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Review Article

Ethnobotanical and pharmacological importance of *Taxus wallichiana* Zucc.

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Abstract

Taxus wallichiana Zucc. or the Himalayan Yew is a gymnosperm growing along the Himalayan region of Indian and adjoining countries. The plant is extensively used by local people for treatment of various diseases such as fever, headache, diarrhea, fractures, problems of nervous system etc. It also finds usage in Unani system of medicine. The plant is rich in various bioorganic compounds natural products such as hydrocarbons, terpene alcohols, terpenoids (including taxoids), organic acids etc. The plant has been explored for anti-inflammatory, analgesic, antipyretic, anticonvulsant, immunomodulatory, hepatoprotective and anticancer activity with satisfactory outcome. The pharmacological activity of the plant is largely due to the presence of large number of terpenoids. The bioactive constituents present in the plant interacts with a large number of biochemical pathways involved in inflammatory processes, cell division cycles and inhibits a number of enzymes to bring about its protective action against various diseases. In this review, an attempt have been made to highlight the beneficial properties of *Taxus wallichiana* in various levels of usage starting from its fundamental ethnobotanical use to pharmacological use involving both *in-vitro* and in-vivo studies. Insights into the molecular mechanisms of action of the active constituents in bringing about the beneficial activity have also been illustrated. The plant can very well become a source of medicine for better management of a large number of diseases including cancer.

Keywords: *Taxus wallichiana*; terpenoids; cancer; anti-inflammatory; Taxol

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Introduction

The Himalayan region has been a rich source of medicinal plants for millions of population inhabiting in around the mountain ranges. The Himalayan region is rich in floral diversity and plants are extensively used by local people for their daily needs ranging from thatching and shelter, fuel, fodder, house hold items, medicines (1, 2). The Indian Himalayan region presently houses 8000 species of vascular plants of which 1748 are known to possess medicinal properties (3). Himalayan mountains are home of 232 families of angiosperms representing 2302 genera and 10452 species while the gymnosperms are represented by 8 families, 20 genera and 51 species (4). *Taxus wallichiana* is one such gymnosperm that grows in the Himalayan region. It is a small to medium sized evergreen tree with a height of 10 m to 28 m (5). The plant is widely distributed in Asia and its

occurrence spans from Afghanistan in the west to Philippines in the east and widely distributed in Himalayan regions of India and adjoining countries (6).

T. wallichiana is traditionally used by local people of Indian subcontinent for the cure of a number of ailments. In India, tincture prepared from aerial part of the plant is traditionally used for treatment of several diseases of central nervous system such as hysteria, grittiness, biliousness, epilepsy and nervousness. The plant also forms one of the components of the popular unani drug 'Zarnab' which is known to possess sedative and aphrodisiac properties (7). *T. wallichiana* is also used indigenously by people of Nepal for curing respiratory problems, bronchitis and cancer (8). The leaves of *T. wallichiana* is also used to prepare herbal tea for cure of epilepsy and indigestion (9). *T. wallichiana* is also reported to have immunomodulatory, anti-bacterial, anti-fungal, analgesic, anti-pyretic and anti-convulsant activities (10). Thus, based on the available reports, an attempt has been made to review the entire domain of beneficial properties of *T. wallichiana* with special emphasis on its chemical constituents, ethnobotanical uses and pharmaceutical applications.

Methodology

Extensive literature survey have been done using internet with PubMed, google scholar forming the search platform. Relevant research papers and review articles were selected in framing the article. The entire review article has been divided into an introductory phase which briefly describes geographical distribution, morphological features and taxonomic classification of *Taxus wallichiana.* Introductory segment is followed by detailed account of ethnobotanical uses of the plant. The article then extends into further insights into the chemical constituents of the plant and pharmacological uses and culminates with the discussion and conclusive remarks on the mode of action of the active principles in bringing about the pharmacological activities. The chemical structures have been drawn using ACD/Chem sketch Freeware 2018.2.5.

Results

Geographical distribution

Taxus wallichiana grows throughout the Himalayan region. In India it is widely distributed in the temperate zone of Himalayan mountains between altitudes of 1800m to 3300m above mean seal level (11). It is a slow growing understory species occurring in forests of Indian Himalayas and grows with *Abies pindrow*, *Quercus semecarpifolia, Q. floribunda, Q. leucotrichophora*, *Betula utilis*, *Acer caesium*, *Pinus wallichiana*, *Rhododendron arboreum* and *Betula alnoides* (12). In the eastern Himalayas, the species grows in moist temperate zones at an altitude between 1600 to 2700 meters in West Kameng, Tawang, Lohit and Dibang Valley districts of Arunachal Pradesh (13). The plant also grows in hilly regions of Manipur and Meghalaya in north-eastern India (14). In Nepal, the plant is found in Rasuwa, Kavre, Kaski, Gorkha, Dolakha, Sindhupalanchok, Lamjung, Myagdi, Sakhuwasabha, Taplejung and Katmandu districts (15). It is also found in the Kanchenjunga landscape, a region of eastern Nepal (parts of Taplejung, Panchthar, Ilam and Jhapa districts), Sikkim and North Bengal (Darjeeling and Jalpaiguri, and recently formed Alipurduar and Kalimpong districts) in India, and western Bhutan (portions of Haa, Chukha, Samtse, Dagana and Paro districts) (16). In Pakistan, the plant is present in Murree hills, Hazara, Swat, Dir, Chitral as undergrowth of other conifers and broad leaved deciduous plants (17). It is also reported from Gilgit-baltistan and also in SWAT valley on the foot hills of Hindukhush range in Pakistan (18, 19).

Description of the plant

Taxus wallichiana is a dioecious tree species (20). The stems are fluted with spreading branches. The barks are thin, reddish brown and scale like (21). Leaves dark grey in colour, glossy green above, paler beneath, linear, 2-3.8 × 0.3 cm in length, coriaceous, flattened, arranged in two vertical opposite rows. Cones are axillary and sessile. Male cones are solitary, axillary, sub-globose, bracts empty, with ten stamens. Female cones are solitary with few imbricate scales surrounding an erect ovule. Ovules are surrounded at base by membranous cup shaped disc. Fruit have bright red disc (Fig 1), succulent, enlarged, 7-8 mm in length. Seeds are olive-green in colour. Seeds dispersed by birds and animals. Growth of the trees are extremely low with 12-14 annual rings per 2.5 cm radius and girth increment 0.4 to 1.3 cm per year (22).

Taxonomic hierarchy (IUCN Taxonomic hierarchy)

Kingdom: Plantae

Division: Tracheophyta

Class: Coniferopsida

Order: Coniferales

Family: Taxaceae

Genus: *Taxus*

Species: *wallichiana* (23)

Synonym: *Taxus baccata* L. ssp. *Wallichiana* Zucc. Pilg.

Fig 1. Picture of *Taxus wallichiana* Zucc. showing ripe female fructification (24)

Population and Conservation status

Populations of *Taxus wallichiana* were existent in Himalayan regions since 3Ma BP (25). The plant is traditionally and extensively used by the locals of Himalayan region largely for primary healthcare purpose (26, 27). This has resulted in unregulated and unscientific harvesting of the plant ultimately leading to the decline and fragmentation of populations (28). The plant is thus marked as 'Endangered' in IUCN redlist with a continuous decrease in population (29). The plant is also included in CITES and negative list of exports of the government of India (30).

Ethnobotanical uses

Taxus wallichiana has been a plant of immense ethnobotanical use amongst the local people dwelling in the Himalayan region. They largely use *T. wallichiana* to cure various diseases. The various ethnobotanical use of *T. wallichiana* across various regions of Himalaya has been compiled in Table 1.

Table.1 Ethnobotanical use of *Taxus wallichiana* Zucc. across various Himalayan regions

#	Region	Vernacular Name	Ethnobotanical uses	Ref.
1.	Asi Ganga sub basin, Uttarakhand, India	Thuner	Bark and seed extract with warm water is given orally for treatment of internal wound.	(31)
2.	Urgam valley, Chamoli Garhwal, Uttarakhand, India	Thuner	Bark extract is used as tea for treatment of high blood pressure.	(32)
3.	Nanda Devi Biosphere Reserve, Uttarakhand, India	Thuner	Bark used as a substitute of tea. The powdered bark is used for the treatment of cold.	(33)
4.	Kedarnath wildlife sanctuary, Garhwal Himalayas, Uttarakhand, India		Bark and bark paste used for the treatment of fractured bones, headache, breast piles	(34)
5.	Niti Valley, Uttarakhand, India	Thuner	Dry powder of bark with salt and ghee is mixed with water to make tea and used for treatment of high blood pressure and cancer. Paste of bark with egg yolk is used as plaster for treatment of fracture.	(35)
6.	Jakholi Block, Rudraprayag district, Uttarakhand, India	Thuner	Juice of leaves are used for the treatment of boils, cuts and wounds	(36)
7.	Mornaula Reserve forest, Kumaon, Uttarakhand, India	Thuner	Bark, oil and leaves are used for treatment of cancer. Bark is also used as fuel.	(37)
8.	Shimla Hills, Himachal Pradesh, India	Thuno, Barmi	Tincture from young shoots are used for treatment of headache, giddiness, feeble and falling pulse, diarrhoea and severe biliousness. Leaves are antispasmodic and used for treatment of nervousness, hysteria, epilepsy and stones.	(38)
9.	Manali wildlife sanctuary, Himachal Pradesh	Rakhal	Barks and leaves are used for treatment of cancer, swelling and as contraceptive.	(39)
10.	Pabbar Valley, Himachal Pradesh	Thuna	Tea prepared from needle and bark are used for treatment of congestion and cough.	(40)
11.	Mandi and Hamirpur district, Himachal Pradesh	Rakhala/ Talispatra	Tea prepared from barks and leaves are used to treat asthma. Bark is used for the treatment of cancer.	(41)
12.	Churah subdivision, District Chamba, Himachal Pradesh, India	Nadgaun/ Brahmi	Bark is used as flavouring agent.	(42)
13.	Shimla water catchment sanctuary, Himachal Pradesh, India	Rakhal	Leaves used to cure cancer. Bark used for preparation of tea.	(43)
14.	Kathua, Jammu & Kashmir, India	Barmi	Decoction of leaves are used to cure asthma, bronchitis, cough, indigestion and epilepsy.	(44, 45)
15.	Rajouri, Jammu & Kashmir, India	Barmi	Decoction of leaves are used to cure asthma, bronchitis and cough.	(46)
16.	Ganderbal, Kashmir, India		Tea prepared from boiling bark in water is used for cure of asthma, giddiness, arthritis, tumour growths.	(47)
17.	Bangus valley, Kashmir, India	Postul	Tea made from bark is used to cure sickness in winter.	(48)
18.	Bandipora, Jammu and Kashmir, India	Postul /Brammi	Bark extract is made into a tea and is used for curing of asthma, headache, giddiness, tumour growths.	(49)
19.	Galliyat, NWFP, Pakistan	Bermi	Decoction of stem is used for treatment of tuberculosis.	(50)
20.	Neelam valley, Muzaffarabad, Pakistan occupied Kashmir	Birmi	Tea from leaves are used to cure asthma and high fever.	(51)
21.	Leepa valley, Muzaffarabad, Pakistan occupied Kashmir	Birmi	Leaf and bark extract is used to treat tumours. Decoction of leaf with honey is used for the treatment of hey fever, flatulation, epilepsy and asthma.	(52)
22.	Kel, Pakistan occupied Kashmir		Decoction of bark is used for treatment of cancer.	(53)
23.	Shogran valley, Pakistan		Plant used for the treatment of cancer, cardiac disorders, head ache, renal disorders and digestive disorders. The plant is antispasmodic, purgative and antirheumatic.	(54)
24.	Manaslu, Sagarmatha and Kanchenjunga region, Nepal		Used for treatment of cancer and jaundice.	(55)

Table 2. Major constituents of different plant parts of *Taxus wallichiana* Zucc

Plant Parts	Nature of Compounds	Name of Compound	Ref.
Essential oil from leaves	Alkane	n-Eicosane; Docosane ; n-Pentacosane.	(67)
	Alkene	Santolinatriene.	
	Alcohol	Geraniol; Globulol; Eugenol; Myrtenol; (E)-Verbenol; n-Hexenol; (Z)-3-Hexenol; (E)-2-Hexenol; n-Hep-tan-2-ol; 1-Hepten-3-ol; (E)-2-Octen-1-ol; (Z)-2-Octen-1-ol; (E)-2-Nonenol; 1-Octanol.	
	Aldehyde	Benzaldehyde; Anisaldehyde; n-Heptanal; n-Octanal; (E)-2-Octenal; n-Nonanal; (E)-2-Nonenal; Dodecanal.	
	Organic acids	Benzoic acid; Hexanoic acid.	
	Organic acid esters	(Z)-3-Hexenyl formate; Octyl formate; (Z)-3-Hexenyl acetate; (E)-2-Hexenyl acetate; (E)-3-Heptenyl acetate; Benzyl acetate; Anisyl acetate;n-Octyl acetate; Sabinyl acetate;(E)-2-Hexenyl-n-hexanoate;Isopropyl-n-octanoate; (Z)-3-Hexenyl benzoate; Methyl benzoate; Geranyl-n-heptanoate; Geranyl benzoate;n-Amyl anisoate; Geranyl tiglate; Methyl salicylate.	
	Terpenes	α-Pinene; β-Pinene; Camphor; β-Caryophyllene; Caryophyllene oxide; (Z)-β-Ocimene; (Z)-Sabinene hydrate; (Z)-Pinene hydrate.	
Leaves	Taxoids (terpenoids)	Taxol; 10-deacetylbaccatin III; baccatin IV; 1-hydroxybaccatin I; 2'-deacetoxydecinnamoyltaxinine J; 2'-deacetoxytaxinine J;2-acetoxybrevifoliol	(68)
		Brevifoliol;2-acetoxybrevifoliol	(68, 69)
		5αO-(3'-dimethylamino-3'-phenylpropionyl) taxinine M;7-O-acetyltaxine A; 2α-acetoxy-2'β-deacetylaustrospicatine.	(70)
		14-β-hydroxy- 10- deacetylbaccatin III;2-Debenzoyl-14β-benzoyloxy-10-deacetylbaccatin III; 14β-Hydroxy-1 0-deacetylbaccatin V.	(71)
		19-debenzoyl-19-acetyltaxinineM; 13-deacetyltaxuspine A; 10-debenzoy1-2a-acetoxybrevifoliol,	(72)
		2-deacetoxytaxinine B	(73)
		Wallifoliol; cephalomannine; 1-O-deacetylbaccatin 11	(69)
Barks	Ketone	4-(4'-hydroxyphenyl)-2-butanone	(56)
	Alcohol	4-(4'-hydroxyphenyl)-2-butanol; 9-hydroxy-4,7-megastigmadiene-3-one-3-oxo-α-ionol	
	Taxoids (Terpenoids)	Taxayuntin E ; Taxayuntin G; Taxayuntin J; Taxinine A;2-Deacetoxy taxinine B; 2-Deacetyl-5-decinnamoyl taxinine E;Taxinine J ; 2-Deacetoxy taxinine J ;5-Decinnamoyl taxinine J; Taxinine M; 19-Debenzoyl-19-acetyl taxinine M; Taxchin A;Taxchin B; 1-Hydroxy-5-deacetyl baccatin I; Baccatin III; Baccatin IV; 10,13-Deacetyl-abeobaccatin IV; 1-Dehydroxy baccatin VI;13-Deacetyl baccatin VI;9-O-Benzoyl-9,10-dideacetyl (15-1)-abeo baccatin VI;9-Benzoyl-9-deacetyl11(15-1)abeo baccatin VI Taxayunnanine C; 2'-Deacetyl austrotaxine, 2-Acetoxy-2',7-dideacetoxy-1-hydroxy austrospicatine; 2-Acetoxy-2'-deacetoxy austrospicatine; 2'-Deacetoxy austrospicatine; 7-Xylosyl-10-deacetyl taxol D; 7-Xylosyl-10-deacetyl taxol;10-Deacetyl taxol; 7-Xylosyl taxol; 7-Xylosyl-10-deacetyl taxol C; N-Methyl taxol C ;10-Deacetyl epi-taxol; 7-Xylosyl taxol C; Epi-taxol; 2-Benzoyloxy-7,9,10, 13-tetraacetoxy-4(20); 11 –taxadiene,2,5,9-Trihydroxy-10, 13-diacetoxy-4(20), 11-taxadiene; 5-Hydroxy-9,10-dia-cetoxy-13-oxo-4(20), 11-taxadiene; 2-Hydroxy-5,10,14-triacetoxy 4(20), 11-taxadiene; 5,7,9,10,13-Pentaacetoxy 4(20), 11-taxadiene;7,9,10,13-Tetraacetoxy-5-(3'-acetylamino3'-phenyl)propionyloxy-4(20), 11-taxadiene;Hydroxy-triacetoxy-5(3'-dimethylamino-3'phenyl)propionyloxy4(20), 11-taxadiene;Taxusin;Yunnanxane.	(74)
Heart wood	Taxoids	Taxusin ; 7-xylosyl-10deacetyltaxol C.	(75)
		13-acetyl-13-decinnamoyltaxchinin.	(76)
Roots	Taxoids	Baccatin III; Baccatin IV; Taxusin; 1β-hydroxybaccatin I; Penta acetoxy taxadiene 7; 7-xyloxyl-10-deacetyl-taxol.	(65)
	Lignans	(-) Seco-isolariciresinol; Taxiresinol; Isotaxiresinol.	
	Lignans	α-Conidendrin; Formosanol; Methyl-α-Conidendral; α-Intermedianol;1, 4-methano-2-benzoxepin-10-methanol, 1, 3, 4, 5-tetrahydro-7-hydroxy-5-(4-hydroxy-3methoxyphenyl)-8-methoxy.	(66)
	Sesquiterpene lactone	Cinnamolide.	
Seeds	Triterpenoid	Ursolic acid.	(77)
	Cyanogenic glucoside	Amygdalin.	
	Phytosterol	β-Sitosterol.	

Chemical constituents

Taxus wallichiana has been a plant of extensive study due to its diverse ethnobotanical uses. The plant also exhibits a wide range of pharmacological properties and detailed investigations about the chemical constituents of the plant have been undertaken by a number of researchers. Essential oil is a major ingredient of

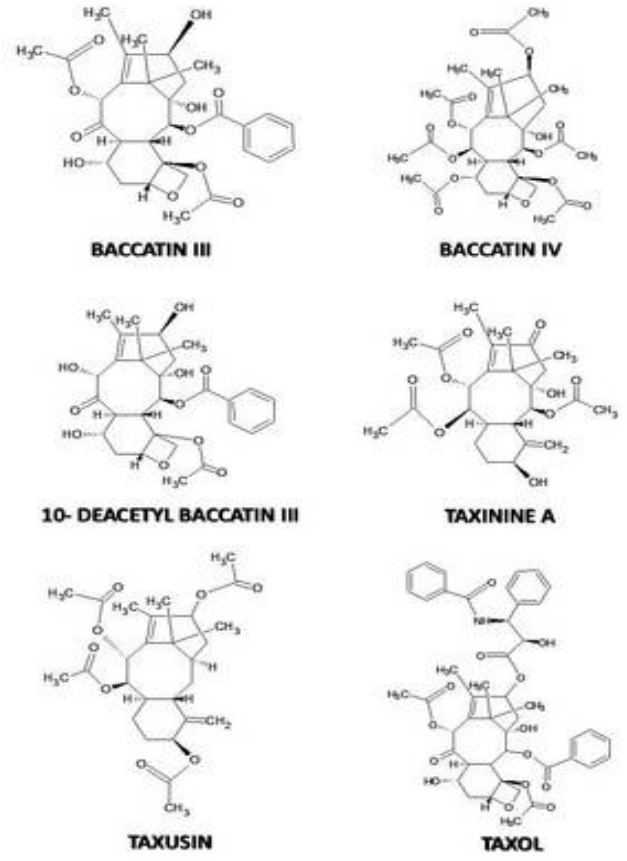

Fig 3. Chemical structures of selected Taxoids present in *Taxus wallichiana* Zucc.

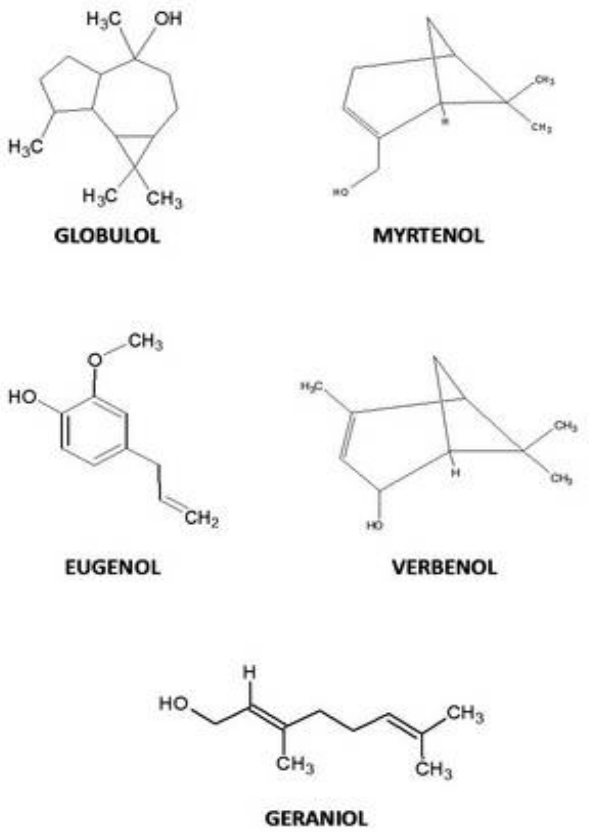

Fig 4. Chemical Structure of selected alcohols present in *Taxus wallichiana* Zucc.

leaves obtained through hydrodystillation process. The major constituents of essential oil are long chain hydrocarbons, terpene alcohols such as geraniol, globulol, eugenol, myrtenol and (E)-verbenol. In addition to it, the essential oil also contains fatty alcohol such with varying chain length, aldehydes, organic acid esters and terpenes (56). These terpenes and terpene alcohols are largely responsible for the characteristic fragrance of essential oil (57). In addition to essential oils, all parts of *T. wallichiana* contains the most unique group of compound by the name taxoids or taxanes. These compounds are largely responsible for pharmacological activities of *Taxus* (58, 59). The taxoids are fundamentally diterpenoids and contains 6/8/6-membered ring skeleton known as taxane skeleton which is chemically a pentamethyl [9.3.1.0] 3, 8 tricyclopentadecane skeleton (60, 61).

The most important and pharmacologically important representative of the taxoids present in *T. wallichiana* is Taxol. The biogenesis of taxol involves the condensation of the three isoprenyl diphosphate (IPP) units with dimethylallyl diphosphate (DMAPP) both of which are produced either through mevalonic (MVA) pathway in the cytosol or via the methyl erythritol phosphate (MEP) pathway in plastids. The first determining step in the biogenetic pathway is cyclization of geranyl geranyl diphosphate (GGPP) to taxadiene followed by eight cytochrome P450-mediated oxygenations, three CoA-dependent acyl/aroyl transfers, an oxidation at C9, and oxetane (D-ring) formation finally resulting in formation of intermediate baccatin III (62, 63) (Fig. 2). The final stage of taxol biosynthesis involves the assembly of C13 side chain appended to the Baccatin molecule (64). Analysis of bark, root and other parts of the plants revealed the presence of taxol, baccatins and other intermediary molecules and taxol analogues (Table 2). Apart from taxoids, *T. wallichiana* also contains lignans such as (-) seco-isolariciresinol, taxiresinol, isotaxiresinol, formasonol, sesqueterpene lactone, triterpenoid cyanogenic glucoside and phytosterols (65, 66). Chemical structures of selected compounds present in *T. wallichiana* is depicted in Figs. 2, 3 & 4 respectively. Most of these compounds have been reported to have pharmacological importance and have been discussed in this article. The various compounds isolated from different parts of *T. wallichiana* are listed in Table 2.

Pharmacological activities of Taxus wallichiana Zucc.

Anti-inflammatory activity

Inflammation is associated with onset of a number of diseases including asthma (78), rheumatoid arthritis (79), atherosclerosis (80), chronic venous insufficiency (81), diabetes (82), cancer (83) etc. Herbal remedies have proven to be an effective way to reduce inflammatory processes (84). In the

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same line *Taxus wallichiana* have proven its efficacy to combat inflammatory processes. Taxusabietane A, isolated from *T. wallichiana* was reported to possess anti-inflammatory potential.

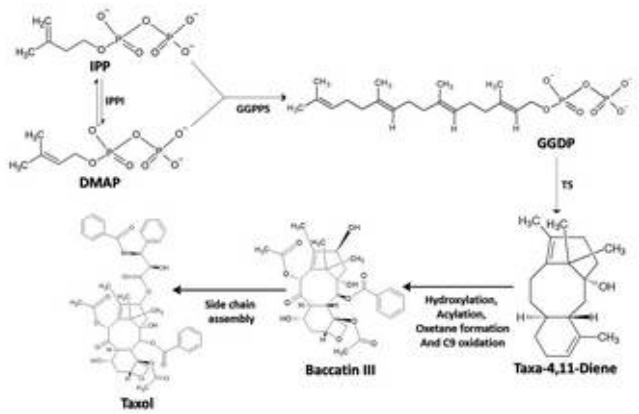

Fig 2. Schematic representation of major steps associated with biosynthesis of taxol

IPP: isopentenyldiphosphate
DMAPP: dimethylallyldiphosphate
GGDP: Geranylgeranyldiphosphate
IPPI: isopentenyldiphosphateisomerase
GGPPS: geranylgeranyldiphosphate synthase
TS: taxadiene synthase

The results obtained from a study indicated that taxusabietane A showed lipoxygenase inhibitory activity with an IC50 value of 57±0.31 µM which was significant in relation to standard compounds baicalein and tenidap sodium with an IC_{50} value of 22.1±0.03 and 41.6±0.02µM respectively. In addition to it, taxusabietane A at 5mg/kg and 10mg/kg dose also inhibited carrageenan induced oedema in adult wistar rats though in this case standard compound indomethacin at same dose exhibited comparatively better result (85). Another study reported that taxoids namely tasumatrol B, 1, 13-diacetyl-10-deacetyl baccatin III (10-DAD) and 4-deacetylbaccatin III (4-DAB), isolated from *T. wallichiana* is significantly effective in bringing down inflammations induced by carrageenan and cotton pellet in rats. At 20mg/kg and 40mg/kg of dose, the tested compounds showed significant inhibition of paw oedema in carrageenan induced inflammation model out of which tasumatrol B was observed to be most effective. In addition to it, cotton pellet test revealed that all the three test compounds showed inhibition against inflammation and granuloma accumulation (86). Two more abietane diterpenoids namely taxusabietane C and taxamairin F have also reported to show lipoxygenase inhibitory effect with 69 ± 0.31 and 73 ± 0.14 µM, respectively (87).

Analgesic, antipyretic and anticonvulsant activity

The analgesic activity of tasumatrol B, a taxane isolated from the bark of *Taxus wallichiana* have been reported, It has been observed that tasumatrol B administered at 40mg/kg in wistar rats significantly decreased the writhing count in acetic acid writhing experiment (86). The analgesic activity of *T. wallichiana* was observed by the formalin test in rats. It was observed that upon treatment with 50, 100 and 200 mg/kg of the extract of *T. wallichiana* to rats previously injected with 0.05 ml of formalin in the plantar surface of hind paw, the pain score severity showed a concentration dependent decline in both early and late phases of analgesia. In addition to it, the antipyretic activity was investigated by injecting 15% aqueous solution of yeast at 10 ml/kg dose to induce pyrexia. This was followed by intraperitoneal injection of 50, 100 and 200 mg/kg of extract. It was observed that all the dose of plant extract showed significant inhibition of pyrexia induced by yeast and the antipyretic effect of 200 mg/kg of extract is comparable to 20 mg/kg of paracetamol between 30 and 60 minute of treatment. The anticonvulsant activity was also tested observing the effect of plant extracts on pentylenetetrazole induced seizers in rats. It was observed that pretreatment of 50, 100 and 200 mg/kg of plant extract significantly reduced mioclonous seizures. Additionally, 100 and 200 mg/kg of extracts significantly delayed the onset of first clonous seizures while 200 mg/kg of extract delayed the tonous seizures. Extracts of all doses protected the rats from tonic-clonic seizures. Diazepam was used as a control and showed remarkable anticonvulsant activity at 7.5 mg/kg. There was also a decrease in percentage of mortality in animals upon pretreatment with extracts (88).

Hepatoprotective activity

The protective action of methanolic extract of *Taxus wallichiana* against carbon tetrachloride induced hepatotoxicity in rats was reported from a recent study. It was observed that CCl_4 treatment resulted in significant elevation of liver enzyme markers namely aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH). Treatment of animals with 1 ml of 100 and 300 mg/kg/body weight resulted in decrease in the levels of liver marker enzymes histopathological investigation of liver of rats treated with plant extracts revealed an almost normal hepatic architecture with less infiltration of fat and absence of necrosis all of which were prominent in rats treated with carbon tetrachloride (89).

Immunomodulatory activity

The immunomodulatory potential of *Taxus wallichiana* was investigated using human lymphocyte as the experimental system. In the experiment, cyclophosphamide was used to supress the proliferation of lymphocytes and this condition was reversed by cotreatment of 1-hydroxy-2-deacetoxy-5-decinnamoyl-taxinine J (1

μg/ml) with concanavaline A (5 μg/ml), an immune stimulant (90).

Anticancer activity

Some studies have been undertaken to investigate the anticancer potential of compounds isolated from *Taxus wallichiana*. A study reported that 1-hydroxy-2-deacetoxy-5-decinnamoyl-taxinine J was cytotoxic to five cancer cell lines namely MCF7, WRL-68, KB, PA-1, Colo 320DM human cancer cell lines as determined by MTT and clonogenic assays (90). Another study have shown cytotoxicity of taxiresinol, a lignan isolated from heartwood of the plant against human liver, colon, ovarian and breast cancer cell lines (91). Taxawallin I, a new taxoid isolated from *T. wallichiana* has been reported to show toxicity against HepG2, A498, NCl-H226 and MDR 2780AD cancer cells (92). Another study reported isolation of four novel taxane derivatives namely N-debenzoyl-N-methyl-N-heptanoyl-taxol, N-debenzoyl-N-me-thyl-N-octanoyl-taxol, N-debenzoyl-N-methyl-N-(4-methylhexanoyl)-taxol, and N-debenzoyl-N-methyl-N-[(4Z)-1-oxo-4-tenenoyl]-taxol from ethanol extract of whole plant. It is also reported that the taxanes were inhibitory towards MCF-7, A549, and 3-AO cancer cell lines and had microtubule stabilizing properties (93).

The term taxane or taxoids refers to all the terpenoid compounds having molecular structure based on baccatin unit, either obtained naturally from *Taxus sp* or are semi synthetically or synthetically prepared exhibiting anticancer properties. Taxanes interacts with the microtubules involved in mitotic process. Taxanes stabilize the microtubules of cells which counteract their depolimerization. Thus, correct separation of two identical sets of chromosomes and their consequent transfer during cell division are inhibited resulting in blockage of cell mitosis ultimately leading to cell death (94). Taxanes promote microtubule polymerization and arrest mitosis through activation of spindle assembly check point and keeping a small number of unattached kinetochores to the microtubules. This delays mitotic metaphase progression and inhibits anaphase prompting complex (95). On a molecular level, binds to a pocket in β- tubulin that faces microtubule lumen and is near the lateral interface between protofilaments thereby affecting normal function and cellular processes (96). The binding of Paclitaxel to β- tubulin subunit results in stabilization of microtubules through induction of conformational changes of the M-loop of β- tubulin which result in more stable lateral interaction between adjacent protofilaments thereby changing microtubule dynamics and inducing mitotic block and triggering apoptosis of cancer cells (97, 98).

Moreover, taxanes also exhibit apoptotic action interacting with various proteins and enzymes which are involved in cell cycle, apoptosis and cell death. Increased reactive oxygen species (ROS) is one of the earliest events of apoptosis and is brought about by taxanes (99). Taxanes also initiate decrease in mitochondrial membrane potential (ΔΨm) and induces opening of mitochondrial membrane permeability pore resulting in release of calcium and cytochrome c from mitochondria (100, 101). Caspases are the family of endoproteases that play an important role in cell inflammation and cell death (102). Taxanes also activates caspases thus initiating cell death and apoptosis (103). B-cell lymphoma (Bcl-2) is the key protein which regulates programmed cell death and apoptosis (104). They may be divided into two major groups namely (a) antiapoptotic protein (BCL-2, BCL-XL, MCL-1, BFL-1, BCL-W, and BCL2L10) and (b) proapoptotic proteins (BAK, BAX) (105). The apoptosis in cancer cells is further induced by phosphorylation and inactivation of antiapoptotic Bcl-2 by taxane (106). Along with Bcl-2, p53 also plays a major role in apoptotic process. It is an important tumour suppressor gene which regulates downstream expression of other genes involved in DNA repair, cell cycle arrest and apoptosis (107). Taxanes are reported to act as a p53 inducers thereby enhancing the apoptotic process (108). The p21 is an universal cyclin dependent kinase (CDK) inhibitor, controlled by p53 and physically interacts and inhibits cyclin-CDK2, cyclin-CDK1, cyclin-CDK4/6 complexes thus regulates progression of cell cycle during G1 and S phases (109). Taxanes results in increase of expression p21 through upregulation of p53 (110).

Toxicity

Toxicity of *Taxus* sp is known to humans since early civilization. Juice of the plant was applied to arrows for hunting and leaves were used for homicide and suicide (111). The main active compounds of the plant include a mixture of diterpenoid alkaloids namely taxine A and B, isotaxine B, taxol B and are responsible for toxicity causing toxicity resulting in the occurrence of symptoms like nausea, vomiting, diffuse abdominal pain, tachycardia (initially) and convulsions, followed by bradycardia and respiratory muscle paralysis (112). The time from ingesting lethal dose of *Taxus* toxin to death usually varies between 2-5 hours with symptoms occurring between 30 minutes to 1 hour after ingestion (113). Generally, the *Taxus* toxins acts by generation of a block in the distal portion of the conduction system of the heart resulting in fatal arrhythmia (114). It is further reported from a study that taxine-B inhibits calcium and sodium transport in myocardial cells and interferes with heart's conducting system thereby acting as a cardiac depressant (115).

Conservational approaches and production of bioactive compounds

The population of *Taxus wallichiana* have taken a toll due to its ever increasing demand among the localites accompanied by high rate of collection. To make the things even more grave, the plant exhibits slow growth and poor regeneration (116). This has resulted in number of conservational

approaches to save the population from extinction. Tissue culture method have been a versatile approach for mass propagation of a number of plants (117). The long dormancy period of the plant can overcome by culture of zygotic embryos which can develop into full grown seedlings in 10-12 weeks (118). A study reported that shoot elongation and root induction through shoot tip culture is feasible and may be applicable for propagation of the plant (119). Another study reported regeneration of *T. wallichiana* plant via shoot organogenesis from callus cultures derived from zygotic embryos (120). Apart from tissue culture techniques, stem cuttings treated with growth promoting substances have also been proved effective for propagation of the plant (121). Another recent study reported that treatment of shoot cuttings of the plant with indole acetic acid (IAA), indole butyric acid (IBA) and napthoxy acetic acid (NAA) resulted in effective initiation of roots (122). Tissue culture techniques are not only used for mass propagation but also for the production of bioactive compounds. There have been reports that extracts from the cell cultures of the plant contains taxol, deacetyl baccatin III and baccatin III (123). Another study detected the presence of taxol (0.8499%) in the callus culture (124). Use of bioreactors for production of taxol and baccatin III from suspension cultures have also been reported from a study (125).

Discussion

The genus *Taxus* or Yew holds immense importance globally from pharmacological point of view. A lot of research work have been undertaken to explore the pharmacological importance of the genus. The most important amongst them is exploration of Pacific Yew or *Taxus brevifolia* . Exploration on the antineoplastic activity of the active constituent of *T. brevifolia* started way back in 1960 (126). It consequently underwent a series of modifications and culminated in commercial production as Paclitaxel by Bristol-Myers Squibb (BMS) in the year 1992 (127). Since then there have been no stopping and a number of variants and semisynthetic analogues such as docetaxel and cabazitaxel were developed all of which possess same anticancer activity. Moreover, studies showed that extracts of European yew, *Taxus baccata*, also have the potential to inhibit Caov-4 and HeLa cancer cell lines (128). It is also reported from a study that the bark extract of *T. baccata* also possess anti inflammatory properties (129). There are also reports that the leaves of *T. baccata* also have bronchodilating properties and beneficial effect on asthma (130). Japanese Yew, *Taxus cuspidata* is also reported to possess inhibitory activity against MCF-7 (breast), PG and A549 (lung), PC-3M-1E8 (prostate), BGC-823 (gastric), WM451 (melanoma), Bel-7402 (hepatocellular), KB (oral squamous), HeLa (cervical), and HL-60 (leukemic) cell lines (131).

Across the world there has been a growing demand of herbal medicines and 80% of the total population are relying on herbal remedies for primary healthcare (132). The figures are similar for Indian population of which 70% relies on plants as source of medicines (133). Now coming back to Himalayan Yew or *Taxus wallichiana*, it is reported that the plant finds extensive use by the people living in various location of Himalayas. The use of plant has been assimilated in their tradition and thus justifies its ethnobotanical and ethnomedicinal significance. Thus, there is a strong requirement for a reverse pharmacological process in which the traditional knowledge and usage pattern of Himalayan Yew can be better explored in the laboratory condition for validation of its pharmacological efficacy. In this regard, the plant has been investigated to some extent for a number of pharmacological activities which has yielded promising outcome. Thus, this species is at par with the other species of *Taxus*, many of which have been extensively investigated for their pharmacological actions. However most of the investigations have been made of cell lines or in animal systems. Experimentation using clinical trials using humans are further required for consequent development of drugs. Additionally, exploration of anticancer potential from *Taxus* sp has become a matter of priority since last 50 years and as mentioned earlier. Paclitaxel is a noble outcome of extensive research on *T. brevifolia*. In a similar manner, the Himalayan Yew can also be explored further for its anticancer potential leading to development and commercialization of drugs required for cancer treatment. In this aspect there are few reports of detection taxol in cell and callus culture of *T. wallichiana*. This avenue also needs further elaboration and investigation with a motive of increasing the concentration and yield of Taxol through modification of ambient culture condition. This would lead to a cost effective approach in the development of anticancer drugs from *T. wallichiana*.

Presently, over exploitation of *T. wallichiana* have resulted in its depletion. Isolation of population through habitat fragmentation leads to restriction of connectivity, resulting in low levels of gene flow between the population with subsequent lower genetic diversity and higher genetic differentiation in and among remaining populations (134). Moreover, smaller isolated populations also undergo frequent inbreeding which often express deleterious alleles leading to reduction in reproductive capacity and low offspring survival (135). Thus, studies on the genetic diversity of the plant is extremely relevant as an important avenue towards conservation as well an indicator of possible extinction. An important approach towards conservation of diminishing population of *T. wallichiana* is to

maintain an effective population size so that the level of genetic variation can be maintained. Thus, proper planning and management of this species in their natural habitat will be extremely fruitful in harvesting pharmacologically active principles from the plant. Techniques of molecular biology and biotechnology should also be involved in the overall process of conservation.

Conclusion

Taxus wallichiana or Himalayan Yew is an endangered gymnosperm that grows in the Himalayan region and is of immense Ethnobotanical importance amongst the people. They contain huge amount of phenolics and terpenoids which forms the backbone of their medicinal potential. The plant has proven its efficacy from pharmacological point of view and can be very well utilised as a cost effective source of medicine for management of diseases of the people of Indian subcontinent. The bioprospection of this plant thus becomes extremely relevant for the benefit of humans.

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Conflict of Interest

Author declares that he has no conflict of interest in the publication.

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