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Pharmacognosy of *Pinus roxburghii*: A Review

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Pinus roxburghii Sarg. (syn. *Pinus longifolia* Roxb.) (Pinaceae), found in the Himalayan region, furnishes an oleo-resin which is used as insecticides, disinfectants and liver disorders. *P. roxburghii* are reported to have wound healing, cytotoxic, antibacterial, antifungal and spasmolytic actions. The plant also shows beneficial effects in the treatment of cough, ulceration and genito-urinary disorders. Folklore reports suggest its use in inflammations, asthma, chronic bronchitis, piles, diseases of the liver and spleen, urinary discharges, toothache, tuberculosis, scabies and epilepsy. This review provides a bird's eye view about geographical distribution, folklore uses, phytoconstituents and pharmacological properties of *P. roxburghii.*

Keyword: *Pinus roxburghii*, Pinaceae**,** oleo-resin, Folklore.

1. Introduction

Pinus roxburghii Sarg. (Pinaceae), commonly known as chir pine, is a tall tree with a spreading crown found in the Himalayan from Kashmir to Bhutan, Afghanistan and in southern Indian hills. It is also planted in the garden for ornamental purpose. The tapping of the stem produces a clear, transparent oleo-resin with the pungent and bitter taste. Distillation of the turpentine oil from the oleo-resin leaves faintly aromatic and transparent rosin (colophony). It is utilized in the manufacturing of fireworks, insecticides and disinfectants and enters into certain lubricating compositions, hair fixing and nail polishing preparations (Anonymous, 2003). It is used in preparation of ointments and plasters and in many products such as chewing gum, polishes, and varnishes, but is a common cause of contact allergy. The resin is applied to cure boils (Rajbhandari, 2001) and administered orally to combat gastric troubles (Manandhar, 2002). The rosin is useful in adhesives, printing ink, electric isolation, paper, soldering flux, varnish and matches. In printing ink industry rosin gives adhesiveness, surface smoothness, hardness, anti-blocking and other properties. Rosin has a good electric isolation, being used as oil in cables for high voltage electricity. In soldering process, rosin is used to get rid of oxide compounds in the surface of metal, synthetic rubber and chewing gums (Wiyono, *et al.*, 2006).

Native Americans have used pine resin to treat rheumatism because of its anti-inflammatory properties. The resin acts to remove the joint inflammation caused by rheumatism, which helps to restore movement and to alleviate pain. The Costanoan Indians gained these benefits by chewing on the gum-like resin. A traditional use for pine resin has been as an external treatment for burns and sores. The pine resin has stimulant, diuretic and laxative properties. In China the resin from a particular pine tree is used to treat abscesses. Resin from the spruce tree was used by colonial Americans as a cold and cough remedy, as well as straight from the tree as a cancer treatment. Physicians in colonial America also recommended tar water, or ground pine resin mixed with water, as a remedy for ulcers, smallpox, and syphilis (Langenheim, 2003). Different parts of the plant are prescribed to treat cough, colds, influenza, tuberculosis, bronchitis, as antiseptic, diaphoretic, diuretic, rubefacient, stimulant and febrifuge (Chopra *et al*., 1986; Puri *et al*., 2011).

1.1 Uses in Folklore Medicine

The plant resin is sweet, bitter, pungent; thermogenic; oleagenous and intestinal antiseptic. Internally, the colophony is used as a stomachic and externally as a plaster and is applied to buboes and abscesses for suppuration. The wood is considered stimulant, diaphoretic and useful in burning of the body, cough, fainting and ulceration. The resin is stimulant both externally and internally. Internally, it acts chiefly on the mucous membrane of the genito-urinary organs, and is, therefore, a very good remedy for gonnorhoea. It has shown diuretic, emmenagogue, purgative and expectorant actions. It is also useful in inflammations, asthma, chronic bronchitis, piles, diseases of the liver and spleen, urinary discharges, earache, toothache, lumbago, tuberculosis, scabies and epilepsy. The gum has shown good effect in diseases of the vagina and uterus (Kirtikar and Basu, 1999; Anonymous, 2003).

1.2 Taxonomy

Kingdom	:	Plantae
Division	:	Pinophyta
Class	:	Pinopsida
Order	:	Pinales
Family	:	Pinaceae
Genus	:	*Pinus*
Species	:	*roxburghii*
Binomial name:		*Pinus roxburghii* Sarg.

1.3 Vernacular Names

The vernacular names of *P. roxburghii* are Bhadradaru, Manojna in Sanskrit; Chil, Chir, Salla in Hindi; Saralgachha in Bengali; Saraladeodara in Gujarati; Salla, Charalam in Malayalam; Simaidevadari in Tamil and Devadaru in Telugu (Kirtikar and Basu, 1999).

1.4 Distribution

A tall tree with a spreading crown is found in the Himalayas from Kashmir to Bhutan and in the Siwalik hills at altitudes of 450-2,400 m. It comes up tolerably well in the plains also and is sometimes planted in gardens for ornamental purposes. The chir pine occurs in the Himalayas almost exclusively in the outer hills and valleys, which receive the bulk of the rainfall during the monsoon (Anonymous, 2003).

1.5 Morphology of *P. roxburghii*

A large tree, branches more or less whorled; bark dark grey, often reddish, deeply fissured, rough, exfoliating in longitudinally elongated plates; leaves in clusters of three, 20-30 cm, long, triquetrous, finely toothed, 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, brown, woody, seeds winged; without wing 7.5-130 mm ×5×6.5 mm; wings long, membranous (Anonymous, 2003).

2. Cultivation

2.1 Natural Regeneration

It takes places through seeds. Under ordinary forest conditions, trees less than 30-year old seldom bear cones. The cones begin to open during April-May of the third year, i.e., about-24 months after their appearance and the seeds get dispersed during April-July. Under natural conditions, the seeds germinate as soon as sufficient moisture is available. The germination commences at the beginning of the monsoon. A number of factors such as light, drought, tonography and soil have considerable influence upon the extent and quality of natural regeneration (Anonymous, 2003).

2.2 Artificial Regeneration

It is required transplanting nursery-raised seedlings or by direct sowing. The mature cones are collected from the trees during March-April and are placed in the hot sun to loosen the scales, and thereafter the seeds are threshed out. The seeds are sown in the nursery during March-April in shallow drills 15 cm apart. The seedlings are picked out in July. One-or two-year old seedlings are usually transplanted at the beginning of the rains (Anonymous, 2003).

2.3 Phytoconstituents of *P. Roxburghii*

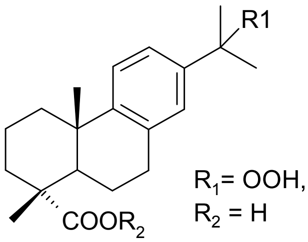
Two new xanthone identified as 1,5-dihydroxy-3,6,7-trimethoxy-8-dimethylallyloxy-xanthone and 1-hydroxy-3,6-dimethoxy-2-β-D-glucopyranoxanthone have been isolated from the methanolic extract of the bark of *Pinus roxburghii* (Rawat *et al*., 2006). The colophony obtained is a transparent golden-yellow solid with the following constants: specific gravity 15.5º, 1.064-1.085; saponification value, 170.0-176.3; acid value, 166.60-173.4; and m.p. 70-72°C. It is composed of mixture of acids, partly in the form of anhydrides; the principal acid is abietic acid. On steam distillation, the needles of *P. roxburghii* are reported to give 0.26 % of colourless, volatile oil known as pine oil. The oil contains *α*-limonene, *α*-phellandrene, borneol, longifolene and *α*-cadinene. Oleoresin from chir is the main source of turpentine oil in the country. The average composition of oil is: *α*-pinene (20-30 %), *β*-pinene (5-10 %), *β*-3-carene (55-65 %), and longifolene and other terpenes (2-10 %). The presence of a *β*-carene, *β*-longifolene and longicycline has also been reported. The bark contains 7-10 % of tannins (Anonymous, 2003). The hydrolyzed fraction of chir pine oleoresin afforded two phenolic acids and a lignan. Ferulic acid, *p*-coumaric acid and pinoresinol were isolated and their structures were established (El-Shaer, 2002).

Diterpene rosin acids isolated from *P. roxburghii* species contained several acids, of which those containing the abietane skeleton have been the subject matter of numerous chemical investigations. Abietic acid is a known inhibitor of lipoxygenase activity, possesses anti-inflammatory properties and binds to phospholipid bilayers. Abietic acid contains a tricyclic perhydro phenanthrene skeleton with a carboxyl group in ring A and a heteroannular trans diene system in rings B and C (Kuchimanchi *et al.*, 2002).

Abietic acid and its methyl ester were investigated under various storage conditions to provide an indication of their preferred oxidation mechanisms and to investigate the most susceptible positions for modification in the abietane skeleton. Six known compounds, methyl 7α, 13β-dihydroxyabiet-8(14)-enoate, methyl-7α, 13α-dihydroxyabiet-8(14)-enoate, methyl-12-oxoabietate, methyl- 7-oxodehydro-abietate, methyl-7α-hydroxydehydroabietate and 13,14-seco-13,14-dioxoabiet-7(8)-enoic acid, and six new oxidation products were identified (Prinz *et al.*, 2002). Pine rosin constitutes a widely available source of abietic acid, from which many derivatives have been synthesized. Studies on the arylation of anilines derived from dehydroabietic acid, the main component of disproportionated pine rosin, are presented. The redox properties of diarylamines were investigated by cyclic voltammerty, and their free radical scavenging activity was tested by reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Three of the diarylamines with lower oxidation potential proved to be as active as isopropyldiphenylamine (IPPD) and superior to tert-butylhydroxytoluene (BHT), both commercially available synthetic antioxidants (Esteves *et al.*, 2001). Oxime ethers of carbonyl compounds derived from longifolene, an abundantly available sesquiterpene from Indian turpenine oil obtained from *P. longifolia* have been synthesized (Sawaikar *et al.*, 1995). A hdyroperoxide, 15-hydroperoxydehydroabietic acid (15-HPDA), with contact allergenic properties has been detected in rosin obtained from *Pinus* species (Shao *et al.*, 1995).

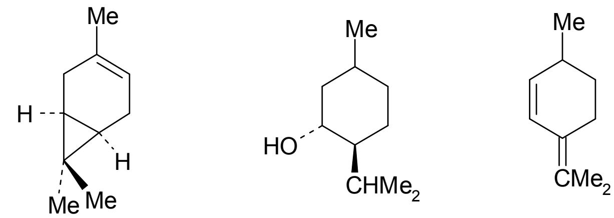
Sugars were extracted from the bark of *P. roxburghii* by 2 methods. Their separation and identification were carried out by thin-layer and paper chromatography. The amount of sugars determined by UV method was: glucose (1.25-

2.49 %), fructose (1.2-2.9 %) and arabinose (1.17-1.87 %).



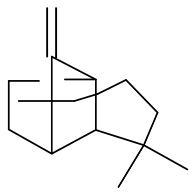
The amounts of total sugar (4.9-6.8%) determined by titrimetric and UV methods are comparable (Ahmad *et al.*, 1990a). Seed oils of *Sesbania aculeata, S. grandiflora, S. aegyptica, Robinia pseudoacacia, Albizia moluccana,* and *P. roxburghii* were analyzed for fatty acid components. All the seed oils resemble the general pattern of oleic-linoleic type. *S. aculeata* and *P. roxburghii* seeds were rich in oil with high linoleic acid oil and polyunsaturated fatty acid contents (Ahmad *et al.*, 1990b). The high value of Stiasny number indicated that the extracts contained large amount of reactable tannins (Ahmad *et al.*, 1989). Three hydroxyacids obtained on hydrolysis of *P. roxburghii* needles wax were identified as 12-hydroxydodecanoic acid, 14-hydroxytetradecanoic acid and 16-hydroxy-hexadecanoic acid by spectral data and chemical studies. The acids were converted to macrocyclic lactones by intramolecular cyclization, under high dilution condition, of the iodoacids with anhydrous K_2CO_3 in EtCOMe or of the parent acids with *p*-toluenesulfonic acid in benzene. These lactones are used in perfumery as fixatives with long-lasting musk-like odor. An estolide composed of two juniperic acid units was also isolated from the reaction product of hydroxyacids with *p*-toluenesulfonic acid in $CHCl_3$ (Dayal *et al.*, 1989).

Turpentine from *P. wallichiana* xylem resin contained around α-pinene (90 %). In addition to small amounts of other terpenes, isomers of undecane, dodecane and tridecane were detected as minor constituents. In contrast, *P. roxburghii* turpentine contained significant amounts of α-pinene, β-pinene, and Δ-3-carene predominating. Abietic and isopimaric acid were the major resin acids of both species but *P. wallichiana* alone contained lambertianic acid (Coppen *et al.*, 1988).

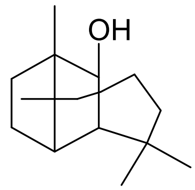


I II III

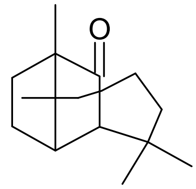
Pine (*Pinus*) rosin afforded the following constituents: abietic acid, abietic acid and dehydroabietic acid mixture, as well as their potassium salts, *d*-pimaric acid and diterpenoid acid salts (Olechnowicz-Stepien and Lamer-Zarawska, 1981). The (+) form of longifolene, which is more common compared to the (-) form, occurs in higher plants, mainly in *P. longifolia.* Longifolene, an important member of the sesquiterpene class of the major mevalonoid group of natural products, was first isolated from Indian turpentine oil from *Chir* pine (Jadhav and Nayak, 1980). The chemical composition of turpentine oil of chirpine grown under the same climatic conditions in New Forest Estate, Dehradun is reported. The turpentine oil obtained from the trees of seed origins lying west of Chakrata contained more β-pinene than those in the east. The maximum (29.67 %) content of β-pinene was found in the oil obtained from the trees of Rawalpindi seed (Verma and Suri, 1978). Essentially complete analyses are reported for the sesquiterpene fractions from the leaf oils of *P. longifolia, P. pinaster, P. thunbergii, P. densiflora, Chamaecyparis nootkatensis,* and *C. obtusa* and of oil from the liverwort *Scapania undulata*. These species have been recorded as sources of longifolene and related compounds, but the present results differ considerably from those of the previous analyses. The best sources of longifolene and its relative compounds were *P. pinaster* and *S. undulata* (16, 46% of the steam volatile oil, respectively) (Banthorpe *et al.*, 1977).



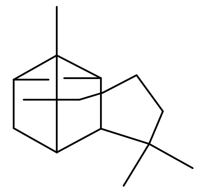
I
Longifolene



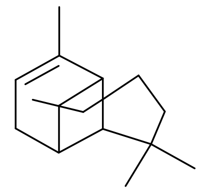
II
Longiborneol



III
Longicamphor



IV
Longicyclene



V
Longipinene

The occurrence of hexacosylferulate in *P. roxburghii* is reported by Chatterjee *et al.*, 1977. Friedelin, ceryl alcohol and β-sitosterol were isolated from the unsaponifiable matter of the petroleum ether extract of the bark of *P. roxburghii* (Beri, 1970).

The essentials oils obtained from the needles of native pines and firs of the following species growing wild in the Indian Himalayan region were studied and the mentioned components identified in *P. excelsa* are α-pinene, *α*-limonene, *α*-phellandrene, dipentene, *α*-carinene, traces of an unidentified carbonyl compound, and azulene, *P. longifolia*, *α*-pinene, *β*-pinene, Δ-3-carene, *α*-limonene, *α*-phellandrene, borneol, bornyl acetate, longifolene, and *α*-cadinene; *Cedrus deodrara: α*-pinene, *β*-pinene, 1-3-carene, *α*-limonene, borneol, boryl acetate, *α*-cadinene, and several unidentified sesquiterpenes (Chaudhary *et al.*, 1960).

Heartwood extraction and fractionation yielded the following stilbene derivatives from *P. longifolia,* pinobanksin (0.02 %), pinocembrin (0.015 %) and pinosylvin monomethyl ether (0.02 %); from *P. gerardiana* are pinobanksin (0.03 %), pinostrobin (0.05 %), pinosylvin monomethyl ether (0.003 %) and *L*-arabinose; from *Pinus khasya* are pinocembrin (0.12 %), pinosylvin monomethyl ether (1.15%) and *L*-arabinose (Ahluwalia *et al.*, 1956).

3. Pharmacological Properties of *P. roxburghii*

3.1 *Wound healing activity of P. roxburghii*

Methanol and aqueous extracts of the leaves of *P. longifolia* were screened for wound healing property on excision, incision wound models in Wistar albino rats. Both the extract showed significant wound healing activity. However, the rate of wound contraction and epitheliazation was faster in methanol extract groups (Kinger *et al.*, 2006).

3.2 *Cytotoxicity activity of P. roxburghii*

Compounds methyl-7-oxodehydro-abietate and methyl-7α-hydroxydehydroabietate, oxidation products of abietic acid and its methyl esters were regarded potent allergens. The cytotoxicity of several compounds against KB cells is evaluated and weak cytotoxicity activity is observed for methyl-7α,13α-dihydroxyabiet-8(14)-enoate, methyl-12-oxoabietate, methyl-7α-hydroxydehydro-abietate and with IC_{50} values of 12.5, 4.5, and 5.8 µg/ml, respectively (Prinz *et al.*, 2002).

3.3 *Antimicrobial activity of P. roxburghii*

A method performed in solid medium in multiwell plates was adapted in order to assess the antimicrobial activity of methyl *cis*-7-oxo-deisopropyldehydroabietate (MCOD) and methyl *trans*-7-oxo-deisopropyldehydroabietate (MTOD), resin acid derivatives obtained from pine rosin. MCOD and MTOD completely or partially inhibited spore germination of filamentous fungi *Mucor racemosus, Syncephalastrum racemosum, Rhizopus stolonifer* and *R. arrhizus* and growth of bacteria *S. aureus* and *E. faecalis.* The combination of both compounds inhibited growth of bacteria *S. aureus* and *E. faecalis.* The combination of both compounds inhibited growth of *Escherichia coli* and *Klebsiella pneumoniae.* Antimicrobial activity of 105 Indian plant species was tested. Among them, 30 showed antibacterial activity; 20 of these exhibited antifungal action as well. Seeds of *Carum copticum*, stem of *P. longifolia*, roots of *Plumbago zeylanica* and *Saussurea lappa*, and rhizome of *Alpinia officinarum* have considerable antifungal activity, especially against pathogenic fungi. Antibiotic activity against a wide variety of microorganisms-pathogenic and nonpathogenic Gram-positive and Gram-negative bacteria, yeast, and fungi-was also noted with leaves of *Lawsonia intermis*, roots of *Plumbago zeylanica*, and fruits of *Tamarindus indica, Terminalia balerica*, and *Emblica officinalis* (Ray and Majumdar, 1976).

3.4. *Antibacterial activity of P. roxburghii*

The stereo-configuration of the molecule seems to play a role in the antibacterial activity of these compounds and a possible synergistic effect seems to increase their antibiotic activity. The technique described in the work showed to be reproducible and sensitive in the evaluation of antimicrobial activity towards fungi and bacteria (Savluchinske Feio *et al.*, 1997).

3.5 *Anti-mosquito activity of P. roxburghii*

Oxime ethers of carbonyl compounds derived from longifolene, a sesquiterpene obtained from *P. longifolia* were evaluated for biological activity against mosquito *Culex quinquefasciatus*. All oxime ethers exhibited 90-100 % mortality due to insect growth regulating (IGR) activity at 1 ppm; some oxime ethers were active at 0.1 ppm showing 80-85 % mortality (Sawaikar *et al.*, 1995).

3.6 Spasmolytic action of *P. roxburghii*

Pharmacological tests have indicated a weak cardiac and spasmolytic action of the abietic acid (Olechnowicz-Stepien and Lamer-Zarawska, 1981).

3.7 Other activities of *P. roxburghii*

The allergenic activity of 15-hydroperoxydehydroabietic acid (15-HPDA) was studied in an experimental sensitization test on guinea-pigs and Pinus shows antiallergenic activities (Shao *et al.*, 1995).

Batta and associates reported a case of a 58-year-old lady having 1-year history of lip and perioral dermatitis. She produced a positive reaction to colophony at 2 and 4 days. She had a positive reaction to her lipstick at 2 days. Colophony was contained in the lipstick pigments. Avoidance of the lipstick resulted in resolution of her symptoms (Batta *et al.*, 1997).

4. Conclusions

This review furnishes the presence of several phytochemical components in *P. roxburghii* and its pharmacological properties. The recent evidences show an effective role of *P. roxburghii* in the development of formulations used for curing skin diseases. The pharmacological activities of *P. roxburghii* are wound healing, cytotoxic and spasmolytic actions that may play important role in the further research on Pinus. The importance of plants in ethnomedicincinal practices provides clues to new area of research and in biodiversity conservation.

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