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Research Article

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In vitro Antibacterial Studies on Essential Oils from *Juniperus communis* L.

Singh Digvijay[1]* and Bhardwaj SV[2]

[1]*Department of Biotechnology, School of Biosciences, Lovely Professional University, Phagwara, Punjab, India*
[2]*Department of Biotechnology, College of Horticulture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India*

ABSTRACT

Juniperus communis belongs to family Cupressaceae, yields 0.25 per cent of essential oil, resin, juniperin and organic acids. The active ingredients are antimicrobial, aphrodisiac and styptic in nature and are useful in treatment of ashthma, chronic bronchitis and diseases of liver and spleen. Shade dried leaves of plant were extracted with petroleum ether, ethanol and methanol and evaporated in vacuo to obtain the crude extract. Zone of inhibition and minimum inhibitory concentration (MIC) of plant extracts were evaluated by Microtiter plate method. Antibacterial assays of extracts showed considerable inhibition capacity and Extracts also displayed remarkable minimum inhibitory concentrations (≥20 µg/ml) which was significant compared to that (≥30 µg/ml) of the reference antibiotic.

Keywords: Antimicrobial activity; Minimum inhibitory concentration; Zone of inhibition; GC-MS

INTRODUCTION

World Health Organization (WHO) remarked that multitude of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are extensively used as medicine and comprise a major source of natural organic compounds. These plants have been assessed for their potential uses as alternative alleviations for the treatment of different infective diseases (Tepe et al., 2004).

Essential oils have been made known to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt, 2004 and Kordali et al., 2005). Some oils from plants have been utilized to treat diseases like cancer and tumor (Sylvestre et al., 2005). More or less, other oils have been used in preservation (Faid et al., 1995), aromatherapy (Buttner et al., 1996) and fragrance industries (Van de Braak and Leijten, 1999). Essential oils are excellent source of biologically active complexes. There has been an elevated concern in identifying at antimicrobial properties of extracts from aromatic plants particularly essential oils (Milhau et al., 1997). Therefore, it is judicious to envisage a variety of plant compounds in these oils with antimicrobial activity and antibiotic potential (Darokar et al., 1998).

Juniperus communis L. commonly known as Juniper belongs to the family *Cupressaceae* and with a circumpolar distribution throughout the cool temperate Northern Hemisphere from the Arctic south in mountains to around 30°N latitude in North America, Europe and Asia between an attitude of 3000-4500m (Stace, 2010). It yields 0.25 per cent of essential oil, resin, juniperin and organic acids (Rushforth, 1987). The active ingredient is aphrodisiac and styptic in nature and is useful in treatment of ashthma, chronic bronchitis and diseases of liver and spleen (Adams, 2004). Juniper berries have long been used as medicine by many cultures including the Navajo people (McCabe et al., 2005). Western American tribes combined the berries of *Juniperus communis* with Berberis root bark in a herbal tea. Native Americans also used juniper berries as a female contraceptive (Tilford, *1997*). [23]

The phytocompounds in *Juniperus communis* L. were observed through GC-MS analysis are terpen- hydrocarbons such as alpha and beta pinene, myrcene, sabinene, thujone, limonene, etc. Oil also contains sesquiterpene

hydrocarbons (caryophyllene, cadinene, elemene) and terpen alcohols (terpinen-4-ol). (Hänsel *et al*., 1999; Leung and Foster, 1996; Pepeljnjak *et al*., 2005).
Despite enormous antimicrobial potential of this plant, still much work has not been done to exploit its antimicrobial compounds and to develop herbal formulation against common bacterial infections. Therefore, present studies evaluate the antibacterial activity of *Juniperus communis* L. using *invitro* approaches.

EXPERIMENTAL SECTION

Material and methods

Media and chemicals:
Nutrient agar media (Hi media, India; final pH 7.3 ± 0.2 at 25°C), was used for the determination of MIC and antibacterial activity. Tetracycline (50 µg/disk) and ampicillin disks (50 µg/disk) were procured from Hi media.

Collection of plant material:
The leaves of *Juniperus communis* L. were collected in the month of March- April from Chitkul site of district Kinnaur, Himachal Pradesh, India and identified by Dr. Ravinder Raina Principal Scientist, Department of Forest Products, College of Forestry, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, H.P., India**.**

Preparation of plant extracts:
Leaves of the plants were thoroughly washed and dried under shade at the room temperature (20 ± 2°C). The dried leaves were then ground to a fine powder in an electric grinder. Stock solutions of the extract were prepared by adding ground leaf powder to 200 ml of each solvent (w/v, 50 g/ 200 ml). Different solvents used for extraction according to polarity were petroleum ether, methanol, ethanol, and water. Prepared extracts were then shaken for at least 6 h for homogenous mixing of ground leaf powder in the solvent. After that each extract was passed through Whatmann filter paper no.1. Final filtrate was then concentrated to 25% crude extract on a rotary evaporator or Soxhlet extraction apparatus under vacuum at 20°C and was utilized for the experiments.

Disc diffusion method:
Antibacterial tests of selected microorganisms were carried out using disc-diffusion method (Bauer *et al*., 1966). Nutrient agar plates (90 mm size) were prepared and cooled down at room temperature (20 ± 2°C). A small sterile cotton swab was dipped into the 24hour old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. This process was repeated by streaking the swab two 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 minutes 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 hour.

GC-MS analysis and compound identification:
GCMS was performed by using GC-MS Shimadzu Model QP-2010 mass spectrometer (Adams, 2000; Davies, 1990). The mass spectrum of unknown components was compared with spectrum of the known components stored in the NIST and Wiley library. Interpretation of mass spectrum of GC-MS was done using data base of NIST library having more than 75,000 compounds. The name, molecular weight and structure of components were then ascertained. The relative percentage was calculated by comparing its average peak area to the total area.

RESULTS AND DISCUSSION

Selection of *Juniperus communis* as test plant against microbes was more than justified because earlier reports by Peplinjale *et al.* in 2005 found that essential oils of Juniper inhibited that growth of Gram +ve bacteria viz. *Bacillus cereus, B.subtilis, Micrococcus flavus, M. luteus, S. aureus, S. epidermides* and *Enterococcus faecalli* and Gram –ve bacteria such as *Serratia*, *Salmonella, Proteus mirabilis, Shigella sonnei* and *Klebsiella oxytoca* and zone of inhibition varied between 10-16 mm. In the present studies, antimicrobial activity of Juniper leaves were analyzed against Gram +ve as well as Gram –ve bacteria. A very high growth inhibition of all the selected bacterial species was observed in methanol and ethanol extracts, whereas, aqueous and petroleum ether extracts showed low growth inhibition. This may be due to the type and polarity of the extract used. As is evident from GC-MS analysis,

majority of the compounds were extracted in methanol and ethanol extract as compared to that of petroleum ether and water. Just like present study, earlier workers have also reported antimicrobial activity of essential oils from Juniper (Hammer *et al*., 1999; Knoblock *et al*., 1989).

The anti-bacterial activity of different concentrations of *Juniperus communis* L. extract against four bacterial species is summarized in Table1 and Figures 1-4. The results revealed that plant extracts showed antibacterial activity with varying magnitudes.

Table 1: Zone of inhibition of plant extracts from *Juniperus communis* L. against bacterial species

Extract / Conc.(ppm)	Methanol			Ethanol			Petro. ether			Aqueous		
	10	20	30	10	20	30	10	20	30	10	20	30
E. coli	12.67	15.33	16.33	13.33	15.33	16.67	7.67	8.33	10.33	7.33	8.67	9.67
B. subtilis	13.33	15.67	16.67	10.67	15.67	16.33	7.33	8.33	9.33	7.33	12.67	13.33
P. aeruginosa	14.33	18.67	19.33	14.67	16.33	17.33	7.33	8.67	10.67	7.33	8.67	10.33
P. putida	20.67	22.33	23.33	12.33	17.67	18.67	8.67	9.33	10.33	9.67	9.33	10.67

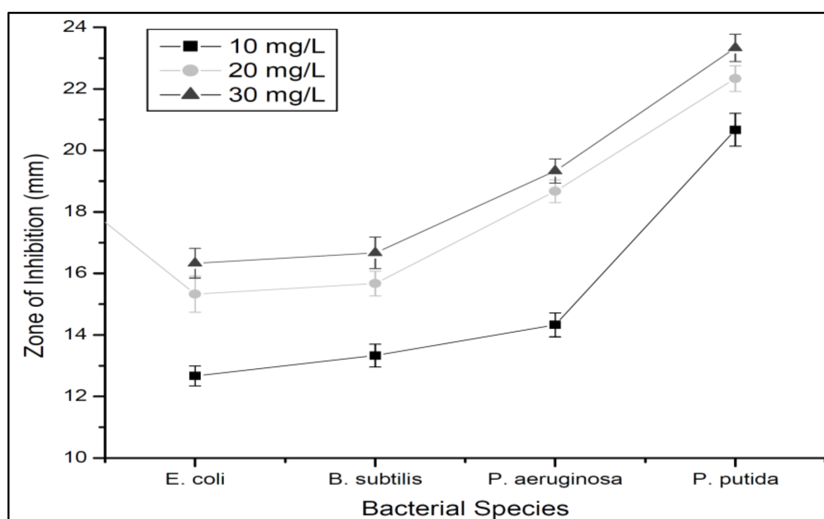

Figure 1: Graph showing zone of inhibition of Methanolic extract against Different bacterial species

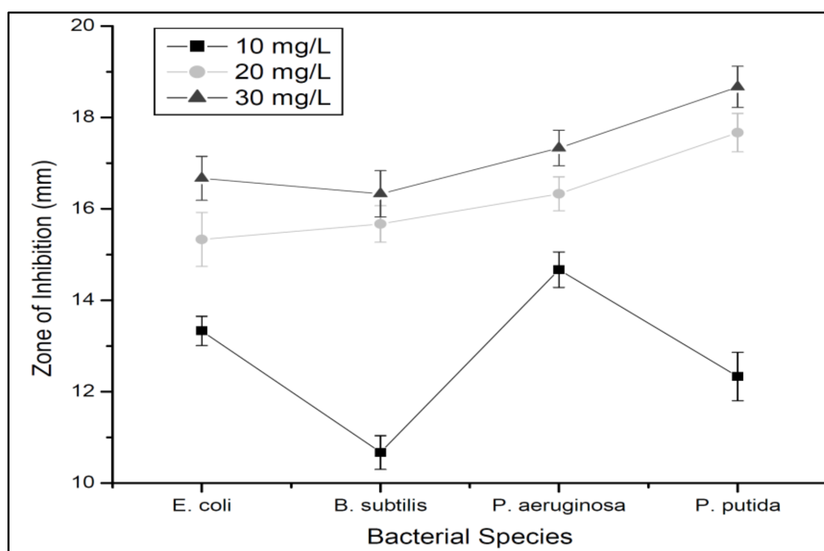

Figure 2: Graph showing zone of inhibition of Ethanolic extract against Different bacterial species

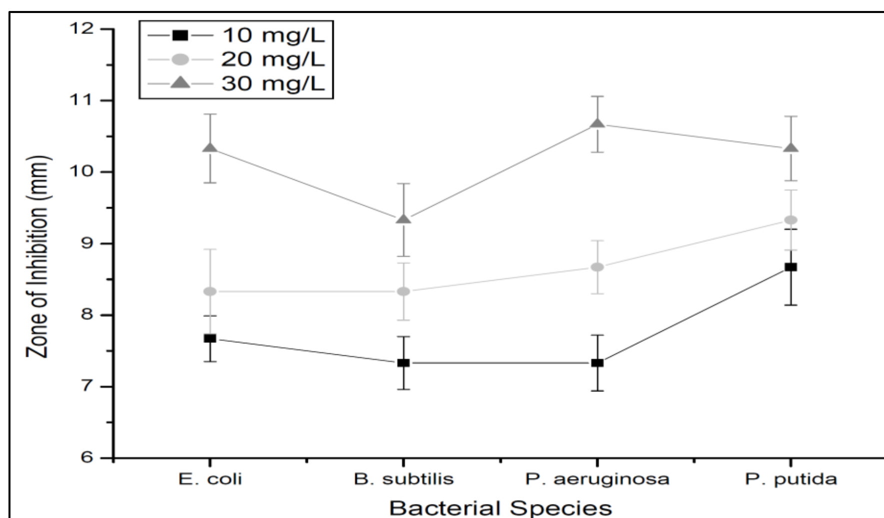

Figure 3: Graph showing zone of inhibition of Petroleum ether extract against Different bacterial species

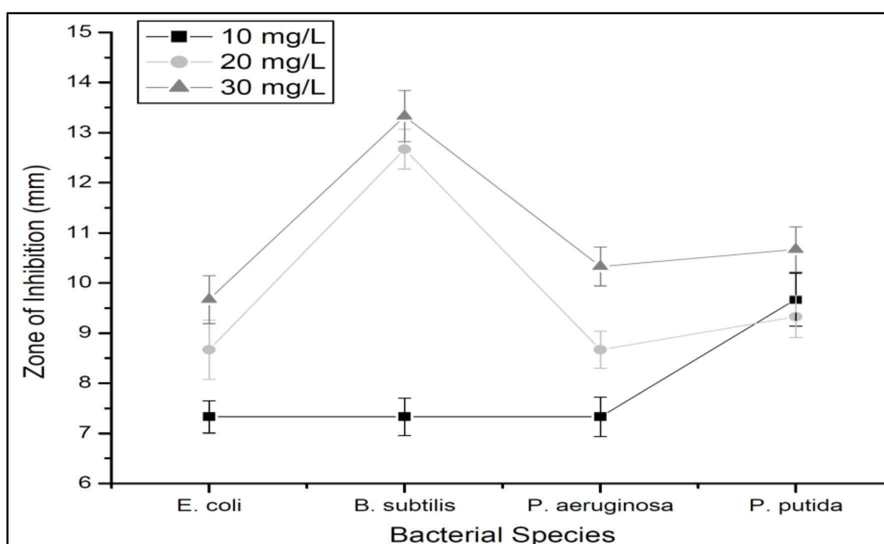

Figure 4: Graph showing zone of inhibition of Aqueous extract against Different bacterial species

The zone of inhibition above 8 mm in diameter was considered as positive result. Generally, most of the tested organisms were sensitive to these plant extracts at selected concentrations. Out of these four extracts, methanolic and ethanolic extracts showed maximum antibacterial activity against tested microorganisms. Methanolic extract at 30 mg/L concentration showed maximum activity against *Pseudomonas putida*. On the other hand, lowest antibacterial activity was shown by petroleum ether and aqueous extract at 10 mg/L concentration which means these extracts have failed to inhibit any of the tested strains. Both gram-positive and gram-negative bacteria were sensitive to the potent Plant extracts. *Pseudomonas putida* was inhibited successfully by all the plant extracts and the maximum value for zone of inhibition was found to be 23.33 mm in methanolic extract followed by *Pseudomonas aeruginosa* (19.33 mm), *Bacillus subtilis* (16.67 mm) and *E. coli* (16.33 mm) (Figure 1). Moderate effects were at 10mg/L and 20 mg/L concentration of these plant extracts. No obvious difference in susceptibility was found between gram-negative and gram-positive bacteria (Figure 2-4). There was no inhibition of growth with the vehicle control (10% DMSO).

Several factors are known to influence the active principle present in the plants. The activity of plant extracts against both gram positive and gram negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Traditional practitioners make use of water preliminary as solvent (Rabe and Van Staden, 1997; Parekh and Chanda, 2007), but present investigations of antibacterial

activities showed that the methanol, ethanol and petroleum ether extract of plant parts were certainly much better and powerful. This may be due to the better solubility of their active components in organic solvents. Using crude extracts of *Juniperus communis* L. (ethanol, methanol, chloroform, petroleum ether and aqueous) against some animal pathogenic bacteria using agar-well method showed significant inhibition of bacterial strains (Kumar *et al.,* 2010). It is also found that chloroform extract of *Juniperus communis* leaves was most effective but in the present study the methanol extract showed highest activity against *Xanthomonas axonopodis* pv. *punicae*. This variation in the results may be as a result of different techniques followed (Disc diffusion method). Some researchers have also conducted antimicrobial activity of isolated compounds of *Juniperus communis* against animal and plant pathogenic bacteria and their results are in agreement with the present studies (Pepeljnjak *et al*., 2005; Rezvani *et al*., 2009). It has however been concluded by Sati and Joshi in 2010 that *J. communis* leaf extracts possess a broad spectrum activity against a panel of bacteria responsible for the most common human and plant bacterial diseases.
The compounds which are observed to be present in high concentration in essential oil of *Juniperus communis* L. plant solvent extracts are terpene hydrocarbons such as alpha and beta pinene, myrcene, sabinene, thujone, limonene, etc. It also contains sesquiterpene hydrocarbons (caryophyllene, cadinene, elemene) and terpene alcohols (terpinen-4-ol) which were previously well documented by various researchers when they extracted the essential oil of *Juniperus communis* L. in different solvent extracts (methanol, ethanol, petroleum ether, n-hexane and water) and further analyzed through GC-MS analysis (Hänsel *et al*., 1999; Leung and Foster, 1996; Pepeljnjak *et al*., 2005).

CONCLUSION

The present study also revealed the presence of similar essential oil components. In the GC-MS chromatogram 91, 69 and 51 peaks were observed corresponding to the presence of similar number of compounds in methanol, ethanol and petroleum ether extracts of *Juniperus communis* L.; of these Phenanthrene carboxylic acid was the major compound (11.44%) followed by Cedrol (9.12 %), 8- Beta cedron-8-ol (7.28%) and Alpha-cadinol (7.04%). Remaining chemical compounds were in trace amounts. The major components and their retention times are summarized in Table 2.

Table 2: Major chemical compounds of *Juniperus communis* L. essential oil (GC-MS analysis)

S.No.	Compound name	Retention time (Min.)	Area (%)
1.	Phenanthrene carboxylic acid	19.02	11.44
2.	Cedrol	19.04	9.12
3.	8- Beta cedron-8-ol	18.77	7.28
4.	Alpha-cadinol	18.76	7.04
5.	Dodecanoic acid	49.92	6.40

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REFERENCES

[1] RP Adams. Junipers of the World: The genus Juniperus, 4th Edition, Trafford Publications, Victoria. **2004**: 49-53.
[2] AW Bauer; WMM Kirby; JC Sherris; M Truck. *Am J Clin Pathology*, **1966**, 45(4), 493-496.
[3] AY Leung; S Foster. Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics, 2nd Edition, John Wiley & Sons, New York, **1996**; 28-35.
[4] B Tepe; D Daferera; M Sokmen; M Polissiou; A Sokmen. *J Agric Food Chem*, **2004**, 52(5): 1132–1137.
[5] CA Stace. New flora of the British Isles, 3rd Edition, Cambridge University Press, Cambridge, **2010**: 54-55.
[6] G Milhau; A Valentin; F Benoit; M Mallie; J Bastide; Y Pelissier; J Bessiere; J. Essent. Oil Res. **1997**, 9(3): 329–333.
[7] G Tilford. Edible and Medicinal Plants of the West, 1st Edition, Mountain Press Publishing Company, Utah, **1997**: 2.
[8] J Parekh; SV Chanda. *Turk J Biology,* **2007**, 31(1): 53-58.

[9] K Knoblock; A Pauli; B Iberi; H Weig Andand; N Weis. *J Essential Oil Research,* **1989**, 1: 119-128.
[10] K Rushforth. Conifers. Helm, Batsford Ltd, Nevada, **1987**: 68-69.
[11] KA Hammer; CF Carson; TV Riley. *J applied microbiol*, **1999**, 86(6): 985-990.
[12] M Faid; K Bakhy; M Anchad; A Tantaoui-Elaraki; Almond paste. *J Food Prod*, **1995**, 58: 547–550.
[13] M McCabe; D Gohdes; F Morgan; J Eakin; M Sanders; C Schmitt. *Diabetes Care*, **2005**, 28 (6): 1534–1535.
[14] M Sylvestre; A Pichette; A Longtin; F Nagau; J Legault. *J Ethnopharmacol*, **2006**, 103(1): 99–102.
[15] MP Buttner; K Willeke; SA Grinshpun. Sampling and analysis of airborne microorganisms. In: CJ Hurst; GR Knudsen; MJ McInerney; LD Stetzenbach; MV Walter. Manual of Environmental Microbiology, 3rd Edition ASM Press, Washington, DC. **1996**: 629–640.
[16] MP Darokar; A Mathur; S Dwivedi; R Bhalla; SPS Khanuja; S Kumar. *Curr Sci*, **1998**, 75:187-189.
[17] N Davies. *J of Chromatography*, **1990**, 503: 1-24.
[18] P Kumar; RP Bhatt; OP Sati; VK Dhatwalia; L Singh. *Int J Pharma and Bio Sciences*, **2010**, 6(1): 1-7.
[19] R Hänsel; O Sticher; E Steinegger. Pharmakognosie –Phytopharmazi*,* 6th Edition, Springer, Berlin, **1999**: 860-861.
[20] RP Adams. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, 4th Edition, Allured Pub Corp, Illinois, **2001**: 455-456.
[21] S Kordali; R Kotan; A Mavi; A Cakir; A Ala; A Yildirim. *J Agric Food Chem*, **2005**, 53: 9452–9458.
[22] S Pepeljnjak; I Kosalec; Z Kaloera; N Blazevic. Antimicrobial activity of Juniper berry essential oil. *Acta Pharmaceutica,* **2005**, 55(4)**:** 417-422.
[23] S Rezvani; M Ali Rezai; N Mahmoodi. *Rasayan J chemistry* **2009**, 2(2): 257-260.
[24] SA Burt. *Inter J Food Microbiol*, **2004**, 94(3): 223–253.
[25] SAAJ Van de Braak; GCJJ Leijten. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, **1999**: 116.
[26] SC Sati; S Joshi. *Afr J Microbiol Res*, **2010**, 4(12): 1291–1294.
[27] T Rabe; J Van. *J Ethnopharmacology*, **1997**, 56: 81-87.