Journal of Ecobiotechnology 2011, 3(9): 04-07
ISSN: 2077-0464
Available Online: http://journal-ecobiotechnology.com/

Phytochemical screening and antimicrobial activity of *Bauhinia variegata* Linn.

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Abstract

The antimicrobial effect of *Bauhinia variegata* Linn. (Caesalpiniaceae) leaf and bark extract was evaluated on microbial strains like Gram positive species *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative species *Escherichia coli* and *Pseudomonas aeruginosa.* The solvent used for extraction of plant were Petroleum ether, Chloroform, Alcohol. The alcoholic extract of leaves of *Bauhinia variegata* shows maximum antimicrobial activity. The *in vitro* antimicrobial valuation was carried out by disc diffusion method. The significant antibacterial activity of active extract was compared with standard antibiotic Amphicillin. The samples of leaves and bark were further used for the phytochemical studies. Results of the phytochemical analysis indicated the presence of alkaloids, oil, fat glycoside, carbohydrates, Phenolics, Tannins, lignin, saponins, flavonoids and Terpinoids. The antibacterial activities of the leaves and bark were due to the presence of various secondary metabolites. The phytochemical screening and antimicrobial activity of *B. variegata* leaves and bark was determined by using the standard method.

Keywords: Antimicrobial, Physico-chemical, Phytochemical, *Bauhinia variegata*

INTRODUCTION

The relatively large *Bauhinia* genus (family: Caesalpiniaceae) consisting of trees, climbers and shrubs is distributed in a wide range of geographic locations. Certain *Bauhinia* species have a long history of traditional medicinal applications (Valdir C.F. 2009).

The plant *Bauhinia variegata* Linn. (Caesalpiniaceae) commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It is widely used in folklore medicine. Its bark, root, leaves, seeds and flowers are used for their medicinal properties. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelmintic (The Wealth of India, 1959).

There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community (Sandhy *et al*., 2006; Ayyanar and Ignacimuthu, 2005; Rajan *et al*., 2002; Natarajan *et al*., 1999 and Ignacimuthu *et al*., 1998). The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin *et al*., 1999). Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research (Samy *et al*., 1998; Hamil *et al*., 2003; Motsei *et al*., 2003). Silver and Bostian (1993) have documented the use of natural products as new antibacterial drugs. There is an urgent need to identify novel substances active towards highly resistant pathogens (Recio, 1989; Cragg *et al*., 1997). In an effort to discover new compounds, many research groups screen plant extracts to detect secondary metabolites with the relevant biological activities. In this regard, several simple bioassays have been developed for screening purposes (Hostettmann, 1991). The present study was carried out on the phytochemical and antibacterial activity of leaf and bark of *B. variegata*.

Received: May 11, 2011; Revised July 21, 2011; Accepted July 21, 2011.

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MATERIAL AND METHODS

Sample collection and Authentication: The fresh, mature healthy leaves and bark of *Bauhinia variegata* Linn. (Caesalpiniaceae) were collected from west land of Dhule away from pollution. The plant materials were identified using the Flora of Dhule and Nadurbar District (Patil D.A., 2003) at Post-graduate Department of Botany, SSVP Sansthas, L.K.Dr.P.R. Ghogrey Science College, Deopur, Dhule-424005 (M.S.) India.

Sample preparation: Fully grown leaves and bark of *B. variegata* were weighed (1kg). The plant samples were shade dried ground and sieved with 2mm copper sieve to form uniform powder and stored in airtight bottles.

Preparation of extract: The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; petroleum ether, chloroform, and alcohol (Vogel, 1988). The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator for prior to use (Beyer and Walter, 1997). Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (Harborne, 1984; Trease and Evans, 1987; Ajaiyeoba, 2000; Edeoga et al., 2005). The positive tests were noted as present (+) and absent (-).

Preparation of microorganism: Isolation of bacterial species of Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and

Gram negative (*Echerichia coli* and *Pseudomonas aeruginosa*) takes place. The cultures of these bacteria were checked for purity by doing gram staining and biochemical test and they were grown in nutrient broth at 37˚C and maintained in nutrient agar slants at 2-8˚C. Nutrient agar medium was used as bacterial culture medium in the antibacterial assays.

Selection of Reference antibiotic: Reference antibiotic Amphicillin was obtained from authorized medical shop Dhule (M.S.). The purity of the antibiotic is 99.8%

Dilutions and Inoculum preparations: The dried plant extracts of *B. variegata* and antibiotic Amphicillin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 10, 20mg/ml. The inoculums of *Staphylococcu aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were prepared in nutrient broth medium and kept incubation at 37˚C for 8 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8˚C for analysis.

Procedure for performing the Disc Diffusion test (Bayer *et al*., 1966): The required amount of Petri plates is prepared and autoclaved at 121˚C for 15 minutes. They were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 10, 20mg/ml of plant extract of *B. variegata* and antibiotic Amphicillin into each separate disc of about 40 µg/ml. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to permit good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

RESULTS AND DISCUSSION

Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant an ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly essential prerequisite (Ramana, 2007).

Petroleum ether, chloroform and alcohol extracts of *B. variegata* leaves and bark were tested against various Gram-negative and Gram-positive bacteria (Table.1). Among the extracts assayed, the alcohol leaf extracts of *B. variegata* exhibited good activity against *S. aureus* at 20mg/ml for example, 15 mm was recorded as diameter zone of inhibition. This was followed by 14 mm *P. aeruginosa*, 10 mm *E. coli* and *B.subtilis* 9 mm respectively. The least activity of bark is 2 mm against *E. coli* and *S. aureus*, whereas 4 mm *S. aureus* at 10mg/ml was recorded by petroleum ether extracts.

The bark extracts (Table.2)of *B. variegata* exhibited good activity against *S. aureus* at 20mg/ml for example, 18 mm was recorded as diameter zone of inhibition. This was followed by 16 mm *P. aeruginosa*, 12 mm *E. coli* and *B.subtilis* 10 mm respectively. The least activity of bark is 3 mm against *E. coli* and *P. aeruginosa*, whereas 3 mm *B. subtilis* at 10mg/ml was recorded by petroleum ether extracts. Activities of the various extracts were comparable to those of standard antibacterial agent Ampicillin.

Amongst the plant species investigated, methanol extract of *Bauhinia variegata* bark showed the most remarkable activity. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. Here, alcohol extracts of *Bauhinia variegata* L. showed remarkable activity against some medically important bacterial strains. In addition such results justify the traditional use of *Bauhinia variegata* L. Further phytochemical studies for identification and elucidation of active constituent in plant material tested in expected to serve as lead in the development of novel bioactive antimicrobial compound.

Table1: Antibacterial efficacy of different solvent extracts of *Bauhinia variegata* leaf

Sr. No.	Microorganism	Strain +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 µg/ml)
1.	*Escherichia coli*	-ve	10	02	04	06	16
			20	05	07	10	
2.	*Pseudomonas aeruginosa*	-ve	10	02	05	05	20
			20	06	07	14	
3.	*Staphylococcus aureus*	+ve	10	04	04	07	25
			20	08	10	15	
4.	*Bacillus subtilis*	+ve	10	03	03	05	15
			20	05	08	09	

Table2: Antibacterial efficacy of different solvent extracts of *Bauhinia variegata* Bark

Sr. No.	Microorganism	Strain +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 µg/ml)
1.	*Escherichia coli*	-ve	10	03	04	07	19
			20	06	08	12	
2.	*Pseudomonas aeruginosa*	-ve	10	05	05	09	22
			20	08	09	16	
3.	*Staphylococcus aureus*	+ve	10	04	05	08	27
			20	09	11	18	
4.	*Bacillus subtilis*	+ve	10	03	04	05	18
			20	06	09	10	

Table 3: Phytochemical Analysis of *Bauhinia variegata*

Phytochemical constituents	Petroleum ether		Chloroform		Alcohol	
	Leaves	Bark	Leaves	Bark	Leaves	Bark
Alkaloids	-	-	-	+	+	+
Oil and Fats	+	+	+	+	+	+
Glycoside	-	-	-	-	+	+
Carbohydrates	-	-	-	-	+	+
Simple phenolics	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Lignin	-	+	-	-	+	+
Saponins	-	+	-	+	+	+
Flavonoids	-	-	-	-	-	+
Terpenoid	-	-	-	-	-	+

Table4: Extractive values of different solvent extracts of *Bauhinia variegata*

Solvent	Leaves	Bark
Methanol	11.50	13.2
Alcohol	17.60	19.00
Benzene	5.84	5.70
Petroleum ether	0.45	0.52
Chloroform	1.10	1.25

Phytochemical evaluation: The results of qualitative screening of phytochemical components in leaves and bark of *B. variegata* revealed the presence of alkaloids, oil, fat glycoside, carbohydrates, Phenolics, Tannins, lignin, saponins, flavonoids and Terpinoids presented in Table 3.

Extractive Values: Extractive values of leaves and bark for different solvents are presented in table 4.

ACKNOWLEDGEMENT

The authors wish to thank Head, Department of Botany, SSVP Sansthas, L.K.Dr.P.R. Ghogrey Science College, Dhule for providing the necessary laboratory facilities.

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