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Review Article

A Review on Ethnobotanical, Phytochemical and Pharmacological Profile of Pinus *wallichiana* A.B. Jacks.

Dwaipayan Sinha*

ABSTRACT

Introduction: *Pinus wallichiana* A.B. Jacks. or the blue pine is one of the important conifer that grows all along the Himalayan range from Afghanistan in the west to Myanmar and China in the east covering the Himalayan regions of Pakistan, Nepal and India. It is found in the upper region of the mountains and often remains associated with other gymnosperms. The plant is of immense ethnobotanical relevance and finds extensive use among the people inhabiting the mountainous region. They are primarily valued for its timber and used for construction and infrastructural purposes. Medicinally the plant is very much important. Throughout the Himalayan region the plant is used for the cure of a number of diseases including treatment of fever, cough and cold, bone fracture, healing of injury and wounds, rheumatic pain, arthritis, inflammations etc. The plant is rich in terpenoids and flavonoids all of which possess strong antioxidant properties. **Methods**: Extensive literature survey was made in the internet with pubmed, google scholar forming the search platform to illustrate the traditional usage of the plant among inhabitants of various regions of Himalayas. Informations of traditional usage, chemical constituents and selected pharmacological activities of the plant were pooled from available research papers to frame the review. **Results and Outcome**: In this review, an attempt has been made to compile the ethnobotanical usage of *Pinus wallichiana*, its phytochemistry and pharmacological activity highlighting its potentiality as a cheap and affordable source of drugs for the benefit of population of Indian subcontinent and adjoining areas.
Key words: Terpenoids, Flavonoids, Conifer, Himalaya, Arthritis, Antioxidant.

Dwaipayan Sinha*

Department of Botany, Government General Degree College, Mohanpur, Paschim Medinipur West Bengal-721436, INDIA.

Correspondence

Dwaipayan Sinha

Department of Botany, Government General Degree College, Mohanpur, Paschim Medinipur, West Bengal-721436, INDIA.

Phone no: 9007709999

E-mail: dwaipayansinha@hotmail.com

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INTRODUCTION

Pinus wallichiana A.B. Jackson or the blue pine is one of the species of *Pinus* that is native to Himalayan ranges, Karakoram range and Hindu Kush Mountains. The extensive and luxuriant growth of this plant is found all along the Himalayan ranges starting from eastern Afghanistan, extending all the way through Pakistan, Indian, Nepal, Bhutan, Myanmar and China with an altitudinal range from 1800 meters to 4300 meters.[1] Globally the species is distributed between 68°E to 100°E longitudes and 25°N to 36°N latitudes. The plant prefers to grow in places with low temperature and occurs in regions having high altitude with low rainfall or low altitude with high rainfall either in form of pure or mixed forests.[2] The plant may form a pioneer species in glacial forelands or may also form the primary species in old growth mixed forests along with *Cedrus deodara* , *Picea smithiana*, *Abies pindrow* in temperate region or may be associated with *Betula utilis* and *Juniperus macropoda* in altitudes beyond 3000 meters near the tree line.[3] The plant prefers to grow on deep moist soils but may also adapt itself well on fertile, well drained sandy clay to sandy clay loamy soil.[4]

Pinus wallichiana is used mainly for timber and stands next to deodar in commercial importance. The sap wood is whitish while the heartwood is light pink to red having dark striations, resinous, straight, even grained with medium fine texture, soft and moderately heavy. The wood acts as a good source of fuel and yields good quality charcoal having calorific value of 4995. The timber is extensively used in making internal fittings of houses, planks, window frames and furniture.[5] In addition to timber, the plant is also exploited for extracting oleoresins which is used for the production of turpentine oil, needle oil and camphor.[6] The plant also finds immense medicinal value among different ethnic communities living in Himalayan region. The resin obtained from the plant is used for wounds and burnt wood called 'Kaalo' is used as antiseptic.[7] The resin is mixed with honey and is used for wound healing, antiseptic, gonorrhea, abscess, ulcer and burning sensation.[8] The fruits and latex of the plants are also applied topically for treating cuts and wounds.[9] The resin also finds application in curing chaffing of heels [10] and is also used with onion paste for treating wounds and cuts.[11]

Thus based on the extensive ethnic usage pattern of the plant, an attempt has been made to summarize the various ethnomedicinal and ethnobotanical uses of *Pinus wallichiana* across various Himalayan regions. In addition to it, a detailed enlisting of various chemical constituents and biological activities of various plant parts have also been summarized in this paper with google scholar and pubmed forming the platform of literature search.

Morphology of the plant

Pinus wallichiana attains a height of more than 50 meters having a straight trunk and short down curved branches which are longer in solitary trees thereby creating a dome like structure (Figure 1A).[12] The trunk is covered by bark which is smooth and resinous when young turning grey and corky with shallow fissures at maturity (Figure 1B).[12] Leaves or needles are bluish green in colour occurring in cluster of five per fascicle and are 10 cm to 20 cm in

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length. Female cones are light brown at maturity and highly resinous. Female cones occur in groups of 1 to 6, 20 cm to 30 cm long. The cone is erect, bluish green when young and becomes pendant, light brown at maturity. The apophysis is pale brown in colour[13] (Figures 1C & 1E).[12] The male cones are ovoid or globose, short lived, comprising of scales or microsporophylls each bearing a pair of pollen sacs on the lower surface. The male cones are 8-10 x 3 mm in dimension. Seeds are ovoid, 6-10 X 5-6 mm in dimension having membranous wings of 1.5 to 3 cm in dimension.[14,12]

TAXONOMIC CLASSIFICATION

Subclass: Pinidae

Order: Pinales

Family: Pinaceae

Genus: *Pinus*

Species: *wallichiana*[15]

TRADITIONAL USE

Pinus wallichiana is deeply assembled within the culture and tradition of the people living in Himalayan and adjoining areas. The used by indigenous people for a variety of purpose which may be grossly divided into non-medicinal and medicinal uses. Non-medicinal uses include the use of plants for thatching and roofing, shelter, fuel wood, construction and infrastructural items and furniture. Medicinal uses include use of various parts of plant or resin for curing of various ailments such as healing, fever, bacterial diseases etc. The detailed non-medicinal and medicinal use of Pinus wallichiana by ethnic people residing in various regions of Himalaya and adjoining mountains are tabulated in Tables 1 and 2.

CHEMICAL CONSTITUENT

Chemical constituents of various plant parts of *Pinus wallichiana* have been worked out in details by various groups of researchers using different solvents and techniques for extraction and detection. It was observed that the essential oils from the needle and turpentine contains terpenes as the major constituent. The alcoholic extract of various parts of plant contains a wide array of compound namely hydrocarbons, terpene acids, organic acids, flavonoids, flavonoid glycosides, terpene alcohols etc. The detailed chemical constituent of various plant parts of *Pinus wallichiana* is tabulated in Table 3 and structure of selected chemical constituent is depicted in Figure 1.

ANTIOXIDANT ACTIVITY

The antioxidant activity of the plant is also studied by various groups of researchers. It has been reported in one study that the crude methanolic extract of leaves and fruits of *Pinus wallichiana* showed strong antioxidant activity when tested with ferric thiocyanate and thiobarbituric acid methods. The results from their study indicated that the leaves possess a free radical scavenging potential and a protective effect towards lipid peroxidation. The activity of the extract was greater

Table 1: Non-medicinal use of *Pinus wallichiana* A.B. Jacks.

S.No.	Region	Vernacular name	Plant part and their uses	References
1.	Bandipore District, Jammu and Kashmir, India	Chi	Seeds are eaten raw by children.	16
2.	District Kinnaur, Himachal Pradesh, India	Chilam, Golda	The tree is used as fuel, timber and for making tools.	17
3.	Shimla Hills, Himachal Pradesh, India	Kail	The wood is used for timber. It yields excellent charcoal.	18
4.	Kashmir Valley, Jammu and Kashmir, India	Kail, Kaeur, Kairo	The timber is used for making buildings, furniture, bridges, viaducts, railway sleepers, street paving blocks, carving, box boards, paper pulps etc. The oleoresin yields turpentine which is the starting material for production of light and heavy tar oil, turpentine pitch, pyroligneous acids and charcoal as residue.	19
5.	Kupwara, Kashmir, India	Kayar	The timber is used for making furniture, and houses. The wood and cones are used as firewoods. Charcoal is obtained from cone, branches and needles.	20
5.	Uttarakhand, India	Kail	The bark yields a black coloured dye.	21
6.	Tones Valley, Garhwal Himalaya, Uttarakhand, India		The Plant is used for household construction.	22
7.	Apatani Plateau, Arunachal Pradesh		Used as Timber, fuel wood, handicrafts, housing and ritualistic material, food.	23
8.	Ayubia National Park and Miandam Valley, Pakistani Hindukush, Pakistan	Kail/ Peyoach	The stem is used for fuel and furniture.	24
9.	Malam Jabba, SWAT, Pakistan	Peoch	Plant is used as fuel wood and making of furniture.	25
10.	Malam Jabba Valley, Swat, Pakistan	Peoch	The plant is used as fuel wood, for making furniture.	26
11.	Utror and Gabral Valleys, District Swat, Pakistan	Peeuch	The tree finds use in timber and furniture industry, match industry, construction of bridges and as fuel wood. It finds use as decoration item.	27

Table 2: Ethnomedicinal use of *Pinus wallichiana* A.B. Jacks.

S.No.	Region	Vernacular name	Plant part and their uses	References
1.	Bangus Valley, Kashmir Himalaya, India.	Kayur	Resin, latex: Stems of plants produce latex called 'kangul' and is applied to heal cracks on heels.	46
2.	Jammu and Kashmir, India.	Kayud	Needles: Needles are fed to the cattle for cure from abdominal worms.	47
3.	Sophian forest area, Jammu and kashmir, India.	Kayod	Used for the treatment of cut, wounds and fractures.	48
4.	Sankaracharya Hills, Jammu and Kashmir, India.	Kayiur, Yar	Resin is used for the treatment of boils, cuts, cracked heels and wounds. It also acts as antiphlogistic and styptic. It is used for the treatment of influenza.	49
5.	Kupwara, Jammu and Kashmir, India.	Kayar	Latex produced from the stem is commonly called Kangul and is applied to cure heel cracks. It is also used to cure wounds and evacuation of pus.	50
6.	Kupwara, Kashmir, India	Kayar	The latex from stem called 'Kangul' is applied to heal cracked heels.	51
7.	Sewa river catchment area, District Kathua, Jammu and Kashmir, India.	Kail	Oleoresin is applied for the treatment of cracked heels.	52
8.	Kashmir Valley, Jammu and Kashmir, India	Kail, Kaeur, Kairo	The leaf oil is used for medicinal purposes. Leaves are weaved to prepare medicinal under clothing. The dark brown viscous and sticky substances called 'killam' is obtained from wood and is applied to arms and legs of farmers for protection against insect bites while working in waterlogged paddy fields.	19
9.	Khistwar, Jammu and Kashmir, India	Chew	The resin from young saplings is used for the treatment of cuts and wounds in skin.	53
10.	Pabbar Valley, Shimla, Himachal Pradesh, India	Kail, Chilta	Resins possess antiseptic properties and used to cure cuts, cracks in feet and wounds. Youngs shoots are used as bandage for treatment of broken joints of humans and cattle.	54
11.	Manali Wildlife Sanctuary, Himachal Pradesh, India	Kail	Used for the treatment of abscesses, dislocation of joints, ulcer and unconsciousness.	55

Table 3: Chemical constituent of various plant part of *Pinus wallichiana* A.B. Jacks.

Plant Part	Chemical constituents	Reference
Needle essential oil	α-Pinene (25.2%), β – Pinene (46.8%), Myrcene (9.5%), α – Terpineol (2.3%), Caryophyllene Oxide (2.1%), Trans Caryophyllene (1.8%), Limonene (1.0%), α- Cadinol (0.9%), Camphene (0.9%), α- Terpinyl Acetate (0.8%), Delta-3-Carene (0.8%), α- Bisabolol (0.6%), α- Humulene (0.5%), α- Phellandrene (0.4%), δ- Cadinene (0.4%), Trans-pinocarveol (0.4%), Geranyl acetate (0.1%).	74
Needle essential oil	α – Thujene (0.1%), Tricyclene(0.1%), α-Pinene (14.8%), α-Fenchene (0.3%), Camphene (1.0%), β – Pinene (34.0%), Myrcene (1.3%), α- Phellandrene (0.3%), α- Terpinene (0.6%) , p-Cymene (0.1%), Limonene (17.8%), α-Pinene oxide (0.6%), Fenchone (0.1%), cis-Limonene oxide (0.3%), trans-Pinocarveol (2.1%), cis-Pinene hydrate (0.3%), trans-Vertbenol (0.5%), Pinocarvone (1.3%), cis-Pinocamphone (0.4%), Terpinen-4-ol (0.2%), α-Terpineol (0.3%), Myrtenal (2.1%), Myrtenol (2.1%), Verbenone (0.3%), trans-Carveol (0.7%), Carvone (0.5%), Undecanone (0.7%), trans-Pinocarvylacetate (0.9%), cis-Pinocarvylacetate (1.9%).	75
Methanolic extract of needle	Dl-Glyceraldehyde dimer (1.38%) , 1,2,3-Propanetriol (Glycerol)(1.64%), Octane, 2,4,6-trimethyl- (0.57%), 2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one (2.07%), Benzoic acid (0.59%), Dodecane (0.94%), 1,2,3-Propanetriol, 1-acetate (0.65%), Tetradecane (1.44%), Hexadecane (0.82%) , 1,3,4,5-tetrahydroxy-cyclohexanecarboxy (quinic acid) (0.95%), Ethyl alpha-d-glucopyranoside (0.83%), Mome inositol (43.27%), 10-methoxy-Nb-alpha-methylcorynantheol (0.56%), (1-butyloctyl) benzene (0.33%), Hexadecanoic acid, methyl ester (0.16%), 1,4-Dioxacyclohexadecane-5,16-dione (0.19%), Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydro (0.35%), Pentadecanoic acid(4.12%), Dibutyl phthalate (3.34%), Eicosane (Icosane) (0.14%), 3,7-Dihydroxy-3-phenyl-4-chromanone (0.22%), 9,12-octadecadienoic acid (Z, Z)- (1.23%), 9,12-octadecadienoic acid (linolelaidic acid) (12.52%), Octadecanoic acid (stearic acid) (1.41%), Ricinoleic acid (0.39%), 2,4a, 8,8-tetramethyl-decahydro-cycloprop (viridiflorol) (2.42%), 1,4,4-trimethyl-8-methylene-1,5-cycloundecaniene (1.45%), 1-phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a, 4b, 5,6, (0.49%), Dehydroabietic acid (1.44%), Acetate, [6-(acetyloxy)-5,5,8a-trimethyl-2-methyleneperhydro-1-napthalenyl] methyl ester (1.25%), 1-phenanthrenecarboxylic acid, 7-ethenyl (levopimaric acid) (0.81%), 10-nonadecanol (0.54%), Stigmast-5-En-3-Ol, (3 beta)-(B sitosterol) (0.78%).	76
n-hexane fraction of aqueous methanolic extract of needle	*β*-Sitosterol, *β*-Sitosterol 3-*O*-*β*-D-glucopyranoside, 5-Hydroxy-7-methoxy-2-(4-methoxy phenyl)-4H-chromen-4-one, Oleanolic acid.	77

Plant Part	Chemical constituents	Reference
Methanolic extract of needle	Isorhamnetin (2.857%), Quercetin (21.426%).	78
Diethyl ether extract of needle	Isorhamnetin (2.857%), Quercetin (5.712%).	78
Turpentine	α- Pinene (90.7%), Camphene (2.5%), β – Pinene/Sabinene/C11 (2.1%), Δ-3-Carene/myrcene (0.4%), α- Terpinene (0.1%), Limonene (0.5%), β- Phellandrene (0.1%), γ- Terpinene (Trace), p-Cymene (0.2%), Terpinolene (0.4%), Longipinene (0.3%), Cyclosativene (Trace), Longicyclene (Trace), Sativene (Trace), Longifolene (0.6%), β- Caryophyllene/ terpinen-4-ol (0.5%), Trans-β-Farnesene/α-humulene (0.1%), α- Terpineol/borneol (0.2%).	79
Resein Acid	Pimaric Acid (0.7%), Levopimaric Acid/Palustric Acid (9.7%), Isopimaric Acid (23.2%), Lambertianic Acid (20.5%), Dehydroabietic Acid (1.7%), Abietic Acid (31.5%), Neoabietic Acid (4.8%).	79
Lipophillic constituents of dry bark	C14:0 Acids (0.34 mg.g^{-1}), C16:0 Acids (1.01 mg.g^{-1}), C17:0 Acids (0.26 mg.g^{-1}), C18:1 Acids (5.40 mg.g^{-1}), C18:2 Acids (3.35 mg.g^{-1}), C18:3 Acids (0.52 mg.g^{-1}), C20:3 Acids (0.56 mg.g^{-1}), C22:0 Acids(0.25 mg.g^{-1}), C24:0 Acids (0.70 mg.g^{-1}), 1,22-dioic-22: acid (0.02 mg.g^{-1}), C22:0 alcohol (0.03 mg.g^{-1}), C24:0 alcohol (0.06 mg.g^{-1}), Thunbergene (0.02 mg.g^{-1}), Diterpene alcohol (0.21 mg.g^{-1}), Sandaracopimaric Acid (0.06 mg.g^{-1}), Isopimaric Acid (2.78 mg.g^{-1}), Palustric Acid (0.04 mg.g^{-1}), Dehydroabietic Acid (1.26 mg.g^{-1}), Abietic Acid (1.45 mg.g^{-1}), Neoabietic Acid (0.27 mg.g^{-1}), C24:0- Monoglyceride (0.06 mg.g^{-1}), Campesterol (0.06 mg.g^{-1}), Sitosterol (0.92 mg.g^{-1}), Sitosterol glucopyranoside (1.49 mg.g^{-1}), Steryl esters (2.07 mg.g^{-1}), Triglycerides (2.31 mg.g^{-1}).	80
Hydrophillic constituent of dry bark	Sugars and sugar alcohols (70.0 mg.g^{-1}), 3,4 –Dihydroxybenzoic Acid (0.66 mg.g^{-1}), Ferulates (4.78 mg.g^{-1}), Secoisolariresinol (0.14 mg.g^{-1}), Monomethyl Pinosylvin (0.48 mg.g^{-1}), Dihydro-monomethyl pinosylvin (0.04 mg.g^{-1}), Resveratrol glycoside (9.09 mg.g^{-1}), Catechin (5.78 mg.g^{-1}), Taxifolin derivative (2.11 mg.g^{-1}), Catechin and gallocatechin derivatives (11.0 mg.g^{-1}).	80
Methanol extract of bark	Kaempferol (2.30%), Rhamnetin (2.08%), Isorhamnetin (2.005%), Quercetin (5.009%), Myricetin (3.0%).	78
Diethyl ether extract of bark	Rhamnetin (2.08%), Isorhamnetin (1.847%).	78
Ethyl acetate extract of bark	Kampherol (1.857%), Quercetin (5.001%).	78

Figure 1: *Pinus wallichiana* A.B. Jacks: (A) Full grown trees in natural habitat, (B) Barks, (C) Unripe female cones, (D) Male Cones with vegetative leaves, (E) Ripe female cones with vegetative leaves.[12]

than Vitamin E and comparable to butylated hydroxy toluene taken as positive control.[82] It was further observed that hydromethanolic extract and aqueous extract of the leaves showed free radical scavenging and nitric oxide scavenging activity. The hydrogen peroxide scavenging activities of the extracts were more than that of standard antioxidant ascorbic acid.[83] The essential oil of *Pinus wallichiana* exhibited free radical scavenging activity as evident from DPPH scavenging assays.[74]

ANTIMICROBIAL ACTIVITY

Pinus wallichiana exhibits antimicrobial properties. It was observed that n-hexane fraction of ethanolic extract of needle inhibited the growth of fungus *Microsporium cannis* with minimum inhibitory concentration of 25 $\mu g.ml^{-1}$. In addition to it, the ethyl acetate fraction brought mortality of *Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis* exhibiting a mortality range of (20-40) at a crude extract concentration of 20 mg per 2 ml of acetone.[84] The needle essential oil also showed inhibitory activity against *Fusarium verticillioides* with minimum inhibitory concentration of 40 ppm exhibiting 25% or less growth than that of the control.[75] It was also reported that the hydromethanolic extract of the needles at concentration of 47.8 $mg.ml^{-1}$ showed highest inhibitory activity against *Pseudomonas aeruginosa* and *Escherichia coli* with a zone of inhibition of 15.66 ± 1.1 mm and 14 ± 0.57 mm respectively.[83] Additionally, the methanolic extract of the needles of the plant showed bactericidal activity against *Bacillus subtilis, Agrobacterium tumefaciens, Xanthmonas phaseoli, Erwinia chrysanthemi* and *Escherichia coli* with activities ranging 54% and 81%.[85] The antimicrobial activity of the plant is tabulated in Table 4.

ANTIPROLIFERATIVE ACTIVITY

The essential oil of *Pinus wallichiana* exhibited a dose dependent antiproliferative activity against THP-1 (Leukemia), A-549 (Lung), HEP-2 (Liver), IGR-OV-1 (Ovary), PC-3 (Prostate) cell lines. The maximum anti-proliferative activity was obtained when the cell lines were treated with 100 $\mu g.mL^{-1}$ of oil with IC50 values of 5.6 ± 1.4, 6.1 ± 0.8, 9.0 ± 1.5, 9.9 ± 1.9, 6.9 ± 1.2 respectively. These values were less than those of standard drugs Paclitaxel and Mitomycin-C taken as positive control.[74]

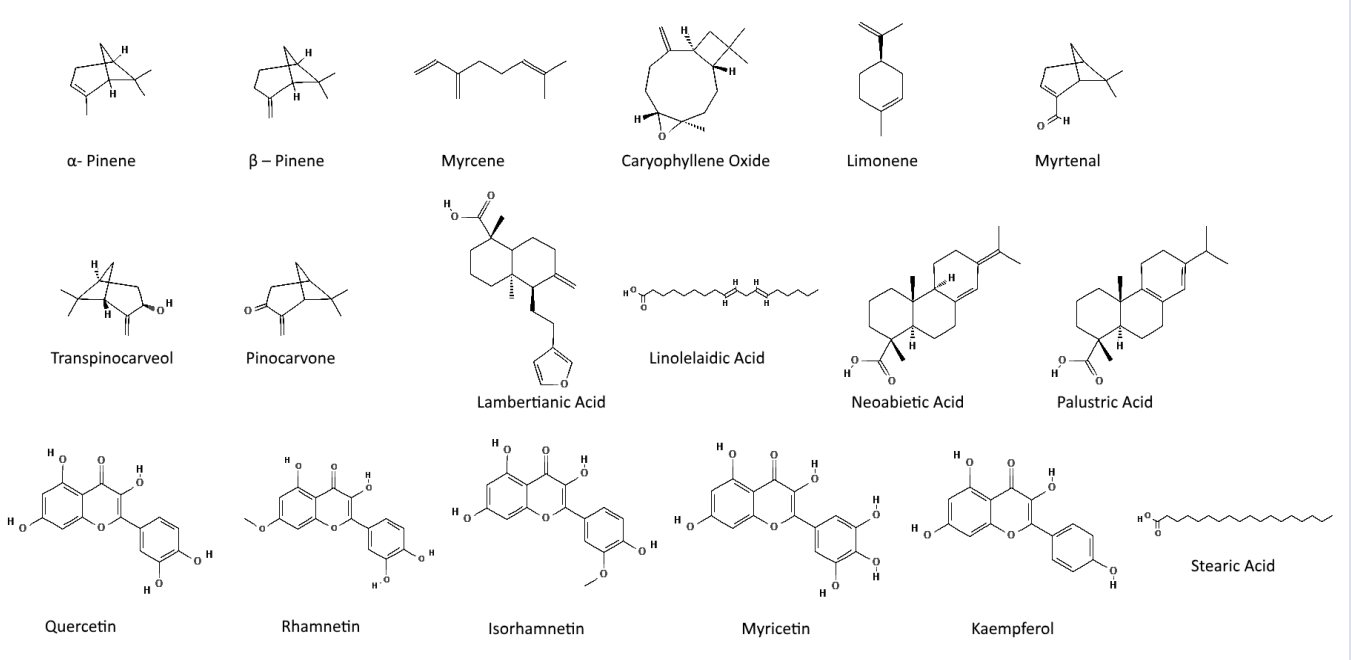

Figure 2: Molecular structures of selected compounds present in *Pinus wallichiana* A.B. Jacks.[81]

Table 4: Antimicrobial activity of *Pinus wallichiana* A.B. Jacks.

Extract	Fungi	Bacteria		Insect	Reference
		Gram Positive	Gram Negative		
Needle essential oil	*Fusarium verticillioides.*				75
Hydro methanolic extract of needle			*Pseudomonas aeruginosa and Escherichia coli.*		83
n-Hexane fraction of ethanolic extract of needle	*Microsporium cannis.*			*Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis.*	84
Methanolic extract of needle		*Bacillus subtilis.*	*Xanthmonas phaseoli, Erwinia chrysanthemi and Escherichia coli.*		85

CONCLUSION

The Himalayan mountain is one of the prominent biodiversity hotspot of the world and boasts of a numerous plants which are of direct use to the local people. The plants growing in the region play a major role in the life of the people living in the Himalayan region and are intricately assembled in their culture and tradition. This has lead to emergence of traditional remedies as an important aspect of their livelihood. It plays a major role in overall health care of the people living in far flung regions of the mountains where conventional health care facility are limiting. This paper projects detailed usage pattern of *Pinus wallichiana* growing in different sectors himalayan region. The plant is extensively used in both non medicinal and medicinal purposes. Though all parts aerial parts of the plants are utilised by the people for a variety of purposes, it is noted that in most cases resins and latex are exploited for cure of variety of ailments by local people. Investigations on the chemical constituents of various plant parts reveal the presence of various terpenoids and flavonoids most of which act as strong antioxidants. Additionally, the antioxidant, free radical scavenging activity and inhibition of lipid peroxidation of the plant have also been investigated by different groups of researchers with a positive outcome from their findings. Further the plant showed inhibitory activity towards fungi, bacteria and insects indicating its antimicrobial potential. The anticancer activity of the plant has also been established by its Antiproliferative activity.

To summarize, the popularity of the plant among local people of Himalayan region as herbal remedy may be due to the presence of terpenoids and flavonoids whose fundamental role is modulation of oxidative balance of the living system and inhibition of microbial activity. Though the plant is widely used by local people proper scientific investigation of its medicinal property is scarce. Thus, investigation of pharmacological activity based on the traditional usage pattern and development of drug through clinical trials would be an effective way of bio prospecting the plant for medicinal use. As the plant is plenty in the Himalayan region, it can act as a cheap source of raw material for development of drugs for efficient management of free radical induced diseases in and around Indian subcontinent.

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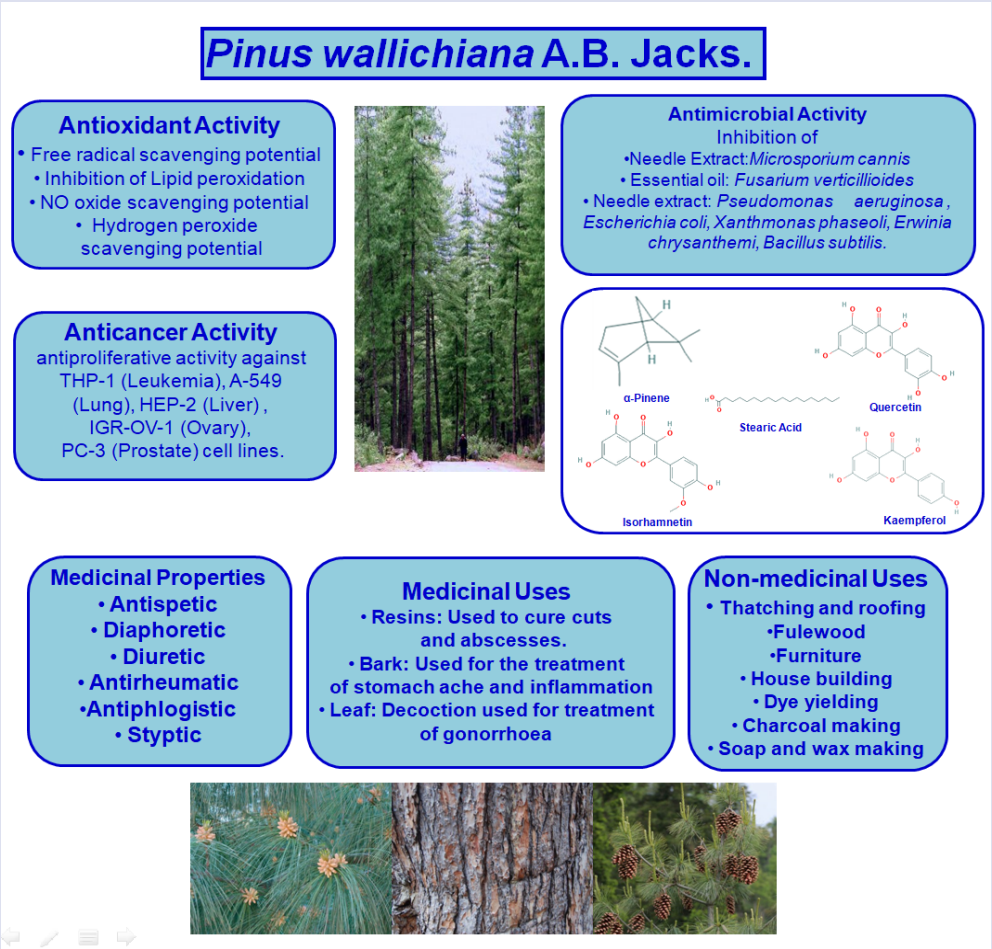
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Dwaipayan Sinha: The author has done his graduation from prestigious Presidency University, Kolkata (then Presidency College) in Botany securing rank in University. He has done his masters from the esteemed department of Botany, University of Delhi, India with specialization in molecular biology and genetics. The author has done his doctorate from CSIR-Indian Institute of Toxicology Research, Lucknow, India on antioxidants using gymnosperm as model system. The author has more than 15 years research experience in gymnosperm, antioxidants, phytochemistry, plant natural products, nutraceuticals and Ethnobotany. The author has done extensive research in various gymnosperm belts of Indian Himalayas and has published several papers in peer reviewed journals. The author is a faculty in Department of Botany, Government General Degree College, Mohanpur, Paschim Medinipur under West Bengal Education Service, Government of West Bengal and has more than 10 years teaching experience to his credit.

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