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Study of antibacterial and antifungal activity of traditional *Cedrus deodara* and *Pinus roxburghii* Sarg

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ABSTRACT

In the present study, the volatile oil, chloroform extract and methanol extract of the woods of the plants *Cedrus deodara* and *Pinus roxburghii* were screened for their antibacterial and antifungal activities *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Candida albicans*, *Aspergillus niger,* and *Aspergillus clavatus* using the Agar diffusion method. The susceptibilities of the microorganisms to the extracts were compared with each other and with a selected standard antibiotic. It was observed that the volatile oil and chloroform extracts showed the significant antibacterial activities while the least antibacterial activity was recorded with the methanolic extracts. The higher Minimum Inhibitory Concentration value of the extracts and oils against fungus suggested that the plants may possess less antifungal activity. Phytochemical analysis and thin-layer chromatography profiling revealed the presence of flavonoids and terpenoids in the oil and chloroform extracts, which could explain the antimicrobial activity. The findings suggest that the *Cedrus deodara* and *Pinus roxburghii* have antimicrobial properties and they can be used in the treatment of infectious diseases. However, further work is required in order to isolate the active constituents of the plants responsible for the antibacterial activity.

Keywords *Cedrus deodara*, *Pinus roxburghii*, antimicrobial, flavonoids, terpenoids, ampicilin

INTRODUCTION

The plants *Cedrus deodara* Loud. and *Pinus roxburghii* Sarg. belonging to family pinacae are commonly known as sedar and chir pine have long been known for their medicinal values (Shah et al., 2006). The chemical constituents obtained from different parts of *Cedrus deodara* include wikstromal, matairesinol, dibenzylbutyrolactol (Rao et al., 2003; Singh et al., 2007), berating, isopimpillin, lignans 1, 4 diaryl butane, benzofuranoid neo lingam, isohemacholone, sesquiterpenes LIII: deodarone, atlantone (Shankaranaryan et al., 1977), deodarin, deodardione**,** limonenecarboxylic acid, α-himacholone, β-himacholone**,** cedrin (6-methyl dihydromyricetin), taxifolin, cedeodarin (6-methyltaxifolin), dihydromyricetin and cedrinoside (Agrawal et al., 1980). The phytoconstituents obtained from *Pinus roxburghii* include friedelin, ceryl alcohol, β-sitostertol, Longifolene, α-pinene, β-pinene, car-3-ene, abietic acid and isopimaric acids.

Cedrus deodara and *Pinus roxburghii* have a long history of numerous traditional and ethnobotanical applications in diverse cultures (Hussain et al., 2006; Kirtikar and Basu, 2006; Kunwar et al., 2009; Shah, 2006). Traditional *Cedrus deodara* has medicinal value and is bitter, hot, pungent, light, oleaginous, diuretic, analgesic, diaphoretic, carminative, and used in belching inflammations, dyspepsia, insomnia, hiccoughs, fever, urinary discharge, epilepsy, skin disease, pulmonary disorder, urinary disorder, bronchitis, itching, elephantitiasis, tuberculous glands, leucoderma, ophthalmia, piles, disorders of the mind and ulcers. All parts of the *Cedrus deodara* are useful in Ayurveda for the treatment of insomnia, disorders of the mind, and diseases of the skin and blood. *Pinus roxburghii* is sweet, bitter, pungent, heating, oleaginous, intestinal antiseptic, purgative, carminative, expectorant, aphrodisiac, fattening, diuretic, anthelmintic, stimulant and analgesic and is used in diseases of the eye, diseases of the liver and spleen, ear, throat and skin, bronchitis, diaphoresis, giddiness, ulcer, inflammation and itching. Many tribes considered it as a cure for all ailments. However, there is not enough scientific data to support the claims made in the ancient literature. The literature survey reveals that some work has already been done on the plants; however, most of the activities are still without scientific backing.

Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agents with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes. The present work was an attempt to evaluate the antibacterial and antifungal activities of the wood oil, chloroform and the methanolic extracts of *Cedrus deodara* and *Pinus roxburghii* and to generate scientifically justified data to support the traditional use.

MATERIALS AND METHODS

Collection and authentication of plant material

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The stem wood of *Cedrus deodara* and *Pinus roxburghii* were collected from naturally growing regions of Pauri Garhwal, Uttarakhand, India. The stem wood of *Cedrus deodara* was authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen has been deposited at the RHMD (NISCAIR/RHMD/Consult /-2011-12/1711/11 dated April 11, 2011). The stem wood of *Pinus roxburghii* was authenticated by Dr. E. Roshni Nayar, Principal Scientist, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. A voucher specimen has been deposited at the NBPGR Herbarium (NHCP/NBPGR/2011-14/7288 dated April 07, 2011).

Drying and communition of plant materials

The wood of *Cedrus deodara* and *Pinus roxburghii* were thoroughly washed and then dried under shade 25 ± 2°C for 10 days. The dried plant samples were grounded well into a fine powder in a mixer grinder and sieved to give a particle size of 50 - 150 mm.

Isolation of volatile oil

The plants material (stem wood of *Cedrus deodara* and *Pinus roxburghii),* were subjected to steam distillation by using a Clevenger apparatus. On cooling, the essentials oil was separated from the aqueous layer, dried over anhydrous sodium sulphate and stored in amber colored glass bottles in a cool place (Handa et al., 2008; Shinde et al., 1999).

Preparation of plant extracts

The powder plants material (stem wood of *Cedrus deodara* and *Pinus roxburghii)* were extracted successively with petroleum ether, chloroform and methanol using a Soxhlet extraction apparatus. The solvents were completely removed under reduced pressure until the semi solid mass was obtained. The extracts were stored in amber colored bottles and kept in a refrigerator until further use.

Phytochemical testing

The wood oils and extracts were subjected to preliminary phytochemical screening for the presence of carbohydrates, alkaloids, amino acids, fats and fixed oils, flavonoids, glycosides, saponin, tannins, proteins, steroids and triterpenoids (Khandelwal 2004; Wagner et al. 2001). The carbohydrate was evaluated by using a Molish and Fehling solution test. The alkaloids content were determined by using Dragendorff's and Hager's tests. The phenolic compounds content were determined by ferric chloride and dilute iodine solution tests. The flavonoids were evaluated by Shinoda, alkaline reagent and zinc hydrochloride tests. The presence of O and C glycosides was determined by using Borntrager's and a modified Borntrager's tests. The presence of fats and fixed oils was determined by a Spot test and a solubility test. The terpenoid contents were determined by a Vanillin sulphuric acid test, 5% phosphomolybdic acid in ethanol test and a 10% ammonium molybdate sulphuric acid test. The protein content was determined by Biuret and ninhydrin tests**.** The presence of steroids was determined by Salkowski, Libermann-Burchard reactions and Liberman reactions**.**

Thin-layer chromatography (TLC) profiling of extracts/volatile oil

For the TLC profile of extracts and oils, the samples were subjected to TLC of silica gel G plates. The plates were developed using various ratios of solvent system: hexane: ethyl acetate (for oils and chloroform extract) and chloroform: methanol (for methanolic extract) as the mobile phase. The spots were visualised using anisaldehyde sulphuric acid at a temperature of 120°C.

Antimicrobial screening of extracts and volatile oil by agar dilution method

Dilution methods were used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents. In the agar dilution tests, microorganisms were tested for their abilities to produce visible growth on a series of agar plates containing dilutions of the antimicrobial agents. The lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism was known as MICs (Barry et al., 1999). The antimicrobial study of extracts and oils was conducted against test microorganisms, including bacteria (gram -ve and gram +ve) and fungi using standard dilution methods. The pure cultures of the gram –ve bacteria (*E. coli, P. aurigenosa), gram +ve bacteria (S. aureus, B. subtilis and S. pyogenes*) and fungi (*C. albicans, A. niger and A. clavatus*) were used for the study. The positive controls used were ampicilin, chloramphenicol and greseofulvin.

RESULTS

Yield of wood oils

The 1000 gm wood powder of *Cedrus deodara* was taken for the extraction of volatile oil by a water distillation method using a Clevenger apparatus. A total of 4.50 ml of volatile oil was obtained. The yield of volatile oil of *Cedrus deodara* was found to be 0.45% v/w. The 1200 gm wood powder of *Pinus roxburghii* was taken for the extraction of volatile oil by a water distillation method using a Clevenger apparatus. A total of 3.80 ml of volatile oil was obtained. The yield of volatile oil of *Pinus roxburghii* was found to be 0.32% v/w.

Yield of wood extracts

The 260 gm wood powder of *Cedrus deodara* was taken for extraction. A total of 17.00 gm of dry chloroform and 8.80 gm of methanolic extracts were obtained. The yield of the chloroform and methanolic extracts were found to be 6.80 and 2.67% w/w, respectively. The 400 gm wood powder of *Pinus roxburghii* was taken for extraction. A total of 8.00 gm of dry chloroform and 4.25 gm of methanolic extract were obtained. The yield of chloroform and methanolic extracts were found to be 2.00 and 1.06% w/w, respectively.

Phytoconstituents of volatile oil

Phytochemical testing showed that the volatile oil of *Cedrus deodara* contains terpenoids, steroids, phenols, fixed oil and fats. Whereas the volatile oil of *Pinus roxburghii* contains

Table 1. Phytochemical constituents present in volatile oil the *Cedrus deodara* and *Pinus roxburghi*

Phytoconstituents	Wood oil	
	Cedrus deodara	***Pinus roxburghii***
Alkaloids	-	-
Glycosides	-	-
Fixed oils and fats	+	+
Phenolic compounds	+	-
Tannins	-	-
Proteins and Amino acids	-	-
Terpenoids	+	+
Steroids	+	+
Flavonoids	-	-

+: present; -: not detected

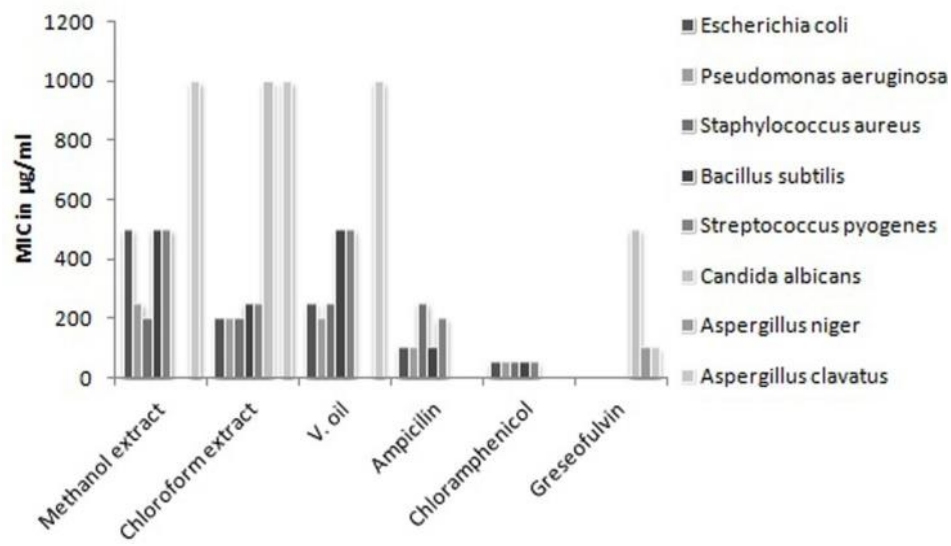

Fig. 1. Antimicrobial activity of methanolic extract, chloroform extract and volatile oil of *Cedrus deodar* by dilution method.

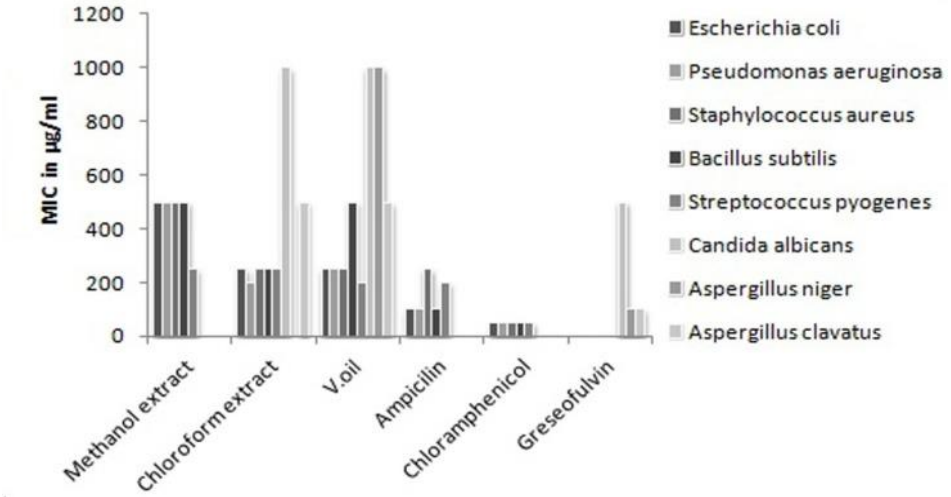

Fig. 2. Antimicrobial activity of methanolic extract, chloroform extract and volatile oil of *Pinus roxburghii* by dilution method.

alkaloids, glycoside, terpenoids, steroids, fixed oil and fats (Table 1).

Phytoconstitents of extracts

Phytochemical testing showed that the chloroform extract of *Cedrus deodara* contains alkaloids, flavonoids, steroid, terpenoid, tannins, glycoside, fixed oil and carbohydrates; whereas the methanolic extract contains alkaloids, flavonoids, steroid, terpenoid, tannins, glycoside and carbohydrates**.** On the other hand, the chloroform and methanolic extracts of *Pinus roxburghii* contain alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins and carbohydrate (Table 2).

Table 2. Phyto-constituents present in extracts of *Cedrus deodara and Pinus roxburghii*

Phytoconstituents	*Cedrus deodara*		*Pinus roxburghii*	
	Chloroform extract	Methanolic extract	Chloroform extract	Methanolic extract
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Tannins	-	+	-	+
Saponins	-	-	+	+
Carbohydrates	+	+	+	+
Proteins	-	-	-	-
Fats and Oils	-	-	-	-

+: present; -: not detected

TLC Profiling of oil and extract

It was evident from the TLC analysis that a large number of compounds may be present in the chloroform extract and volatile oil of *Cedrus deodara* and *Pinus roxburghii*, in comparison to the methanol extract.

Antimicrobial screening of extracts and volatile oil

The effect of the chloroform extract of *Cedrus deodara* on gram –ve (*Escherichia coli*, *Pseudomonas aeruginosa),* gram +ve (*Staphylococcus aureus, Bacillus subtilis*) bacteria and fungus (*Candida albicans*) were more as compared to the methanolic extract and volatile oil (Fig. 1). The effect of the chloroform extract and oil of *Pinus roxburghii* on gram –ve (*Escherichia coli*, *Pseudomonas aeruginosa),* gram +ve (*Staphylococcus aureus, Bacillus subtilis*) bacteria and fungus (*Candida albicans*) were more as compared to the methanolic extract (Fig. 2). Whereas the antimicrobial effect of the extracts and volatile oil on bacteria and fungi were less as compared to the standards. The MIC data (µg/ml) of volatile oil and extracts of *Cedrus deodara* and *Pinus roxburghii* were given in table 3 and 4, respectively.

DISCUSSION

It has been concluded that the volatile oil and chloroform extract of *Cedrus deodara and Pinus roxburghii* showed the maximum antibacterial activity. Literature showed that flavonoids were reported to possess biological activity against microbes (Cushnie and Andrew, 2005). The preliminary phytochemical testing and TLC profiling of extracts and oils showed the presence of higher concentrations of flavonoids, saponins, glycosides, alkaloids and terpenoids in the oil and chloroform extracts of *Cedrus deodara* and *Pinus roxburghii.* The antibacterial activities of *Cedrus deodara and Pinus roxburghii as* recorded in this study may therefore be due mainly to the presence of flavonoids and terpenoids in the extract. The methanolic extracts possessed less antibacterial

Table 3. Antimicrobial activity of methanolic extract, chloroform extract and volatile oil of *Cedrus deodar* by dilution method

Micro organism	Minimum Inhibitory Concentration (MIC) in µg/ml					
	Methanol extract	Chloroform extract	Volatile oil	Ampicilin	Chloramphenicol	Greseofulvin
Escherichia coli	500	200	250	100	50	NA
Pseudomonas aeruginosa	250	200	200	100	50	NA
Staphylococcus aureus	200	200	250	250	50	NA
Bacillus subtilis	500	250	500	100	50	NA
Streptococcus pyogenes	500	250	500	200	50	NA
Candida albicans	>1000	1000	>1000	NA	NA	500
Aspergillus niger	-	-	>1000	NA	NA	100
Aspergillus clavatus	1000	1000	1000	NA	NA	100

-: not detected; NA: not applicable

Table 4. Antimicrobial activity of methanolic extract, chloroform extract and volatile oil of *Pinus roxburghii* by dilution method

Micro organism	Minimum inhibitory Concentration (MIC) in µg/ml					
	Methanol extract	Chloroform extract	Volatile oil	Ampicilin	Chloramphenicol	Greseofulvin
Escherichia coli	500	250	250	100	50	NA
Pseudomonas aeruginosa	500	200	250	100	50	NA
Staphylococcus aureus	500	250	250	250	50	NA
Bacillus subtilis	500	250	500	100	50	NA
Streptococcus pyogenes	250	250	200	200	50	NA
Candida albicans	>1000	1000	1000	NA	NA	500
Aspergillus niger	-	-	1000	NA	NA	100
Aspergillus clavatus	-	500	500	NA	NA	100

-: not detected; NA: not applicable

activity; this might be due to the presence of fewer quantities of flavonoids and terpenoids ingredients in the methanolic extract. The higher MIC value of the extracts and oils of plants against *Candida albicans, Aspergillus niger, and Aspergillus clavatus* suggests that the plants may possess less antifungal activity in comparison to the standard.

Our findings suggest that the *Cedrus deodara* and *Pinus roxburghii* have antimicrobial properties and they can be used in the treatment of infectious diseases. Further work is required in order to isolate the active constituents of the plants responsible for the antibacterial activities.

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CONFLICT OF INTEREST

The authors have no conflict of financial and personal interests with other people or organization.

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