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Full Length Research Paper

Antibacterial potential of leaf extracts of *Juniperus communis* L. from Kumaun Himalaya

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The sensitivity of five pathogenic multi drug resistant bacteria (*Bacillus subtilis*, *Erwinia chrysanthemi, Escherichia coli*, *Agrobacterium tumefaciens* and *Xanthomonas phaseoli*) was tested against the crude leaf organic extracts (methanol, ethanol, chloroform and hexane) and aqueous extracts of a Kumaun Himalayan gymnospermous plant *Juniperus communis* (Cupressaceae), employing disc diffusion method. All the extracts of *Juniperus communis* were found effective by showing a mark zone of inhibition except aqueous extract. The hexane extract showed maximum inhibition against the test microorganisms (ZOI, 16 – 21 mm) followed by ethanol, methanol and chloroform extract (ZOI, 6 – 17 mm). The inhibitory activity of these extracts was found very effective as compared to Ampicillin (10 mcg) and Erythromycin (15 mcg) standard antibiotics which were used as positive control against these tested microorganisms.

Key words: Kumaun Himalaya, antibacterial activity, *Juniperus communis*, gymnosperm, crude extract, ampicillin, erythromycin.

INTRODUCTION

The biological diversity in the Indian Himalayan Region (IHR) especially in Kumaun Himalaya has been a source of medicine for millions in the country and elsewhere (Tripathi, 2007). At present, the pharmaceutical sector is using nearly 280 medicinal plant species, out of which 175 are from the IHR (Dhar et al., 2000). Various plants being the effective source of both traditional and modern medicines are genuinely useful for primary health care. Ethanobotanist and natural drug pharmacist have reported that a number of medicinal plants are capable of curing various diseases (Dhar et al., 1968; Basu, 2002). Interest in plants with antimicrobial properties has revived as a result of many problems associated with the use of antibiotics (Abu-Shanab et al., 2004). Therefore, new prototype antimicrobial agents are needed to address this situation.

Juniperus communis L., a gymnosperm, belonging to the family Cupressaceae, (common name-Juniper) is a high altitude shrub which occurs at 2000 to 2400 m in Kumaun Himalaya. These species vernacular names are *Jhora, Betar, Pama,* etc. It is well documented for its medicinal value for diarrohea, abdominal pain, tumors, piles, bronchitis and indigestion in traditional system of medicine (Kirtikar and Basu, 1935).

Juniper berries (mature female cones) have long been used as flavouring agents in foods and alcoholic beverages such as gin (Clutton, 1972). It contains about 2% volatile oil, juniperin, resins (about 10%), proteins and formic, acetic and malic acids. The dried ripe fruits contain oil of juniper, pinene, cadinenes, camphene and a number of other diterpene acids. Dried berries of juniper and juniper decoction have been evaluated into recent animal studies (Swanston et al., 1990). In herbal medicine, juniper oil has been used as a carminative, diuretic and as a steam inhalant in the management of bronchitis. It has also been used in arthritis as well as antioxidant (Takacsova et al., 1995). Berries are also recommended in cough, infantile tuberculosis and diabetes (Zaman et al., 1970), whereas, ash of the bark is used for certain skin diseases (Baquar, 1989) . In Kumaun Himalaya there is paucity of information on the antimicrobial activity of gymnosperm plants and no record is available on *J. communis.* Therefore, the present study

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was carried out to examine the antibacterial activity of methanolic, ethanolic, chloroformic, hexane and aqueous extracts of leaves of *J. communis,* occurring at high altitude in Kumaun Himalaya, India against a wide range of pathogenic bacterial organisms.

MATERIALS AND METHODS

Collection of plant material

The leaves of *J. communis* (Cupressaceae) were collected in the month of October from Nainital, Kumaun Himalaya, India and authenticated by the Department of Botany, Kumaun University, Nainital. A voucher specimen was deposited in the herbarium of the department (KU 202).

Extraction procedure

Leaves of the plant were thoroughly washed and dried under shade at the room temperature (20 ± 2°C). The dried material was powdered in an electric grinder.

To prepare stock solution 50 g of this powder was added to 200 ml of solvents (w/v, 50 g/ 200 ml). Solvents used for extraction were methanol, ethanol, chloroform, hexane and water. Each extract was shaken for at least 6 h and after that each extract was passed through Whatman filter paper no.1 and the final filtrate as 25% crude extract thus concentrated on a rotary evaporator under vacuum at 20°C and was utilized for the experiments.

Microorganisms used

Five (gram +ve and -ve) bacteria (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No.40, *Agrobacterium tumefaciens* MTCC No.609, borrowed from Institute of Microbial Technology, Chandigarh, India and *Xanthomonas phaseoli* and *Erwinia chrysanthemi* were obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India) were used in this investigation.

Screening of antibacterial activity

Antibacterial tests of selected microorganisms were carried out using disc-diffusion method (Bauer et al., 1966). Nutrient agarplates (90 mm size) were prepared and cooled down at room temperature (20 ± 2°C). A small sterile cotton swab was dipped into the 24 h old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. This process was repeated by streaking the swab 2 or more times rotating the plates approximately 60° each time to ensure even distribution of inoculum. After inoculation the plates were allowed to dry at room temperature (20 ±2°C) for 15 min in laminar chamber for settle down of inoculum. The filter paper discs (5 mm) loaded with 40 µl of extract were placed on the surface of the bacteria seeded agar plates and it was allowed to diffuse for 5 min then these plates were incubated at 37 ± 1°C for 24 h.

Gentamycin (30 mcg), erythromycin (15 mcg) and ampicillin (10 mcg) were placed into agar plates used as positive control and respective solvent were also used as negative control. After 24 h of incubation, the diameter was observed for inhibition zones (measured in mm including disc size). Tests were performed in triplicates and observed values of ZOI are expressed as mean value with standard error of means (SME).

RESULTS

The results obtained in this investigation for the antibacterial activity of *J. communis* using disc diffusion method showed that all the bacterial strains are sensitive to tested extracts (methanol, ethanol, chloroform and hexane) except aqueous extract (Table 1). Their activity order was: hexane > ethanol > methanol > chloroform extract against all the tested microorganisms. Their zone of inhibition was 21 ± 0.6 mm for *E. coli,* 19 ± 0.6 for *A. tumefaciens,* 19 ± 1.0 for *X. phaseoli,* 18 ± 0.8 for *E. chrysanthemi,* and 16 ± 0.2 for *B. subtilis* (Table 1) . All the bacterial strains were found more sensitive to the tested extracts in comparison to commercially available susceptibility discs of erythromycin and ampicillin used as positive control (Plate 1).

DISCUSSION

As evident from the available literature, *J. communis* L. is well documented for its use for remedies of various ailments. Due to stressful climatic and geophysical conditions, Kumaun Himalayan region plants offer greater possibilities of having novel molecules and even larger quantities of active compounds (Dhawan, 1997; Kaul, 2010). Though the earlier workers in Kumaun have investigated *J. communis* for its ethnobotanical uses and chemical composition (Pandey and Pandey, 1999; Pandey and Mathela, 2000), the present investigation is the first attempt to explore the antibacterial potentiality of high altitudinal Kumaun Himalayan gymnospermous plant *J. communis.*

Relying upon the results obtained in the present investigation, it is clear that almost all extracts of leaves of *J. communis* were effective against the pathogenic bacteria except aqueous extract. The effectiveness of different extracts of *J. communis* was different. The hexane fraction showed more activity followed by ethanol and methanol extract. This might be due to the various substances that show activity against bacteria are more soluble in organic solvents than aqueous medium and therefore, not present in aqueous extract as suggested by Boer et al. (2005).

In a related study Clark et al. (1999) reported effectiveness of crude methanol and hexane extract of *Juniperus* sps. (heart wood and leaves) against animal pathogenic bacteria using agar-well technique. They observed hexane extract with highest activity against the tested bacteria thus supporting the present findings, hexane extract as the most effective fraction. Similarly, Kumar et al. (2010) also used crude extracts of *J. communis* (ethanol, methanol, chloroform, petroleum ether and aqueous) against some animal pathogenic bacteria using agar-well method. They found the chloroformic extract of *J. communis* leaves was most effective but in the present study the chloroform extract showed lowest

Table 1. Antibacterial activity of different extracts of *J. communis.*

	Diameter of inhibition zone(mm)*							
Microorganisms	**C**	**H**	**E**	**M**	**W**	**E**	**G**	**A**
A. tumefaciens	9.0 ± 0.6	19 ± 0.6	17 ± 0.3	11 ± 1.8	na	na	32 ± 4.0	na
E. coli	9.0 ± 0.6	21 ± 0.6	17 ± 0.2	12 ± 1.4	na	na	29 ± 2.9	na
E. chrysanthemi	13 ± 2.4	18 ± 0.8	11 ± 2.3	14 ± 2.8	na	na	28 ± 3.6	na
X. phaseoli	10 ± 0.2	19 ± 1.0	16 ± 1.5	11 ± 1.1	na	na	26 ± 2.4	na
B. subtilis	6.0 ± 0.2	16 ± 0.2	12 ± 0.2	13 ± 0.6	na	na	28 ± 2.8	na

* All the values are mean ± Standard Error of Mean of three determinations, C, H, E, M, W- Chloroform, Hexane, Ethanol, Methanol, Aqueous extracts, E, G, A**-** Erythromycin, Gentamycin, Ampicillin (+control), na**-** not active.

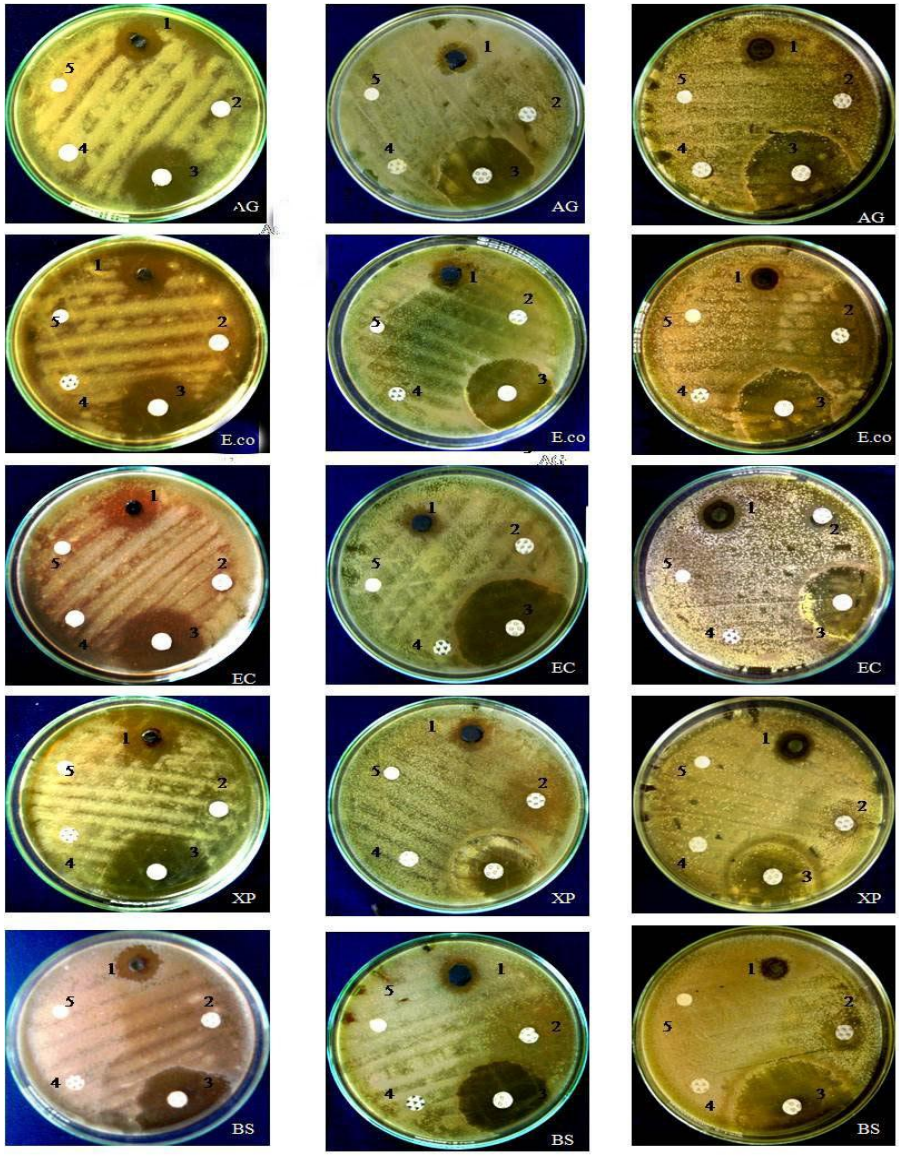

Plate 1. - Antimicrobial activity of ***J.*** *communis* extracts against some pathogenic bacteria. (A) - Hexane extract (B) - Methanol extract (C) - Ethanol extract. AG- *Agrobacterium tumefaciens,* E. co- *Escherichia coli* EC- *Erwinia chrysanthemi,* XP- *Xanthomonas phaseoli* BS- *Bacillus subtilis* 1- xtract, 2, 3, 4- positive controls (erythromycin, gentamycin, ampicillin), 5- negative control (solvent).

activity against all the strains. This variation in the results may be as a result of different techniques followed (Disc diffusion method). The non effectiveness of aqueous extract to all the bacterial strains supports the findings of Kumar et al. (2010). Other researcher such as Pepeljnjak et al.(2005) and Rezvani, (2009) conducted antimicrobial activity of isolated compounds of *J. communis* against animal pathogenic bacteria and their result is in agreement with this our study.

Majority of the previous workers tested the antibacterial activity of *J. communis* extracts/compounds employing agar-well method which requires comparatively larger amount of extracts/compounds. The present study was conducted by using disc-diffusion technique with a small fraction of extracts nevertheless; the results indicate that small fractions of *J. communis* extract are sensitive against the tested bacteria.

On the basis of available literature it was observed that there is no previous record on the sensitivity of these plant pathogenic bacterial strains *E. chrysanthemi, A. tumefaciens* and *X. phaseoli* which are responsible for various plant diseases like crown gall, leaf blight, leaf spot and rot disease. It concludes that *J. communis* leaves extracts possess a broad spectrum activity against a panel of bacteria responsible for the most common human and plant bacterial diseases.

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