

Full Length Research Paper

Antibacterial activity of Kumaun Himalayan *Biota orientalis* L. leaf extracts

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The antibacterial property of methanol, ethanol, chloroform and hexane extracts of Kumaun Himalayan gymnospermous plant *Biota orientalis* leaves were investigated against some pathogenic Gram-positive and Gram-negative bacterial strains (*Agrobacterium tumefaciens*, *Bacillus subtilis*, *Escherichia coli*, *Erwinia chrysanthemi* and *Xanthomonas phaseoli*) using disc diffusion method. Methanol extract showed the highest inhibitory activity against *B. subtilis* (ZOI, 16 mm), followed by ethanol extract (14 to 16 mm), chloroform extract (12 to 15 mm) while lowest inhibition was recorded in hexane extract. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined and recorded in the range 62.5 to 500 µg/ml and 125 to 500 µg/ml, respectively. The lowest MIC value 62.5 µg/ml was recorded against *B. subtilis* and *E. chrysanthemi* in the methanol extract whereas the lowest MBC value 125 µg/ml was recorded against *B. subtilis* in the same extract. The aqueous extract was found totally inactive against all the pathogens tested.

Key words: *Biota orientalis*, antibacterial activity, disc diffusion, gymnosperms and plant extracts.

INTRODUCTION

Plants and plant extracts have been utilized as medicines since time immemorial (Samuelsson, 2004). World Health Organisation (WHO) estimates that 80% populations of developing countries depend on the traditional medicine for their primary health care needs, while 85% people in third world use plants or their extracts as the active substances in health care system (Shome et al., 1996; Sheldon et al., 1998). Indian subcontinent is vast repository of medicinal plants which are used in different part of traditional medicinal treatments (Chopra et al., 1956; Sati and Joshi, 2011). Resistance towards prevailing antibiotics have become widespread among bacteria and fungi, therefore, new class of antimicrobial substances are urgently required. It is well known that plants, although lacking the typical immune response, have an in-built system for protection against biotic and abiotic stress conditions. Several studies indicated the presence of many compounds with antimicrobial properties in plants or plant parts (Darokar et al., 1998; Joshi and

Sati, 2012).

Biota previously known as *Thuja* is a small genus belongs to the Cupressaceae family comprising five species, two native to North America and three native to Eastern Asia. *Biota orientalis* L. an evergreen gymnosperm is widely cultivated as a common ornamental timber tree (Assadi, 1998; Sharad et al., 2008). During the last few years most of the phytochemical studies on this plant species focused on the terpene composition of the essential oil, where α -pinene and α -cedrol have been reported as the major constituents of the leaf oil (Chen et al., 1984; Li and Liu, 1997; Nickavar et al., 2003). In folk medicine *B. orientalis* has been used to treat bronchial catarrh, cystitis, urine carcinomas, amenorrhea and rheumatism.

The major constituent of the oil, the monoterpene thujone, is used pharmacologically as an active ingredient in the production of nasal decongestants and cough suppressants, perfumes etc. The present investigation is

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Figure 1. (a) *Biota orientalis* plant; (b) *B. orientalis* leaves powder.

an attempt to evaluate the antibacterial efficacy of aerial parts of Kumaun Himalayan gymnospermous plants *B. orientalis*.

MATERIALS AND METHODS

Collection of plant materials

B. orientalis L. (Cupressaceae) is a beautiful ornamental plant commonly known as Morpankhi (Figure 1a). The green leaves were collected from Nainital, Kumaun Himalaya, India and authenticated by the Department of Botany, Kumaun University, Nainital. A voucher specimen was deposited in the herbarium.

Extraction procedure

The leaves of *B. orientalis* were thoroughly washed and dried at room temperature. The dried material was powdered in an electric grinder (Figure 1b). To prepare a stock solution, 50 g of this powder was added to 200 ml of solvents (w/v). Solvents used for extraction

were methanol, ethanol, chloroform, hexane, and water. Powdered leaf was dissolved in different solvents separately in 250 ml Erlenmeyer flask and kept in a shaker for 6 to 10 h. The prepared extracts were filtered through Whatman filter paper number 1. The final filtrates were concentrated on a rotary evaporator under vacuum at 20°C and utilized for antibacterial assessment (Mohanta et al., 2007).

Microorganisms used

Five (Gram +ve and -ve) bacteria *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 40, *Agrobacterium tumefaciens* MTCC 609, were obtained from Institute of Microbial Technology, Chandigarh, India whereas *Xanthomonas phaseoli* and *Erwinia chrysanthemi* were obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India.

Screening of antibacterial activity

Antibacterial tests of selected microorganisms were carried out using disc-diffusion method (Bauer et al., 1966). A small sterile

cotton swab was dipped into the 24-hour-old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. After inoculation the plates were allowed to dry at room temperature in laminar chamber. The filter paper discs (5 mm) loaded with 40 µl of extract were placed on the surface of the agar plates. After 5 min the plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 h. Gentamycin (30 mg), erythromycin (15 mg), and ampicillin (10 mg); were used as positive controls and the respective solvent were taken as negative control. After 24 h of incubation, the dishes were observed for bioactivity (Plate 1) and the diameter was observed for zone of inhibition (ZOI). All tests were performed in triplicate and observed values of ZOI are expressed as mean value with standard error of means (SEM).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

All the fractions were tested for the minimum inhibitory concentration and minimum bactericidal concentrations. MIC was performed at five concentrations of extracts (500, 250, 125, 62.5 and 31.25 µg/ml) following serial dilution technique. All the discs showing no visible growth of microorganisms were sub cultured and incubated at 37°C for overnight. The highest dilution showing 100% inhibition was recorded as MBC.

RESULTS

The antibacterial activities of *B. orientalis* methanol, ethanol, chloroform and hexane extracts against 5 test microorganisms are presented in Table 1. The MIC and MBC for each fraction are presented in Table 2. As evident from Table 1, the methanol extract had the highest inhibitory activity among all the tested extracts. The activity order of used extracts was: methanol > ethanol > chloroform > hexane extract against all the tested microorganisms. The maximum zone of inhibition was 16 mm each for *B. subtilis* and *X. phaseoli*, while 15 mm each for *E. Chrysanthemi* and *A. tumefaciens* and 14 ± 0.6 mm for *E. coli* (Table 1). MIC and MBC values for the microorganisms sensitive to the methanol extract were in the range of 62.5 to 125 µg/ml and 125 to 500 µg/ml, respectively. In the present investigation, lowest MIC value 62.5 µg/ml was recorded against *B. subtilis* and *E. chrysanthemi* in the methanol fraction and the lowest MBC value 125 µg/ml was recorded against *B. subtilis* (Table 2). The ethanol and chloroform extracts showed MIC and MBC values in the same range that is, 125 to 250 µg/ml and 250 to 500 µg/ml, respectively. For hexane extract the MIC values were in the range of 250-500 µg/ml whereas no MBC was recorded.

DISCUSSION

In the present investigation antibacterial screening at 1000 µg/ml leaf extract of *Biota orientalis* suggests that it is active against all the test bacterial pathogens and their activity was found comparable with that of erythromycin and ampicillin (Figure 2). The methanol fraction showed

its highest activity against all the pathogens as compared to other extracts. This confirms the findings of earlier workers that methanol is a better solvent for consistent extraction of antimicrobial substances (Ahmad et al., 1998; Karaman et al., 2003). It is interesting to note that in the MIC and MBC analysis the lowest value of MIC (62.5 µg/ml) and MBC value (125 µg/ml) are observed for methanol extract against *B. subtilis* and *E. chrysanthemi* (Table 2).

B. orientalis is well documented for its use for remedies of various ailments (Guleria et al., 2008). The antioxidant activity of its ethanol fraction of aerial part was evaluated by Dubey and Batra, (2009). Essential oil of *B. orientalis* has been widely used in steam bath. The thujone-rich fraction separated from crude ethanolic extract of *Biota* had been reported to possess anticancer potential (Biswas et al., 2011).

Antibacterial, antifungal, analgesic, antiinflammatory, anthelmintic and pharmacological effects of this plant has also been studied by some workers (Ezzat, 2001; Guleria et al., 2008; Bissa et al., 2008; Bhan et al., 2011). Guleria et al. (2008) determined the chemical composition of *B. orientalis* collected from north-western Himalaya and they found α -pinene, α -cedrol, caryophyllene, limonene, α -terpinolene and α -terpinyl acetate, active compound in leaf extract.

Chen et al. (1989) reported the antibacterial activity of *B. orientalis* against *Streptococcus mutans*. Similarly Sharma et al. (1990), also studied antibacterial activity of various extracts of *B. orientalis* against 9 animal pathogens and found positive results. Hafez and Abdel-Salama (2004), investigated the chemical composition and antimicrobial activity of the volatile constituents of *B. orientalis*. Later, studies on pharmaceutical, pharmacological and clinical properties of this plant were done by Naser et al. (2005). Bissa et al. (2008) reported the antibacterial activity of *B. orientalis* against *A. tumefaciens* and *E. coli* using disc diffusion method and found aqueous extracts better than organic extract but in this study, the aqueous extracts were found totally inactive against all the tested microorganisms. This might be due to that active substances are more soluble in organic solvents than aqueous medium and hence, could not be presents in aqueous extract as suggested by Sati and Joshi, (2010). However, non effectiveness of aqueous extract to the bacterial strains supports the finding of Kumar et al. (2010).

This is the first report on the sensitivity of plant pathogenic bacterial strains *E. chrysanthemi*, *A. tumefaciens* and *X. phaseoli* against the Kumaun Himalayan *B. orientalis* as all the previous workers tested the antibacterial activity of extracts against animal pathogen only. It is interesting to note that these strains are responsible for various plant diseases like crown gall, leaf blight, leaf spot and rot disease; therefore the present findings would be useful in curing various human and plant bacterial disease.

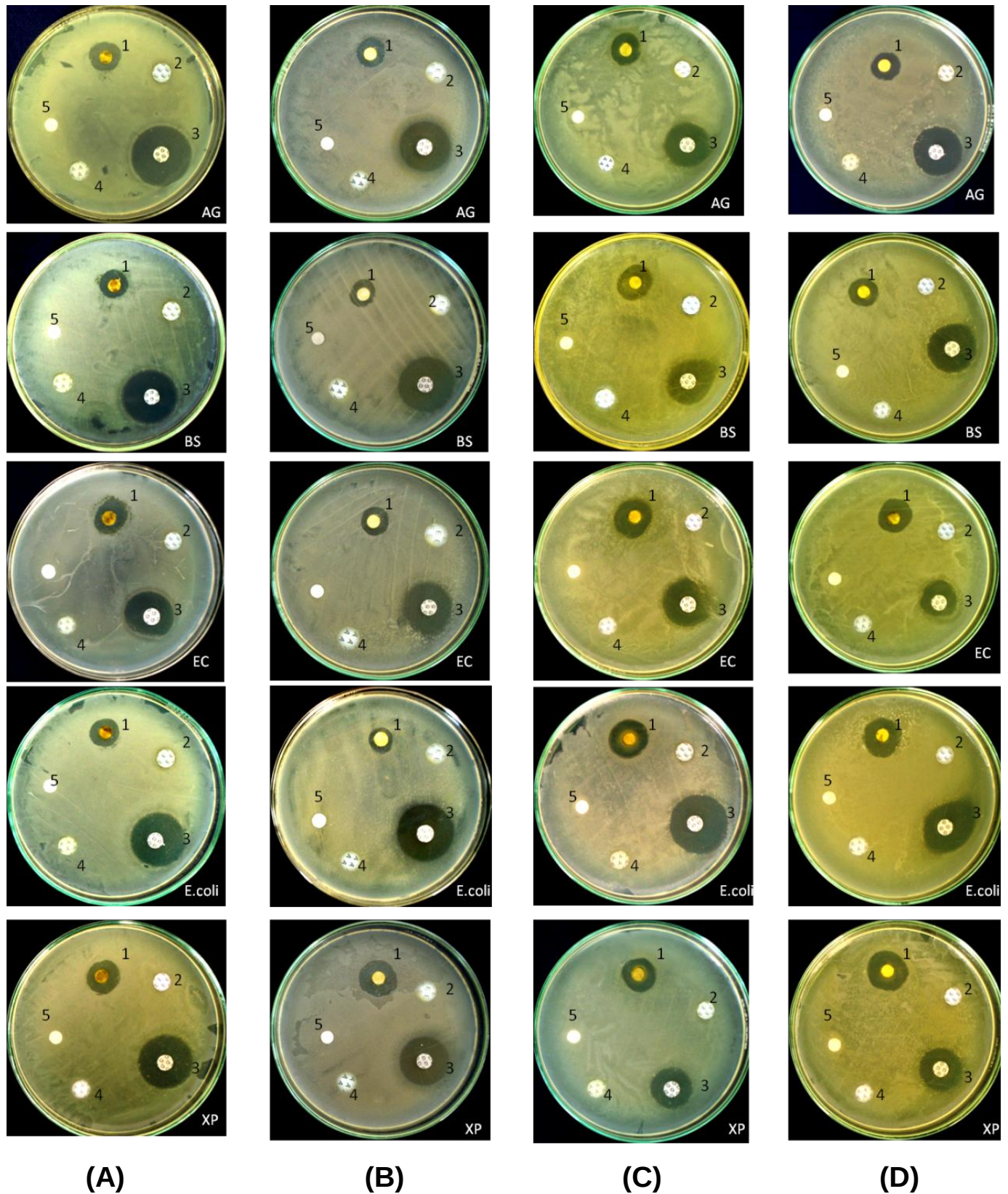


Plate 1. Antibacterial activity of *Biota orientalis* extracts against pathogenic bacteria. **(A)** Hexane extract, **(B)** chloroform extract, **(C)** ethanol extract, **(D)** methanol extract. **AG**, *Agrobacterium tumefaciens*, **E. co.**, *Escherichia coli*, **EC**, *Erwinia chrysanthemi*, **XP**, *Xanthomonas phaseoli*, **BS**, *Bacillus subtilis*; 1, extract, 2, 3, 4- positive controls (erythromycin, gentamycin, ampicillin), 5- negative control (solvent).

Table 1. Antibacterial activity of different extracts of *B. orientalis*.

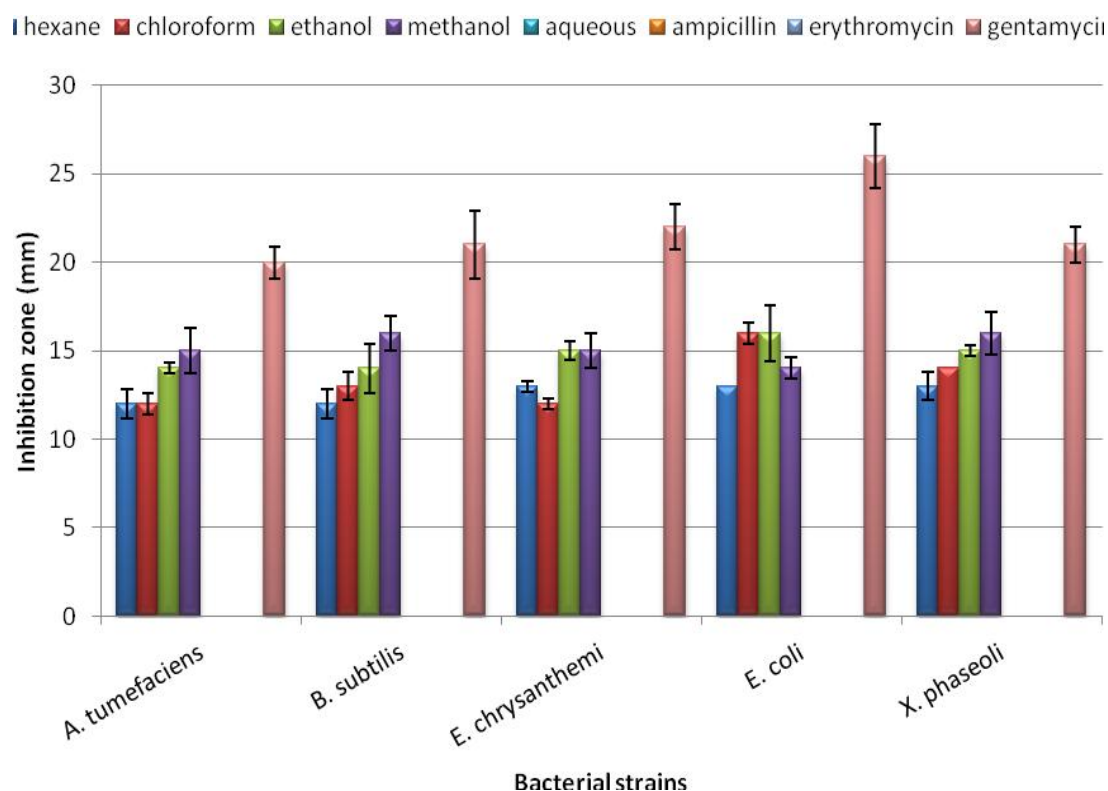
Microorganism	Diameter of inhibition zone (mm)*							
	H	C	E	M	Aq	A	E	G
<i>A. tumefaciens</i>	12 ± 0.8	12 ± 0.6	14 ± 0.3	15 ± 1.3	na	na	na	20 ± 0.9
<i>B. subtilis</i>	12 ± 0.8	13 ± 0.8	14 ± 1.4	16 ± 1.0	na	na	na	21 ± 1.9
<i>E. chrysanthemi</i>	13 ± 0.3	12 ± 0.3	13 ± 0.5	15 ± 1.0	na	na	na	22 ± 1.3
<i>E. coli</i>	13 ± 0.0	16 ± 0.6	16 ± 1.6	14 ± 0.6	na	na	na	26 ± 1.8
<i>X. phaseoli</i>	13 ± 0.8	14 ± 0.0	15 ± 0.3	16 ± 1.2	na	na	na	21 ± 1.0

*All the values are mean ± Standard Error of Mean (SEM) of three determinations H, C, E, M, Aq- Hexane, chloroform, ethanol, methanol, aqueous extract. A, E, G - Ampicillin, erythromycin, gentamycin, (+control), na- not active.

Table 2. MIC and MBC evaluation of *B. orientalis*.

Microorganism	Concentration (µg/ml)							
	Hexane		Chloroform		Ethanol		Methanol	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>A. tumefaciens</i>	500	na	250	500	250	na	125	na
<i>B. subtilis</i>	500	na	250	na	250	na	62.5	125
<i>E. chrysanthemi</i>	250	na	125	500	125	500	62.5	250
<i>E. coli</i>	250	na	125	250	125	250	125	na
<i>X. phaseoli</i>	500	na	250	na	125	500	125	500

na: no activity

**Figure 2.** Inhibition zones of different leaf extracts of *B. orientalis* against five bacterial strains.

Recently, Srivastava et al. (2012), reviewed biological properties of *B. orientalis* and concluded that it has the great potentiality against a number of health problems namely: bacterial, fungal and worm infections. Relying upon the present findings it is clear that all extracts had very promising results against the tested bacterial pathogenic strains in comparison to synthetic drugs Ampicillin and Erythromycin. Moreover, plant extracts are not only effective but also eco-friendly and therefore, further study on *Biota orientalis* is required to isolate and identify the active principles from the extracts of this gymnospermous plant.

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