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Efficacy of Oleoresin obtained from Bore-Hole Method in Chir-Pine for Potential Antimicrobial Activity

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ABSTRACT

Plant-based natural products have served as a source of medicine for curing many diseases and ailments since the existence of life. As per the recent investigation, approximately 25000 products from plants have been obtained which are being used in various diseases. Plants are recently being used in different biological activities due to their efficacy in treating serious diseases. So the study present has been planned to elucidate the chemical profiling and antimicrobial activity of Oleoresin from Chir-pine from Narendranagar forest division under Garhwal Himalaya. Oleoresin obtained from Chir-pine has been tested against Escherichia coli, Listea monocytegenes, and Staphylococcus aureus at three different concentrations Viz. 50 mg/ml, 100 mg/ml and 200 mg/ml. It was recorded that Oleoresin was active against all three tested bacterial cultures. We have tested all the Oleoresin extract in 50 mg/ml, 100 mg/ml, and 200 mg/ml of DMSO against Escherichia coli, Staphylococcus aureus, and Listea monocytegenes which showed significant activity. High activity was recorded in 200 mg/ml concentration with an inhibitory zone of 2.0 ± 0.34 mm against Staphylococcus aureus whereas minimum activity was recorded against Listea monocytegenes with an inhibitory zone of 1.2 ± 0.30 mm.

Keywords: *Oleoresin, Chir-pine, Antimicrobial activity, Bore-hole, Rill method*

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INTRODUCTION

Indian Himalaya harbors 3,500 species of well-known medicinal plant species that are being used for various purposes. It has been reported that aromatic and medicinal plants have huge antimicrobial activity. As per recent data, the Indian Himalaya produces 80% of medicinal and aromatic plants in its vicinity. Due to its huge floral wealth, India is an emerging part of the global market for herbal drugs and formulation and also could serve raw materials to the groups that are dealing with new drug development (Parveen, 2013). In the Ayurveda system, medicinal plants root issued to treat rheumatism, asthma, dysentery, skin problems, ringworm as well and epilepsy (Navneet *et al*., 2020a; Navneet *et al*., 2020b). Uttarakhand Himalaya includes huge forested areas having large repositories for biodiversity conservation VizRajaji Tiger Reserve, Corbett Tiger Reserve, Nanda Devi National Park, and other forest divisions like Narendranagar Forest Division, Tehri Forest Division, etc. These protected areas support a large number of medicinal and aromatic plants that act as sources of natural remedies (Bahshwan & Aljehany, 2020). It is imperative for the future generation to scientifically explore the phyto-diversity, and design the constructive plan and strategies for the conservation and sustainable utilization of forest flora (Dar *et al*., 2002; Mir & Shafi, 2017).

Pinus roxburghii Sargent (Pinaceae) commonly known as "Chir Pine" is one of the most important species of the Uttarakhand Himalaya which covers an area of 8900 km^2 in India (Sharma, 2002). Chir-pine grows between altitudes of 450-2300 above mean sea level (AMSL). In India, Chir-pine occupies an area of 412,000 ha in Uttarakhand, 158,813 ha in Jammu and Kashmir, and 1,36000 ha in Himachal Pradesh (Anonymous, 1990). Oleoresin mainly consists of terpenoids, gum, rosin, and turpentine oil (Bohlmann & Keeling, 2008). Turpentine can be separated into α-pinene and β-pinene. α-pinene is used as an insecticide and for the preparation of synthetic oils, flavors, and a fragrance ingredient whereas β-pinene is used mainly in the Pharma industry in the manufacturing of various products (Stubbs *et al*., 1984; Komov *et al*., 2021). In *Pinus, the* resin is produced and a complex network of radial and axial resin ducts is responsible for its storage and through which it flows (Vázquez-González *et al*., 2020; Miri *et al*., 2021). Pinenes an important component also act as a natural antifungal agent for treating various fungal diseases (Chang *et al.,* 2008; Matan *et al*., 2012; Oran & Cezayirlioglu, 2021). Rosin is used for coating and in the preparation of rubber and adhesives (Stubbs & Smith, 1984; Stubbs *et al*., 1984; Rodrigues-corrêa *et al*., 2012, 2013). Therefore, the present study aims to characterize the phytochemical characteristics and antimicrobial efficacy of

Oleoresin obtained through the Bore-hole method from Chir-pine in Narendranagar forest division under Uttarakhand Himalaya.

MATERIALS AND METHODS

Tested microorganism

In the present study, *Escherichia coli* (MTCC No. 118) *Listea monocyte genes* (MTCC No. 657)*, and Staphylococcus aureus* (MTCC No. 7433) were used. 50 mg/ml and 100 mg/ml concentration of Oleoresin extract in DMSO were used for testing on a Mueller Hinton agar plate (MTCC No. 7433).

Oleoresin extraction from Chir-pine

In the first phase, Bore-hole was implemented as the trial base in the Narendranagar forest division. *Pinus* forest was divided into different diameter ranges of 20-30, 30-40, 40-50, 50-60, 60-70, and 70-80 cm. Hand-driven drill bits of 1.00 inch, and 1.25 inch were made for drilling bore-hole. The chemical stimulant was sprayed and small pipes were fixed tightly in the holes which are attached to the plastic bags on which the Oleoresin collected.

Antimicrobial activity

The antibacterial activity test was carried out using the disk diffusion method (Kaul, 1997).

Phytochemical analysis of Oleoresin

Gas chromatography is used to determine all the quality parameters of Oleoresin obtained from *Pinus*. In Gas chromatography, Agilent gas chromatography is mainly used and all the parameter changes should be completed through 'Star' software via computer. All the content of α –Pinene and β –Pinene Careen was recorded. For the determination of turpentine sample 0.2µl in a syringe and injected in G.L.C, after 10 min the % of α–Pinene and β–Pinene and carene was recorded. Analysis of variance (ANOVA) and Pearson correlation was done for statistical analysis by using the SPSS Version 20 (Statistical Package for Social Science).

RESULTS AND DISCUSSION

Antimicrobial efficacy

In the present study, the antimicrobial activity of Oleoresin from Chir-pine was tested against *Escherichia coli, Listea monocyte genes, and Staphylococcus aureus* at three different concentrations Viz. 50 mg/ml, 100 mg/ml and 200 mg/ml. It was recorded that Oleoresin was active against all three tested bacterial cultures which showed its huge activity against all the tested pathogens.

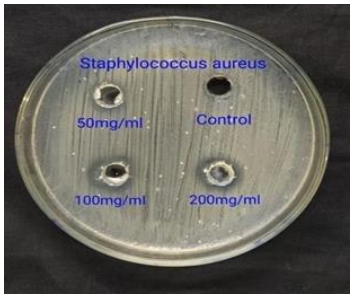
The result from the present study indicated that the DMSO extract of Oleoresin showed significant antimicrobial activity against the tested pathogens thus inhibiting the high inhibitory zone of *Escherichia coli, Listea monocytopgenes, Staphylococcus aureus* on the cultural plate. We have tested all the Oleoresin extract in 50 mg/ml, 100 mg/ml, and 200 mg/ml of DMSO against *Escherichia coli, Staphylococcus aureus, and Listea monocytopgenes* which showed significant activity. High activity was recorded in 200 mg/ml concentration with an inhibitory zone of 2.0 ± 0.34 mm against *Staphylococcus aureus* whereas minimum activity was recorded against *Listea monocytopgenes* with an inhibitory zone of 1.2 ± 0.30 mm.

In the present study, the antimicrobial activities in 50mg/ml concentrations of Oleoresin extract were active against different bacteria like *Escherichia coli, Staphylococcus aureus Listea monocytopgenes* with the inhibitory zone 1.6 ± 0.30 mm, 1.3 ± 0.34 mm, and 1.2 ± 0.30 mm. On the other hand, 100 mg/ml concentration of Oleoresin was active against all three tested pathogens with the inhibitory zone of 1.8 ± 0.30 mm, 1.8 ± 0.35 mm, and 1.6 ± 0.35 mm. It was further observed that Oleoresin at 200 mg/ml concentration in DMSO extract showed huge antimicrobial activity against *Staphylococcus aureus* with 2.0 ± 0.34 mm, *Escherichia coli* with 1.9 ± 0.30 mm and *Listea monocytopgenes* with 1.9 ± 0.32 mm activities. On the other hand, at all three concentrations, DMSO as negative has been recorded with no activity.

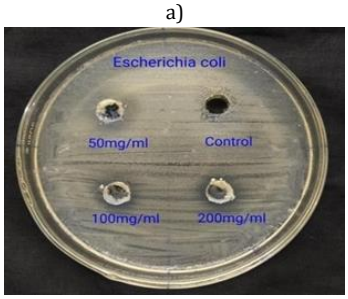
The concentration-dependent activity was recorded in the present study as we tested all the plant extracts at 100 mg/ml and 200 mg/ml. Further, it was also observed from various studies that DMSO extract of resin from different species of Pine showed different activities at different concentrations. At lower concentrations, lesser antimicrobial compounds result in low activity. However, in the present study, we have tested all the plant extracts at 100 mg/ml and 200 mg/ml against the selected pathogens in which the DMSO extract of Oleoresin has shown great antibacterial activity **(Table 1 and Figure 1)**.

Table 1. Antimicrobial activity of Oleoresin against the tested pathogens (Zone of inhibition in Cm)

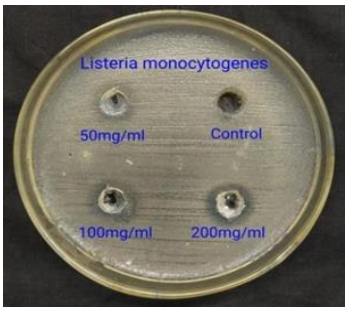
Tested microorganism	50 mg/ml	100 mg/ml	200 mg/ml	Negative control (DMSO)
Escherichia coli	1.6 ± 0.30	1.8 ± 0.30	1.9 ± 0.30	-
Staphylococcus aureus	1.3 ± 0.34	1.8 ± 0.35	2.0 ± 0.34	-
Listea monocytopgenes	1.2 ± 0.30	1.6 ± 0.35	1.9 ± 0.32	-



a)



b)



c)

Figure 1. Antimicrobial potential through disc diffusion assay showing the zone induced by Gentamicin (control) (a) *S. aureus*, (b) *E. coli*, (c) *L. monocytogenes*

Phytochemical screening of Oleoresin

Phytochemical screening of Oleoresin revealed the presence of Carbohydrates, Phenolic compounds, and Steroids along terpenoid whereas other tested phytochemicals likeSaponin glycosides, Flavoniodes, Insulin, and Amino acid were absent. Further, we have also compared the Oleoresin in the Bore-hole method with the traditional method. It was observed that there was significant variation in the Rosin %, α-Pinene, β-Pinene, Carene, and Turpentine Oil % in all the years both in Rill and Bore-hole methods. On the other hand, the presence of Carbohydrates, Phenolic compounds, and Steroid terpenoids, like Saponin glycosides, Flavoniodes, Insulin, and Amino acids have been recorded in both methods **(Figure 2)**.

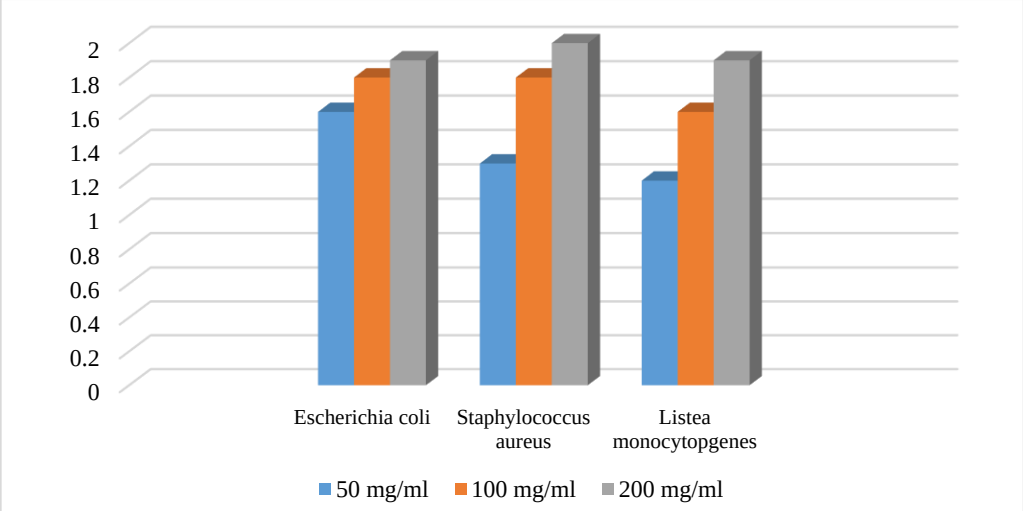


Figure 2. Antimicrobial efficacy of Oleoresin against the tested pathogens

Comparative analysis of Oleoresin obtained in Bore-hole with traditional methods

The quality of the rosin, turpentine, and other products is much better than the Oleoresin obtained from the Rill method hence sold at a high price. The present study recorded the best quality of Oleoresin in the Bore-hole method as compared to the Rill method. In 1 Kg of Oleoresin, about 70.00-72.20% of Rosin was obtained in the Rill method whereas, in the Bore-hole method, approximately 74.10% of Rosin was obtained which was best among all the three years of the Rill method. On the other hand, α-Pinene and β–Pinene were simultaneously recorded with 18.55-19.89 and 3.20-3.25 in all three years but in Bore-hole α-Pinene and β–Pinene were 20.80 and 03.40 which was best as compared to the three-year data of Rill method. It was further observed in the present study that the quality of Turpentine Oil and Carene in the Rill method varied from 14.00-16.20% and 50.62-54.62 in all the studied three years which was less compared to the study Borehole method where the quality of Turpentine Oil was 18.50% and Carene was 55.02. So there were huge differences recorded in both methods while observing the quality and characteristics of Oleoresin. The borehole method was found to be very effective in terms of quality parameters Viz. Rosin %, α-Pinene, β-Pinene, 3-Carene and Turpentine Oil. It was also observed that the extra inappropriate material like impurities (dirt, bark, debris, leaves, etc.) and water content also vary in both the method of resin tapping. About 8% of water content was recorded in 01 kg of resin in the Rill method whereas only 7% of water content was recorded in the Bore-hole method. Impurities which mainly included, bark, debris, leaves, etc. constitute 15-20% of the resin (01 kg) in the Rill method whereas in the Bore-hole method, it constitutes 1-2% of the total resin tested. So all the parameters were found to be suitable for the use of the Bore-hole method as compared to the traditional Rill method **(Tables 2-4)**.

Table 2. Phytochemical Screening of Oleoresin obtained in the Bore-hole method

Test	Inference
Froth formation (Saponin glycosides)	-
Carbohydrates	+
Phenolic compounds	+
Flavonoids	-
Insulin	-
Amino acid	-
Steroid and terpenoid	+

Table 3. Comparative analysis of Oleoresin quality

Parameters	Rill method (01 Kg Raw Resin)			Bore-hole method (01 Kg Raw Resin)
	2018	2019	2020	2020
Rosin %	70.00	71.1	72.20	74.10
Turpentine Oil %	14.00	15.00	16.20	18.50
α - Pinene	18.55	19.20	19.89	20.80
β - Pinene	03.20	03.05	03.25	03.40
Carene	50.62	52.50	54.62	55.02

(P > 0.05)

Table 4. Other inappropriate content in Oleoresin

Parameters	Rill method (01 Kg Raw Resin)	Bore-hole method (01 Kg Raw Resin)
		2020
Water content	8%	7%
Impurities (dirt, bark, debris, leaves, etc.)	15-20%	1-2%

Indian Himalayan regions have great potential for medicinal plant wealth in states like Uttarakhand, Jammu, and Kashmir, Himachal Pradesh is known as a center of folk medicine which has old evidence (Pandith *et al.,* 2018). The plant provides various medicinal and non-timber forest products which are the main source of livelihood and sustainability in these hilly states. A recent study has explored various species of plants Himalayas for their effective efficacy against various diseases and remedial ability. As the Himalayas are fully occupied with medicinal and aromatic flora species like Chir-pine are a huge source of Oleoresin and wood for household activities in Uttarakhand Himalaya. In the present study, we have tested the antimicrobial efficacy and phytochemical screening of Oleoresin obtained from the Bore-hole method in Narendranagar forest division which is an essential part of Garhwal Himalaya. The antimicrobial efficacy was tested against three important pathogens *Escherichia coli, Listea monocytopgenes, and Staphylococcus aureus.* High antimicrobial activity was recorded in 200 mg/ml concentration with an inhibitory zone of 2.0 ± 0.34 mm against *Staphylococcus aureus* whereas minimum activity was recorded against *Listea monocytopgenes* with an inhibitory zone of 1.2 ± 0.30 mm.

Oleoresin for the present study was obtained from the Borehole method in Narendranagar forest division and it was tested for further antimicrobial activities against the three bacterial pathogens. The bore-hole method for Oleoresin extraction and its antimicrobial activity was first demonstrated in the Narendranagar forest division under the supervision of DFO. The present study showed ample evidence from the results obtained and favored the Oleoresin from Bore-hole as an efficient antimicrobial agent.

Plants have been a source of medicine since the existence of life on Earth for the treatment of various human ailments. Due to the presence of various antimicrobial agents on their leaves, stems, and roots, they can be a source of novel drug development. As all plants possess certain antimicrobial activities, so medicinal and aromatic plants serve as herbal bliss for mankind. These have huge potential in the treatment of diseases due to a large number of antimicrobial agents and secondary metabolites which lead to the discovery of novel drugs and safety for a better life (Sofowora *et al.*, 2013; Kesharwani *et al.*, 2019; Salmerón-Manzano *et al.*, 2020). It was revealed from the study that all the pines are capable of producing good quantity and quality of resin. Further, resin yield varied across the season (Lombardero *et al*., 2000; Hood & Sala, 2015; Neis *et al*., 2018; Zas *et al.,* 2020). As per the study of Hood and Sala (2015) and Rodriguez-Garcia *et al*., (2013) groove-to-groove variation in resin yield is also affected by accumulation of induced response and wounds on the bark. As medicinal plants provide great efficacy in drug development against various pathogens in different ways, so they become an essential part of human life (Pan *et al.*, 2014). In the present study, the results of the phytochemical extraction of Oleoresin obtained from the Bore-hole method showed the presence of various compounds like tannins, Rosin, Turpentine Oil, α–Pinene, β–Pinene, Carene which can be used in anti-microbial and anti-hyperglycemic activities as well as antioxidant activity. It was observed in studies that the amount of phytochemical in a particular plant depends upon the polarity of compounds, the extraction method, and the tested samples as well as the polarity of the solvent.

It was estimated from recent records that about 350,000 plants have huge importance which included gymnosperms including Chir-pine, angiosperms, ferns, and pteridophytes (Pan *et al*., 2014; Koparde *et al.*, 2019). All of these pants have a large number of secondary metabolites but a maximum of the compounds have great bioactive constituents like phenol, alkaloid, and tannin (Ravi Shankar & Shukla, 2007). In the present study, the presence of Carbohydrates, Phenolic compounds, Steroid terpenoids and Saponin glycosides, Flavoniodes, Insulin, and Amino acids has been recorded in both methods.

At present, the use of herbal medicine has increased due to its efficacy and less or no side effects. Secondary metabolites like alkaloids, flavonoids, and phenols have a great role in the living system which also reduces the risk of serious diseases like cancer, diabetes, etc. Secondary metabolites have huge potential for the living body (Kumbhar & Godghate, 2015), further, they also prevent cancer or reduce its risk. At the same time, flavonoids and phenols also show various effects like anti-inflammatory and anticarcinogenic. In the present study, the presence of phenolic compounds, Flavonoids, and Insulin from Oleoresin could be a source of disease curing and as an anti-inflammatory and anticarcinogenic agent but more studies need to be done in favor of the present study. Studies from various workers showed that millions of plants have been explored for potential phytochemical screening and antimicrobial activity which has great potential due to the presence of biological compounds (Mir *et al*., 2017; Wagey *et al*., 2018; Koparde *et al.,* 2019). In the present study, we have explored Rosin, Turpentine Oil, α–Pinene, β–Pinene, and Carene from the Oleoresin from Chir-pine which can be a source of medicine and antimicrobial agents for various diseases.

It is imperative for the new generation to scientifically explore floral diversity, and design constructive strategies for sustainable utilization and conservation of forest flora (Mir & Shafi, 2017). It is well known that knowledge of folk traditional medicinal plants as antimicrobial agents can play a significant

role in modern medicinal systems for disease curing and drug development. The use of folk medicine use is widespread nowadays across the world as a remedial measure. Plants like Chir-pine can play a very important role in modern medicinal systems due to their novel compounds for potential antibacterial, anti-inflammatory, anticancer, and antifungal activities.

CONCLUSION

In the present study, we have tested all the Oleoresin extracts at 100 mg/ml and 200 mg/ml. It was the first study in Garhwal Himalaya that tested the Bore-hole method of Oleoresin extraction along with its antimicrobial activity against bacterial pathogens. Further, it was also observed from the present study that DMSO extract of resin from Pine showed different activities at different concentrations. At lower concentrations, lesser antimicrobial compounds result in low activity. High activity was recorded in 200 mg/ml concentration with an inhibitory zone of 2.0 ± 0.34 mm against *Staphylococcus aureus* whereas minimum activity was recorded against *Listeamonocytopgenes* with an inhibitory zone of 1.2 ± 0.30 mm. Phytochemical screening of Oleoresin revealed the presence of tannins, saponins, flavonoids, terpenoids, Phenols, and carbohydrates. Although antibacterial activities at some concentrations provided lower or no inhibitory zone which further needs to be clarified in different concentrations of plant extract.

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ETHICS STATEMENT: None

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