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Review Article

A Review on Himalayan Pine Species: Ethnopharmacological, Phytochemical and Pharmacological Aspects

Aditi Sharma, Lalit Sharma, Rohit Goyal*

ABSTRACT

Introduction: Ever since ancient times, medicinal plants recognized as major source of therapeutics, as rescue for human diseases and maintain health. There is an exponential increase in usage of green medicines due to less cost and fewer side effects. The family Pinaceae, is largest conifer in species diversity. Pinus is the largest genus of monoecious, resiniferous, evergreen trees commonly known as Pines. Three species of pines occur wild in Indian Himalayas *Pinus roxburghii*, *Pinus wallichiana* , *Pinus gerardiana*. In addition, the constituents present in these plants are beneficial for the purpose of treatment of various ailments. **Methods:** Information was collected from scientific journals, books, and reports via electronic search tools(Medline, Pubmed etc.) **Results:** This review summarizes the existing information on three species of Pinus in relation to their pharmacognostic properties, phytochemistry, ethanopharmacology and pharmacological activities.

Key words: Pinus, Himalayas, *Pinus roxburghii*, *Pinus wallichina* , *Pinus gerardiana,* Phytochemistry, Pharmacology, Ethanopharmacology.

Aditi Sharma, Lalit Sharma, Rohit Goyal*

School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh, INDIA.

Correspondence

Dr. Rohit Goyal

School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh, INDIA.

Phone no : +91-98160-62679

E-mail: rohit_pharm@yahoo.co.in

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INTRODUCTION

Medicinal plants are recognized as major source of therapeutics, throughout human history to fight illness and maintain health. The usage of natural product in treatment of diseases has been increased because of its natural source and comparatively lesser side effects as compared to the complexity in formulating chemical based drugs, as well as uprising cost has led worldwide researchers to focus on the medicinal plant research. The plant extracts possess medicinal properties and are often used as sweetening agent, colouring agent, preservatives in many medicinal formulations.[1] India has a rich diversity of medicinal as well as aromatic plants and holds a unique place in the world in the traditional system of medicine thus called medicinal Garden of the world. India is one of the twelve mega biodiversity center having over 45000 plant species.[2] Pinus is the most common genus of the family *Pinaceae*, which in turn is the largest family within the coniferous. It is a large genus with over 110 species worldwide.[3] The genus is divided into two subgenera: Strobus (Haploxylon, soft pines) and Pinus (Diploxylon, hard pines).[4] Five species of pines are indigenous to India viz.*P. roxburghii (Chir pine), P.wallichiana* (Blue pine), *P. kesiya* (Khasi pine), *P. gerardiana* (Chilgoza pine) *and P. merkussi* (Teriasserian pine).[5] Among all *P. roxburghii,P. wallichiana* and *P. gerardiana* are found in the Himalayas, whereas *P. kesiya* and *P.merkussi* are indigenous to Assam (India) and Burma.

The Indian Himalayan region, a birthplace of Ayurveda and alternative therapies, covers about 18% of India and extends more than 2,800 km long and 220-300 km wide with altitudes of 200-8000m. India fulfills 80% demand of Ayurvedic medicine, 46% of Unani drugs and 33% of allopathic drugs.[6,7,8] The unique climatic conditions enable a rich array of growth of various medicinally useful plants.[8] Pinus species are important forest primarily for timber interests and source of gum oleoresins. Three species of Pinus plants are abundantly found in Himachal Pradesh *i.e. Pinus roxburghii, Pinus wallichiiana* and *Pinus gerardiana*. *P. roxburghii* Sarg (chir pine) is a tall tree with spreading crown grows at an altitude of 450-2400m from Kashmir to Bhutan and Siwalik hills.[9] *P. wallichiana* (blue pine) found at an altitude 2000-3500 m whereas *P. gerardiana*, commonly (Chilgoza pine) found at an altitude of 1600-3000m in district Kinnaur of Himachal Pradesh (H.P.). The present review was aimed to aware the researchers about the potential of Pinus species from Himalayas and to fully explore the scientific basis for the medicinal uses of these plant species.

Pinus roxburghii

Habitat and morphology

Pinus roxburghii Sarg. (Pinaceae) is an older terrestrial ornamental plant in the world. It is the most important pine of North Western Himalayas and an important resin and timber yielding species. In India it is found in Himachal Pradesh, Kashmir and Uttaranchal.[10] It is a large tree with spreading crown

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reaching 30-50 m with a trunk diameter of up to 2 m. It is found at the height of 500 to 2,500 m above sea level and grows gregariously.[11] It is a large tree with branches in more or less whorled, bark dark gray, often reddish, deeply fissured, rough, exfoliating in longitudinally elongated plates, leaves in clusters of three, 20-30 cm, long, triquetrous, finely toothed, needle like, light green, persisting on an average for a year and a half; male flowers about 1.5 cm long, arranged in the form of cones, female cones, solitary or 2-5 together, ovoid, 10- 20 cm×7.5×13 cm when ripe.[9] The tapping of the stem produces clear, transparent oleo-resin with the pungent and bitter taste. Taxonomy and common names of *Pinus roxburghii* Sarg are shown in Table 1.[12]

Phytochemical constituents

P. roxburghii is known to be a rich source of terpenoids, flavonoids, tannins, xanthones other compounds shown in Table 2. Structure of different bioactive compounds are given in Figure 1a, 1b, 1c.

Ethnopharmacological uses

Pinus roxburghii has been widely used as a traditional remedy by the local tribes in various parts of Northern India. The wood oil is antiseptic, diaphoretic, rubefacient, aromatic and carminative in nature. It is used as nerve tonic and expectorant and as remedy for diseases of the eye, ear, pharynx, hemorrhages, worm infestations and skin.[20] The bark paste is applied in burns, scalds and ulcers. The timber is largely used for various purposes e.g., matchbox industry, sports goods, musical instruments, house building, furniture, tea chests etc. The volatile component of resin known as turpentine oil is the most important basic raw material for the synthesis of terpene chemicals widely used as adhesives, lubrication, solvents, plasticizers, paints and varnishes, antiseptic and expectorant. It is included in the Indian Pharmaceutical Codex as *Oleum terebintinae* for treatment of chronic bronchitis.[21] Turpentine oil is applied externally as rubefacient in lumbago and arthritis. It is also used as remedy for neuralgia, minor hemorrhages of tooth sockets and also recommended in gangrene of lungs.[22] It is used to arrest minor haemorrhages in tooth sockets and nose. In the form of enema used in obstinate constipation. Inhaling the vapors of turpentine is useful in bronchitis. Resin (*Biroja*) is obtained as solid residue in the distillation of turpentine oil from oleoresin. It is used for bangles, varnish, paints, polish industries, ingredient of printing inks, batteries. To heel cracks boiled resin (*khaida* or *leesa*) are used. The carbon is collected from the burnt resinous wood (*doi*) of *P. roxburghii* mixed with mustard oil and is made into a paste (*kajal*), which is applied inside the lower eyelids to keep the eyes clean and attractive.[23] The resin mixed with the ash of *Betula utilis* is commonly applied over sprains and plastered on fractured bone for quick recovery, soften scar tissue and consumed as remedy in worm infestation and gastric trouble.[24] The resin is a stimulant and used internally as stomachic and in gonorrhea. The bark has tannins used for coloring the leather. Leaves are used in construction of roofs as *"Channana"*. The needles of *Pinus roxburghii* are ground and mixed with water and given to patients suffering from measles. The oil obtained by the distillation of the needles is used in muscular pains and as expectorant.[25] The seeds from the female cones are edible and are consumed in treatment of bronchitis, tuberculosis and urinary bladder infections.[25]

Table 1: Taxonomy of *Pinus roxburghii*.

Taxonomic classification	Synonym	Common names
Kingdom: Plantae	*Pinus longifolia*	English: Long leaved Pine or chir pine
Division: Pinophyt		Hindi: Chil, Chir, Salla
Class: Pinopsida		Sanskrit: Manojna
Order: Pinales;		Gujrati name: Teliyodeodaro
Family: Pinaceae		Bengali: Saralgachhai
Genus: *Pines*		Malyalam: Salla, Charalam
Subgenus : *Pines*		Tamil :Simaidevadari
Species: *roxburghii*		Telgu: Devadaru

Table 2: Bioactive constituents in *Pinus roxburghii*.

Part of plant	Bioactive constituents
Needle essential oil [14,15]	α-Pinene (22.8%), camphene (0.4%), β-pinene(14.1), Δ3-carene (50.6%), α-phellandrene (0.1%),α-terpinene (0.4%), limonene (0.9%), β-phellandrene (0.7%), γ-terpinene (0.5%), *p*-cymene (tr), longipinene (0.2%), cyclosativene (tr), sativene (0.1%), longifolene (3.4%), β-caryophyllene (0.2%), α-terpeinyl acetate (0.3%), longicyclene (0.2%), terpinolene (3.8%), reported in resin. Zafar *et al* 2010 reported α-Pinene (29.3%), β-myrcene (1.1%), 3-carene (14.2%), terpinyl acetate (1.0%), α-terpineol (4.5%), borneol acetate (2.2%), α-longipinene (1.2%), caryophyllene (21.9%) and caryophyllene oxide (3.1%) in needle essential oil.
	Caryophyllene, 3 Carene, Alpha humulene, alpha pinene, Beta pinene, alpha terpineol, Car-3-ene, Longifolene, camphene, limonene, alpha ternene, alpha terpineol, alpha borneol.
Wood essential oil [13]	Caryophyllene (16.75), Thunbergol(16.29), 3 carene (14.95%), Cambrene(12.08%), alpha thujene (10.81%), terpinolen (7.17%), alpha pinene(4.8%), alpha caryophyllene (3.7%) , sabinene (3.79%), Verticol (1.84%), 4 terpineol (1.79%), myrcene (1.28%)
Bark essential oil [13]	alpha pinene(31.29%), 3 carene (28.05%), Cambrene(4.86%), Longifolene (4.42%), Thunbergol(4.11), beta pinene (2.99%), sylbestrene (2.4%), terpineol (2.05%), terpinolen (2.03), terpinyl acetate(1.56%), elemol(1.46%), Methyl dihydro abtate (1.3%), myrcene (1.36%), Bornyl acetate (1.1%), alpha cadinol (1.08%)
Stem and needle extract [14,16]	Quercetin, resin acid (abietic acid, neoabietic acid), taxifolin, catechin, quercetin derivative, taxifolin derivative, catechin and gallocatechin, kaempferol, rhamnetin isorhamnetin, myricetin, 3,4-dihydroxybenzoic acid, 3,4-dihydroxycinnamic acid, monomethyl pinosylvin, dihydromonomethyl pinosylvin, resveratrol, glycoside, pinoresinol, secoisolariciresinol.
Petroleum ether extract [17]	Friedelin, ceryl alcohol and β-sitosterol
Bark [17, 18]	1,5-diliydroxy-3,6,7-triniethoxy-8-allyloxyxanthone, 1-hydroxy-3,6-diinethoxy-2-β glucopyranoxanthone, friedelin, ceryl alcohol, b-sitosterol, taxifolin, quercetin, catechin, kaempferol, rhamnetin, 3,4-dihydroxybenzoic acid, 3,4-dihydroxycinnamic acid, pinosylvin, pinoresinol, resin acid, sterols, gallocatechin and tannins
	Hexacosylferulate
P. roxburghii needles wax [19]	12-hydroxydodecanoic acid, 14-hydroxytetradecanoic acid and 16- hydroxy-hexadecanoic acid

Commercial uses

Pinus roxburghii is majorly a timber yielding plant and hence possess high commercial value. The heartwood of the plant is used in the making of furniture and building houses while the softwood is used in packaging cases and tea chest.[26] The bark is rich in tannins and finds its application in tanneries. The resin is commonly used to repair broken ceramic

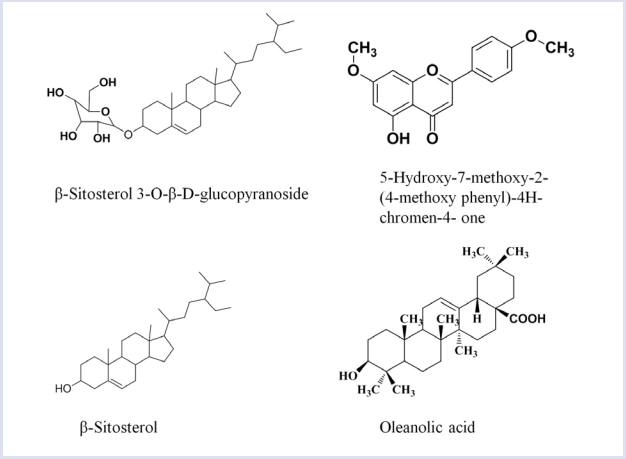

Figure 1a: Structure of different bioactive compounds from Turpentine oil of *Pinus roxburghii.*

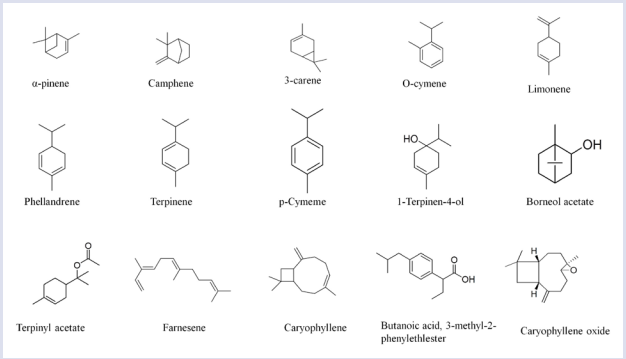

Figure 1b: Structure of different bioactive compounds from *Pinus roxburghii* Needles.

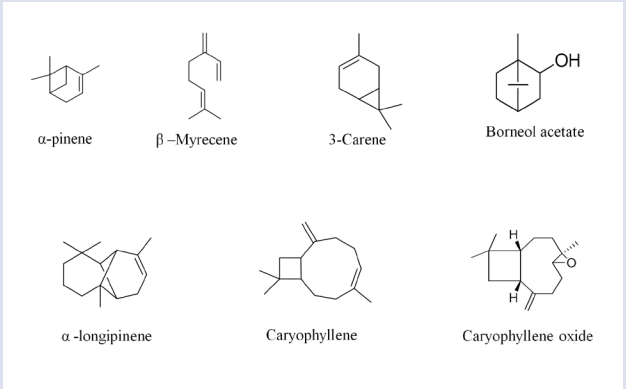

Figure 1c: Structure of different bioactive compounds from *Pinus roxburghii* Bark.

pottery. It is also used in protective coatings, varnishes and printing ink. When destructively distilled, resin produces a viscous liquid called rosin oil which is used as lubricating greases.[27] Turpentine oil is commercially important as it is a major component in varnishes, thinners, sealing wax, soaps and disinfectants.[27]

Pharmacological uses

Hepatoprotective Activity

Imran *et al*, 2012 studied hepatoprotective activity of wood oil of *Pinus roxburghii* at doses of 200, 300 and 400 mg/kg on rat liver damage induced by carbon tetrachloride and ethanol. The substantially elevated enzymatic levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and decreased level of reduced glutathione (GSH) and total protein were significantly restored to normal levels.[10]

Analgesic and Anti-inflammatory Activities

Alcoholic extract of *Pinus roxburghii* bark exhibited anti-inflammatory and analgesic activityat the doses of 100, 300 and 500 mg/kg (analgesic activity was evaluated by acetic acid-induced writhing and tail immersion tests in swiss albino mice. Acute and chronic anti-inflammatory activity was evaluated by carrageenan-induced paw oedema and cotton pellet granuloma in wistar albino rats. These activities were due to the presence of polyphenolic compounds present in the extract.[28]

Anticonvulsant Activity

Kaushik *et.al.,* 2012 reported alcoholic extract of *Pinus roxburghii* extract at doses of 100, 300 and 500 mg/kg was effective against generalized tonic-clonic and partial seizures using maximal electroshock induced seizure model in rats.[29]

Antiasthmatic activity

The alcoholic extract of *P. roxburghii* was evaluated as antiasthmatic using guinea pig ileum preparation (*in-vitro*), histamine-induced bronchospasm in guinea pigs and catalepsy in mice (*in-vivo*). Anti-allergic activity of the plant was evaluated using milk-induced leukocytosis in mice and passive paw anaphylaxis in rats (*in-vivo*).[30]

Antioxidant and Antidyslipidemic Activities

Pinus roxburghii needle extract possesses significant potential to lower the level of plasma lipid profile followed by a beneficial effect on high density lipoproteins (HDL) in high fat diet fed hyperlipidemic golden Syrian hamster model. Antioxidant activity of n- butanol fraction and alcoholic extract was found to be significant when assessed by trolox equivalent antioxidant capacity (TEAC) assay. Sharma *et.al.*, 2016 described plant extract of *Pinus roxburghii* bark posses significant antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide assays.[11,31]

Anticancer activity

Petroleum ether, ethyl acetate, chloroform and ethanol extract of *Pinus roxburghii* Sarg. was evaluated for anticancer activity on IMR-32 Human Neuroblastoma cancer cell line and implicit the observation that petroleum ether and Chloroform extracts having promising activity. Cone essential oil of *P. roxburghii* showed notable cytotoxic activity on MCF-7cells at 100 µg/ml.[32,33]

Antibacterial Activity

The plant extract shows the antimicrobial potential against a wide variety of microorganisms**.** Bissa *et.al.,* 2008 studied antibacterial activity of aerial parts of *Pinus roxburghii* against *E.coli*, *Enterobacter aerogenes*, *Agrobacterium tumefaciens*.[25] Sharma *et. al.*, 2016 reported significant antimicrobial

activity of *Pinus roxburghii* bark extract against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* and promising antifungal activity against *Candida albicans* [31] Aqueous and alcoholic extracts from *P. roxburghii* stem, leaves, bark, female cone and male cone showed growth-inhibitory activity against the bacterial plant pathogen *Agrobacterium tumefaciens*.[34]

Antidiabetic activity

Ethanolic extract of *Pinus roxburghii* bark at dose of 100, 300, 500 mg/kg possesses significant antidiabetic activity in alloxan-induced-diabetic rats.[35] In an in-silico study by Kaushik *et. al.*, 2014, it was observed that secoisoresinol, pinoresinol, and cedeodarin showed the best docking results on different diabetic receptors.[36] In an another study Kaushik *et. al.*, 2015 showed that the extracts from the bark of *Pinus roxburghii* by bioassay guided fractionation have good antidiabetic activity when tested through α-amylase inhibitory assay *in-vitro*.[37]

Pinus gerardiana

Habitat and morphology

Pinus gerardiana, known as the chilgoza pine, *(noosa*, or *neoza)* is a pine native to the northwestern Himalayas. There are about 29 species of pine which produce edible nuts those are utilized by indigenous tribal cultures in the world. In India, out of six species of pine, *Pinus gerardiana* is the only species which produces edible and highly nutritious nuts.[38] This species is distributed not only in India but also in Afghanistan, Tibet, Baluchistan (Pakistan) between 2000 and 3350 m elevation.[39,40,41] In India, it is distributed only in Himachal Pradesh (Kinnaur and Chamba Districts) and Jammu and Kashmir.

The branches are slightly ascending, and usually not whorled. The bark exfoliates in irregular thin flakes, gray in colour. The leaves are needle like, stiff, dark green, and are arranged in clusters of three. Male cones are long, and female cones are oblong ovoid with thick woody scales. Seeds are cylindrical, elongated, dark brown pointed at the tip, measure and bear a rudimentary wing. *Pinus gerardiana* is well known for its edible seeds (Chilgoza), rich in carbohydrates and proteins. These "nuts" are known and sold locally under the name of Chilgoza, "Neja" (singular) or "Neje" (plural). Chilgoza is only pine which is of immense social forestry importance because it is an income source for tribal peoplein the Kinnaur district of Himachal Pradesh.[42] They are either eaten raw or roasted and are also included as an ingredient in a variety of traditional dishes, such as breads, candies, sauces and cakes, as well as in vegetable and meat dishes. In general, pine nuts are known to be a good source of nutrients. Taxonomy and common names of *Pinus gerardiana* are given in Table 4.

Phytochemical constituents

The nuts are considered to be rich source of various nutrients including proteins, carbohydrates, fibers, minerals besides its higher amount of oil. Its oil is of very good quality, free of cholesterol and a rich source of fatty acids like Stearic acid (0.3%), Linoleic acid (Omega-6) (51.3 %), Linolenic acid (Omega-3) (1.5%) Oelic acid (Omega-9) (39.7% Arachidic acid (2.1%) Palmitic acid (7.2%).[43] Hoon *et. al.*, 2014, reported approximately 50% fat, 30% protein, 10% carbohydrate, 4% ash and 6% moisture in Pine nuts.[44] The seeds with edible kernels are obtained from ripe cones credited with carminative stimulant and expectorant properties. Analysis of kernels possesses delicate terbinthine flavor, moisture (7.5%), protein (15.9%), fat (49.9%), carbohydrates (21.6%), fibre (2.2), and mineral matter (2.9%). On pressing kernels yielded a transparent clear oil having pale yellow. Low molecular weight components in pine nuts from *Pinus pinea* includes glucose, fructose, sucrose, and raffinose, several soluble carbohydrates, saccharides (galactose, maltose, and planteose) and cyclitols (pinitol, galactinol, galactopinitol A1, fagopyritol B1, and other glycosyl-inositols). Most abundantly found cyclitols are *chiro*inositol, fagopyritol B1, and pinitol.[45] Structure of different bioactive compounds from *Pinus gerardiana* are shown in Figure 3.

Ethnopharmacological uses

The cones and wood are used as timber and firewood. Branches are commonly used as roof thatching materials in houses. Wood is also used as building material and making of huts and wooden boxes.[46] The bark of the tree is made into baskets and also into rough buckets forfetching water.[47,48] Tan or green dye is obtained from the needles. The needles contain a substance called terpene, released when rain washes over the needles and it has a negative effect on the germination of some plants, including wheat.[49,50] The resins are obtained by tapping the trunk, or by destructive distillation of the wood. Turpentine consists of an average of 20% of the oleoresin separated by distillation. Turpentine has a wide range of uses, including as a solvent for waxes etc, for making varnishes.

Table 3: ***Pharmacological activities of Pinus roxburghii.***

Activity	Parts used	Dose	Methods
Hepatoprotective [10]	Wood oil	(200, 300, 500) mg/kg	Carbon tetrachloride and ethanol induced hepatotoxicity
Analgesic [28]	Bark	(100, 300, 500) mg/kg	Acetic acid induced writhing and tail immersion test in Swiss albino rats
Anti inflammatory[28]	Bark	(100,300,500) mg/kg	Carrageenan induced paw oedema and cotton pellet granuloma in wistar albino rats
Anti convulsant activity [29]	Bark	(300, 500) mg/kg	Maximal electroshock(MES) and Pentylenetetrazole(PTZ) induced seizures in wistar albino rats .
Anti asthmatic activity [30]	Whole plant	100mg/kg	Histamine induced broncospasm in guinea pig and catalepsy in mice
Antidyslipidemic [31]	Needle	100mg/kg	High fat diet fed hyperlipidemic golden Syrian hamster
Anticancer [32,33]	Bark	(100, 200, 400, 800)μg/ml	IMR-32 Human neuroblastoma cancer cell line
	Cone oil	100μg/ml	MCF- 7 cells
Antibacterial and antifungal [25, 31,34]	Needles and female cones	5mg/ml	*E. coli*, *Enterobacter aerogenes*, *Agrobacterium tumefaciens*, *Pseudomonas aurignosa*, *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Candida albicans*
	Bark	(500, 1000, 1500) μg/ml	
Antidiabetic [37]	Bark	(100,300,500) mg/kg	Alloxan induced diabetic rats

Table 4: Taxonomy of *Pinus gerardiana*.

Taxonomic classification	Synonym	Common names
Kingdom: Plantae	Pinus gerardii	English :Gerrard's Pine, Chilgoza Pine
Division: Pinophyt		Hindi: Chilgoza, Neoza
Class: Pinopsida		Sanskrit: Dhanu,priyalam
Order: Pinales;		Gujrati name: Chaolli
Family: Pinaceae		Bengali: Chirongi
Genus: *Pinus*		Malyalam: Mungaper
Subgenus : Ducampopinus		Tamil:Sarra payer
Species: *Pinus gerardiana*		Telgu: Morrihettu

Figure 3 : *Pinus roxburghii* (Chir Pine) a) Cone b) Tree c) Needles d) Trunk.

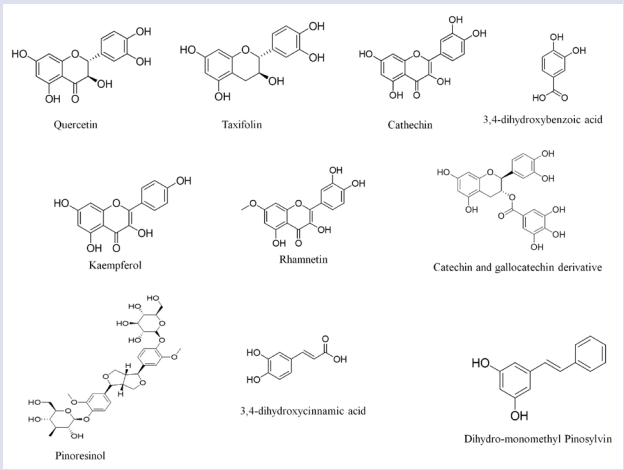

Figure 2: Structure of different bioactive compounds from *Pinus wallichiana*.

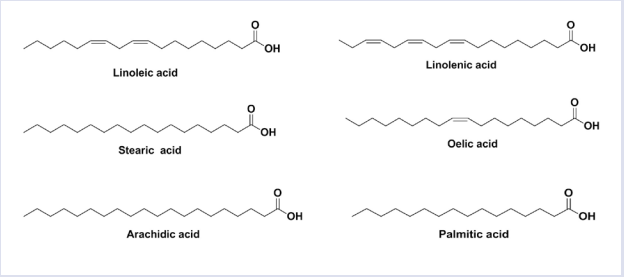

Figure 4: Structure of different bioactive compounds from *Pinus gerardiana*

Rosin is the substance left after turpentine is removed. This is used by violinists on their bows and also in making sealing wax, varnish etc. Pitch can also be obtained from the resin and is used for waterproofing, as a wood preservative etc.[51] Chilgoza is considered as one of the important dry fruits of the tribal area having carminative, stimulant and expectorant properties.

Commercial uses

The main economic use is its edible, oil-rich seeds (neoza in Hindi), which are harvested by knocking the cones from the trees in autumn and during early winter. The Chilgoza pine on tapping yield oleoresin of good quality, but owing to its limited availability and avoidance of destruction of trees for obtaining most valuable seeds the species has not been exploited commercially for timber. In traditional systems, sufficient cones are usually left on the tree to ensure that some seed is available for natural regeneration.[42]

Pharmacological uses

Antioxidant assay

Hoon *et. al.* 2014, studied total antioxidant capacity and the presence of antioxidant compounds in *Pinus gerardania*. Antioxidant compounds such as gallocatechin, catechin, lutein, lycopene, caretenoids and tocopherols are present in *P. gerardania*. Gallocatechin had the highest presence out of all the compounds.[44] Sharma *et. al.* 2016, revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and saponins in *P. gerardania*. Quantitative phytochemical analysis of plant extracts showed the presence of phenolics, flavonoids, tannins, beta-carotene and lycopene. Bark extract showed significant antioxidant activity against DPPH, nitric oxide and H_2O_2 free radicals scavenging assays.[31]

Cardiovascular disorders and thromboembolism

P. gerardiana nut oil caused blood clot lysis *in-vitro, in vitro* whole blood coagulation was also seen reduced. *P. gerardiana* nut oil has no effect on blood cell indices *in-vivo*. It was found effective in treatment of cardio-vascular disorders and thrombo embolism.[52]

Table 5: ***Pharmacological activities of Pinus gerardiana.***

Activity	Parts used	Dose	Methods
Antioxidant assay [31,44]	Chilgoza	(62.5, 125, 250, 500, 1000) mg/kg	DPPH, ABTS, radical cation scavenging assay.
Cardiovascular disorders and thromboembolism [52]	Nuts oil	30μl/ml, 60μl/ml 200μl/ml	Fibrinolytic activity, Epinephrine and collagen activated platelet aggregation
Antiinflamatory [31]	Bark	500, 1000, 1500, 2000, 2500 μg/ml	HRBC membrane stabilization assay, Albumin denaturation assay
Antimicrobial assay [31]	Bark	500, 1000, 1500 μg/ml	S. aureus, *E. coli*, *P. aeruginosa*and *K. pneumonia*, *Candida albicans*

Table 6 : Taxonomy of *Pinus wallichiana.*

Taxonomic classification	Synonym	Common names
Kingdom: Plantae	*Pinus excels*	Botanical name: Pinus wallichiana
Division: Pinophyta	*Pinus griffithii*	English :Himalayan pine
Class: Pinopsida	*Pinus chylla*	Hindi: Kail
Order: Pinales;		Kashmir Yiro, kail,kaiar
Family: Pinaceae		Bhutan TongschiLamshing
Genus: *Pinus*		German :Tranenkiefer
Subgenus :Strobus		
Species: *Pinus wallichiana*		

Table 7: Bioactive constituenets in *Pinus wallichiana.*

Plant part extract	Bioactive constituents
Needle extract [59]	Quercetin (21.426 %) , Isorhamnetin (2.857 %)
	β-sitosterol,β-sitosterol-3-O-β-d-glucopyranoside,5-hydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one ,Oleanolic acid
Stem bark methanolic extract [60]	Kampherol (2.300%), Rhamnetin (2.08 %), Myrcetin (3.0%), Isorhamnetin (2.005%), Quercetin (5.009)
Turpentine [61]	α-pinene(90%), Abietic and isopimaric acid , lambertianic acid.

Antiinflammatory activity

Hydroalcoholic extract of *P. gerardiana* stem bark at 500, 1000, 1500, 2000, 2500 ug/ml showed *in-vitro* anti-inflammatory activity, carried out using albumin denaturation and HRBC membrane stabilization assays.[31]

Antimicrobial and antifungal activity

Hydro-alcoholic extract of *P.gerardiana* at 500, 1000, 1500 ug/ml possessed potent antibacterial activity against Gram positive (S. aureus) and Gram negative (*E. coli*, *P. aeruginosa*and *K. pneumoniae*) bacteria and showed prominent antifungal activity against *Candida albicans.*[31]

Pinus wallichiana

Habitat and morphology

Pinus wallichiana (Bluepine/Kail), is coniferous evergreen tree native to Himalayas, Karakoram and Hindu Kush mountains from eastern Afghanistan, east across northern Pakistan and India. The species grows naturally along the entire length of temperate Himalayas usually at altitudes ranging 2000 to 3,500 meter above mean sea level.[53] Tree is 50+ m tall with straight trunk and short downcurved branches. Branches longer in solitary trees, creating a dome-like crown.[40] Bark is smooth and resinous in young stems, turning grey and corky with shallow fissures on mature trees with height of 36m. Bluish green leaves are needle like and are in clusters of five, 10-20 cm long, cones are light brown and when ripe are very resinous and easily distinguishable from other pines. Female cones are in groups of 1-6, 20-30 cm long, erect when young but later pendant, bluish-green when young, maturing to light brown with pale brown apophyses.[40] Of the Indian pines, it is the finest pines of north-western Himalayan region, is well known for its commercial and ecological importance, the wood of the blue pine is considered to be the best and stands next to deodar in value.[54,55,56] Though exploited mainly as a source of timber, the species is good source of oleoresin also, which is used for the production of turpentine oil, rosin, needle oil and camphor[57,58] Taxonomy and common names of *Pinus wallichiana* are shown in Table 6:

Traditional and commercial uses

Many species of Pinus yield valuable timber used for making furniture, door, window frames, paper pulp.[42] The wood is moderately hard, durable and highly resinous. It is a good firewood but gives off a pungent resinous smoke. Pine timber is divided in hard pines of two or three needled species and soft pine of five needled species. In India *P. wallichiana* (Kail) and *Pinus roxburghii* yield commercial timber. It is a commercial source of turpentine which is superior quality than that of *P. roxburghii* but is not used so freely.[42] It is of similar timber properties and quality to *P. strobus* and *P. monticola* in North America, with tall, straight trees producing wood of good strength.

Phytoconstituents

Sharma *et. al.*, 2016, reported the presence of alkaloids, flavonoids, tannins, phenols, lycopene and carotenoids in hydroalcoholic extracts of *Pinus wallichiana* stem bark extract. [31] Presence of other constituents are shown in Table 7. Structure of different bioactive compounds are given in Figure 5.

Pharmacological Activities

Antimicrobial activity

Sharma *et. al.*, 2015 reported hydroalcoholic needle extract of *Pinus wallichiana* have significant antibacterial activity against *P. aeruginosa* and *E. coli*. Hydroalcoholic stem bark extract of *Pinus wallichiana* possess significant antibacterial activity against *Pseudomonas aurignosa*, *S. aureus*, *K. pneumonia* and possesses potent antifungal activity against *Candida albicans.*[31] In another study by Rahman *et. al.*, 2016 *Pinus wallichiana* showed potent antibacterial and antifungal activity against *Escherichia coli, Bacillus subtilis, Shigella flexenari* (clinical isolate), *Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi*, the fungal strains were *Trichophyton longifusis* (clinical Isolate), *Candidaalbicans*, *Aspergillus flavus*, *Microsporum canis* and *Candida glaberata.*[62]

Figure 5: *Pinus gerardiana* (Chilgoza pine)a) Cone b) Tree c) Needles d) Trunk e) Kernels

Figure 6: *Pinus wallichiana* (Blue Pine) a) Cone b) Tree c) Needles d) Trunk.

Insecticidal assay

Different fractions of *Pinus wallichiana* extract were tested against various insects *Rhyzopertha dominica, Tribolium castaneum* and *Callosobruchus analis*. The The *n*-hexane fraction showed 20 % activity against *R. dominica,* while the ethyl acetate showed 20 % activities against all the strains. The chloroform revealed 20 % activity against *R. dominica,* while the aqueous showed 20 % and 40 % activity against *R. dominica* and *C. analis.*[60]

Phototoxic activity

The *n*-hexane, ethyl acetate, chloroform, aqueous fractions of *Pinus wallichiana* needles showed significant phytotoxicity activity at 500μg/ml, while at low concentration 50μg/ml and 5μg/ml showed moderate activity.[60]

Antioxidant activity

Hydroalcoholic extract of leaves and stem bark of *Pinus wallichiana* possess significant *in-vitro* antioxidant activity when tested in DPPH, nitric oxide radical scavenging, hydroxyl radical scavenging and hydrogen peroxide radical scavenging assays. Presence of phenolics, flavonoids, tannins, carotene were also reported in *Pinus wallichiana.*[62,31]

Table 8: Pharmacological activities *of Pinus wallichiana*.

Activity	Parts used	Dose	Methods
Antimicrobial activity[60,62]	Needles	24mg/ml	Agar well diffusion method
Insecticidal activity[60]	Needles	20mg/kg	Insects: *Rhyzopertha dominica, Tribolium castaneum* and *Callosobruchus analis*.
Antioxidant[31]	Bark	(10,20,40,80,100) μg/ml	DPPH, Nitric oxide and Hydroxyl radical scavenging assay
Anti inflammatory[31]	Bark	(500, 1000, 1500, 2000, 2500) μg/ml	Albumin denaturation, HRBC membrane stabilization

Table 9: Marketed formulation of Pinus species.

Name of formulation	Plant part used	Pharmacological activity
Polyherbal oil extract [63]	Oleo resin of *Pinus roxburghii*	Analgesic and anti-inflamatory
Rumalaya gel [64,65]	Resin from *Pinus roxburghii*	Joint and Bone pain associated with various orthopedic ailments

Anti-inflammatory activity

Hydroalcoholic stem bark extract of *Pinus wallichiana* possesses significant *in-vitro* anti-inflammatory activity when tested through albumin denaturation and RBC membrane stabilization assays.[31]

Marketed formulations

A summary of commercial formulations of Pine species is given in Table 9.

CONCLUSION

This review furnishes the presence of several phytochemical components and pharmacological properties of *Pinus roxburghii*, *Pinus wallichiana* and *Pinus gerardiana*. The pharmacological activities reported in the present review confirm that the therapeutic value of these plants is very high, having a leading capacity for the development of a new, safe, effective and cheaper drug in future. But there is need for more elaborative study, pharmacological investigations, clinical trials, more exploration and public awareness for the best utilization of medicinal properties of these pine species. Even the industrial entrepreneurs also should come forward with new concepts and steps towards the best use of these potential medicinal pine species. Lets hope that in future herbal products will be competing modern medicines with added advantages of more safety and lower costs.

CONFLICT OF INTEREST

The authors have declared no competing interest.

ABBREVIATIONS

HDL: High Density Lipoprotein; **DPPH:** 2,2,diphenyl picrylhydrazyl; **TEAC:** Trolox equivalent antioxidant activity; **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6sulphonic acid.

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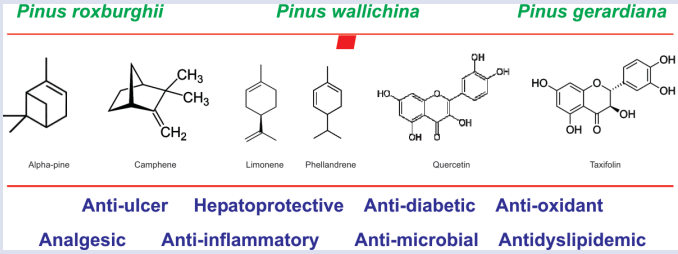
GRAPHICAL ABSTRACT



SUMMARY

- A pine is any conifer in the genus Pinus of the family Pinaceae.
- Pinus is the largest genus of monoecious, resiniferous, evergreen trees commonly known as Pines.
- *Pinus roxburghii* , *Pinus wallichiana, Pinus gerardiana* are native species of Pine from Himalayas.
- The constituents present in these pinus plant species are beneficial for the purpose of treatment of various ailments.
- The phytoconstituents and pharmacological activities of the pinus plant species confirm that the therapeutic value of these plants is very high, having a leading capacity for the development of a new, safe, effective and cheaper drug in future.

ABOUT AUTHORS

Aditi Sharma: (M.Pharm, Pursuing PhD) is working as an Assistant Professor (Pharmacology) in School of Pharmaceutical Sciences, Shoolini University.

Lalit Sharma: (M.Pharm, Pursuing PhD) is working as an Assistant Professor (Pharmacology) in School of Pharmaceutical Sciences, Shoolini University.

Dr. Rohit Goyal : (M.Pharm, PhD) Dr. Goyal is working as an Associate professor (Pharmacology) in School of Pharmaceutical Sciences, Shoolini University.

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