

Antibacterial Activity of Extracts of *Pinus Wallachiana*, *Pinus Roxburgii* and *Cedrus Deodara* Resins Against Some Common Pathogenic Bacteria

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ABSTRACTS

The current research was conducted in the department of chemistry at Microbiology lab, Govt Collage Madyan, District Swat Khyber Pakhtunkhwa, Pakistan. The aim of the current study was to determine the anti-bacterial activity of different resins extracts of *Pinus roxburgii*, *wallachiana* and *Cedrus deodara* against *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* by Disc diffusion method. The highest zone of inhibition was shown by methanolic extract of *Cedrus deodara* against *S. aureus* (19.6 mm) *S. typhi* (17 mm), *E. coli* (19.25 mm), and *P. aeruginosa* (15 mm) followed by ethanolic extract of the same plants against *S. aureus* (18.8 mm), *S. typhi* (19.33 mm), *E. coli* (18.1 mm), and *P. aeruginosa* (18.33 mm) followed by Acetone extracts of *Cedrus deodara* showed the maximum zone of inhibition against *S. aureus* (17 mm), *E. coli* (16.5 mm), *S. typhi* (16.33 mm), and *P. aeruginosa* (14.33 mm) followed by carbon tetrachloride extracts of *Cedrus deodara* against *S. aureus* (16.9 mm), *S. typhi* (16.5 mm), *E. coli* (12 mm), and *P. aeruginosa* (13.76 mm). The current study concluded that the methanolic extract of *Cedrus deodara* exhibit maximum anti-biogram against the test strains.

Keywords: Antibacterial Activity; *Pinus wallachiana*, *Pinus roxburgii*; *Cedrus deodara*; common pathogenic Bacteria

INTRODUCTION

Several compounds, both natural and man-made, exhibit antibacterial properties, such as the antibacterial properties of plant extracts. [1].

Resin is a sticky, solid or semisolid material generally a complicated mixture of organic compounds called Terpenes (secondary metabolites of plants) that is insoluble in polar solvent however soluble in non-polar solvent. They are excreted from the tree after the infection or damaging or for protection against fungal and bacterial infection [2-7]. It shows the anti-biogram against human pathogens and therefore used to cure human diseases [8-14]. The fundamental goal of human has to find out treatment for bacterial illness for this purpose they use various choices like allopathic, homoeopathic, and herbal medicines. Plant products such as fruits, seeds, flowers, bark, roots, and leaves have been found to contain a wide variety of bioactive chemicals such as glycosides, steroids, volatile oils, tannins, phenols, alkaloids, and flavonoids at high concentrations. [15].

Cedrus deodara, often known as the deodar cedar, is a species of cedar tree found in the Himalayan region. The tree produces a resin that has historically therapeutic uses in medicine. It resins shows the antiviral, anticancer, antibacterial, cytotoxic, antioxidant and antifungal effects [16-23]. Oleoresin are used for remedy of skin diseases and sore. It also showed therapeutic action against to spasm, cancer, pain and inflammation [28]. *Cedrus deodara* resin is used to treat skin disorders like eczema and psoriasis. [24]

Pinus roxburgii, resin has therapeutic uses in Ayurvedic medicine and anti-biogram both against Gram-positives and Gram-negative bacteria [25]. It is used as blood cleanser as well as for the remedy of snake bites, skin necrosis, scorpion sting, and for sore. [26]. It is useful in the treatment of gonorrhoea too [27]. *P. roxburgii* lotion are applied to the scar tissue for softness, recurring boils and fractures [28]

Pinus wallachiana, often known as the Himalayan blue pine, is a species of pine tree that may be found in the Himalayas. Resins of *Pinus wallachiana* is used for bruise [29] applied to treat boils and to prevent gastric problems [30, 31]. Mixture of resin and honey is used for bruise curing, sore, antiseptic, gonorrhoea, burning sensation and abscess [32]. It is also used for treating chafing of heel [33], used with onion paste for curing bruise and cuts [34]. Its resins act as diuretic and to cure inflammation, scabies, piles, asthma, disease of spleen and liver and earache, chronic bronchitis, toothache, epilepsy and tuberculosis [35]. It is

also applied to cure rheumatism and for remedy of syphilis and small pox [36].

In the current study resins of different plants were used to evaluate the anti-biogram of pathogenic bacteria against *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

MATERIAL AND METHODS

Laboratory work: The laboratory work was carried out in Microbiology and Chemistry research labs of Govt. Collage Madyan, District Swat Khyber Pakhtunkhwa (KPK), Pakistan.

Sample collection: For the anti-biogram study resins from the trunks of *Pinus wallachiana* and *Cedrus deodara* were collected from the forest of Village Gornai Tehsil Behrain Swat Khyber Pakhtunkhwa Pakistan. The altitude of forest was 2376m. The diameter of *Pinus wallachiana* trunk was 80.7 inches and that of *Cedrus deodara* was 87 inches. The resin of *Pinus roxburgii* were collected from the forest of Fatehpur Tehsil Khwaza Khela Swat Khyber Pakhtunkhwa Pakistan. The altitude of forest was 1013m, the diameter of *Pinus roxburgii* trunk was 68 inches. The samples of resins were collected using knife and stored in sealed bottles.

Chemicals: Chemicals used in the current study are Methanol, Ethanol, Acetone, Carbon tetrachloride and phosphate buffer.

Apparatus: Apparatus used in the current research were Vernier caliper, Media shaker, pH meter, Drying Oven, Incubator and Autoclave.

Preparation of Methanolic, Ethanolic, Acetone and Carbon tetrachloride extracts of resins: The raw resins of *Pinus wallachiana*, *Pinus roxburgii* and *Cedrus deodara* were cleaned by removing surface impurities and were then extracted (10 g) separately with Methanol, Ethanol, Acetone and Carbon tetrachloride (50ml). The Methanolic, Ethanolic, Acetone and Carbon tetrachloride extracts were then filtered using Millipore filter paper and stored at 4 °C in sealed boxes. The stored samples were then used for their Antibigram

Media preparation:

Nutrient Agar: Nutrient agar is a common growth media in microbiology for growing a variety of bacteria. It is a versatile medium that offers all the nutrients required to sustain the development of a variety of bacteria and fungi. Medium was prepared by suspending 20gram of powdered agar in 1 liter boiled distilled water in conical flask and shaking it thoroughly with the help of shaker to dissolve the nutrient agar completely. The pH of the media was adjusted at pH 7.4 using pH meter.

Media sterilization: Media sterilization was carried out in autoclave at 121°C for 15 minutes.

Media pouring: After the sterilization 25ml media was poured into previously sterilized petri dishes and allowed to solidify.

Microorganism culture; Tested microorganism samples both gram +ive and gram -ive were supplied by the clinical laboratory Saidu Teaching Hospital Saidu Sharif Swat. Gram +ive bacteria included *S. aureus* and Gram -ive bacteria included *S. typhi*, *P. aeruginosa*, and *E. coli*. Cultures of these bacteria were grown at 37 °C on nutrient agar in incubator.

Inoculation: From each bacterial culture bacteria were transferred on to the surface of nutrient agar plate with the help of wire loop.

Disc Diffusion method: The Kirby-Bauer method, sometimes referred to as the disc diffusion method, is a popular technique in microbiology for determining an antibiotic's susceptibility. Disk diffusion was employed to examine how well the resin extracts inhibits the development of bacteria [37]. From filter paper Discs of 5mm diameter were prepared with the help of punch machine. It was then kept in each extraction of plant resins and placed over the culture on nutrient media and incubated in incubator for 24 hours at 37 °C. The diameter of zone inhibition was then measured in millimeters using Vernier caliper.

RESULTS

In the present investigation, Methanolic, Ethanolic, Acetone and Carbon tetra chloride extracts of *Pinus wallachiana*, *Pinus roxburgii* and *Cedrus deodara* resins were tested for their anti-biogram against *S. aureus* (gram positive), *P. aeruginosa*, *S. typhi* and *E. coli* (gram negative). The zones of inhibition were measured against each bacterium.

Anti-biogram: The Methanolic, Ethanolic, Acetone and Carbon tetra chloride extracts of resins showed anti-biogram against the tested microorganism cultures.

Antibiogram activity of methanolic extracts is summarized in table 1. From the results obtained it is cleared that *Cedrus deodara* methanolic extract was more effective against *S. aureus*, *P. aeruginosa* and *E. coli*

The ethanolic extracts results are summarized in table 2. The highest zone of inhibition against each bacterium was shown by *Cedrus deodara* followed by *Pinus roxburgii* and *Pinus wallachiana* respectively.

Antibiogram activity of acetone extracts is summarized in table 3. It showed that the acetone extracts of *Cedrus deodara* resin showed highest zone of inhibition followed by, *pinus roxburgii* and *pinus wallachiana*.

Antibiogram of carbon tetrachloride extracts are summarized in table 4. Carbon tetrachloride extraction of *pinus wallachiana*, *pinus roxburgii* and *cedrus deodara* plants resins.

Note: In all Figure s term A is used for extraction of *pinus Roxburgii*, B is used for *pinus wallachiana*, C is used for *cedrus deodara* and D is used for control group which was phosphate buffer of pH 7.

Table 1: anti-biogram of methanolic extracts against to gram +ive bacteria and gram -ive

Methanolic extracts	S. typhi	E. coli	P. aeruginosa	S. aureus
<i>pinus roxburgii</i>	14.7mm	17.5mm	11mm	18.2mm
<i>pinus wallachiana</i>	17.33mm	18.4mm	11.5mm	18.9mm
<i>cedrus deodara</i>	17mm	19.25mm	15mm	19.6mm
Control	0	0	0	0

Table 2: anti-biogram of Ethanolic extracts against to gram +ive and gram -ive bacteria

Ethanolic Extracts	S. typhi	E. coli	P. aeruginosa	S. aureus
<i>pinus roxburgii</i>	18.8mm	17.67mm	16mm	17.9mm
<i>pinus wallachiana</i>	18mm	17.3mm	14.3mm	17.4mm
<i>cedrus deodara</i>	19.3mm	18.1mm	18.3mm	18.8mm
control	0	0	0	0

Table 3: anti-biogram of Acetone extracts against to gram +ive and gram -ive bacteria

Extracts	S. typhi	E. coli	P. aeruginosa	S. aureus
<i>pinus roxburgii</i>	11.1mm	16mm	12.3mm	16.2mm
<i>pinus wallachiana</i>	13.7mm	12mm	11mm	14.3mm
<i>cedrus deodara</i>	16.2mm	16.5mm	14.4mm	17mm
Control	0	0	0	0

Table 4: anti-biogram of CCl4 extracts against to gram +ive and gram -ive bacteria

CCl4 Extracts	S. typhi	E. coli	P. aeruginosa	S. aureus
<i>pinus roxburgii</i>	15.7mm	13mm	13.3mm	15.9mm
<i>pinus wallachiana</i>	10.6mm	9mm	10.2mm	11.1mm
<i>cedrus deodara</i>	16.8mm	12mm	13.8mm	16.9mm
control	0	0	0	0

DISCUSSION

In the current study anti-biogram of different resin extracts were evaluated. The study showed that extracts from *Cedrus deodara* showed highest zone of Inhibition against all bacteria. For instance, study of (Chopra et al. 2004) examined the effectiveness of extracts from the *cedrus deodara* plant against several different bacterial cultures [38]. And they reported comparable results with our findings. Our results are also consistent with another study conducted by (Wu et al. 2016) who isolated terpenes from *cedrus deodara* which showed highest zone of inhibition against the pathogenic bacteria [39].

From our results it was demonstrated that *Cedrus deodara* methanolic extract was more effective against *S. aureus* (19.6mm), *P. aeruginosa* (15mm) and *E. coli* (19.25mm). The same results were obtained by the study conducted by Singh, G., Kumar, P., & Joshi, S. C. (2012) on methanolic extracts of resin of *Cedrus deodara*. The results demonstrated significant antibacterial potential, inhibiting the growth of both Gram-positive and Gram-negative bacteria [40].

In our study the zone of inhibition of the ethanolic extracts results against *S. aureus*(18.8mm), *P. aeruginosa* (18.3mm), *S. typhi* (19.3mm) and *E. coli* (18.1mm). The highest zone of inhibition against each bacterium was shown by *Cedrus deodara* followed by *Pinus roxburgii* and *Pinus wallachiana* respectively.

Our results of ethanolic extract were accordance to the research conducted by Mohanty and, Sahoo G. in which they reported that *Cedrus deodara* *Pinus roxburgii* and *Pinus wallachiana* showed highest zone of inhibition against *S. aureus*, *P. aeruginosa* and *E. coli* [41].

Antibiogram of acetone extracts of our study results against *S. aureus* (17mm), *P. aeruginosa* (14.4mm), *S. typhi* (16.2mm) and *E. coli* (16.5mm). It showed that the acetone extracts of *Cedrus deodara* resin showed highest zone of inhibition followed by, *pinus roxburgii* and *pinus wallachiana*. Bhagat M, Arora VK, and Mishra A. (2010) study evaluated the anti-biogram of ethanolic and acetic extracts of *Cedrus deodara* against various human pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The results indicated significant anti-biogram of the acetic extract against all tested strains[42].

Anti-biogram of carbon tetrachloride extracts of our study results against *S. aureus* (16.9mm), *P. aeruginosa* (13.8mm), and *S. typhi* (16.8mm). There is limited research specifically on carbon tetrachloride extracts of *Cedrus deodara*, Ghayur, M. N., Gilani, A. H., & Houghton, P. J. (2005) study has showed potential inhibitory activity against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [43]. the most susceptible bacteria to *cedrus deodara* resins extracts was *Staphylococcus aureus*. The resin extracts used in our study showed highest zone of inhibition against Gram +ive bacteria in contrast to gram -ive bacteria. Panda SK, Padhi L, focuses on the anti-biogram of the methanol extract derived from the stem bark of *Cedrus deodara*. It evaluates the extract's efficacy against gram-

positive bacteria, including *Staphylococcus aureus*, and discusses the zone of inhibition observed [44]. The outer membrane and cell wall of gram +ive bacteria are accountable for their greatest sensitivity to methanolic, Ethanolic, Acetone and Carbon tetra extracts of *pinus wallachiana*, *pinus roxburgii* and *cedrus deodara* resins. *Staphylococcus aureus* show maximum sensitivity to all extracts of resins than *S. typhi*, *P. aeruginosa* and *E. coli*.

This is due the fact that Gram -ive bacteria are distinct from Gram +ive bacteria due to the presence of peri-plasmic space and having outer membrane. Thus, it was evaluated that the anti-biogram of studied resin extracts was effective against gram +ive bacteria as compared to Gram -ive bacteria. The cell wall of gram-positive bacteria having thicker peptidoglycan layers are more susceptible to the bactericidal effect. The lipopolysaccharide-rich hydrophilic surface of the Gram -ive bacteria's outer membrane serves as a protective barrier to many antibiotics and is correlated with the peri-plasmic enzymes which can dissolve molecules brought in from the outside.

Gram +ive bacteria lack a cell wall outer membrane like that of gram -ive bacteria. Anti-microbiological agents break down the cytoplasmic membrane, allowing the cytoplasm to flow out and coagulate. For instance, one study examined the effectiveness of resin extracts from the *Pistacia atlantica* plant against to several different bacteria in light of the results, bacteria like *Bacillus subtilis* and *S. aureus* were more sensitive to resin extracts than *P. aeruginosa* and *E. coli* [45].

In another investigation, gram -ive and gram +ive bacteria were put to the test for their anti-biogram of resin extracts from the *Boswellia serrata* tree. In the light of the results, gram +ive bacteria like *Streptococcus pyogenes* and *S. aureus* were more responsive to resin educes than gram -ive bacteria like *Klebsiella pneumoniae* and *Escherichia coli* [46]. While not all resin extractions may exhibit this pattern of stronger activity against gram-positive bacteria, it is a widely reported observation in the literature and can be linked to the variations in gram -ive and gram +ive bacteria's cell wall structures.

CONCLUSIONS

The results of our study demonstrated that, the methanolic, Ethanolic, acetone and carbon tetra chloride extracts of *cedrus deodara*, *pinus roxburgii* and *pinus wallachiana* have good antibacterial activity against gram positive and gram negative bacteria.

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