

Short Communication

***In vitro* antibacterial activity of alkaloid extract from stem bark of *Mahonia manipurensis* Takeda**

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The genus *Mahonia* belongs to one of the primitive groups of plant family Berberidaceae. A number of species from this genus are widely used in traditional and folk medicine systems in different parts of the world. *Mahonia manipurensis* crude alkaloid extract was obtained from the stem bark by extracting from 80% of methanol extract. *In vitro* antibacterial activity of the alkaloid extract was screened against five different pathogenic bacteria of clinical origin including two species of gram-negative and three species of gram-positive bacteria. Using agar well diffusion method, growth inhibition was observed in four of the five tested pathogens at two different concentrations (5 and 2.5 mg/ml). The result indicates that the alkaloid extract exhibited significant antibacterial activity. Similar antimicrobial activity was also reported from other species of this genus by previous workers. Minimum inhibitory concentration (MIC) was also determined using agar dilution method, following two-fold serial dilution and observed that the lowest value was exhibited by *Bacillus cereus* and *Enterococcus faecalis* each corresponding to 256 µg/ml concentration.

Key words: *Mahonia manipurensis*, antibacterial activity, alkaloid extract, Minimum inhibitory concentration.

INTRODUCTION

Mahonia manipurensis Takeda belongs to the family Berberidaceae. The plant is endemic to the Northeastern region of India in the states of Manipur and Nagaland. Although there is merger report about the local used of this plant against diarrhoea, fever and jaundice, however, a number of species of this genus are widely used as folk medicine in different parts of the world. For instance the dried stems of *Mahonia bealei* and *Mahonia fortunei* are commonly used in traditional Chinese medicine against fever, swelling, inflammation, jaundice, dysentery and constipation (Pharmacopoeia Committee, 1990). Extract from the stem of *Mahonia acanthifolia* is given against dysentery, diarrhoea and jaundice by traditional practitioners in Darjeeling Himalaya (Pranay and Ritu, 2009). Alkaloids are the main active compounds in

Mahonia plants (Ji et al., 2000). Berberine, palmatine and jatrorrhizine are the principal alkaloids found in this genus and these compounds are known to have *in vitro* antibacterial and antifungal activities (Schiff, 1987). Considering the important applications of some plant species belonging to this genus in folk and traditional medicine systems, the present study was designed to investigate the antibacterial properties of *M. manipurensis* Takeda against certain pathogenic bacterial strains of clinical origin.

MATERIALS AND METHODS

Plant material collection and identification

The stem bark of *M. manipurensis* and herbarium specimens were collected from Mao area, Senapati District, Manipur in the month of April-2009, identified from Flora of Manipur 1: 410-411, 1993 and verified from Kew Herbarium, Edinburgh 6: 222. 1917. A voucher specimen (Collection No. 188) was prepared from the collected

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plant and deposited in the herbarium of the Department of Botany, NEHU, Shillong, India.

Detection of alkaloid

The presence of alkaloid in plant sample (stem bark) was detected following the standard qualitative detection methods as described by Culvenor and Fitzgerald (1963) and Kapoor et al. (1969) using alkaloidal precipitating reagents such as Mayer's, Dragendorff's and Silicotungstic acid.

Extraction of alkaloids

The plant stem bark was removed, dried in oven at 35°C for overnight and ground into fine powder using grinder. About 100 g of the powder plant sample was extracted with 800 ml of 80% methanol in 1.5 L beaker with stirring at interval in room temperature for 36 h. The extract is filtered using Whatman No. 42 filter paper. The filtrate was then concentrated to 1/10th of the original volume in a Buchi rotavapor under reduced pressure at 40°C. The concentrated extract was then used for extraction of alkaloid following Harborne principle (1998) and yielded 1.12 g crude alkaloid extracts corresponding to 1.12% in terms of dry weight starting materials. The alkaloid extract thus obtained is stored at 4°C until further use.

Preparation of test extracts

About 25 mg of alkaloid extract is dissolved in 5 ml of 10% DMSO (% v/v) solution and filtered through 0.22 µm nylon-66 membrane filter (Axiva). 1 ml of this solution which corresponds to 5 mg/ml concentration is diluted with 1 ml of autoclaved sterile distilled water to make another solution of 2.5 mg/ml concentration. The two different concentrations of the solution thus prepared freshly are used to test for antibacterial activity.

Experimental microorganisms

Five different pathogenic bacterial strains were obtained from Institute of Microbial Technology, Chandigarh, India. Three Gram-positive (*Bacillus cereus* MTCC 430, *Enterococcus faecalis* MTCC 439 and *Enterobacter cloacae* MTCC 7408), two Gram-negative (*Escherichia coli* MTCC 116 and *Shigella flexneri* MTCC 1457) bacteria were used for the study.

Chemicals and culture media for antibacterial assay

The culture media Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB) and Nutrient Agar (NA) are used for evaluating the antimicrobial activity of the alkaloid extract. These culture media are obtained from Himedia (MHA) and SRL (MHB and NA), Mumbai, India. The standard antibiotics disc Streptomycin 25 mcg (Himedia) was used as positive control against tested bacterial strains. All other reagents and solvents used are of analytical grade purchased from Sd. Fine Chem. Mumbai and CDH, New Delhi, India.

Susceptibility test method

Standardisation of bacterial suspension

A cell suspension of about 1.5×10^6 CFU/ml was obtained from a McFarland 0.5 BaSO₄ turbidity standard. The cell suspension was

standardised by adjusting the optical density (OD) to 0.11 at 625 nm using Lambda-35 UV-VIS Spectrophotometer. 1 ml of this standardised cell suspension culture (within 30 min) was then used to inoculate by spreading the surface of overnight prepared NA plates and incubated at 37°C (MHA and at 30°C for *E. cloacae*) for 24 h.

Agar well diffusion assay

The antibacterial diffusion assay was carried out using Agar well diffusion method as described by Perez et al. (1990) and Ahmad et al. (1998). One Streptomycin antibiotic drug standard disc (Himedia) of concentration 25 mcg was placed in the centre of each plate as positive control. The assessment of antibacterial activity of the plant alkaloid extract was based on the measurement of diameter of inhibition zone (IZ) in mm formed around the well.

Each well was loaded alternately with 100 µl one with 5 mg/ml and the other with 2.5 mg/ml concentration. The assay was carried out in triplicates and the result thus obtained is taken as the mean of the three readings (Table 1) for each concentration and not statistical tools were used to measure the standard deviation.

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was carried out using Agar dilution assay (Irith et al., 2008) following the twofold serial dilution. Concentrations of 2.48, 1.24, 0.512, 0.256 and 0.128 mg/ml of the crude alkaloid extract were prepared separately by dissolving the sample in 10% DMSO (v/v) autoclaved distilled water and filter sterilized through 0.22 µm nylon-66 membrane filter. The MIC was recorded as lowest concentration of the alkaloid extract showing no visible growth of the test bacterial strains on the agar plate after 24 h incubation.

RESULTS

The total alkaloid extract showed significant zone of inhibition against Gram-positive bacteria *B. cereus*, *E. cloacae* and *E. faecalis* and one Gram-negative bacterium *S. flexneri* (Table 1). The other Gram-negative bacterium *Escherichia coli* were not observed inhibition at these two different concentrations (5 and 2.5 mg/ml). Also observation of the result from the table indicates that there is not significant variation of the inhibition zone for all the four bacteria which are tested with positive inhibitions. The solvent 10% aqueous DMSO (% v/v) used for dissolving the alkaloid extract always gave negative inhibition showing that it did not influence the antibacterial activities observed for the test sample of the plant extract. Further MIC was determined again all the four bacterial strains that showed positive inhibition and found that the lowest value was exhibited by *B. cereus* and *E. faecalis* each corresponding to 256 µg/ml concentration.

DISCUSSION

The alkaloids are known to have antimicrobial and antiparasitic properties. Verpoorte (1998) have reported

Table 1. Antibacterial activity of alkaloids extract from *M. manipurensis* stem bark.

Bacterial strains	Concentration (mg/ml)	Diameter zone of inhibition (mm)				With Streptomycin standard (mm)	MIC in µg
		R ₁	R ₂	R ₃	Mean		
<i>B. cereus</i> MTCC 430	5	18.5	19	19	18.8	25	256
	2.5	15	14.5	15	14.8		
<i>E. coli</i> MTCC 116	5	NI	NI	NI	NI	16.5	O
	2.5	NI	NI	NI	NI		
<i>E. cloacae</i> MTCC7408	5	18	18	18	18	24.5	512
	2.5	15	15	14.5	14.8		
<i>E. faecalis</i> MTCC 439	5	19	19	19.5	19.2	28	256
	2.5	15	15	15	15		
<i>S. flexneri</i> MTCC 1457	5	19.5	20	20	19.8	27	512
	2.5	16	16	16	16		

NI = No inhibition, NO = No observed and R = reading.

about 300 alkaloids showing such activity. Similar results on antibacterial activity were reported on related species of the genus *Mahonia* by Duraiswamy et al. (2006), Livia et al. (2004) and Li et al. (2007). Generally, the plant extracts inhibited the Gram-positive bacteria better than the Gram-negative ones. This is in agreement with reports on plant extracts by Tomas-Barberan et al. (1988), Vlietinck et al. (1995), Rabe and Van Staden (1997). The reason could be attributed to the presence of extra outer membrane in their cell wall acting as barrier for the compound(s) to diffuse into the bacterial cells. The alkaloids sanguinarine, berberine, jatrorrhizine and palmatine are known to inhibit the multiplication of bacteria, fungi and viruses (Schiff, 1987; Schmeller et al., 1997). Therefore the antibacterial activity observed in the present investigation is attributed to the alkaloids berberine, palmatine and jatrorrhizine which have been widely known to occur in different species of this genus.

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