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In-vitro antibacterial activity of *Juniperus communis* L. against bacterial pathogens

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Abstract

The present study was conducted to investigate antimicrobial activity of *Juniperus communis* against seven bacterial species. Aqueous extract either cold or water does not have any activity against all tested bacteria strains. However methanolic, ethanolic, chloroform, petroleum ether was active against *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus* but has no activity against *Salmonella typhi* and *Klebsiella pneumoniae*.

Keywords:- *Juniperus communis, Antimicrobial, Pathogens*

Introduction

Juniperus communis L. belong to family *Cupressaceae* grows well in heavy clay soils, tolerates a pH range from 4 to 8, succeeds in light woodland but dislikes heavy shade. Established plants are very tolerant of drought (Beckett and Beckett, 1979). Although the fully dormant plant is cold-tolerant throughout Britain, the young growth in spring can be damaged by late frosts. All parts of the plant are very aromatic (Genders, 1994). Juniper is a very polymorphic species that has a long history of culinary and medicinal use (Phillips and Foy, 1990). It is frequently grown in the ornamental and herb garden. Juniper fruits are commonly used in herbal medicine, as a household remedy, and also in some commercial preparations.The fully ripe fruits are strongly antiseptic, aromatic, carminative, diaphoretic, strongly diuretic, rubefacient, stomachic and tonic (Chiej, 1984; Launert, 1981; Lust, 1983; Uphof, 1959; Chopra *et al*., 1986). They are used in the treatment of cystitis, digestive problems, chronic arthritis, gout and rheumatic conditions. They can be eaten raw or used in a tea, but some caution is advised since large doses can irritate the urinary passage. Externally, it is applied as a diluted essential oil, having a slightly warming effect upon the skin and is thought to promote the removal of waste products from underlying tissues (Chevallier, 1996). It is, therefore helpful when applied to arthritic joints etc. The fruits should not be used internally by pregnant women since this can cause an abortion. The fruits also increase menstrual bleeding so should not be used by women with heavy periods (Chevallier, 1996). The plant has a variety of local uses. The dried fruit is used as flavouring in sauerkraut, stuffing and it is an essential ingredient of gin. The fruit is generally used in herbal medicine as a household remedy. They are especially useful in the treatment of digestive disorders, kidney and bladder problems (Grieve, 1984). The ripe fruits are used in the treatment of cystitis, digestive problems, chronic arthiritis, gout and rheumatic condition. It is applied as diluted essential oil, having a slightly warming effect upon the skin and is thought to promote the removal of waste products from underlying tissues.

In present study antibacterial activity of different fraction of *J. communis* leaves were tested against seven bacterial strains i.e. *Micrococcus luteus, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Listeria monocytogenes, E. coli* and *Staphylococcus sp.*

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Materials and Method

Collection of plant material

The leaves of *J. communis* was collected from Alkapuri base region of Garhwal Himalayas, Uttarakhand and identified by Botanical Survey of India, Dehradun. Leaves are shade dried and powdered using mortar pestle.

Extraction of plant material

100 gm of air dried powdered leaves were extracted with different solvent i.e methanol, ethanol, chloroform, petroleum ether, cold water and hot water. After extraction process was completed filtrate, which was obtained by the extraction, were concentrated in Rotary Evaporator (Butchi Type) till all the solvent evaporates. If it is not possible then extract were taken out in preweighed beaker (100 ml) and evaporate under water bath with porcelain particle or glass bead to avoid bumping of solvent and temperature should be maintained under boiling temperature of the solvent. Before putting the antibacterial activity all plant extract methanolic, ethanol, petroleum ether, ethyl acetate, chloroform, cold water and hot water extract were stored at the temperature of 4 °C. Bring out all the extract at room temperature when required at the time of antibacterial activity.

Antibacterial Assay

Bacterial strains

A total seven bacterial strains were used for this study i.e. *Micrococcus luteus*, *Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Listeria monocytogens, E. coli and Staphylococcus aureus* were obtained from IMTECH, Chandigarh.

Preparation of Inoculum

The ideal inoculum after overnight incubation gives the even semi confluent growth. Too heavy inoculum may reduce the size of inhibition zone by many antimicrobial agents from plant source. Using a straight wire touch 5-10 well isolated colonies of particular microorganism against which antimicrobial activity to be tested. Inoculate on the Nutrient Broth Medium. Incubate at 35-37°C for 4 – 6 hour. The density of the inoculums is adjusted to 10^8 cfu/ml by comparing with that of 0.5 Mc Farland Standard.

Agar well diffusion

0.1 ml of the original cultures (about10^6-10^7 cells) were added into sterile duplicate sets of Petri dishes and 25 ml of the molten (45° C) Mueller Hinton Agar (HiMedia, Ltd.) was poured into Petri dishes. The methanol extract (0.1 ml) were placed in wells (8 mm diameter) cut in the agar media and plates were incubated at 37 °C. The resulting inhibition zones obtained with bacteria were recorded after 24 hour.

Results and Discussion

Result of antibacterial activity is given in Table-1 aqueous extract either cold or water does not have any activity against all tested bacteria strains. However methanolic, ethanolic, chloroform, petroleum ether are active against *M. luteus*, *Ps. aeruginosa*, *L. monocytogens*, *E.coli* and *Staphylococcus* but has no activity against *Salmonella typhi* and *Klebsiella pneumoniae.*

Most active fraction in all the extract was methanolic followed by chloroform, ethanol and petroleum ether. Many author reported the antibacterial and antifungal activity of *Juniper* sp. against bacterial pathogen. Karaman *et al.* (2003), reported the antifungal and antibacterial activity of *J. communis* against 56 bacterial species and 31 isolates of 5 fungi species based on the inhibition zone using the disc-diffusion assay, minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values. The aqueous extract of Juniper sp. had no antimicrobial effect against the test microorganisms whereas the methanol extract had inhibitory effects on the growth of 56 strains of 24 bacterial species in the genera of *Acinetobacter, Bacillus, Brevundimonas, Brucella, Enterobacter, Escherichia, Micrococcus, Pseudomonas, Staphylococcus*, and *Xanthomonas*. In addition 11 *Candida albicans* isolates at a concentration of 31.25-250 micro g/ml were also inhibited. Earlier investigations on *J. procera* leaves and stem bark have yielded several antimicrobial diterpenes, including totarol, ferruginol, 4-*epi*-abietic acid, 4-*epi*-abietol, *E*-communic acid and *Z*-communic acid, of

Table-1: Effect of Different fraction of *J. communis* leaf against Bacterial species

Microorganism	Zone of Inhibition (in mm)					
	MeOH	EtOH	$CHCl_3$	PtEt	CW	HW
M. luteus	18.50±0.40	18.00±0.60	18.50±0.40	17.50±0.90	NA	NA
Ps. aeruginosa	16.50±0.30	16.00±0.20	16.50±0.20	14.50±0.60	NA	NA
S. typhi	NA	NA	NA	NA	NA	NA
K. pneumoniae	NA	NA	NA	NA	NA	NA
L. monocytogenes	16.00±0.60	16.10±0.10	17.00±0.50	17.50±0.20	NA	NA
E. coli	15.50±0.50	15.20±0.80	15.50±0.40	16.50±0.40	NA	NA
S. aureus	15.50±0.20	15.50±0.20	15.50±0.40	14.00±0.20	NA	NA

which totarol and ferruginol exhibited potentiating activities of INH against four typical mycobacteria: *M. intracellulare*, *M. smegmatis*, *M. xenopei* and *M. chelonei* (Mossa *et al.*, 1992, 2004; Muhammad *et al.*, 1992, 1995, 1996). Stefanovic *et al.* (2007), studied the antibacterial and antifungal activity of *J. communis* essential against *Agrobacterium tumefaciens, Bacillus subtilis, E. coli* and *P. fluorescens* and showed that essential oil of *J. communis* L. possess significant antibacterial activity in vitro, that can be attributed to the presence of various substances, mainly the phenolic monoterpene. The potential for developing antimicrobials from higher plants appear rewarding as it will lead to the development of phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effect that are associated with synthetic antimicrobials (Iwu, 1999). A scientific evidence has brought about the possibility of the utilization of plant extracts in the treatment of fungal and bacterial infections and the development of antibacterial and antifungal products (Famsworth, 1984). Furthermore, antimicrobial activity has also made a better understanding of the use of traditional medicine as potential drugs in addition to contemporary drugs (Cooposamy and Magwa, 2007). India is perhaps the larger producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in tradition, folk and herbal medicine, representing about 75% of the medicinal needs. More research should be done to find other plant sources to combat the treatment of deadly diseases.

In conclusion *J. communis* leaf extracts possess a broad spectrum of activity against a panel of bacteria responsible for most common bacterial disease.

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