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Full Length Research Article

Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark

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ABSTRACT

Bauhinia variegata L. bark powder was defatted with petroleum ether. The non-defatted plant material as well as the defatted plant material was then individually extracted in different solvents with increasing polarity viz. 1,4-dioxan, acetone, methanol, dimethylformamide (DMF) and distilled water respectively. The extractive value of B. variegata L. for non-defatted extracts ranged from (0.7-13%) and for defatted extracts the extractive value ranged from (1-10.5%). The antibacterial activity of all the extracts (non-defatted and defatted) of Bauhinia variegata L. bark was determined by agar well diffusion method at three different concentrations i.e., 10 mg/ml, 5 mg/ml and 2.5 mg/ml. The antibacterial activity of defatted extracts of Bauhinia variegata L. was more than those without defatting. Maximum activity was observed at highest concentration i.e. 10mg/ml. Defatted acetone and methanol extracts of Bauhinia variegata L. were most active as compared to other extracts against all the studied organisms. Petroleum ether extracts of Bauhinia variegata L. was inactive against all microorganism ***(Afr. J. Biomed. Res. 9: 53 – 56, 2006)***

Keywords: *Antibacterial activity, Bauhinia variegata L. non-deffated extracts, defatted extracts.*

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INTRODUCTION

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001) i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999).

The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. In particular, the search for components with antimicrobial activity has gained increasing importance in recent times, due to growing world wide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Davies, 1994). Hence, there is a constant need for new and effective therapeutic agents. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants are active against microorganism are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction (Vilegs *et al*., 1997).

The research on the medicinal plants should be extended with the identification of the active principles in the plants. Scientific examination of the remedies could lead to standardization and quality control of the products to ensure their safety. It is after such evaluations that they can be approved for use in the primary health care. Such research activities could also lead to the development of new drugs as in the past.

Bauhinia variegata L. (Caesalpiniaceae) having Kachnar as the local Indian name, was evaluated for the preliminary antibacterial activity and phytochemical analysis. The different extracts of *Bauhinia variegata* L. were screened for potential antibacterial activity against some medically important bacterial strains.

MATERIALS AND METHODS

Plant material: *Bauhinia variegata L. (Caesalpiniaceae) were collected from Shrikant Pharmacy, College Road, Keshod. The material was collected in the form of dried parts, which were then powdered and preserved in airtight bottles for further studies.*

Microorganisms : *The test organisms included the gram-positive bacteria Bacillus cereus (ATCC 11778), Staphylococcus aureus (ATCC 25923) and gram-negative bacteria Klebsiella pneumoniae (NCIM 2719), Escherichia coli (ATCC 25922), Pseudomonas pseudoalcaligenes (ATCC 17440). All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.*

Extraction: *Bauhinia variegata* L. bark powder (100 g) was defatted with petroleum ether (1000 ml). Residue was collected, air dried and separated in five batches, each of 20 g. Each batch of the defatted plant material was individually extracted in 200 ml of different solvents with increasing polarity viz. dioxan, acetone, methanol, dimethylformamide (DMF) and distilled water respectively. The dried plant material was also extracted in all the solvents without defatting with petroleum ether. All the extracts were kept overnight on rotary shaker, filtered and centrifuged at 5000 rpm for 5 min. Supernatant was dried under reduced pressure to yield a solid residue. Each extract was preserved in vials and kept at 4 °C before use. The yields of all the extracts corresponding to the initial dry plant material are shown in the Table 1.

Preliminary Phytochemical analysis: Qualitative phytochemical analysis of *Bauhinia variegata* L. bark powder was tested as follows: Tannins (200 mg plant material in 10ml distilled water, filtered). A 2ml filtrate + 2ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10ml methanol, filtered). A 2ml filtrate + 1% HCl + steam, 1ml filtrate + 6 drops of Wagner's reagent. Browinsh-red precipitate indicated the presence of alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water. Frothing persistence meant Saponin present). Cardiac Glycosides (Keller-kiliani test: 2 ml filterate + 1 ml glacial acetic acid+ $FeCl_3$ + conc. H_2SO_4). Green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-

Burchard reaction: (200 mg plant material in 10ml chloroform, filtered). A 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered). A 2 ml filtrate + conc. HCl + magnesium ribbon. Pink-tomato red color indicated the presence of flavonoids (Harbone, 1973).

Microbial assay: Antibacterial activity of all the extracts of *Bauhinia variegata* L. bark was determined by agar well diffusion method (Nair and Chanda, 2005) at three different concentrations i.e., 10 mg/ml, 5 mg/ml and 2.5 mg/ml. using Mueller Hinton agar No.2 (Hi Media). The defatted extracts as well as those without defatting were diluted in dimethylsulphoxide (DMSO). Pure DMSO was taken as the control. The experiment was performed three times. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented in Fig 1-2.

RESULTS AND DISCUSSION

Bauhinia variegata L. bark contained tannins, alkaloids and saponins. The extractive value of *B. variegata* for non-defatted extracts ranged from (0.7-13%) and for defatted extracts the extractive value ranged from (1-10.5%). The details are shown in table 1.

Table 1
Extractive values and % Yield of the extracts of *Bauhinia variegata L.* in different solvents with increasing polarity

Extracts	Extractive values (g)	% Yield (w/w)
B1	0.104	0.52
B2	1.044	5.22
B3	1.755	0.77
B4	2.546	12.73
B5	2.536	12.68
B6	1.541	7.7
B7	1.794	8.97
B8	2.052	10.26
B9	0.376	1.88
B10	0.889	4.44
B11	1.455	7.27

B1: petroleum ether B7: (defatted) 1,4-dioxan
B2: 1,4-dioxan B8: (defatted) acetone B3: acetone B9: (defatted) methanol B4: methanol B10: (defatted) DMF
B5: DMF B11: (defatted) distilled water B6: distilled water

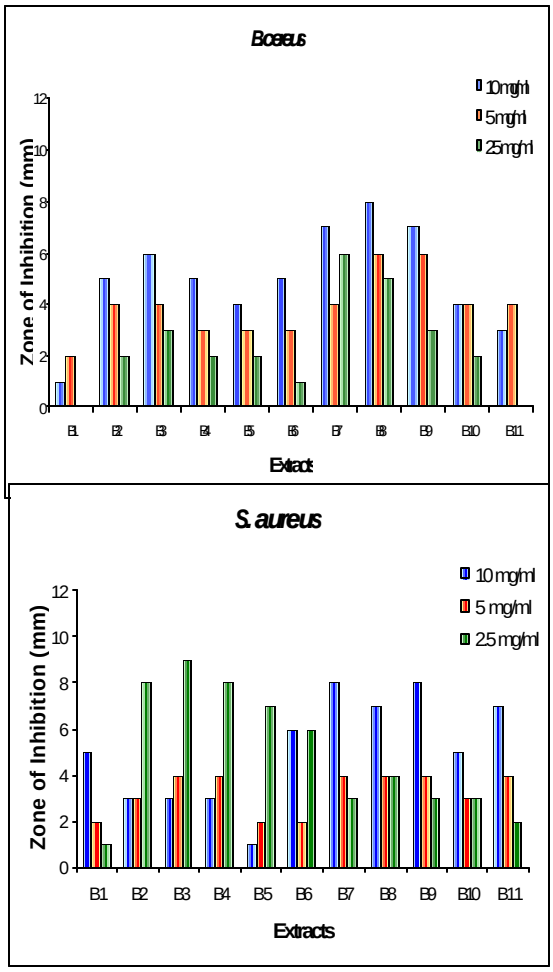

Fig.1: *Antibacterial activity of different extracts of Bauhinia variegata L. at three concentrations viz.10 mg/ml, 5 mg/ml and 2.5 mg/ml against B. cereus and S. aureus B1: petroleum ether, B2: 1,4-dioxan, B3: acetone, B4: methanol, B5: DMF, B6: distilled water. B7-B11 are defatted extracts, B7: 1,4-dioxan, B8: acetone, B9: methanol, B10: DMF, B11: distilled water*

The extracts of *Bauhinia variegata* L. (B1-B11) had a concentration dependent antibacterial activity with more sensitivity for gram negative bacteria than gram positive bacteria used in the study (Figs. 1 and 2). Medicinal plants can be poisonous if wrong plant parts or wrong concentrations are used (Forhne, 1999). Herbal medicines are assumed to be harmless. Although, herbal extracts need to be assured for its quality control and efficacy for a particular dose.

All the extracts of *Bauhinia variegata* L. showed considerable antibacterial activity at all the three concentrations (10 mg/ml, 5 mg/ml and 2.5 mg/ml) with certain variations and minimum activity was exhibited by petroleum ether extract (B1) at all the three concentrations against the investigated microorganisms in comparison to

the other extracts (Figs. 5 and 6). Further, petroleum ether extract (B1) and deffated water extract (B11) did not exhibit any activity against *B. cereus* at the lowest concentration viz. 2.5 mg/ml (Fig. 1).

The extracts B1-B11 were most susceptible to *P. pseudoalcaligenes* followed by *E. coli* (Fig. 2) while *B. cereus* was resistant in comparison with other bacterial strains exhibiting comparatively less antibacterial activity (Fig. 1).

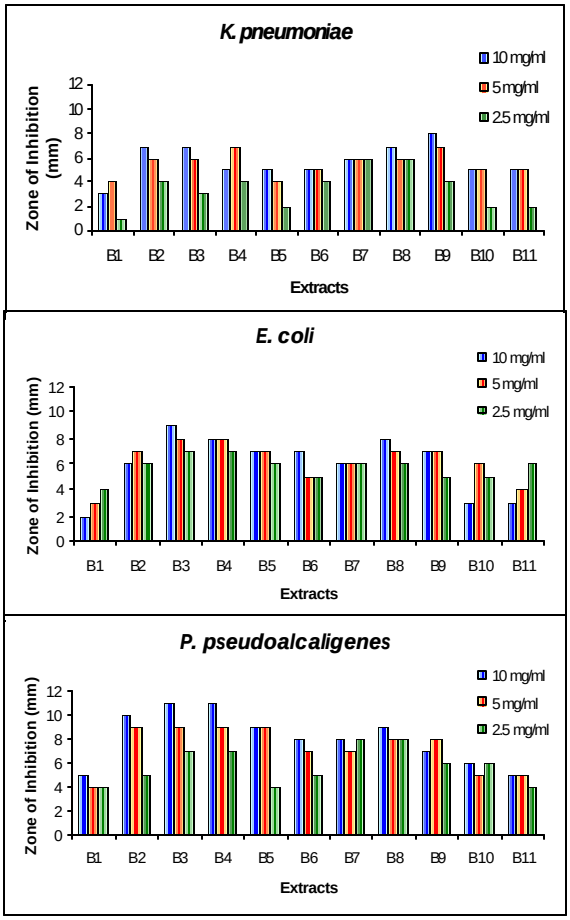

Fig. 2: Antibacterial activity of different extracts of Bauhinia variegata L. at three concentrations viz.10 mg/ml, 5 mg/ml and 2.5 mg/ml against K .pneumoniae, E. coli and P. pseudoalcaligenes B1: petroleum ether, B2: 1,4-dioxan, B3: acetone, B4:methanol, B5: DMF, B6: distilled water. B7-B11 are defatted extracts, B7: 1,4-dioxan, B8: acetone, B9: methanol, B10: DMF, B11: distilled water

Amongst all the extracts of *Bauhinia variegata* L. (B1-B11), the acetone and methanol extracts exhibited maximum antibacterial activity against the studied gram positive and gram negative bacteria. However, the trend with which these extracts inhibited gram positive bacteria was different from that of gram negative bacteria with certain variation with regard to three different concentrations (10 mg/ml, 5 mg/ml and 2.5 mg/ml). The deffated extracts were more active than the extracts without deffating against gram positive bacterial strains while against gram negative bacterial strains, the deffated extracts exhibited either similar or less activity than the extracts without deffating (Figs. 1 and 2).

Defatted extracts (B7-B11) were more active than the extracts without defatting (B2-B6). Petroleum ether extract exhibited negligible activity against *B. cereus.* Maximum activity was observed at highest concentration (10 mg/ml) followed by the intermediate concentration (5 mg/ml) and then the lowest concentration (2.5 mg/ml).Acetone extract was most active amongst all the extracts against B. *cereus* (Fig. 1). This differential activity might be due to the presence of some oils, wax, resins or fatty acids in the plant material which may block the plant extract from entering the bacterial cell wall.

The extracts without deffating (B2-B6) exhibited maximum activity against *S. aureus* at the lowest concentration (2.5 mg/ml) while at the same concentration defatted extracts showed minimum antibacterial activity.Moreover, deffated extracts exhibited maximum activity at the highest concentration (10 mg/ml) against *S. aureus* (Fig. 1). All the extracts (B1-B11) followed similar trend of antibacterial activity against tested gram negative bacterial strains viz. *K.pneumoniae, E. coli* and P. *pseudoalcaligenes*. Maximum activity was observed at the concentration 10 mg/ml followed by concentrations 5 mg/ml and 2.5 mg/ml. However, for *E. coli,* petroleum ether extract (B1) and deffated water extract (B11) exhibited maximum activity at the lowest concentration (2.5 mg/ml) followed by intermediate concentration (5 mg/ml) and the highest concentration (10 mg/ml) (Fig. 2).

The overall results of the antibacterial activity of various extracts of *Bauhinia variegata* L. at three concentrations studied reveal that deffated extracts showed better activity than those without deffating. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. Here, acetone and methanol 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 it also supports some of the phytochemical and pharmacological investigation of this plant carried by many researchers. The results suggest that traditional folk medicine could be used as a guide in our continuing search for new natural products with potential medicinal properties.

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