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Screening of *Bauhinia* Species Crude Extract against Clinically Infectious Bacteria

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ABSTRACT

This study was carried out to investigate the anti-bacterial activity of the crude extract of the leaves of four *Bauhinia* species, namely *B. purpurea*, *B. galpinii*, *B. roxburghii* and *B. vahlii* using the agar-well diffusion method. The methanolic extract of the leaves was tested against Gram-positive strains like *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative strains like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The activity was more pronounced against Gram-positive bacteria than against Gram-negative ones. *B. purpurea* showed good inhibition zones against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *K. pneumoniae* in decreasing order. Whereas *B. galpini* showed less inhibition zones, even less range of inhibitory zones were found in the case of *B. roxburghii* and *B. vahlii*. The inhibitory effect of the extracts was compared with standard antibiotic Ciprofloxacin. Our results show that *Bauhinia* spp. can be a promising source of natural products with potential antibacterial activity.

Keywords: *Bauhinia* species, anti-bacterial, methanol extract, pathogenic strains

INTRODUCTION

Plants, being a major source of natural therapeutic remedies, have been used in various parts of the world to treat various infectious diseases (Vahidi *et al*. 2002). To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified manner (Roja and Rao 2000; Awadh Ali *et al.* 2001; Nitta *et al.* 2002). The emergence of resistance among key microbial pathogens, including *S. aureus*, to conventional antimicrobials is a serious problem that scientists face all around the world (Tanaka *et al.* 2006). Large-scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary step in the isolation and characterization of the active principle further leading to drug development (Farhana *et al.* 2009). Multidrug resistance is a world-wide problem mainly attributed to the extensive use of antibiotics, selection pressure on bacterial strains, and lack of new drugs, vaccines, and diagnostic aids. These shortcomings lead to an urgent global call for new antimicrobial drugs, particularly from natural resources (Habeeb *et al.* 2007).

The aim of the present study was to determine the effect of methanolic extract of leaves of *Bauhinia* species on some pathogenic strains. *Bauhinia* species are widely distributed in the tropics and are important for animal nutrition because of their high protein content. Plants of the genus *Bauhinia*, commonly known as cow's-paw or cow's hoof, are widely distributed in most tropical countries and have been used frequently in folk medicine to treat different kinds of pathological disorder particularly diabetes, infections, as well as pain and inflammation (Filho 2009).

B. purpurea Linn. is a medium sized deciduous tree, grown and cultivated as an avenue tree in India. Traditionally this plant has been used in the treatment of dropsy, pain, rheumatism, convulsions, delirium, septicemia, etc. (Asolker *et al.* 2000). The bark of the plant is used as an astringent in the treatment of diarrhoea (Muralikrishna *et al.* 2008). Its decoctions are recommended as a useful wash for ulcers (Kirthikar and Basu 2001). *B. galpinii* is a straggling and prostrate shrub, is a native of south and tropical Africa. This is grown in the garden as an ornamental plant for its bright scarlet flowers. *B. roxburghii* Cor. is mainly found in Malabar and Travancore and near the coast. It is a very long climber with curious stems alternately twisted one way and the other between the straight margins. *B. vahlii* Wight & Arnott. is a leguminous multipurpose liana, which occurs in the tropics and sub tropics. The leaves provide an excellent source of fodder in the Central Sub-Himalayan region and are also used as a material for making a variety of wrappers (Upreti and Dhar 1996). It is an indigenous, multipurpose species in Kumaun Himalaya, most suitable for plantation programmes in mined, industrial waste and marginal lands as it is useful for increasing soil fertility (Dhar and Upreti 1999). The leaves are used as plates and for many other purposes; the seeds are roasted and eaten. The bark yields fibre which is used for making ropes.

MATERIALS AND METHODS

Plant materials

The leaves of four *Bauhinia* plant species viz. *B. purpurea*, *B. galpinii*, *B. roxburghii* and *B. vahlii* were collected from Lalbagh in Bangalore during January 2006 and identified at the Dr. S. G. Reddy college by botanist Dr. S. Sundara Rajan and Voucher specimens SRBP40, SRBG41, SRBR42 and SRBV43 were deposited at Dr. S. G. Reddy College, Bangalore.

Extraction

Freshly collected leaves of four *Bauhinia* plant species were shade-dried and then powdered using a mechanical grinder. 100 g

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of each pulverized material was extracted separately with 500 ml of methanol (LR grade, Merck, India) using Soxhlet apparatus. At the end of extraction, extracts were filtered under vacuum through a Whatman No. 1 filter paper and the process was repeated until all soluble compounds had been extracted. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). A portion of the residue was used for the antibacterial assay.

Bacterial culture

The bacterial strains used in this study were clinical isolated from different infection status of patients presenting symptoms of *S. aureus*, *B. subtilis*, *P. aeruginosa* and *K. pneumoniae*-associated diseases. The isolates were identified by a standard method (Cowan and Steel 1993). The standard strains used were *S. aureus* (ATCC-29737), *B. substilis* (ATCC-6059), *P. aeruginosa* (ATCC-20852) and *K. pneumoniae* (MTCC-618). The organisms were maintained on nutrient agar slope at 4°C and sub-cultured into nutrient broth by a picking-off technique (Aneja 2003) for 24 hrs before use.

Antibacterial assay

The agar-well diffusion method was used to test antibacterial activity of the extract against taken bacterial strains (Nair *et al.* 2005). Nutrient agar (Hi Media, India) was used as the bacteriological medium. Solutions of known concentration of the test samples were made by dissolving measured amount of the samples in 10% aqueous dimethylsulfoxide (DMSO). Pure DMSO was taken as the negative control and Ciprofloxacin (50 mg/ml) as the positive control. 100 µl of inoculum was aseptically introduced to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculum. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter. 100 µl (containing 25, 50, 100 and 200 µg) of test samples and 100 µl control compound were introduced in the well. The same procedure was used for all the strains. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The diameter of the zone of inhibition produced by each agent was measured with a ruler, expressed in millimeter (Bauer *et al*. 1966) and compared with those produced by the commercial antibiotic Ciprofloxacin.

Statistical analysis

The values were expressed as mean ± S.E.M. The data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple pair-wise comparison tests to assess the statistical significance. $P < 0.01$ was considered as statistically significant, using software ezANOVA ver. 0.98.

RESULTS AND DISCUSSION

The four species of *Bauhinia* viz. *B. purpurea*, *B. galpini*, *B. roxburghii* and *B. vahlii* were investigated for their antimicrobial potential. In the course of our screening for the antimicrobial activity the leaf extracts of the plants were evaluated against ciprofloxacin as standard. Activity was determined against the four strains which included Gram-negative and Gram-positive bacteria (**Tables 1-4**). The plant extracts differ significantly in their activity. It was observed that the antimicrobial activity of the studied plant extracts was exhibited mainly against the Gram-positive bacteria, *S. aureus* and *B. subtilis* even at the least concentration than Gram-negative bacteria, *P. aeruginosa* and *K. pneumoniae*. The susceptibility of Gram-positive bacteria supports earlier reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria (Vlietinck *et al.* 1995; Rabe and Van Staden 1997). This could be attributed to the fact that the cell wall in Gram-positive bacteria has a single layer, whereas the Gram-negative cell wall is a multi-layered structure (Yao and Moellering 1995; Ozcelik 1998), acting as a barrier to many environmental substances, including antibiotics (Tortora *et al.* 2001). *Staphylococcus aureus* isolated from mucus sample was the most susceptible bacteria amongst all the bacterial strains investigated in the present work. The inhibitory activity against *S. aureus* could be unspecific and due to the presence of flavonoids which occurred in almost all species of *Bauhinia*, and has been observed by other authors (Filho 2009). Inhibitory activity against *Bacillus substilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* was almost always associated with activity against *Staphylococcus aureus*, which is to be expected; because *S. aureus* is more susceptible to most antibiotics (Rovira *et al.* 1999).

The most pronounced activity with greater inhibition zones was found in the methanolic extract of the plant *B. purpurea* which was followed by *B. galpini*, *B. roxburghii* and *B. vahlii* at different concentrations, which supports the earlier report of Kumar *et al*. (2005). The activities observed in the crude extract could be due to the presence of more than one bioactive compound. Hence, *B. purpurea* is considered to be the most effective plant which demonstrated the greatest antimicrobial effect against all tested microorganisms. Overall, it seems that similar but not identical activity patterns were observed in four different species of *Bauhinia*. The antimicrobial activity observed in four species coincides nicely with reports of the ethnomedicinal use of these species. For example, *Bauhinia* species are used in the treatment of diarrhea, and our study was found to be active against *S. aureus*. Ciprofloxacin, which was used as a positive experimental control against all bacterial strains assayed, produced a good zone of inhibition, while no inhibitory effect could be observed for DMSO used as negative control.

The present results offer a scientific basis for the therapeutic potency of *Bauhinia* plant species used in traditional medicine. Natural products are in great demand due to their extensive biological properties for providing source for the discovery of many types of effective bioactive compounds (Nalina and Rahim 2007). Structurally diverse secondary metabolites in *B. purpurea* were reported such as bauhinoxepin, bauhibenzofurin, bauhispirorin and bauhinol which prove that the *Bauhinia* species are a rich source of bioactive compounds (Surat *et al*. 2007). Although a number of chemical components described for the genus *Bauhinia* are also found in other species, the secondary metabolites produced by this genus, particularly the flavonoids make these plants an important source of potential phytotherapeutic and medicinal agents (Filho 2009).

In conclusion, the results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants estimated. The obtained results could form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. *B. purpurea*, *B. galpini*, *B. roxburghii* and *B. vahlii* could be a source for antibacterial drugs against Gram-positive bacteria, especially against *Staphylococcus aureus*. The results of the investigation do not reveal which chemical compound is responsible for the aforementioned activity. Further studies are needed to isolate the exact active component, which are responsible for the antimicrobial activity. In addition, *in vivo* studies are necessary to determine the toxicity of the active constituents, their side effects, circulating levels, pharmacokinetic properties and diffusion in different body sites. Scientific knowledge on the biological properties and active principles of these plants has progressed significantly in recent years. Further studies are required for designing a potentially active antibacterial synergized agent of plant origin.

Table 1 Antibacterial activity of *Bauhinia purpurea* leaf extract against clinically important bacterial strains.

Bacterial strains tested and source	*Bauhinia purpurea*				Ciprofloxacin
	25 µg	50 µg	100 µg	200 µg	
Sa ATCC 29737	20.00 ± 0.58*	22.00 ± 0.58	23.00 ± 0.58	23.80 ± 0.42	27.00 ± 0.58
Sa Pimples	20.33 ± 0.18*	22.40 ± 0.35	23.27 ± 0.64	23.67 ± 0.35	25.53 ± 0.29
Sa Pus	20.07 ± 0.48*	22.33 ± 0.67	23.07 ± 0.41	23.73 ± 0.24	24.93 ± 0.52
Sa Mucus	20.27 ± 0.18*	22.00 ± 0.81	23.27 ± 0.37	24.07 ± 0.18	26.00 ± 0.12
Sa Wound swab	20.40 ± 0.35*	22.27 ± 0.66	23.33 ± 0.24	23.53 ± 0.29	24.87 ± 0.47
Bs ATCC 6059	19.00 ± 0.58*	21.53 ± 0.29	22.27 ± 0.27	22.40 ± 0.23	22.53 ± 0.29
Bs Stool	18.33 ± 0.18*	22.13 ± 0.59	23.13 ± 0.24	23.33 ± 0.18	23.67 ± 0.24
Bs Stool	19.47 ± 0.29	21.60 ± 0.23*	22.53 ± 0.24	22.67 ± 0.33	24.00 ± 0.12
Bs Stool	18.53 ± 0.24*	21.80 ± 0.42	22.80 ± 0.12	22.87 ± 0.24	23.27 ± 0.37
Bs Stool	18.73 ± 0.37*	21.67 ± 0.18	22.87 ± 0.47	23.27 ± 0.24	23.60 ± 0.31
Pa ATCC 20852	16.00 ± 0.58	18.00 ± 0.58*	19.33 ± 0.33	20.87 ± 0.47	21.33 ± 0.18
Pa Sputum	16.67 ± 0.24*	18.80 ± 0.12	19.80 ± 0.42	21.33 ± 0.57	21.80 ± 0.12
Pa Stool	16.40 ± 0.35*	18.40 ± 0.69	19.53 ± 0.29	20.33 ± 0.33	20.67 ± 0.18
Pa Pus	15.80 ± 0.23*	18.40 ± 0.60	20.20 ± 0.31	21.47 ± 0.24	21.87 ± 0.47
Pa Ear swab	16.20 ± 0.12*	18.20 ± 0.20	19.60 ± 0.42	20.73 ± 0.18	21.73 ± 0.64
Kp MTCC 618	9.33 ± 0.24	12.13 ± 0.24*	15.47 ± 0.24	17.27 ± 0.18	18.53 ± 0.29
Kp Urine	9.47 ± 0.18	12.60 ± 0.12*	15.53 ± 0.18	17.80 ± 0.12	18.20 ± 0.12
Kp Urine	9.47 ± 0.24*	12.47 ± 0.24	15.07 ± 0.18	17.20 ± 0.12	18.00 ± 0.58
Kp Urine	9.00 ± 0.12	11.87 ± 0.18	15.40 ± 0.12*	17.40 ± 0.31	18.80 ± 0.12
Kp Urine	9.27 ± 0.18	12.47 ± 0.18*	15.40 ± 0.23	17.33 ± 0.24	18.67 ± 0.33

The values are the mean of three experiments ± S.E. *P<0.01
Abbreviations: Sa, *Staphylococcus aureus*; Bs, *Bacillus substilis*; Pa, *Pseudomonas aeruginosa*; Kp, *Klebsiella pneumoniae*.

Table 2 Antibacterial activity of *Bauhinia galpini* leaf extract against clinically important bacterial strains.

Bacterial strains tested and source	*Bauhinia galpini*				Ciprofloxacin
	25 µg	50 µg	100 µg	200 µg	
Sa ATCC 29737	11.00 ± 0.58	13.00 ± 0.58*	17.00 ± 0.58	19.00 ± 0.58	27.00 ± 0.58
Sa Pimples	10.93 ± 0.55	13.00 ± 0.12*	16.33 ± 0.31	17.33 ± 0.67	25.53 ± 0.29
Sa Pus	11.60 ± 0.12	12.33 ± 0.33*	16.33 ± 0.33	18.73 ± 0.37	24.93 ± 0.52
Sa Mucus	10.80 ± 0.23	12.80 ± 0.20	16.67 ± 0.33	17.00 ± 0.58*	26.00 ± 0.12
Sa Wound swab	11.20 ± 0.23	13.00 ± 0.53*	16.67 ± 0.67	17.67 ± 0.33	24.87 ± 0.47
Bs ATCC 6059	9.60 ± 0.31	12.73 ± 0.18*	16.33 ± 0.88	17.67 ± 0.88	22.53 ± 0.29
Bs Stool	10.40 ± 0.23	12.77 ± 0.38*	16.00 ± 1.00	18.67 ± 0.67	23.67 ± 0.24
Bs Stool	9.87 ± 0.24	12.20 ± 0.12	15.33 ± 0.33*	16.67 ± 0.67	24.00 ± 0.12
Bs Stool	9.60 ± 0.23	11.93 ± 0.07	15.67 ± 0.33*	19.33 ± 0.67	23.27 ± 0.37
Bs Stool	9.60 ± 0.35	12.27 ± 0.18	15.73 ± 0.47*	19.00 ± 0.58	23.60 ± 0.31
Pa ATCC 20852	8.93 ± 0.52	11.87 ± 0.59	13.67 ± 0.33	16.00 ± 0.00*	21.33 ± 0.18
Pa Sputum	9.00 ± 0.58	11.53 ± 0.24	13.33 ± 0.33*	15.00 ± 0.58	21.80 ± 0.12
Pa Stool	9.93 ± 0.18	11.73 ± 0.37	13.53 ± 0.29	14.00 ± 0.00*	20.67 ± 0.18
Pa Pus	9.13 ± 0.57	11.47 ± 0.24	14.00 ± 0.23*	15.33 ± 0.67	21.87 ± 0.47
Pa Ear swab	8.93 ± 0.55	12.00 ± 0.12*	13.33 ± 0.67	13.73 ± 0.29	21.73 ± 0.64
Kp MTCC 618	5.40 ± 0.23	7.60 ± 0.31	9.53 ± 0.29	11.27 ± 0.18*	18.53 ± 0.29
Kp Urine	5.27 ± 0.29	7.80 ± 0.12	9.53 ± 0.18	11.53 ± 0.18*	18.20 ± 0.12
Kp Urine	5.00 ± 0.12	7.47 ± 0.18	9.20 ± 0.12*	11.47 ± 0.24	18.00 ± 0.58
Kp Urine	5.60 ± 0.12	7.27 ± 0.18	9.73 ± 0.18	11.40 ± 0.23*	18.80 ± 0.12
Kp Urine	5.13 ± 0.24	7.73 ± 0.18	9.47 ± 0.18	11.33 ± 0.24*	18.67 ± 0.33

The values are the mean of three experiments ± S.E. *P<0.01
Abbreviations: Sa, *Staphylococcus aureus*; Bs, *Bacillus substilis*; Pa, *Pseudomonas aeruginosa*; Kp, *Klebsiella pneumoniae*.

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Table 3 Antibacterial activity of *Bauhinia roxburghii* leaf extract against clinically important bacterial strains.

Bacterial strains tested and source	*Bauhinia roxburghii*				Ciprofloxacin
	25 µg	50 µg	100 µg	200 µg	
Sa ATCC 29737	6.93 ± 0.18	10.33 ± 0.88	12.00 ± 0.58*	15.33 ± 0.67	27.00 ± 0.58
Sa Pimples	6.40 ± 0.23	9.67 ± 0.33	12.33 ± 0.33*	14.00 ± 1.15	25.53 ± 0.29
Sa Pus	7.53 ± 0.24	11.00 ± 1.00	11.67 ± 0.67	14.00 ± 0.00*	24.93 ± 0.52
Sa Mucus	7.00 ± 0.58	10.33 ± 0.33	11.67 ± 0.33	13.33 ± 0.67*	26.00 ± 0.12
Sa Wound swab	7.67 ± 0.44	10.00 ± 1.00	12.33 ± 0.67*	14.67 ± 0.67	24.87 ± 0.47
Bs ATCC 6059	5.93 ± 0.58	9.00 ± 0.58	11.07 ± 0.48	13.67 ± 0.33*	22.53 ± 0.29
Bs Stool	5.87 ± 0.13	8.67 ± 0.67	11.33 ± 0.33	13.00 ± 0.58*	23.67 ± 0.24
Bs Stool	5.47 ± 0.29	9.33 ± 0.67	11.67 ± 0.44	13.33 ± 0.67*	24.00 ± 0.12
Bs Stool	6.13 ± 0.44	9.33 ± 0.33	10.67 ± 0.67*	12.67 ± 0.67	23.27 ± 0.37
Bs Stool	6.20 ± 0.12	9.87 ± 0.13	11.00 ± 0.58	12.33 ± 0.33*	23.60 ± 0.31
Pa ATCC 20852	4.47 ± 0.24	7.33 ± 0.33	9.67 ± 0.33	10.67 ± 0.67*	21.33 ± 0.18
Pa Sputum	5.00 ± 0.12	7.67 ± 0.33	9.67 ± 0.67*	12.67 ± 0.67	21.80 ± 0.12
Pa Stool	4.40 ± 0.31	7.67 ± 0.67	10.67 ± 0.33*	12.67 ± 1.33	20.67 ± 0.18
Pa Pus	5.00 ± 0.31	8.00 ± 0.58	10.33 ± 0.33*	12.00 ± 1.15	21.87 ± 0.47
Pa Ear swab	4.33 ± 0.24	8.33 ± 0.33	10.00 ± 0.58*	12.33 ± 1.20	21.73 ± 0.64
Kp MTCC 618	4.00 ± 0.12	6.33 ± 0.24	8.00 ± 0.12	10.20 ± 0.12*	18.53 ± 0.29
Kp Urine	4.27 ± 0.18	6.33 ± 0.18	8.33 ± 0.24	10.60 ± 0.12*	18.20 ± 0.12
Kp Urine	4.53 ± 0.18	6.40 ± 0.12	8.47 ± 0.18*	10.47 ± 0.24	18.00 ± 0.58
Kp Urine	4.40 ± 0.12	6.47 ± 0.24	8.20 ± 0.23	10.27 ± 0.18*	18.80 ± 0.12
Kp Urine	4.47 ± 0.24	6.00 ± 0.12	8.60 ± 0.12	10.40 ± 0.12*	18.67 ± 0.33

The values are the mean of three experiments ± S.E. *P<0.01
Abbreviations: Sa, *Staphylococcus aureus*; Bs, *Bacillus substilis*; Pa, *Pseudomonas aeruginosa*; Kp, *Klebsiella pneumoniae*.

Table 4 Antibacterial activity of *Bauhinia vahlii* leaf extract against clinically important bacterial strains.

Bacterial strains tested and source	*Bauhinia vahlii*				Ciprofloxacin
	25 µg	50 µg	100 µg	200 µg	
Sa ATCC 29737	5.67 ± 0.24	7.67 ± 0.33	11.00 ± 0.58	11.67 ± 0.33*	27.00 ± 0.58
Sa Pimples	5.60 ± 0.35	7.33 ± 0.33	11.13 ± 0.57	11.53 ± 0.24*	25.53 ± 0.29
Sa Pus	5.80 ± 0.35	7.60 ± 0.31	11.53 ± 0.24	11.80 ± 0.12*	24.93 ± 0.52
Sa Mucus	5.33 ± 0.33	7.67 ± 0.18	11.40 ± 0.23	12.00 ± 0.00*	26.00 ± 0.12
Sa Wound swab	5.73 ± 0.27	7.33 ± 0.67	11.00 ± 0.00	11.33 ± 0.44*	24.87 ± 0.47
Bs ATCC 6059	4.80 ± 0.12	5.40 ± 0.31	7.60 ± 0.31	9.73 ± 0.37*	22.53 ± 0.29
Bs Stool	4.27 ± 0.18	5.93 ± 0.07	7.33 ± 0.33	9.60 ± 0.23*	23.67 ± 0.24
Bs Stool	5.00 ± 0.12	5.33 ± 0.18	7.67 ± 0.33	9.60 ± 0.35*	24.00 ± 0.12
Bs Stool	4.60 ± 0.31	5.67 ± 0.33	7.60 ± 0.12	10.33 ± 0.24*	23.27 ± 0.37
Bs Stool	4.67 ± 0.33	6.00 ± 0.12	7.80 ± 0.42	9.40 ± 0.23*	23.60 ± 0.31
Pa ATCC 20852	4.33 ± 0.18	4.33 ± 0.33	6.60 ± 0.12	9.13 ± 0.24*	21.33 ± 0.18
Pa Sputum	4.53 ± 0.18	4.60 ± 0.31	6.40 ± 0.31	8.93 ± 0.48*	21.80 ± 0.12
Pa Stool	4.40 ± 0.12	4.20 ± 0.12	6.40 ± 0.12	8.53 ± 0.24*	20.67 ± 0.18
Pa Pus	4.33 ± 0.07	4.60 ± 0.12	6.80 ± 0.12	8.80 ± 0.61*	21.87 ± 0.47
Pa Ear swab	3.73 ± 0.07	3.53 ± 0.29	5.60 ± 0.23	7.53 ± 0.29*	21.73 ± 0.64
Kp MTCC 618	0.00 ± 0.00	0.00 ± 0.00	3.27 ± 0.18	4.93 ± 0.07*	18.53 ± 0.29
Kp Urine	0.00 ± 0.00	0.00 ± 0.00	3.60 ± 0.12	5.13 ± 0.18*	18.20 ± 0.12
Kp Urine	0.00 ± 0.00	0.00 ± 0.00	3.47 ± 0.24	5.33 ± 0.18*	18.00 ± 0.58
Kp Urine	0.00 ± 0.00	0.00 ± 0.00	3.40 ± 0.12	5.40 ± 0.12*	18.80 ± 0.12
Kp Urine	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 0.18	4.87 ± 0.52*	18.67 ± 0.33

The values are the mean of three experiments ± S.E. *P<0.01
Abbreviations: Sa, *Staphylococcus aureus*; Bs, *Bacillus substilis*; Pa, *Pseudomonas aeruginosa*; Kp, *Klebsiella pneumoniae*.

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