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Phytochemical and biological activities of *Pinus wallichiana*: A short review

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Abstract
Pinus wallichiana A.B. Jacks is known as the blue pine and it 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 this plant is very 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 with terpenoids and flavonoids and has strong antioxidant effect and other biological activities.

Keywords: *Pinus wallichiana*, chemical compounds, plants, bioactivities

Introduction
Plants have been an important source in cancer drug discovery. The medicinal values of plants lie in their phytochemicals, which makes specific physiological actions on the human body. Phytochemicals are compounds found in plants that are utilized as food and medicine top reserve against illness and to ensure human health [1]. Since ancient time, people have been using various natural compounds in the forms of medicines for the treatment of various diseases. Many of recent therapies are based on this ancient system of medicine. Documentation of plants for their ethnopharmacological properties is reported from the 1000 years. Around 50% of modern drugs are *Pinus wallichiana* (Common names: Kail, Biar) is a famous conifer for its timber quality. The plant is useful for its gum, resin and fuel wood purposes and also suitable for various medicinal purposes [2]. It is habituate to grow in the altitude range of 1800-4300 m above sea level. Plants grow naturally in Himalayan range, India, Pakistan, Nepal, China, Afghanistan and Bhutan [3]. Bark and needles extract of plant has shown the presence of a good amount of phenolics and flavonoids along with antioxidant properties [4, 5]. HPLC analysis of the needles has shown the presence of quercetin, rhamnetin, isorhamnetin, and kaempferol [6]. We extracted the phytoconstituents of needle of *P. wallichiana* with methanol using accelerated solvent extractor (ASE) at room temperature and high pressure to increase the yield and diversity of phenolics. In upcoming years, gas chromatography-mass spectroscopy (GC-MS) has emerged as a promising method for the analysis of bioactive constituents. It has been employed to identify the various bioactive constituents e.g., non-polar compounds, long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds with volatile oil, fatty acids and lipids [7, 8]. This review mentioned phytochemicals and bioactivities of the plant.

Traditional Use
It 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 usesinclude use of various parts of plant or resin for curing of various ailments such as healing, fever, bacterial diseases [9].

Biological activities

Antioxidant Effect

The crude methanolic extract of leaves and fruits of the plant proved strong antioxidant activity when tested with ferric thiocyanate and thiobarbituric acid methods. The results proved that the leaves had a free radical scavenging potential and a protective effect towards lipid peroxidation where the activity of the extract was greater than Vitamin E and comparable to butylated hydroxy toluene taken as positive control [10] It was further observed that hydromethanolic extractand 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 antioxidantascorbic acid [11]. The essential oil of *P. wallichiana* exhibited free radical scavenging activity as evident from DPPH scavenging assays [12].

Antimicrobial Effect

It showed 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 µg.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 [13]. 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 [14]. 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. 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% [15].

Antiproliferative Activity

The essential oil of the plant showed 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 µg.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 [12].

Chemical compounds

Chemical constituents of various parts of the plant have been worked out in details by various groups of researchers using different solvents and techniques for extraction and detection. The essential oils from the needle and turpentine has terpenes as the major constituent. The alcoholic extract of various parts of plant has a wide array of compound namely hydrocarbons, terpene acids, organic acids, flavonoids, flavonoid glycosides, terpene alcohols etc.

Needle essential oil

It contained 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%) [12].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%) [12].

Methanolic extract of the 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%) [16].

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 (17).

Diethyl ether extract of the needle

Isorhamnetin (2.857%), Quercetin (5.712%) [18].

Lipophillic constituents of dry bark

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) [19].

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) [19].

Methanol extract of bark

Kaempferol (2.30%), Rhamnetin (2.08%), Isorhamnetin (2.005%), Quercetin (5.009%), Myricetin (3.0%) [18].

Ethyl acetate extract of bark

Kampherol (1.857%), Quercetin (5.001%) [18].

Conclusion

The plant is rich with interesting bioactive constituents that contributed to the bioactivity of the plant as antitumor, antioxidant, antimicrobial effects and others.

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