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Antibacterial activity of selected *Dendrobium* species against clinically isolated multiple drug resistant bacteria

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Dendrobium **species are widely used in traditional medicine as remedies for tonic to nourish the stomach, promote the production of body fluids and decrease fever. In this study, the antibacterial activities of four traditionally used *Dendrobium* species were tested against clinically isolated multiple drug resistant (MDR) bacteria. Hexane, chloroform, acetone, ethanol and methanol extracts of *Dendrobium amoenum, Dendrobium crepidatum*, *Dendrobium moniliforme* and *Dendrobium longicornu* were tested for antibacterial activity against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Acinetobacter baumannii* by using the well diffusion method. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined using the serial dilution method. For *D. amoenum*, hexane, chloroform and acetone extracts showed antibacterial activity against *S. aureus* (ZOI: 11.33, 12.00 and 11.00 mm respectively), acetone extract against *A. baumannii* (ZOI: 13.00 mm) and methanol extract against *P. aeruginosa* (ZOI: 12.00 mm). For *D. crepidatum*, hexane, chloroform, acetone, ethanol and methanol extracts showed antibacterial activity against *S. typhi* (ZOI: 10.00, 11.67, 12.00, 9.67 and 12.67 mm respectively), hexane, ethanol and methanol extracts against *P. aeruginosa* (ZOI: 10.00, 9.67 and 15.00 mm respectively), choloroform and acetone extracts against *S. aureus* (ZOI: 9.67 mm) and ethanol extract against *E. coli* (ZOI: 11.00 mm). For *D. moniliforme*, chloroform extract showed antibacterial activity against *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *A. baumannii* (ZOI: 12.67, 12.00, 11.67 and 13.00 mm respectively), acetone extract against *S. aureus* (ZOI: 11.00 mm) and *A. baumannii* (ZOI: 12.67 mm). For *D. longicornu*, chloroform extract showed antibacterial activity against *A. baumannii* (ZOI: 12.00 mm). MIC and MBC of these extracts of plants showed they have moderate antibacterial activity against the bacterial strains used. In conclusion, these *Dendrobium* species can be used as antibiotic agents.**

Key words: *Dendrobium*, extracts, minimum bactericidal concentration (MBC), multiple drug resistant (MDR) bacteria, minimum inhibitory concentration (MIC), zone of inhibition (ZOI).

INTRODUCTION

Orchids are the largest and most diverse family of angiospermic plants; most orchids are widely used in traditional medicine as remedies for severe diseases (Pant and Raskoti, 2013). Several *Dendrobium* species

are used in traditional medicine as tonics to nourish the stomach, promote the production of body fluids and decrease fever (Ng et al., 2012; Xu et al., 2013). They produce a variety of secondary metabolites, such as phenolic compounds (Hu et al., 2010; Li et al., 2009a; Zhao et al., 2003), bibenzyl derivatives (Bi et al., 2004; Chen et al., 2010; Hu et al., 2008; Li et al., 2009b, 2009c, 2014; Majumder et al., 1999; Majumder and Chatterjee, 1989), lignin glycosides and phenanthrenes (Hu et al., 2008), and alkaloids (Elander et al., 1973; Kierkegaard et al., 1970; Li et al., 2008; Venkateswarlu et al., 2002). Such metabolites are responsible for these species' wide variety of medicinal properties (Chand et al., 2016; Paudel et al., 2015, 2017).

Bacterial infections are a global concern due to development of resistance towards various types of antibiotics. Multiple drug resistant (MDR) pathogens has been increasing, causing nosocomial and community-acquired deadly infections mainly due to the extensive utilization of antibiotics (Köck et al., 2010; Mongalo et al., 2013). Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Acinetobacter baumannii* have inherent resistant to most available antibiotics (Chambers, 1997; Kim et al., 2005; Patzer and Dzierzanowska, 2007).

The use of medicinal plant extracts to treat infectious disease is an age-old practice which rely on traditional medicine (Zaiden et al., 2005). According to the World Health Organization (WHO), approximately 80% of the world population rely on plants derived products for their treatment. There are many plants which have been reported to have antibacterial activities. New therapeutic antibacterial agents are greatly needed to treat emerging MDR bacterial infections. Therefore, the present study aimed to evaluate the antibacterial activity of selected *Dendrobium* species: *Dendrobium amoenum*, *Dendrobium crepidatum*, *Dendrobium longicornu* and *Dendrobium moniliforme* extracts against MRSA *S. aureus*, and MDR strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi* and *A. baumannii*.

MATERIALS AND METHODS

Plant materials

Whole stems of selected *Dendrobium* species (*D. amoenum*, *D. crepidatum*, *D. longicornu* and *D. moniliforme*) were collected from Chitlang and Daman of Makawanpur district, central Nepal, and voucher specimens were deposited at the Tribhuvan University Central Herbarium (TUCH), Kathmandu, Nepal (Specimens number: M05, M02, M03, M01 and M04, respectively).

Preparation of plant extracts

Plant materials were prepared by grinding air-dried stems and extracted with hexane, chloroform, acetone, ethanol and methanol in the ratio of 1:10 (w/v) using a Soxhlet extractor (Jones and Kinghorn, 2005). The solvent was evaporated under room temperature to obtain dry extracts. Each extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a 1 mg/ml stock solution.

Microorganisms

The bacterial strains used in this study were clinical isolates and ATCC strains obtained from the National Public Health Laboratory, Kathmandu, Nepal: *S. aureus* (MRSA), *E. coli* (ATCC 25922), *A. baumannii* (ATCC 17978), *K. pneumoniae*, *P. aeruginosa* and *S. typhi*. The standard antibiotic drugs ciprofloxacin (for *S. aureus* and *E. coli*), chloramphenicol (for *K. pneumoniae*), gentamicin (for *P. aeruginosa*), azithromycin (for *S. typhi*) and meropenem (for *A. baumannii*) were used as positive controls.

Antibacterial activity

Individual bacterial strains were streaked on nutrient agar (Hi-Media, India) plates and incubated at 37°C for 24 h. Pure cultures were obtained, transferred to nutrient broth (Hi-Media, India) and incubated in a shaker incubator at 37°C and 120 rpm overnight. The turbidity of bacterial suspensions was adjusted to the 0.5 McFarland standards for antibacterial testing. Single bacterial strains from the appropriate bacterial suspensions were inoculated on Mueller Hinton agar plates with a sterile cotton swab. The antibacterial activities of the plant extracts were determined by using the well diffusion method. Five wells with a diameter of 7 mm were prepared on nutrient agar for each bacterial strain. Each well was filled with 100 µl of plant extract (1 mg/ml), DMSO (the negative control) or the standard antibiotics (the positive control). The plates were incubated overnight at 37°C. After incubation, the clear zone of inhibition around the point of application of each sample solution was measured in millimeters (mm).

Minimum inhibitory concentrations (MICs) were determined by using the serial dilution method (Abu-Shanab et al., 2006). The extracts were serially diluted in nutrient broth medium. Each dilution (1 ml) was inoculated into 1 ml of sterile nutrient broth containing 0.25 ml of the tested bacterial strain and incubated at 37°C for 24 h. Pure plant extracts of each assessed concentration in 1 ml of nutrient broth were used as the positive control, and 0.25 ml of bacteria culture in 2 ml of nutrient broth was used as the negative control. The MIC was equal to the lowest concentration of extract (mg/ml) for which there was no detectable growth of bacteria.

The minimum bactericidal concentrations (MBCs) were determined by sub-culturing test dilutions of the extracts onto fresh medium and incubating for an additional 24 h. The lowest concentration of the extract (mg/ml) that did not result in the appearance of a single bacterial colony on the solid medium was regarded as the MBC (Abu-Shanab et al., 2006).

Statistical analysis

All data are presented as the means ± standard deviation (SD) of triplicate samples. Values for the extracts were tested by using Chi-

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Table 1. Antibacterial effect of different solvent extracts of four *Dendrobium* species and standard antibiotics as positive control at 1 mg/ml concentration against different bacterial strains.

Plant name	Extract	Zone of inhibition (ZOI) (mm) [mean ± SD]					
		***Staphylococcus aureus* (MRSA)**	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Acinetobacter baumannii***
	Hexane	11.33±1.15	-	-	-	-	-
	Chloroform	12.00±0.00	-	-	-	-	-
D. amoenum	Acetone	11.00±0.00	-	-	-	-	13.00±0.00
	Ethanol	-	-	-	-	-	-
	Methanol	-	-	-	12.00±0.00	-	-
	Hexane	-	-	-	10.00±0.00	10.00±2.00	-
	Chloroform	9.67±0.58	-	-	-	11.67±1.15	-
D. crepidatum	Acetone	9.67±0.58	-	-	-	12.00±0.00	-
	Ethanol	-	11.00±1.00	-	9.67±0.58	9.67±0.58	-
	Methanol	-	-	-	15.00±0.00	12.67±0.58	-
	Hexane	-	-	-	-	-	-
	Chloroform	-	-	12.67±0.58	12.00±0.00	11.67±0.58	13.00±0.00
D. moniliforme	Acetone	11.00±0.00	-	-	-	-	12.67±1.15
	Ethanol	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	12.00±0.00
D. longicornu	Acetone	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-
	Ciprofloxacin	23.67±1.53	11.33±0.58	-	-	-	-
	Chloramphenicol	-	-	25.00±0.00	-	-	-
Positive control	Gentamicin	-	-	-	13.33±0.58	-	-
	Azithromycin	-	-	-	-	17.67±2.52	-
	Meropenem	-	-	-	-	-	15.00±0.00
	Chi-square	58.39	58.72	58.48	58.13	58.36	58.70
	df	20	20	20	20	20	20
	p	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

square test with a significance threshold of $p \leq 0.05$.

RESULTS

The antibacterial activity of different solvent extracts of the four *Dendrobium* species were tested by using the well diffusion method. The result of antibacterial activity of four *Dendrobium* species is shown in Table 1. For *D. amoenum*, hexane, chloroform (Figure 1a) and acetone extracts showed antibacterial activity against *S. aureus* (ZOI: 11.33, 12.00 and 11.00 mm respectively), acetone extract against *A. baumannii* (ZOI: 13.00 mm) and methanol extract against *P. aeruginosa* (ZOI: 12.00 mm). For *D. crepidatum*, hexane, chloroform, acetone, ethanol and methanol extracts showed antibacterial activity against *S. typhi* (ZOI: 10.00, 11.67, 12.00, 9.67 and 12.67 mm respectively), hexane, ethanol and methanol extracts against *P. aeruginosa* (ZOI: 10.00, 9.67 and 15.00 mm respectively), choloroform and acetone extracts against *S. aureus* (ZOI: 9.67 mm) and ethanol extract against *E. coli* (ZOI: 11.00 mm). For *D. moniliforme*, chloroform extract showed antibacterial activity against *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *A. baumannii* (Figure 1b) (ZOI: 12.67, 12.00, 11.67 and 13.00 mm respectively), acetone extract against *S. aureus* (ZOI:

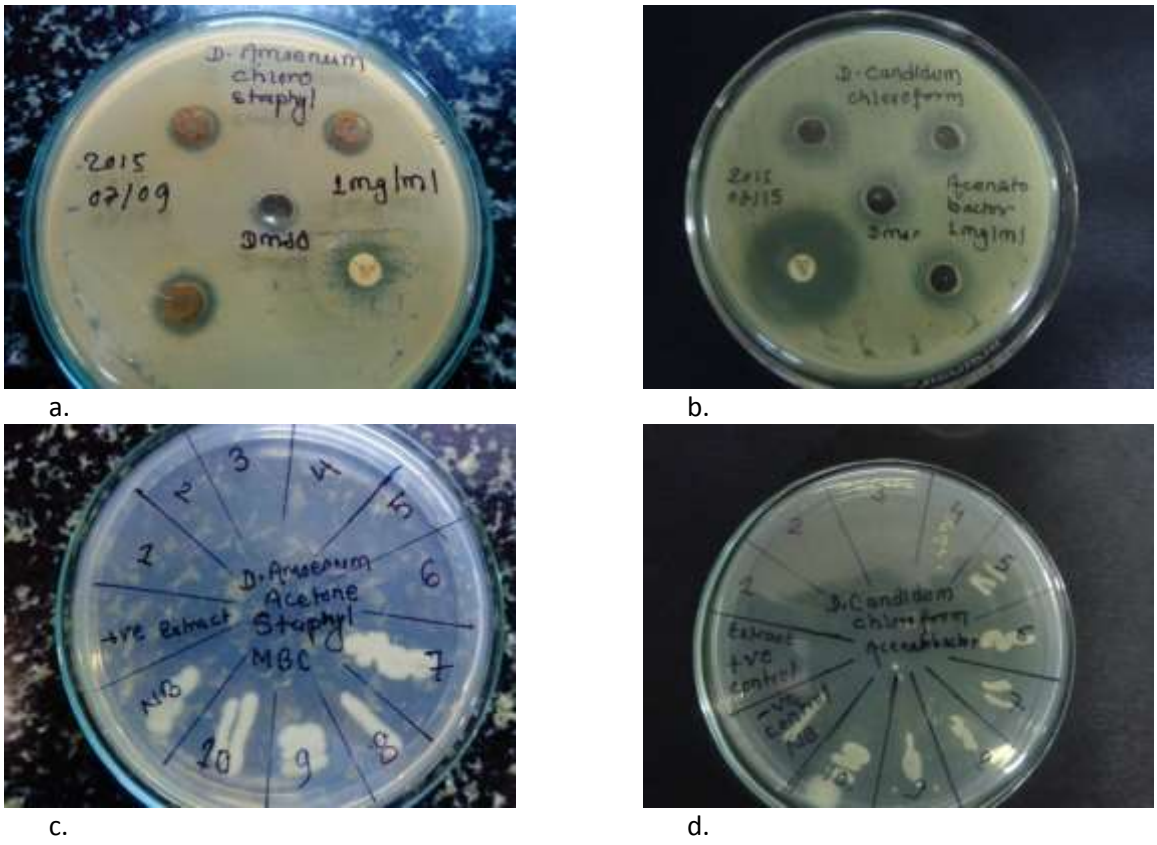

Figure 1. Antibacterial activities of different extracts of *Dendrobium* species against different bacterial strains. a) ZOI of *D. amoenum* chloroform extract against *S. aureus*; b) ZOI of *D. moniliforme* chloroform extract against *A. baumannii*; c) MBC of *D. amoenum* acetone extract against *S. aureus;* d) MBC of *D. moniliforme* chloroform extract against *A. baumannii*

11.00 mm) and *A. baumannii* (ZOI: 12.67 mm). For *D. longicornu*, chloroform extract showed antibacterial activity against *A. baumannii* (ZOI: 12.00 mm).

The antibacterial activity and potencies of the extracts were quantitatively assessed by determining MIC and MBC (Table 2). The values MIC and MBC of the extracts of four *Dendrobium* species indicate that they have moderate antibacterial activity against the bacterial strains used. For *D. amoenum*, acetone extract (Figure 1c) showed lowest MIC and MBC against *S. aureus* (MIC and MBC: 0.39 mg/ml), acetone extract against *A. baumannii* (MIC: 1.56 mg/ml, MBC: 3.12 mg/ml) and methanol extract against *P. aeruginosa* (MIC: 0.78 mg/ml, MBC: 3.12 mg/ml). For *D. crepidatum*, acetone extract showed lowest MIC and MBC against *S. typhi* (MIC: 0.78 mg/ml, MBC: 6.25 mg/ml), hexane extract against *P. aeruginosa* (MIC: 0.78 mg/ml, MBC: 6.25 mg/ml), choloroform extract against *S. aureus* (MIC: 0.78 mg/ml, MBC: 6.25 mg/ml) and ethanol extract against *E. coli* (MIC: 3.12 mg/ml, MBC: 6.25 mg/ml). For *D. moniliforme*, chloroform extract showed moderate antibacterial activity against *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *A. baumannii* (Figure 1d) (MIC: 3.12, 1.56, 0.39 and 1.56 mg/ml respectively, MBC: 3.12 mg/ml), acetone extract against *S. aureus* (MIC: 0.39 mg/ml, MBC: 6.25 mg/ml) and *A. baumannii* (MIC: 1.56 mg/ml, MBC: 3.12 mg/ml). For *D. longicornu*, chloroform extract showed moderate antibacterial activity against *A. baumannii* (MIC: 0.39 mg/ml, MBC: 6.25 mg/ml).

DISCUSSION

Drug-resistant bacterial infection treatment has been stimulated by investigations on natural compounds as alternative medicine (Abu-Shanab et al., 2004; Adwan and Mhanna, 2008; Denis et al., 2009). In the present study, analyses of growth inhibition activity performed by using the well diffusion method showed that all the tested plants, which are commonly used by traditional medical practitioners, exhibited moderate activity against clinical isolates of bacterial strains at extract concentrations of 1 mg/ml. The results are in agreement with those obtained in prior studies that have investigated medicinal plants for antibacterial activity (Abu-Shanab et al., 2004, 2006; Adwan and Mhanna, 2008; Aliyu et al., 2008; Habib et al.,

Table 2. MIC (mg/ml), MBC (mg/ml) and ZOI (mm) of different solvent extracts of four *Dendrobium* species against different bacterial strains.

Plant names	Extracts	*Staphylococcus aureus* (MRSA)			*Escherichia coli*			*Klebsiella pneumoniae*			*Pseudomonas aeruginosa*			*Salmonella typhi*			*Acinetobacter baumannii*		
		MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI	MIC	MBC	ZOI
	Hexane	1.56	6.25	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	0.39	6.25	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. amoenum	Acetone	0.39	0.39	16	-	-	-	-	-	-	-	-	-	-	-	-	1.56	3.12	18
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	0.78	3.12	15	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-	-	-	-	0.78	6.25	15	1.56	6.25	13	-	-	-
	Chloroform	0.78	6.25	14	-	-	-	-	-	-	-	-	-	1.56	6.25	13	-	-	-
D. crepidatum	Acetone	1.56	3.12	18	-	-	-	-	-	-	-	-	-	0.78	6.25	13	-	-	-
	Ethanol	-	-	-	3.12	6.25	11	-	-	-	1.56	6.25	13	3.12	6.25	13	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	3.12	6.25	17	3.12	12.5	12	-	-	-
	Hexane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	3.12	3.12	13	1.56	3.12	17	0.39	3.12	13	1.56	3.12	19
D. moniliforme	Acetone	0.39	6.25	13	-	-	-	-	-	-	-	-	-	-	-	-	1.56	3.12	14
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.39	6.25	15
D. longicornu	Acetone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

2011; Nitta et al., 2002; Voravuthikunchai and Kitpipit, 2005).

Infections caused by multiple drug resistance, including resistance to β-lactam, are among the most difficult infections to treat (Abu-Shanab et al., 2004, 2006; Kim et al., 2005). In this study, the growth of *S. aureus, E. coli, K. pneumoniae, S. typhi* and *A. baumannii* were markedly inhibited by some extracts of *D. amoenum, D. crepidatum, D. moniliforme* and *D. longicornu*. Therefore, it appears likely that the antibacterial compound(s) extracted from these *Dendrobium* species (data not shown here) may inhibit bacteria via a different mechanism than that associated with currently used antibiotics and may have therapeutic value as antibacterial agents against bacterial strains with multiple drug resistance.

Overall, the screening of extracts from all tested plants showed that Gram-positive bacteria were more susceptible than Gram-negative bacteria. This phenomenon may be attributable to a distinctive feature of Gram-negative bacteria: the presence of a thick outer murein membrane. This outer membrane prevents certain drugs and antibiotics from entering the cell. In contrast, Gram-positive bacteria may be more susceptible to the tested extracts because the peptidoglycan

that constitutes the outer layer of such bacteria is not an effective permeability barrier to bioactive compounds. Thus, Gram-negative bacteria have more complex cell wall than Gram-positive bacteria; this distinction partially explains why Gram-negative bacteria are generally more resistant to antibiotics than Gram-positive bacteria (Behera et al., 2013; Zaiden et al., 2005). Extracts of the tested plants do not have the types of antibiotic compounds that can readily enter the cell and kill Gram-negative bacteria.

The MIC and MBC values obtained for the extracts against the bacterial strains support the findings obtained by using the well diffusion method. One concern is that, for the extracts obtained in this study, MIC values were lower than MBC values, thus suggesting that these extracts are bacteriostatic at lower concentrations and bactericidal at higher concentrations (Abu-Shanab et al., 2006; Nitta et al., 2002; Ross et al., 2011).

In conclusion, some of the extracts of the selected plants showed moderate antibacterial activity against the bacteria tested. The findings indicated that these species that are frequently used in traditional medicine and as tonic may be potential sources of antimicrobial agents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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