

Antioxidant and anticandidal activity of *Juniperus communis* L.

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Received: September 11, 2009 | Accepted: January 27, 2010 | Online: April 4, 2010

Abstract

Different solvent extract methanolic, ethanolic, Petroleum ether, Chloroform, Hot and cold aqueous extract of *Juniperus* leaves was tested against *Candida albican*. It was found that all the tested solvent extract Aqueous extract both cold and hot water of *J. communis* showed no activity against *C. albican*. However ethanol and petroleum ether showed maximum inhibitory action against *C. albican*. The MIC value of Petroleum ether extract was 25mg/ml, while in case of ethanol it was 50 mg/ml. Antioxidant activity of *Juniperus* leaves was evaluated by three methods. All method used showed that leaves of *Juniperus* has antioxidant activity.

Keywords: *C. albican* | *J. communis* |

Antioxidant activity

Introduction

Candida albicans, opportunistic pathogen, remains leading cause of fungal diseases, frequently mortal in immunocompromised individuals. There is no licensed vaccine yet, despite the fact that candidiasis occur at 75% of all females during the lifetime (Sobel, 1988). Prolonged antifungal therapy, to prevent recurrence, cannot be without side effects. The serious problem is increased drug resistance (Wingard, 1994). Search for alternative way of fighting with the disease is very necessary. The dimorphic fungus *C. albican* is both a commensal and an opportunistic pathogen in humans. Depending on the underlying host defect, this microorganism is able to cause a variety of infections that range from mucosal candidiasis to life-threatening invasive infections. The frequency of the latter has increased in recent years as a result of an expanding immunocompromised population (Calderone, 2001; Garber, 2001).

The genus *Juniperus* includes 60 to 70 species of aromatic evergreen plants native to North Europe, Asia and North America. The plant bear blue or reddish fruits variously described as berries or berries like cones, juniper widely used as ornamental trees. The cone is a small

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green berry during its first year of growth and turn blue black during the second year. The flower blooms from May to June.

Juniperus communis L. which is known as common juniper is evergreen coniferous shrub or small tree occurring through out the northern hemisphere from Europe to Siberia and grow upto the height of 10m; it can be either prostrate or erect. Its preferred habitat is heath, moorland and chalk downs, but is also found as undergrowth in mixed open forest. Juniper berry oil has been used as diuretic. This activity is due to the presence of terpinen-4-ol, which is known to increase glomerular filtration rate. The effect of Juniper oil has been reported against urinary tract infection (Schilcer, 1995). Pepeljnjak *et al.* (2005) studied the antibacterial activity against sixteen bacterial species seven yeast-like fungi, three yeast and four dermatophyte strains. Juniper essential oil showed similar bactericidal activities against Gram-positive and Gram-negative bacterial species, with *MIC* values between 8 and 70% (V/V), as well as a strong fungicidal activity against yeasts, yeast-like fungi and dermatophytes, with *MIC* values below 10% (V/V). The strongest fungicidal activity was recorded against *Candida* spp. (*MIC* from 0.78 to 2%, V/V) and dermatophytes (from 0.39 to 2%, V/V).

Materials and Methods

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 pre weighed 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.

Anticandidal activity

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 10⁸ cfu/ml by comparing with that of 0.5 Mc Farland Standard.

Agar Well Diffusion

0.1 ml of the original cultures (about 10⁶-10⁷ cells) was spreaded with help of sterile non toxic swab on Mueller Hinton Agar (HiMedia,

Ltd) plates. After drying the different solvent extract (0.1ml) was placed in wells (8mm diameter) cut in the agar media and plates were incubated at 37° C (Kıvan and Akgül, 1986). The resulting inhibition zones obtained with bacteria were recorded after 24 hour.

Antioxidant activity

Superoxide dismutase (SOD) activity assay

The assay of superoxide dismutase was done according to the procedure of Das *et al.* (2000). In this method, 1.4ml aliquots of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer of pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM Hydroxylamine hydrochloride and 0.1ml of 50 mM EDTA) was added to 100 ml of the sample extract and incubated at 30°C for 5 minutes. 80 ml of 50 mM riboflavin was then added and the tubes were exposed for 10 min to 200 W fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanimide in 5% phosphoric acid) was added and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions

Peroxidase activity

The assay was carried out by the method of Addy and Goodman (1972). The reaction mixture consisted of 3ml of buffered pyrogallol (0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5 ml of 1% H₂O₂. To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol).

Glutathione peroxidase Activity

Glutathione peroxidase was assayed according to the procedure of Rotruck *et al.* (1973) with some modifications. The reaction mixture consisting of 0.4 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H₂O₂, 0.2 ml of water and 0.5 ml of enzyme was incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5 ml of 10% TCA and after centrifugation; 2 ml of the supernatant was added to 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). The color developed was read at 412 nm and the enzyme activity is expressed in terms of mg of glutathione utilized/min/mg protein.

Results and Discussion

Anticandidal activity

In the present study the anticandidal activity of six extracts of *J. communis* (Methanolic, ethanolic, Petroleum ether, Chloroform, cold water and hot Water) was evaluated against *Candida albican* (Table 1). Aqueous extract both cold and hot water of *J. communis* showed no activity against *C. albican*. However ethanol and petroleum ether showed maximum inhibitory action against *C. albican*, zone of inhibition was found 19.0 ± 1.2 mm and 22.0 ± 0.5 mm respectively (Fig.1). The MIC value of Petroleum ether extract was 25mg/ml, while in case of ethanol it was 50 mg/ml. In case of Methanolic and chloroform extract of *J. communis* almost equal inhibition was obtained, zone of inhibition was 16.0 ± 0.5mm and 16.5 ± 1.0 mm respectively. MIC value in both extract was 50 mg/ml (Table 2). Anticandidal activity of *Juniperus sp.* was also reported by Karaman *et al.* (2003).



Fig. 1. Anticandidal activity of Different solvent extract of Juniperus leaves.

- 1. Methanolic**
- 2. Ethanolic**
- 3. Chloroform**
- 4. Petroleum ether**
- 5. Hot water**
- 6. Cold water**

In addition, these results confirmed the evidence in previous studies reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents, such as water, ethanol and hexane (Ahmad et al., 1998; Eloff, 1998; Lin et al., 1999).

The antifungal activity of the extracts from unground leaves was strong against *Cryptococcus neoformans* and *T. mentagrophytes* from a number of taxa. *Juniperus osteosperma* and both varieties of *J. occidentalis* were particularly active against *C. neoformans* (hexane extract). The hexane extracts of these taxa, which are active against *C. neoformans*, are noticeably ineffective against *T. mentagrophytes*. The methanol (polar) extracts of *J. californica* and *J. osteosperma* showed activity against *T. mentagrophytes* (Clark et al., 1990).

Methanol and dichloromethane extracts of leaves and stems of *Juniperus sp. oxycedrus* (from Spain) have been found to reduce the blood pressure of normotensive rats (Bello et al., 1997), to inhibit the response to histamine, serotonin and acetylcholine (Moreno et al., 1997), and to exhibit significant anti-inflammatory activity (Moreno et al., 1998). Several extracts of leaves, resins, barks and fruits of *Juniperus sp.* (from Turkey) were found to inhibit the growth of several bacteria.

Antioxidant Activity

The antioxidant enzymes and free radical scavengers may provide a defensive mechanism against the deleterious actions of ROS (Reactive Oxygen Species). Some of the antioxidant enzymes that are found to provide a protection against the ROS are superoxide dismutase, catalase, peroxidase, glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase and ascorbate oxidase (Bandyopadhyay et al., 1990). The non-enzymatic antioxidants which act as scavengers are glutathione, vitamin A, vitamin E, and vitamin C (Acker et al., 1993).

Antioxidant activity of *J. communis* leaves was determined by three enzymatic methods viz Superoxide dismutase (SOD), Peroxidase and Glutathione peroxidase which have been presented in Table 2. Results showed that the leaves of *Juniperus* have significant antioxidant activities. Antioxidant activity of *Juniperus* leaves were observed to be 2.9 unit/mg by Superoxide dismutase enzymatic method and 0.9×10^6 and 160 international units by using, Peroxidase and Glutathione Peroxidase activity methods respectively. Brits et al. (2001) also reported the antioxidant activity of *juniperus* sp.

	Zone of Inhibition (in mm)					
	MeOH	EtOH	CHCl ₃	PtEth	Cold water	Hot water
<i>Candida albican</i>	16.0 ± 0.5	19.0 ± 1.2	16.5 ± 1.0	22.0 ± 0.5	NA	NA

Table 1. Anticandidal activity of *J. communis* leaf extract.

	Minimum Inhibitory Concentration					
	MeOH	EtOH	CHCl ₃	PtEth	Cold water	Hot water
<i>Candida albican</i>	50 mg/ml	50 mg/ml	50 mg/ml	25mg/ml	NA	NA

Table2. Minimum inhibitory concentration of *J. communis* leaf extract

S.No.	Sample	SOD	Peroxidase Activity (IU/L)	Glutathione peroxidase
1	Juniperus communis leaves	2.9 unit/mg	0.9X 10 ⁶	160
			1 unit- micro moles pyragallol oxidized/min	I unit- mg of GSH utilized/min

Table 3. Antioxidant activity of *J. communis* leaves.

References

- Acker, V., Koymans, L.M.H. and Bast, A. (1993) Molecular pharmacological importance of vitamin E, structural aspects of NADP free radical. *Biol. Med.* 5, 311-328.
- Addy, S.K. and Goodman, R.N. (1972). Polyphenol oxidase and peroxidase in apple leaves inoculated with a virulent or an avirulent strain for *Ervinia amylovora*. *Ind. phytopath.* 25, 575-579.
- Ahmad, I., Mehmood, Z., Mohammad, F., 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology* 62, 183–193.
- Bandyopadhyay, U., Das, D and Banerjee, R. (1990) Reactive oxygen species: Oxidative damage and pathogenesis. *Curr. Sci.* 77, 658-666.
- Bello, R., Moreno, L., Beltra'n, B., Primo-Yu' fera, E., Esplugues, J. 1997. Effects on arterial blood pressure of methanol and dichloromethanol extracts from *Juniperus oxycedrus* L. *Phytotherapy Research* 11, 161-162.
- Burits M., Asres, K and Bucar, F. 2001. The Antioxidant Activity of the Essential Oils of *Artemisia afra*, *Artemisia abyssinica* and *Juniperus procera*. *Phytotherapy Research.* 15: 103-108.
- Calderone, R.A., 2001. *Candida* and candidiasis, ASM Press, New York,
- Clark, A.M., McChesney, J.D and Adams, R.P. 1990. Antimicrobial Properties of Heartwood, Bark/Sapwood and Leaves of *Juniperus* Species. *Phytotherapy Research.* 4 (1):15-19.
- Das, K., Samanta, L. and Chainy, G.B.N. (2000) A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. *Ind. J. Biochem. Biophys.* 37, 201-204.
- Eloff, J.N., 1998. Which extract should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60, 1–8.
- Garber, G. 2001. An overview of fungal infections, *Drugs* 61 (Suppl. 1):1–12.
- Karaman, I, Sahin, F., Güllüce, M., Ö'gütçü H., Sengül, M., Adıgüzel, A. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *Journal of Ethnopharmacology* 85 (2003) 231–235.
- Kıvanç, M. and Akgül, A. 1986. Antibacterial activities of essential oils from Turkish spices and citrus" *Flavour and Fragrance Journal.* 175-179.
- Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager, A.K., van Staden, J., 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology* 68, 267–274.
- Moreno, L., Bello, R., Beltra'n, B., Calatayud, S., Primo-Yu' fera, E., Esplugues, J. 1998. Pharmacological screening of different *Juniperus oxycedrus* L. extracts. *Pharmacology and Toxicology* 82, 108-112.
- Moreno, L., Bello, R., Primo-Yu' fera, E., Esplugues, J. 1997. In vitro studies of methanol and dichloromethanol extracts of

- Juniperus oxycedrus* L. Phytotherapy Research 11, 309- 311.
- Pepeljnjak, S., Ivan, K., Zdenka, K., Era, N. 2005. Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L., *Cupressaceae*). *Acta Pharm.* 55 (2005) 417-422.
- Rotruck, J.T., Pope, A.L. and Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. (1973) Selenium: Biochemical role as a component of glutathione peroxidase. *Science.* 179, 588-590.
- Schilcher H 1995. Juniper berry oil in diseases of the efferent urinary tract?] *Med Monatsschr Pharm.* 18(7):198-9
- Sobel, J.D., 1988. Pathogenesis and epidemiology of vulvovaginal candidiasis, *Ann. NY Acad. Sci.* 544:547-557.
- Wingard J.R.1994. Infections due to resistant *Candida* species in patients with cancer who are receiving chemotherapy, *Clin. Infect.Dis.* 19 (Suppl. 1) (1994) S49-S53.

