

Test for Reducing Sugars: Each extract were shaken with distilled water and filtered. The filtrate was boiled with few drops of Fehling's solution A and B. An orange red precipitate indicates the presence of sugars [15].

Test for Saponins: 0.2 g of each extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows presence of saponins [16].

Test for Flavonoids: 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCl were added. Yellow solutions that turn colorless indicate the presence of flavonoids [17].

Test for Phlobatanins: 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2%HCL solution. Red precipitate indicates the presence of phlobatanins [18].

Test for Steroids: 2 ml of acetic anhydride was added to the mixture of 0.5 g of each extract and H_2SO_4 (2 ml). The color from violet to green in some samples indicates the presence of steroids [19].

Test for Terpenoids: 0.2 g of each extract was mixed with 2ml of chloroform and concentrated (3ml) H_2SO_4 was carefully added to form a layer. The formation of reddish brown coloration at the interface indicates the presence of terpenoids [16].

Test for Cardiac Glycosides: 2 ml of each extract, 1ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated H_2SO_4 were added. The greenish blue colour indicates the presence of cardiac glycosides [17].

Test for Anthraquinones: 0.5 g of each extract was boiled with 10% HCl for few min. The reaction mixture were filtered and allowed to cool. Equal volume of chloroform was added to each filtrate. Few drops of 10% ammonia was added and heated. Rose-pink color indicates the presence of anthraquinones [14].

Antioxidant Activity: The hydrogen atom or electron donation abilities of the corresponding extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to standard protocol [18-20] 10 mg DPPH was dissolved in 100 ml MeOH to obtain a concentration of 100 mg/ml. The stock solution of the fractions/extracts was prepared by dissolving 0.25 mg in 50 ml MeOH. Dilutions of the stock solutions of the crude extracts were prepared to obtain concentrations of 10 mg, 20 mg, 40 mg, 60 mg, 80 mg, 100 mg, 250 mg and 500 mg, while the control was prepared by dissolving 1ml DPPH in 4 ml MeOH (without sample). 1 ml DPPH was added to each solution and the solution were kept in dark and allowed to stand for exist 30 min. The UV absorption of each solution was recorded at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. The experiment was carried out in triplicated and the average absorption was noted for each concentration. Scavenging of free radicals by DPPH as percent radical scavenging activities was calculated as follows.

% DPPH = Control absorbance – extract absorbance X 100 / control absorbance

Micro-organism Assortment and Preservation: Three selected bacterial strains *(Staphylococcus aureus Bacillus subtilis* and *Klebsiella pneumonia*) were obtained from the stock culture of Centre of Biotechnology, University of Peshawar, Pakistan and stored in Muller-Hinton agar at low temperature (4°C) prior to subculture.

Microbial Assay: The tests for susceptibility were done using agar well diffusion methods as early discussed [21-23] to test the antimicrobial activity of the extracts and fractions. The Muller-Hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 hours. The broth culture of the test organism was placed in sterile petri-dish to which 20 ml of sterile molten MHA was added. Wells were bored into the medium using 0.2 ml of extracts (*n*-hexane, chloroform). Streptomycin (2 mg/ml) as standard antimicrobial was used. Incubation was done for 1 hour to make possible the diffusion of the antimicrobial agent into the medium. The inoculation plates were incubated at 37°C for 24 hours and the diameter of the zone of inhibition of microbial growth were measured in millimeters (mm).

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the crude extract and fractions of *B. alba* and *B. variegata* flowers are given in table 1 and table 2, respectively, which showed the presence of bioactive secondary metabolites such as tannins saponins sugars, flavonoids, terpenoids, alkaloids, phlobatanins and steroids.

The crude extracts and fractions were also evaluated for their antioxidant potential. The antioxidant activity was performed by DPPH radical scavenging assay. Different fractions showed activity at different level (Table 3 and 4). Generally the lowest antioxidant activity was found in chloroform fraction. The ethyl acetate, methanol and *n*-hexane fractions showed moderate scavenging activity as compared to standard querceitin. Interestingly the methanol extract of *B. alba* (Table. 4) showed highest activity among the fractions of the same plants. The present investigation revealed that the flowers of *B. alba* and *B. variegata* can be used in less quantity in order to reduce the risk of various diseases causes due to free radicals. The pharmacological activity of *B. alba* and *B. variegata* was confirmed from the antioxidant and antibacterial assay of the crude extract and fractions.

The extracts and fractions were further evaluated for their antibacterial potential against selected bacterial strain (Staphylococcus aureus, *Bacillus subtilis* and *Klebsiella pneumonia*).

Generally *n*-hexane, ethyl acetate and methanolic fractions of *B. alba* were found active against selected bacterial strain (Table 5) and displayed high inhibitory zone of (12 nm) at the tested concentration of (22 mg/ml). While chloroform and methanolic fractions of *B.variegata were* found active against *S. aureus, K. pneumonia, B. subtilis* (Table 6) and showed high inhibitory zone of (14 nm) at the same concentration.

Table 1: Phytochemical profiling of *n*-hexane, chloroform, ethyl acetate, methanolic fraction and crude extract of *B. alba* flowers

Chemical components	*n*-hexane	Chloroform	EtOAc	MeOH	Crude extract
Alkaloids	-	-	-	-	-
Terpenoids	+	+	+	+	+
Flavonoids	+	-	+	+	+
Anthraquinones	-	-	-	-	-
Tannins	-	-	-	+	+
Phlobatanins	-	-	-	-	-
Saponins	+	+	+	-	+
Glycosides	-	-	-	-	-
Reducing sugars	-	+	-	+	+
Steroids	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+

Key words: present: +, absent: -

Table 2: Phytochemical profiling of *n*-hexane, chloroform, ethyl acetate, methanolic fraction and crude extract of *B. variegata* flowers

Chemical components	*n*-hexane	Chloroform	EtOAc	MeOH	Crude extract
Alkaloids	-	-	-	-	-
terpenoids	+	+	+	+	+
flavonoids	-	+	+	+	+
anthraquinones	-	-	-	-	-
tannins	-	-	-	+	+
phlobatanins	-	-	-	-	-
saponins	+	+	+	-	+
glycosides	-	-	-	-	-
Reducing sugars	-	+	-	+	+
steroids	+	+	+	+	+
cardiac glycosides	+	+	+	+	+

Key words: present: +, absent: -

Table 3: DPPH radical scavenging activities of the crude extract and various fractions of *B. alba* flowers

n-Hexane fraction		Chloroform fraction		EtOAc fraction		Methanolic fraction		Crude extract	
Conc. mg/ml	% DPPH	Conc. mg/ml	% DPPH	Conc. mg/ml	% DPPH	Conc. mg/ml	%. DPPH	Conc. mg/ml	% DPPH
20	4.59	20	10.51	20	25.4	20	20.94	20	30.87
40	5.27	40	10.20	40	30.1	40	37.79	40	16.44
60	12.01	60	7..99	60	40.5	60	44.7	60	22.52
80	12.07	80	12.53	80	46.3	80	55.06	80	28.53
100	15.8	100	11.67	100	55.9	100	60.08	100	45.44
250	41.39	250	12.16	250	60.2	250	70.8	250	37.4
500µg/ml	78.5	500µg/ml	15.61	500µg/ml	68.3	500µg/ml	77.7	500µg/ml	83.1

Table 4: DPPH radical scavenging activities of the crude extract and various fractions of *B. variegata* flowers

n-Hexane fraction		Chloroform fraction		EtOAc fraction		Methanolic fraction		Crude extract	
Conc. mg/ml	% DPPH	Conc. mg/ml	% DPPH	Conc.mg/ml	% DPPH	Conc.mg/ml	% DPPH	Conc.mg/ml	% DPPH
20	20.01	20	2.5	20	22.4	20	15.94	20	30.06
40	20.07	40	4.97	40	26.1	40	20.79	40	93.58
60	12.62	60	5.10	60	30.5	60	26.7	60	18.97
80	24.27	80	9.03	80	46.3	80	33.06	80	93.41
100	26.27	100	12.0	100	59.9	100	45.08	100	49.65
250	52.48	250l	17.36	250	68.2	250	60.8	250	65.42
500	73.40	500l	29.89	500	80.3	500	66.7	500	76.94

Table 5: Antimicrobial activity of the crude extract and fractions of *B. alba*

Microorganism	*n*-Hexane	Chloroform	Ethyl acetate	Methanol
Staphylococcus aureus	12	10	10	12
Klebsiella pneumonia	10	0	12	10
Bacillus subtilis	0	0	0	0

Key: 0 = Not active; Well size: 6 mm

Table 6: Antimicrobial activity of the crude extract and fractions of *B. variegate*

Microorganism	*n*-Hexane	Chloroform	Ethyl acetate	Methanol
Staphylococcus aureus	0	14	10	12
Klebsiella pneumonia	0	12	0	12
Bacillus subtilis	0	10	0	10

Key: 0 = Not active; Well size: 6 mm
S. aureus, K. pneumonia, B. subtilis

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