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Antibacterial effects of *Cedrus deodara* oil against pathogenic bacterial strains in-vitro approaches

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Abstract

Pakistan has a great wealth of *Cedrus deodara* have pharmacological potential with a great value and usage as traditional medicine. Crude oil extractions from old branches of *C. deodara* were chopped and oil was obtained by traditional method. The present study was carried out in vitro to determine Antibacterial Effects of *C. deodara* oil against Five Pathogenic Bacterial Strains. Antimicrobial activities of *C. deodara* oil was evaluated by well and disc diffusion methods and erythromycin was used as a positive control. Results revealed that *C. deodara* oil exhibit excellent inhibitory effects against *E. coli* showed 32mm and 24mm and erythromycin 19mm zone of inhibition, *S. typhimurium* showed 19mm and 14mm and erythromycin 18mm zone of inhibition, *P. aeruginosa* showed 19mm and 16mm and erythromycin 15mm zone of inhibition, *E. faecalis* showed 20mm and 18mm and erythromycin 12mm zone of inhibition and *B. subtilis* showed 25mm and 13mm and erythromycin 20mm zone of inhibition with well and disc diffusion method respectively. This research summarized that *C. deodara* oil has board range antimicrobial activity. This study reported about *C. deodar* oil which is benefits for science and Pharmaceuticals Industries who involved in modern health care concept. Further studies should be done to find out the active compound responsible for antibacterial effects to use in modern drugs developments.

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Introduction

Nature has provided an entire store-house of remedies to treat all ailments of mankind. Plants and plant products use as medicines could be traced as far back as the foundation of human civilization. Antimicrobial activity of therapeutic plant has turn out to be a worldwide concern. There is a continuous and urgent need to find out new antibacterial compounds for new communicable diseases. Consequently, researchers are increasingly turning their attentiveness to conventional medicine and probing for new leads to develop enhanced drugs against broad range microbial infections including bacterial and fungal [1, 2].

Cedrus deodara is a species of Pinaceae family native to ancient Greek and Latin. *C. deodara* is an evergreen conifer tree getting up to 85 meters in height with approximately rough black, furrowed bark and scattering branches, shoots dimorphic, leaves 2-5,- 5-8 cm needle like Triquetrous, sharp, piercing, flowers generally monoecious, but some trees or branches normally Bear flowers of one sex [3] Cedrus is a genus of Pinacea with mostly tropical and subtropical worldwide distribution; the genus is consisted of trees which are occasionally cultivated either for their worth to conventional cultures or for ornamental purposes. Seeds are discarding in season of winters. A tree of deodara can survive up to 600 years. Flowers emerge in September and October [4]. *C. deodara* (Deodar cedar or Himalayan cedar), is resident to Eastern Afghanistan, Western Himalayas in, Western Nepal, North Pakistan, North Central India, and south western-most Tibat. It is third most frequent (10%) after Abies pindrow Royal (60%) and Picea simthiana (15%) in Pakistan's part of Himalayan forests, Recorded *C. deodara* from 23 localities from Hindukush and Himalayan Moist Temperate forests of Pakistan [5].

C. deodara has been confirmed to have great pharmacological prospective with a huge utility and usage as tradition medicine. This analysis summarized the plant distinctiveness with chemical composition and their pharmacological activities. various studies indicated that C. deodara have many qualities, such as immunomodulatory, antitumor and anti-inflammatory, properties, as fighting fit as exerting an effect on the nervous system, neuroleptic effect, cytotoxic effect antioxidant property. Bark of the herb is a good remedy for remittent and inters remittent fever, diarrhea and dysentery [4].

The leaf and cone extract obtain with chloroform, methanol and acetone are used against certain strains of bacteria which shows different zones of inhibition. Volatile oil obtained by steam distillation also shows antibacterial activity against both gram positive and gram negative. The ethanolic extract used against three gram negative such as *Escherichia Coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and three gram positive such as *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus* which shows a very good antibacterial activities [6]. Both the volatile oil and ethanolic extract from root, leaves and stem shows anti *E. coli* activity [7]. In case of fungi the essential oil of *Cedrus deodara* used in amount of 150µg/ disc against *Candida albican* and *Aspergillus fumagatus* which shows potent activity against *Aspergillus fumagatus* but have no activity against *Candida albican* [8]. It also possesses the activity against *Fussarium Oxysporum and Alternaria porri* [9].

Aims and objectives of the study were to explore the possibility of "Antibacterial Effects of *Cedrus deodara* oil against Pathogenic Bacterial Strains *In-Vitro*." For this purpose following objectives were carried out: *C. deodara* oil extraction, Antibacterial activities of *C. deodara* oil against Gram positive and Gram negative bacteria.

Materials and methods

The crude technique of oil extraction is broadly trained amid the farmers of Bhaderwah Tehsil of district Doda. Aged and rather reddish colored twigs are crushed into small pieces and carefully put in the sandy pot having small holes (bored out) in the center and at the base. The pot is load up in such a manner that the base hole of the primary pot is precisely at

the center of mouth of following (lower pot). These identical pots, one over another are sited in soil, keeping one third of lower pot dug in the soil. The upper pot containing wood pieces is covered with lid and heated by burning wood from above; taking proper care that heat doesn't dissipate much to lower pot. Heating burns the wood pieces where by oil is released in process which is in collected in the lower pot kept inside the soil. The oil thus extracted, has multiple uses in agriculture allied areas [10].

Five bacterial strains were used in the research (antibacterial activity of *C. deodara* wood oil). These microbial strains included: *Escherichia coli* ATCC 74250, *Salmonella typhimurium* ATCC 33582, *Pseudomonas aeruginosa* ATCC 74303, *Enterococcus faecalis* ATCC 35824 and *Bacillus subtilis* ATCC 41742.

To determine the activity of different plant extracts microorganisms were grown on Nutrients agar media. Media preparation was done according to manufacturer's instructions. *Nutrient broth was an enrichment fluid medium (NB) for the growth of microorganisms*. Medium was prepared by adding 13g of dehydrated powder using electrical balance (SHIMADZU) into 1 liter of distilled water. pH was adjusted by electrical pH meter (JENWAY, 3305) at 7.4 and was boiled to dissolve completely. The major use of Mueller Hinton Agar was for Antimicrobial Susceptibility Testing (AST). Its performance was specified by the NCCLS (National Committee for Clinical Laboratory Standards, 2000). *Medium was prepared by* adding 38g to 1 liter of distilled water. pH was adjusted to 7.3 and was boiled to dissolve completely. All Media were sterilized by using automatic autoclave (REXALL, CO) at 121°C for 15 minutes. Media was poured in pre-sterilized glass petri plates of 90mm in Laminar Flow Hood (ESCO) which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

Antimicrobial activity of *C. deodara* oil was tested using agar well diffusion method. With the help of sterile micropipette tips *C. deodara* 100µl were poured into the wells as described by Adeniyi *et al.*, (1996) Wells of 6mm diameter with sterile cock borer were aseptically punched in the 90mm MHA agar plates. The plates were incubated at 37°C for 24h. After incubation, the diameter of the resulting zone of inhibition was measured with the help of Digital Vernier Caliper (Mitutoyo) and the average values were recorded. Each antimicrobial assay was performed three times. Mean values were reported in this paper. All data were measured average value of three replicates and standard error (±). Results and figures were subjected to Microsoft excel 2007 and through SPSS 16.0 using Chi square test.

Results

In the present research *C. deodara* wood oil was used for antimicrobial activity. Crude oil was extracted from *C. deodara* wood pieces by traditional method of burning. The oil was collected in the lower pot at the end shown in the Figure 1.

Table 1. Zone of inhibitions (mm) produced by *C. deodar* wood oil against Gram negative bacterial strains in comparisons with standard antibiotics discs.

Bacterial strains	Zone of inhibitions against *C. deodar* oil		Positive control
	Well diffusion method	Disc diffusion method	Erythromycin
E. coli	32 mm	24 mm	19 mm
S. typhimurium	19 mm	14 mm	18 mm
P. aeruginosa	19 mm	16 mm	15 mm

Gram positive (*E. faecalis* ATCC 35824, *B. subtilis* ATCC 41742) and Gram negative bacteria (*E. coli* ATCC 74250, *P. aeruginosa* ATCC 74303, *S. typhimurium* ATCC 33582) were used for antibacterial activity. The (Figure No. 2, 7 and 9) showed that the *C. deodara* oil formed zone of

inhibition (32 mm with well diffusion and 24 mm with disc diffusion method) against *E. coli*, *S. typhi*, *C. deodar* oil formed zone of inhibition (19 mm with well diffusion and 14 mm with disc diffusion method) shown in (Figure No.3, 7 and 9). *C. deodar* oil is also formed (19 mm with well diffusion and 16 mm with disc diffusion method) zone of inhibition against *P. aeroginosa* (Figure No.4, 7, 9 and Table 1). *E. faecalis* showed (20 mm with well diffusion and 18 mm with disc diffusion method) zone of inhibition (Figure No.5 and 8) and *B. subtilis* also showed zone of inhibition (25 mm with well diffusion and 13 mm with disc diffusion method) against *C. deodar* oil (Figure No.6, 8, 9 and Table 2).

Table 2. Zone of inhibitions (mm) produced by *C. deodar* wood oil against Gram positive bacterial strains in comparisons with standard antibiotics discs.

Bacterial strains	Zone of inhibitions against *C. deodar* oil		Positive control
	Well diffusion method	Disc diffusion method	Erythromycin
E. faecalis	20 mm	18 mm	12 mm
B. subtilis	25 mm	13 mm	20 mm

Fig. 1. *C. deodara* Oil extraction, Zones inhibition at different methods of *C. deodar* oil against *E.coli.*

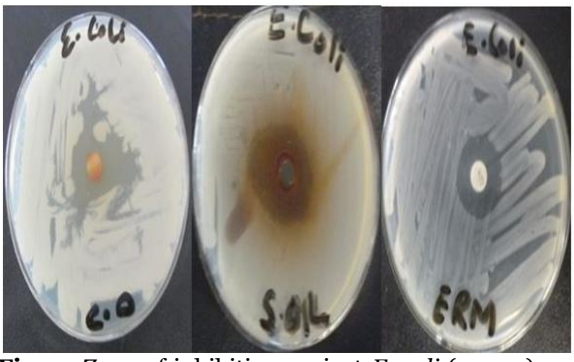

Fig. 2. Zone of inhibition against *E. coli* (24mm) on Disc diffusion method, (32mm) on Well diffusion method and (19mm) on Erythromycine (Antibiotic discs). Zones inhibition at different methods of *C. deodar* oil against *S. typhi.*

Discussion

The aim of this research was to find antibacterial effects of *Cedrus deodara* oil extract against Gram positive and Gram negative bacteria. β Himachalenes a major compound of *C. deodara* that have antimicrobial activity [6, 7].

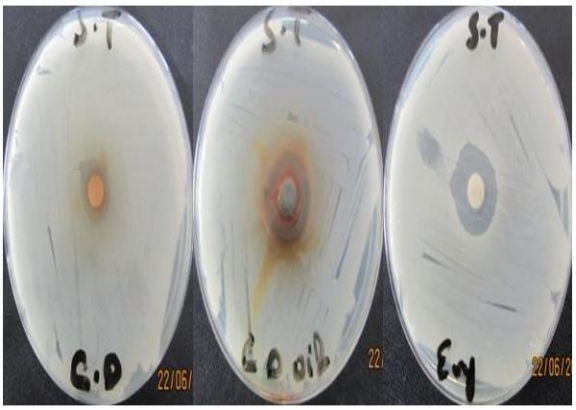

Fig. 3. Zone of inhibition (14mm) against *S.typhi on* Disc diffusion method, (19mm) on Well diffusion method and (18mm) on Erythromycine (Antibiotic discs). Zones inhibition at different methods of *C. deodar* oil against *P. aurigenosa.*

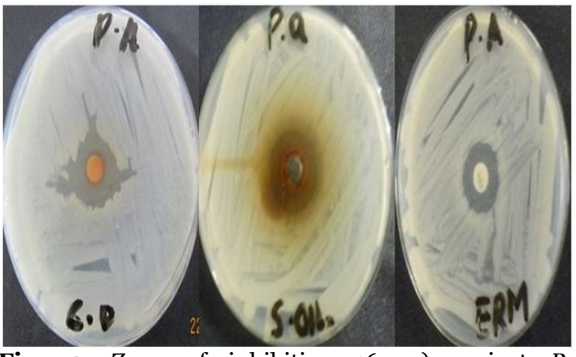

Fig. 4. Zone of inhibition 16mm) against *P. aeruginosa* on Disc diffusion method, (19mm) on Well diffusion method and (15mm) on Erythromycine (Antibiotic discs). Zones inhibition at different methods of *C. deodar* wood oil against *E. fecalis.*

During the last two decades, several groups have sought application of *C. deodara* oil and extracts used for antimicrobial activity against different bacteria [6, 7] and fungi [8, 9]. In case of β himachalenes present in *C. deodara* [11], *E. coli* [12], *P. aeruginosa, E. faecalis and B. subtilis* has board range of antibacterial effects.

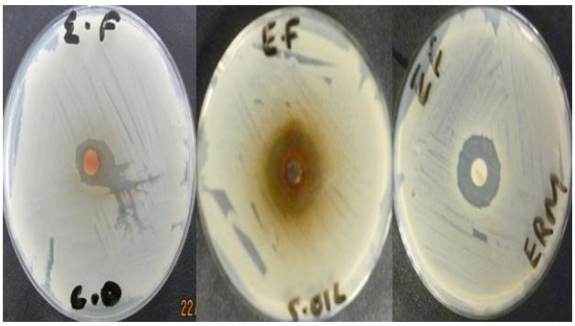

Fig. 5. Zone of inhibition (18mm) against *E. faecalis* on Disc diffusion method, (20mm) on Well diffusion method and (12mm) on Erythromycine (Antibiotic discs). Zones inhibition at different methods of *C. deodar* oil against *B.subtilus*.

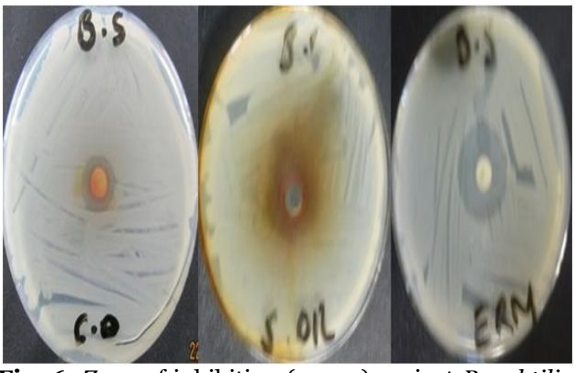

Fig. 6. Zone of inhibition (13mm) against *B. subtilis* on Disc diffusion method, (25mm) on Well diffusion method and (20mm) on Erythromycine (Antibiotic discs).

In the present study *C. deodara* was used evolution of antimicrobial activities against *E.coli* showed zone of inhibition 32mm with well diffusion and 24mm with disc diffusion method and erythromycin was used as positive control with 19mm zone of inhibition (Table 1, Figure 2 and 7), *S. typhimurium* showed zone of inhibition 19mm and 14mm and erythromycin 18mm (Table 1, Figure 3 and 7), *P. aeruginosa* also showed zone of inhibition 19mm and 16mm and erythromycin 15mm (Table 1, Figure 4 and 7), while Gram positive bacteria *E. faecalis* showed zone of inhibition 20mm and 18mm and erythromycin 12mm (Table 2, Figure 5 and 8) and *B. subtilis* showed zone of inhibition 25mm and 13mm and erythromycin 20mm (Table 2, Figure 6, 8 and 9).

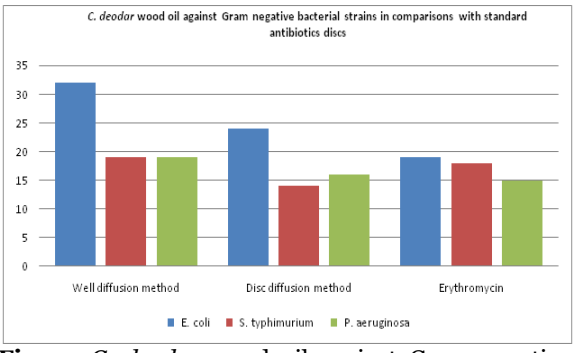

Fig. 7. *C. deodar* wood oil against Gram negative bacterial strains in comparisons with standard antibiotics discs**.**

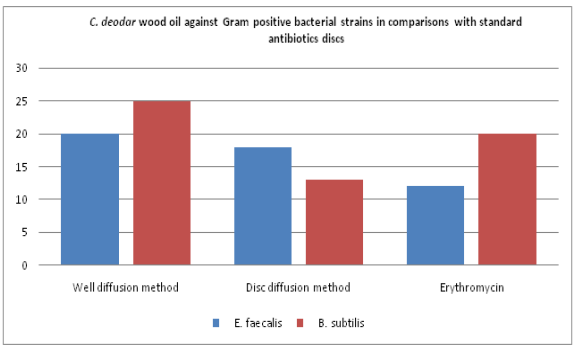

Fig. 8. *C. deodar* wood oil against Gram positive bacterial strains in comparisons with standard antibiotics discs.

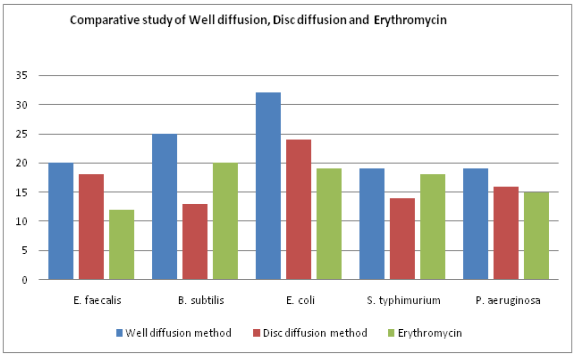

Fig. 9. Comparative study of Well diffusion, Disc diffusion and Erythromycin Antibiotic discs.

Antimicrobial agents presently available in the market are inadequate due to their toxicity, low effectiveness and prove expensive in case of prolonged treatment. The discovery of a potent remedy from plant origin will be a great advancement

in microbial disease therapies. Therefore, there is needed to develop new antimicrobial agents which can satisfy the present demand.

Conclusion

This research summarized that *C. deodara* oil has board range antimicrobial activity. This study reported about *C. deodar* oil which is benefits for science and Pharmaceuticals Industries who involved in modern health care concept. Further studies should be done to find out the active compound responsible for antibacterial effects to use in modern drugs developments.

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Competing interests

The author and co-authors have no competing interests.

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