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Research Article

Deodar Cedar (*Cedrus Deodara*): Efficacy for Potential of Secondary Metabolites and Antibacterial Activity

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Abstract

The setback of microbial resistance is growing and the use of antimicrobial drugs in the future is still uncertain. Infectious diseases are accountable for millions of global deaths annually. Therefore, necessary steps need to be taken to reduce this problem. In the present study, crude extracts of leaves, bark and resins of *Cedrus deodara* in methanol was investigated for secondary metabolites (flavonoids, glycosides, phenols, saponins, tannins, terpenoids) and antibacterial effect of *Cedrus deodara* was evaluated on multidrug resistant (MDR) strains of *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) by agar well diffusion method. Ciprofloxacin was used as standard.

The methanolic extracts of leaves of *C. deodara* tested positive for glycosides, flavonoids, phenol, tannins and terpenoids. The Bark was tested positive for glycosides, flavonoids, phenol, tannins but resins was tested positive for terpenoids. Leaves of *C. deodara* showed zone of inhibition with all the five strains of microorganisms, which were used. *C. deodara* resin methanolic extract showed maximum zone of inhibition with *Bacillus subtilis* which are 25 ± 0.1 mm and bark showed maximum zone of inhibition with *Staphylococcus aureus* 21 ± 0.6 mm. The potency shown by these extracts recommends their use against multidrug resistant microorganisms. Time-kill curve showed a fast and sharp antimicrobial activity. Based on the research experiments, it was identified, that the methanol extract of *Cedrus deodara* exhibited quite high antimicrobial activity as well as secondary metabolites and this quality together with lots of other values must be considered in green landscape planning of contemporary urban environment and for the other purposes too.

Key words: cedrus deodara; antibacterial; agar well diffusion assay; multidrug resistant; microorganisms

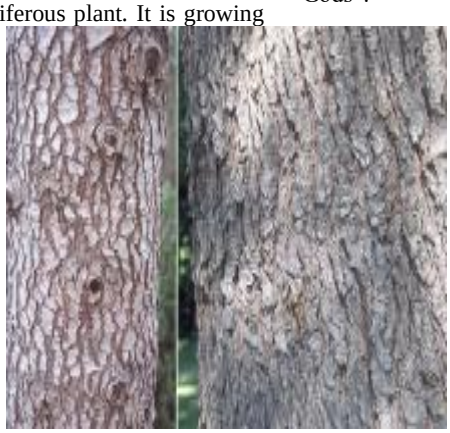
Introduction

Exploration in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as a substitute solution to the health problems in cities. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important. Medicinal herbs are known to fabricate certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth. Medicinal herbs have always received a high priority among the wild plant resources, for their exploitation, management and conservation. As for plant diversity, medicinal plants have been considered to have the highest relative value in societies (Hamilton 2004). Herbal medicine coming into vogue world-wide due to a growing admiration of natural products being cheaper and safer, has elevated the degree of commination (Pareek 1996). Higher plants have been shown to be an inherent source for new antimicrobial agents. The utilization of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The flora used, as drugs are fairly innocuous and relatively free from toxic effects or were Natural products perform various functions and many of them have interesting and useful biological activities (Geo et al 1998 and B. Mahesh et al 2008). There are more than 35,000 plants species have been used in various human cultures around the world for medicinal purpose. Biologically active compounds present in herbal plants have always been of great interest to scientists working in this field (H S Muhammad et al 2005, Singh et al 2005 and Prakash J et al 2005). Indispensable oils from spices and herbs are the most promising natural antimicrobials, because they do not cause microbial resistance due to the diversity of mechanisms of action. They are generally regarded as safe for human consumption without limitations on intake and commonly accepted by consumers (Dobre et al., 2011). The side effects and high cost of treatment and non-availability of medicines to the poor populations, who live in remote areas, are also the reasons for the demand for herbal medicine (Tamilarasi & Ananthi, 2012).

Botany and Uses

The present study was designed to evaluate the antibacterial activity, phytochemical properties of important medicinal plant such as *Cedrus deodara (Fam. Pinaceae).* The best drought and moisture-tolerant tree-plant bearing -25˚C frost and any type of undesirable conditions is the Himalayan Cedar (*Cedrus deodara*). Cedars are very popular ornamental trees used mostly used in horticulture. True cedar trees are indigeneous to the northern and western mountains of the Middle East countries. Himalayan Cedar, *Cedrus deodara,* is a coniferous plant. It is growing naturally in East Asia, North-western part of Himalayas, mountains of Afghanistan, Pakistan, India, Nepal. The Himalayan cedar (Figure 1) has exceptionally reddish brown strong, firm and at the same time soft and aromatic bark. Beautiful needles are clustered as Because of valuable bark and extremely valuable "Cedar oil", it has been widely used in various ways for many centuries since BC till today, in naturally spreading areas. In India, it is considered to be "a divine tree". Its name is derived from "Deodara", a Sanskrit word – "Devaradu" meaning "the forest of the Gods".

(a) Cedrus deodara Tree



(b) Cedrus deodara Bark

(c)Cedrus deodara Leaves

(d)Cedrus deodara Resin

Figure 1: *(a) Tree, (b) bark, (c) Leaves and (d) resin of Cedrus deodara*

All parts of the *Cedrus deodara* are useful in Ayurveda for the treatment of insomnia, psychological disorders and dermatological issues and blood. 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. The present work was an attempt to evaluate the antibacterial and phytochemical activities of the methanolic extracts of *Cedrus deodara* to generate scientifically justified data to support the traditional use.

In our previous study we investigated about the nutraceutical, antimicrobial properties of ghaf (AlGhais et al 2020 a, b, c and Bhardwaj V, 2021d, e). Also, we investigated that ghaf and mangrove has potential of antioxidant and antimicrobial properties (Bhardwaj V, 2021a, b, c). Therefore, to continue our further research, we explored natural sources for new antimicrobial agent and also to meet the increasing demand of antimicrobial agent, alternative strategies, this study have been considered recently. Therefore, Hence, the objectives of the study were to seek the antimicrobial activity of methanolic extract of *Cedrus deodara* and also secondary metabolites. This probe was carried out as an awareness of medicinal value of *Cedrus deodara*.

Material and Methods

Plant Material Collection

Three different parts of Deodar cedar tree were collected in plastic bags from Chail in Himachal Pradesh, India in month of November 2021, at the altitude 2250 m above sea level. Leaves, bark and resin parts of *C. deodara* were washed with water, dried at 45 °C for 6 h, and then were crushed into powders with a mixer (Bhardwaj. V 2021b).

Preparation of the Extracts

The powdered samples 5 g was extracted with 25.0 ml of methanol followed by continuous hot extraction method. Stirred well and kept for incubation in closed container. Centrifuged the tubes at 4000 rpm for 30

min. Transferred the supernatant extract for drying for 10 min and finally got residue of leaves sample. Weighed accurately 0.1 gm of residue in test tube and added 1.0 mL of methanol [10 % (w/v) solution]. The final concentration of extracts used for further experiment. All the extracts were then stored at 4°C in refrigerator for further analysis as crude methanolic extracts (AlGhais et al 2020b; Bhardwaj. V 2021d).

Chemicals

The chemicals used in the present investigation were of analytical grade and of high purity from Merck. Standard kits and reagents used for analysis were purchased from Germany and USA.

Test Organisms

In the present study, the bacterial strains used were *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) obtained from the American Type Culture Collection (ATCC) to determine the antibacterial activity of Scypha. The bacterial strains were procured from LTA srl Italia. Pure culture of bacteria was maintained at 4 °C on nutrient agar slants.

Methodology for Detection of Antibacterial Activity

Inoculums Preparation

The bacterial isolates were first grown in 5 ml of nutrient broth in to sterile test tubes for 18 h before use.

Agar Well Diffusion Assay

The antibacterial activity of methanolic extracts of *C. deodara* (leaves, bark and resin) was tested against isolates by agar-well diffusion method. An aliquot of 100 µl inoculum for each bacterial isolate was evenly spread by a sterile glass spreader onto Muller Hinton Agar using sterilized cotton swab and was allowed at room temperature. A Cork borer of 6 mm diameter was used to punch well in agar plates to cut uniform wells. Wells were bored in agar plates. The concentration of the extracts was 10% (w/v), prepared using methanol as solvent. Subsequently, 30 µl of extracts (leaves, bark and resin) were poured into the wells. Ciprofloxacin 30 µg was used as positive control. Then the plates were kept at 2-8 °C in a refrigerator to allow diffusion of the extracts in to the agar and further incubated at 37 °C for 24 h. The diameter of zone of inhibition was measured to the nearest millimeter (Sohel 2010; Uddin et al 2007). The formation of clear inhibition zone of ≥7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract (Okwori 2007). The effect was compared to those of antibiotic discs. The tests were performed in triplicates and the mean was taken. The whole experiments were performed under strict aseptic conditions.

Phytochemical analysis

Test for Flavonoids (Ammonia test)

1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of conc. sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

Test For Glycosides (Keller Kilian Test)

5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1 ml of conc. sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Test For Phenols (Ferric Chloride Test)

0.5 ml of the extract was added with few drops of neutral ferric chloride (0.5%) solution. Formation of dark green color indicates the presence of the phenolic compounds.

Test For Saponins (Froth Test)

1 ml of the extract was taken in a test tube and distilled water (2 ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of froth formation during warming confirms the presence of saponins.

Test For Tannins (Ferric Chloride Test)

1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

Test for Terpenoids (Salkowski test)

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3ml of conc. sulfuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Time kill Curve

The selected Deodar cedar extracts that showed highest bactericidal effect against the selected microorganisms were used, and time – kill curve was plotted. A 16- h culture was harvested; the suspension was adjusted using the McFarland standard and was then further diluted in saline 0.85% to achieve approximately 1.5×10^8 CFU/ml. The selected cedar extracts were added to aliquots of 1mL Müller Hinton broth in amounts that would achieve the bactericidal concentrations for the selected bacteria followed by the addition of 1ml of the inoculum. Further samples were taken from each tube to monitor growth by measuring the absorbance (optical density) at 600nm wavelength at time intervals (0, 2, 4,6,8,10,12,14 and 16h) (Yin et al., 2002).

Statistical Analysis

The tests were performed in triplicates. Data are expressed as mean. Pair wise comparisons were performed. Experimental error was determined for triplicate and expressed as standard deviation (SD).

Results and Discussion

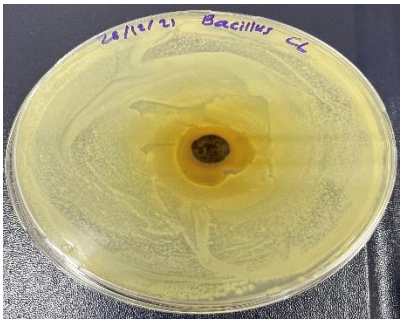
The objective of this research was to seek the antimicrobial activity of methanolic extract of *Cedrus.deodara* and also secondary metabolites. This probe was carried out as an awareness of medicinal value of *Cedrus deodara*, for their activity against selected bacterial pathogens. Three different extracts of different parts of Cedrus deodar (leaves, bark, and resin) were treated using methanolic extraction. Methanolic extracts were found to be more potent against human pathogens. Similar results were reported by Derwich et al. (2010) who reported that the essential leaves of *Cedrus altantica* were active against *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis, Bacillus sphericus* and *Staphylococcus intermedius*. In the present study phytochemical analysis of the Deodar cedar extracts was done to explore their composition. The results revealed the presence of terpenoids, flavenoids, glycosides, phenols, saponins and tannins. These results are similar with the results obtained by Devmurari, (2010) who reported that the phytochemical studies of *Cedrus deodara* revealed the presence of alkaloids, glycosides flavonoids, triterpenoid, tannins, proteins and fixed oil. In particular, the terpenoids substances are the secondary metabolites that characterize *C. libani* derived products (Kizil et al., 2002; Yilmaz et al., 2005; Loizzo et al., 2008).

Antibacterial Activity of Deodar Cedar Extract against Human Pathogenic Bacteria

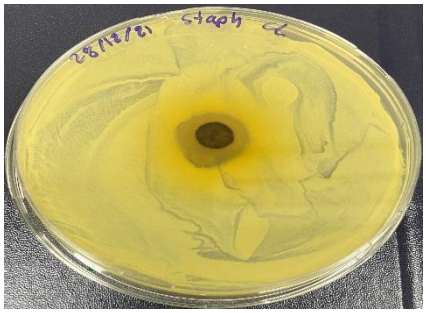
Table 1 summarizes the results of antibacterial activities of extracts of marine sponge, which was evaluated on multidrug resistant (MDR) strains of *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) by agar well diffusion method. Leaves (Figure 2) of *C. deodara* showed zone of inhibition with all the five strains of microorganisms, which were used. *C. deodara* resin (Figure 3) methanolic extract showed maximum zone of inhibition with *Bacillus subtilis* which are 25 ± 0.1 mm and bark showed maximum zone of inhibition with *Staphylococcus aureus* 21 ± 0.6 mm. (Figure 4). All tested extracts of Cedar resin and bark showed no activity against *E. coli*, *Salmonella.enterica*, *Pseudomonas aeruginosa* (Table 1).

S No.	Microorganisms	Zone of Inhibition (mm) Cedar Leaves methanolic extract	Zone of Inhibition (mm) Cedar Resins methanolic extract	Zone of Inhibition (mm) Cedar Bark methanolic extract
1	*Bacillus subtilis* (ATCC 6633)	18 ± 0.0	25 ± 0.1	17 ± 0.5
2	*E. coli* (ATCC 8739)	2 ± 0.5	No zone	No zone
3	*Salmonella enterica* (ATCC 14028)	17 ± 0.2	No zone	No zone
4	*Staphylococcus aureus* (ATCC 6538)	19 ± 0.2	20 ± 0.2	21 ± 0.6
5	*Pseudomonas aeruginosa* (ATCC 27853)	1 ± 0.1	No zone	No zone

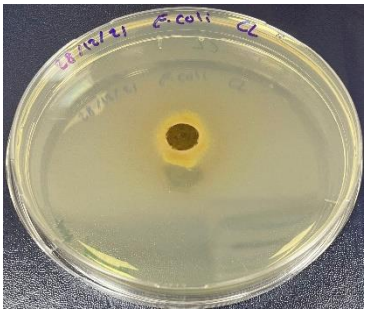
Table 1: *Diameters of the inhibition zone to extracts of C. deodara*



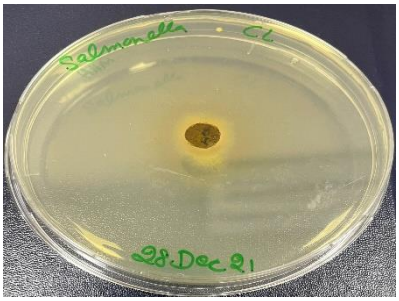
Bacillus subtilis (ATCC 6633)



Staphylococcus aureus (ATCC 6538)



E.coli (ATCC 8739)



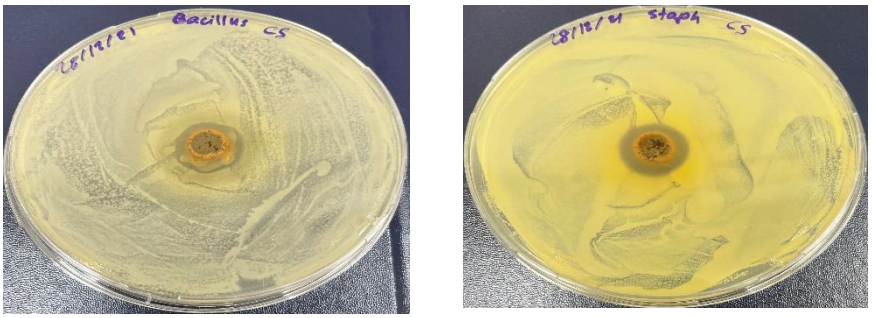
Salmonella enterica (ATCC 14028)

Pseudomonas aeruginosa (ATCC 27853)

Figure 2: *extracts of cedar leaves showed antibacterial activity as indicated by the zone of inhibition against different microorganism's strain*

Bacillus subtilis (ATCC 6633) | *Staphylococcus aureus (ATCC 6538)*

Figure 3: *Extracts of Cedar Resin showed antibacterial activity as indicated by the zone of inhibition against different microorganism's strain*



Bacillus subtilis (ATCC 6633) | *Staphylococcus aureus (ATCC 6538)*

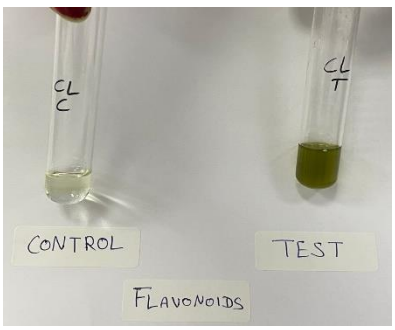
Figure 4: *Extracts of Cedar Bark showed antibacterial activity as indicated by the zone of inhibition against different microorganism's strain*

Phytochemical Screening of Deodar Cedar (Leaves, Bark, Resins)

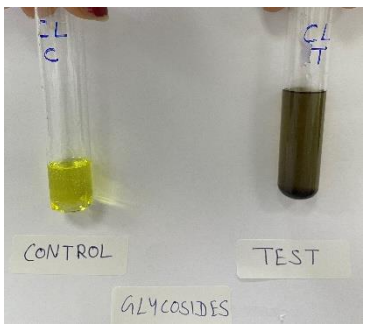
Six phytochemicals were screened for this research work (tannins, phenolic, terpenoids, glycosides, saponins and flavonoids) as seen in Table 2, from the crude extracts obtained from marine species exhibiting bioactivity. Cedar leaves (Figure 5) methanol crude extract tested positive for the presence of Flavonoids, terpenoids, phenol, tannins and glycosides. Similarly, cedar bark (Figure 6) showed the presence of phytochemicals Flavonoids, phenol, tannins and glycosides. But, in case of Cedar resin (Figure 7) methanol crude extract did not test positive for the existence of any of the other mentioned phytochemicals, except terpenoids, which are tested positive (Table 2).

SNo.	Phytochemicals	Cedar Leaves methanolic extract	Cedar Resins methanolic extract	Cedar Bark methanolic extract
1	Flavonoids	+	-	+
2	Glycosides	+	-	+
3	Phenol	+	-	+
4	Saponins	-	-	-
5	Tannins	+	-	+
6	Terpenoids	+	+	-

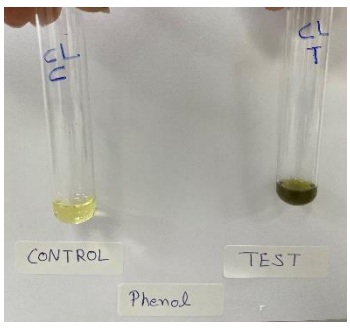
Table 2: *Phytochemicals present in methanolic crude extracts of Cedar leaves, bark and resins (C. deodara)*



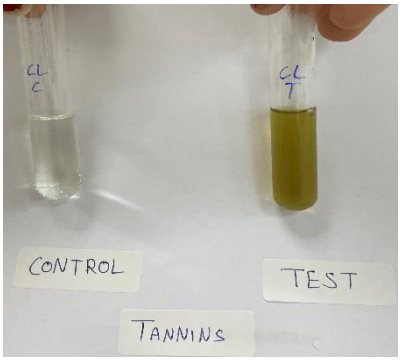
Flavonoids



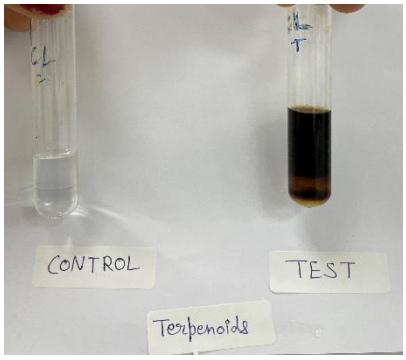
Glycosides



Phenol

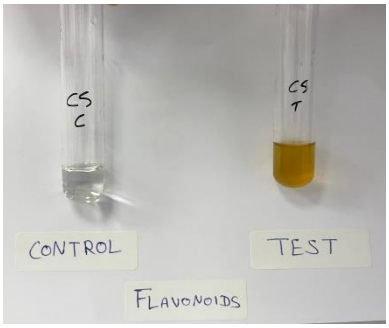


Tannins

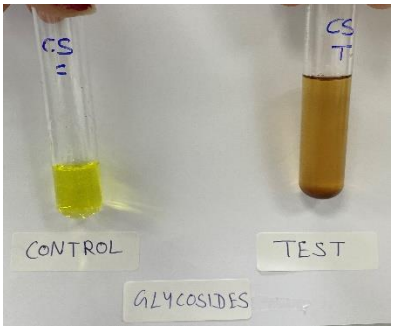


Terpenoids

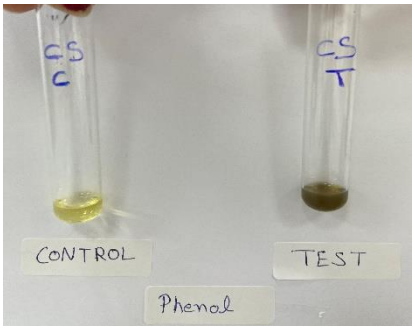
Figure 5: *Showing the confirmation of Phytochemicals present in crude extract of Cedar Leaves*



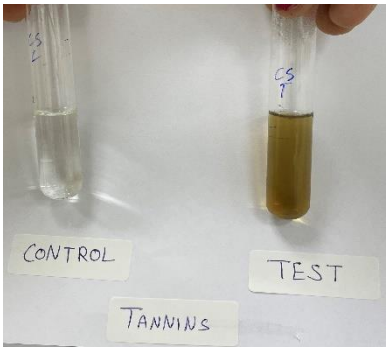
Flavonoids



Glycosides

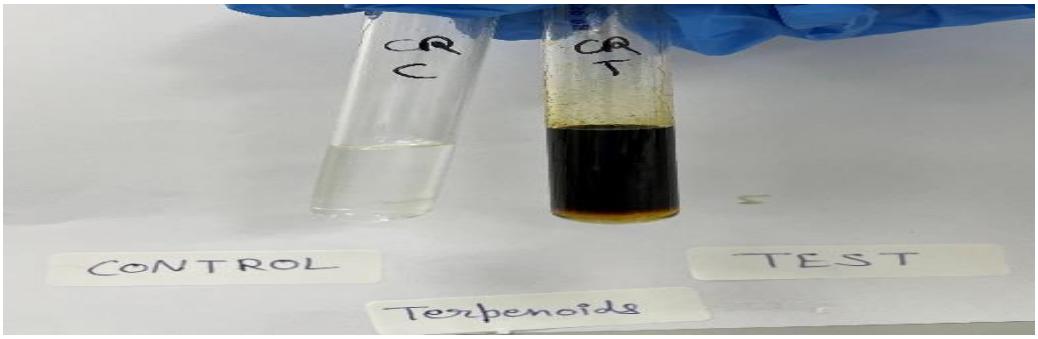


Phenol



Tannins

Figure 6: *Showing the confirmation of Phytochemicals present in crude extract of Cedar Bark*

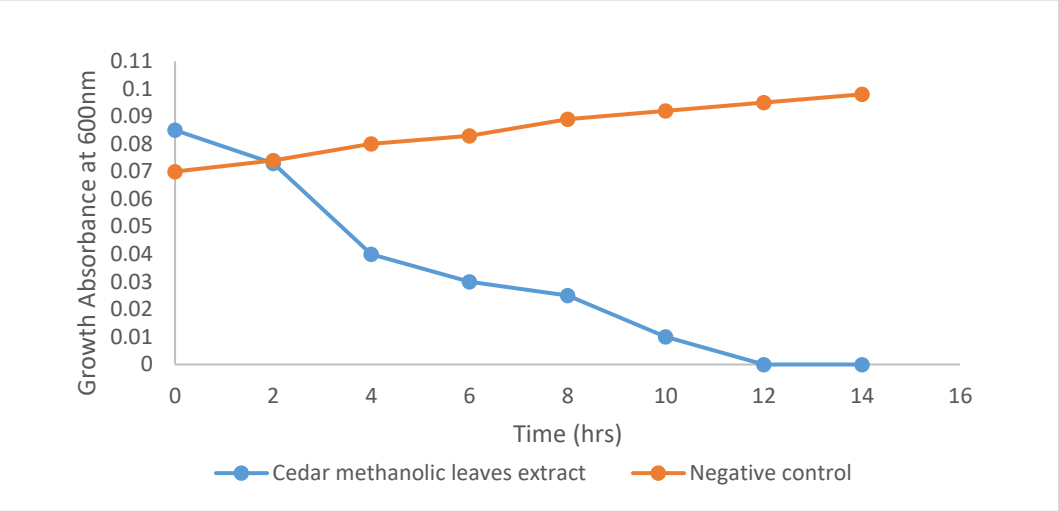


Terpenoids

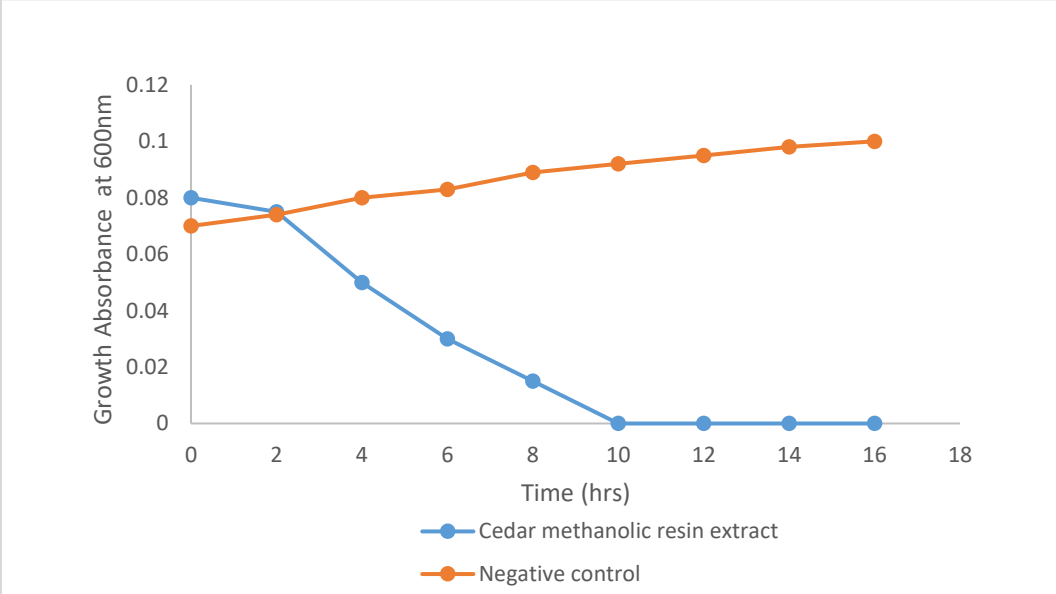
Figure 7: *Showing the confirmation of Phytochemicals present in crude extract of Cedar Resin*

Time Kill Curve of Deodar Cedar (leaves, bark, resins)

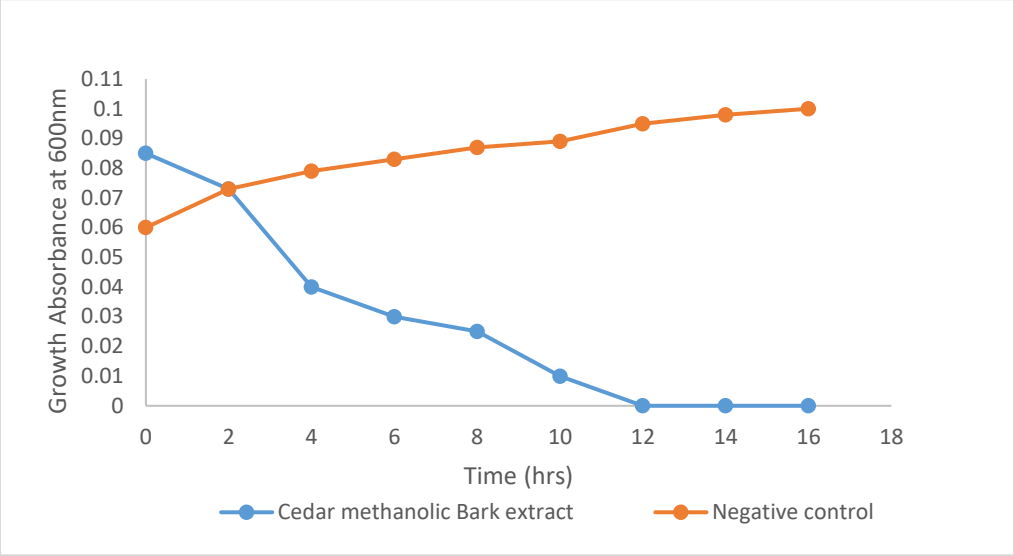
The selected Deodar cedar extracts that showed highest bactericidal effect against the selected microorganisms were used, and time – kill curve was plotted. In case of Cedar leaves extract Time-kill curve was plotted against the selected pathogen (*Staphylococcus. aureus*) in comparison to a negative control (Graph 1). Similarly, Cedar Resin extracts time kill curve was plotted against the most potent pathogen *Bacillus.subtilis* in comparison to a negative control (Graph 2). Moreover, Cedar bark (Graph 3) extracts time kill curve was plotted against the selected pathogen *Bacillus.subtilis*. Negative control was without methanol extract.



Graph 1: *Time-kill curve of the most potent Cedar leaves extract against the selected pathogen (Staphylococcus. aureus) in comparison to a negative control.*



Graph 2: *Time-kill curve of the most potent Cedar Resin extracts against the selected pathogen (Bacillus.subtilis) in comparison to a negative control.*



Graph 3: *Time-kill curve of the most potent Cedar bark extracts against the selected pathogen (Bacillus.subtilis) in comparison to a negative control.*

Conclusion

This probe was carried out as an awareness of medicinal value of *Cedrus deodara*, for their activity against selected bacterial pathogens. The Deodar cedar extract particularly the methanolic extract, obtained from different parts of the tree (leaves, bark and resin), showed an antimicrobial effect against human pathogens which suggests that it could be considered as a safe antimicrobial agent. The broad spectrum antibacterial activity of Deodar Cedar seemed to be due to the presence of terpenes, glycosides detected in the bioactive fractions. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds as well as secondary metabolites.

Abbreviations

SD, standard deviation; **MDR**, Multidrug resistant; **ATCC**, American Type Culture Collection; **E**, Extract; **h**, hours; **C**, ciprofloxacin; ***C. deodara***, *Cedrus deodara*

Ethics Approval and Consent to Participate

Not applicable.

Consent for publication

Not applicable.

Availability of Data and Materials

The relevant data and materials are available in the present study.

Competing Interests

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

Funding

Not applicable.

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Authors' Contributions

VB performed all the experiments. VB analysed the data and wrote the manuscript.

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