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ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF THE PLANT *BAUHINIA VARIEGATA*

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ABSTRACT

Antibacterial activity of petroleum ether, chloroform, methanol and aqueous extracts of leaves of *Bauhinia variegata* was determined against gram positve and gram negative bacteria. Penicillin and gentamycin were used as reference standards for comparison of activity. The activities were found to be concentration dependent for all different samples tested.

It was found that aqueous extract has antibacterial activity against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* while methanol extract has this activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with minimum inhibitory concentration as compared with standard (Penicillin 10 μg/mL and gentamycin 80 μg/mL). The petroleum ether extract and chloroform extracts were active against *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration for aqueous extract was 10 μg/mL, 10 μg/mL, 50 μg/mL and 50 μg/mL, respectively against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis,* and *Pseudomonas aeruginosa)*, for methanol extract was 500 μg/mL (*Staphylococcus aureus)* and 50 μg/mL (*Pseudomonas aeruginosa)*, for petroleum ether extract was 100 μg/mL (*Staphylococcus aureus)* and 250 μg/mL (*Escherichia coli)*, for chloroform extract was 250 μg/mL (*Escherichia coli)* and 500 μg/mL (*Staphylococcus aureus).*

Key word: *Bauhinia variegata Linn,* Antibacterial activity, Kanchanar

INTRODUCTION

Bauhinia variegata L. is also known as Kanchanar in Hindi and belongs to a family leguminosae. A medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India[1]. Leaves are 10-15 cms long, as broad as or rather

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broader than long, cleft ¼ to 1/3 of the way down into 2 obtuse lobes, pubescent beneath when young, the pubescence nerves persisting along[2]. The chemical constituents found are β-sitosterol, lupeol, kaempferol-3-glucoside and tannins[3]. Leaves of *Bauhinia variegata* extract contains carbohydrates, amides, reducing sugars, vitamin C[3], crude protein, fibers[4], calcium and phosphorus[5]. The crude protein content of leaves, collected from Izatnagar and Palampur, was reported to be 15.80 and 13.18%, respectively on dry matter basis[6]. Quercetin, rutin, quercitrin, apigenin and apigenin-7-O-glucoside were isolated from the leaves of *Bauhinia variegata*[7]. Two new long chain compounds heptatriacontan-12,13-diol and dotetracont-15-en-9-ol were isolated from the leaves of *Bauhinia variegata*[8]. The leaves of the plant are widely used for stomatitis[9] and also inhibit the activity of peptic enzymes[10]. The present study is therefore undertaken to study the antibacterial activity of various extracts of *Bauhinia variegata L.* leaves. Petroleum ether, chloroform, methanol and aqueous extracts were evaluated against *Staphylococcus aureus, Bacillus subtilis* (gram positive) and *Pseudomonas aeruginosa, Escherichia coli* (gram positive). Gentamycin and penicillin were used as the standard.

EXPERIMENTAL

Plant material

Bauhinia variegata L. leaves were obtained from Regional Research Institute, Bangalore and authenticated by Dr. Shiddamallyya N. (authentication reference No. RRI/BNG/SMP/Drug Authentication/2007-08/15 dated 09/05/2007). Voucher specimen was deposited at the Regional Research Institute, Bangalore (RRCBB account No.1630).

Preparation of extract

The powdered plant material was successively extracted by using Soxhlet extractor. Solvent was recovered, extract concentrated and percentage yield was calculated. The solvents were recovered by using simple distillation method. The charged drug from the central compartment was removed, dried, recharged and extracted by using next solvent of higher polarity than the first solvent. By using Soxhlet extractor, exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity are petroleum ether, chloroform, methanol and finally water. The percentage yield of each extracts was 4.2%, 0.71%, 13.72%, 17.64% w/w on dried plant material basis, respectively. Standard methods[11,12] were used for preliminary phytochemical screening of the extract to know the nature of phytoconstituents present in them. From the phytochemical tests, it was

found that the extracts contain steroids, tannins, alkaloids, cardiac glycosides, flavonoids and sugars.

Preparation of sample

Different semi-solid fractions of extract were dissolved in corresponding solvent and different concentrations were expressed in terms of μg/mL of the solvent.

Drugs used

Penicillin (10 μg/mL) and gentamycin (80 μg/mL) were used as reference standard for antibacterial activity.

Microorganisms used

Four different organisms were used for the determination of Minimal Inhibitory Concentration (MIC) of the extracts. Four organism included two gram positive *(Staphylococcus aureus, Bacillus subtilis)* and two gram negative *(Pseudomonas aeruginosa Escherichia coli*. The organisms were procured from Department of Microbiology, The Oxford College of Science, Bangalore. All organisms were maintained at 4^0C on slant of nutrient agar and in nutrient broth.

Preparation of inoculum

Stock cultures were maintained at 4°C on slant of nutrient agar. For the experiments, inoculum was prepared by subculturing each culture from slants to flask in sterile nutrient broth and incubated at 37^0C for 24 hrs.

Preparation of media

Nutrient agar medium (NA) was used for preliminary antibacterial study. The medium was prepared by dissolving different ingredients of nutrient agar medium in distilled water and sterilized by autoclaving at 121^0C for 15 minutes.

Antibacterial activity

Determination of Minimal Inhibitory Concentration (MIC)

The disc diffusion method[13] was used for the determination of minimum inhibitory concentration. *In vitro* antibacterial activity was screened by using nutrient agar (NA). The NA plates were prepared by pouring molten media into sterile petriplates. The plates were

allowed to solidify and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts of different concentrations were loaded on 3 mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^0C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter and the lowest concentration of each extract, which is showing inhibition of growth of bacteria, was determined. These studies were performed by using standard drugs (10 μg/disc penicillin and 80 μg/disc gentamycin).

RESULTS AND DISCUSSION

The results indicate that the all extracts possess the antibacterial activity. The MIC of the aqueous extract showed the effectiveness of the extract at low concentration against against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* while methanol extract has this activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed minimum inhibitory concentration as compared with standard (Penicillin 10 μg/mL and gentamycin 80 μg/mL). The petroleum ether extract and chloroform extract were active against *Staphylococcus aureus* and *Escherichia coli*. Various extracts were compared for their antibacterial potential with the standard.

The objectives of the present paper were to study antibacterial activity of various extracts of the leaves of *Bauhinia variegata Linn*. The aqueous extract showed MIC against all the organisms at concentration (10 μg/mL, 50 μg/mL) as shown in Table 1. Methanol extract was effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MIC being 50 μg/mL and 500 μg/mL (Table 1).

Table 1: MIC (μg/mL) values of different extracts of *Bauhinia variegata*

Extracts	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
Petroleum ether	100	250	-	-
Chloroform	500	250	-	-
Methanol	500	-	-	50
Water	10	10	50	50

The petroleum ether extract (MIC being 100 μg/mL and 250 μg/mL, respectively) and chloroform extract (MIC being 500 μg/mL and 250 μg/mL, respectively) were active against *Staphylococcus aureus* and *Escherichia coli* and this activity may be due to presence of glycoside, alkaloid and tannins. From the zone of inhibition and MIC calculated for the extracts, it was observed that aqueous extract showed prominent antibacterial activity against all the four microorganisms.

Table 2: Zone of inhibition values (mm) in MIC of different extracts of *Bauhinia vaieigata*

Extracts	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
Petroleum ether	6	7	-	-
Chloroform	8	7	-	-
Methanol	9	-	-	4
Water	9	7	5	6
Penicillin (10 μg/mA)	6	6	6	6
Gentamycin (80 μg/mL)	16	16	9	15

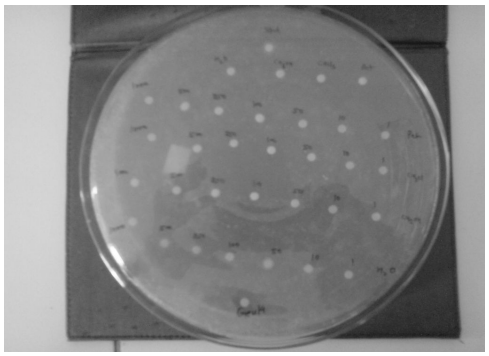

Fig. 1: *E. Coli*

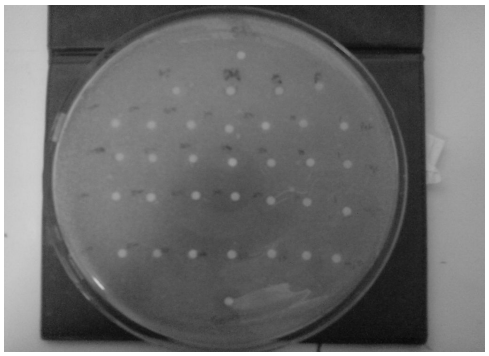

Fig. 2: *Bacillus subtilis*

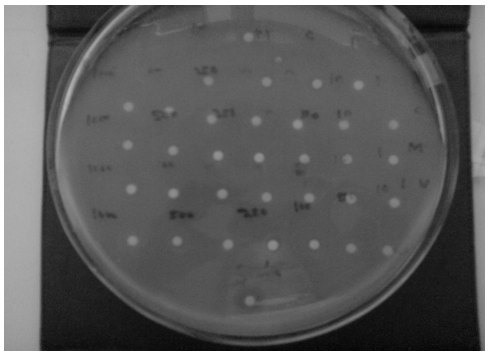

Fig. 3: ***P. aeruginosa***

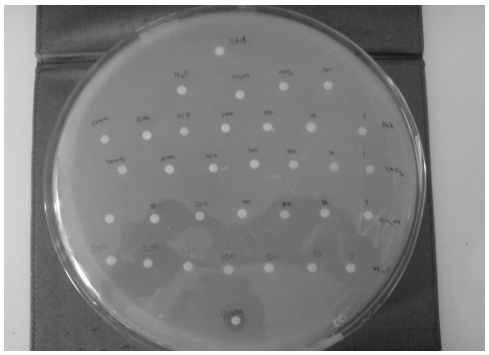

Fig. 4: *Staphylococcus aureus*

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