Ethanobotany, Phytochemical and Pharmacological Aspects of *Thuja orientalis* : A Review

Neetu Jain* and Meenakshi Sharma
Laboratory of Microbiology, Department of Botany, University of Rajasthan, Jaipur
*Corresponding Author E-mail: neetugodika@yahoo.co.in
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**ABSTRACT**

*Thuja orientalis* commonly known as white cedar belonging to family Cupressacae is an monoecious small tree or shrub. It is also known as Tree of life and Orientale Arbor-Vitae in English. *T. orientalis* has an effective natural origin that has a tremendous future for research as the novelty and applicability are still hidden. *Thuja* is used for treatment of bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea, rheumatism, asthma, skin infections, mumps, bacterial dysentery, arthritic pains and premature blandness. Different parts of plant are exhibited extensively biological activities like hair growth-promoting, antiviral, anti-allergic, anti-epileptic, anti-inflammatory, antibacterial, antioxidant, and antifungal activities. Apart of these effects, it can be used as nematicidal, insecticidal and molluscicidal activity against various pests. The present review article give comprehensive information about various medicinal and traditional utility of the chemical composition and pharmacological activity of the plant and its constituents.

**Key words:** *Thuja orientalis*, Biological Activities, Bioactive Constituents, Ethanomedicinal use.

**INTRODUCTION**

*Thuja orientalis* commonly known as arbor vitae or white cedar or morpankhi belonging to family Cupressacae is well known medicinal plant. *T. orientalis* is naturally distributed and cultivated in large parts of Asia as described in history. It is assumed to have originated from northern and north-eastern China, Korea, Siberia, Japan, Taiwan and Central Asia. Most of the places it grow like an ornamental plant. Plant is usually 10-200 feet tall, with stringy surfaced reddish brown bark. Leaves are usually 1-10 mm long, needle like in first year, and become scale like in later. These leaves are arranged in alternate decussate pairs in four rows along the twigs. The flower are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. The male and female flowers are usually borne on separate twigs or branchlets. They are tiny, terminal, cone-like bodies. The male cones rounded and reddish or yellowish, the female very small and green or tinged with purple.
Mature cones are solitary, egg shaped or oblong, 8 to 16 millimeters (about 1/2 inch) long, with 4 to 6 (but sometimes 3 or as many as 10) pairs of thin, flexible scales that terminate in thickened ridges processes. Seed flattened, ovoid 5-7mm and 3-4 mm wingless. It is extensively cultivated as an ornamental tree in cool and moist places for its attractive dense foliage and bush like habit of growth. It is also grown as a hedge plant in India.

**Phytochemical constituents of T. orientalis:**
Biochemical studies reveal that fresh plant contains essential oil, reducing sugar, water-soluble polysaccharides, water-soluble minerals, free acid, tannic agents, flavonoids, saponins, glycosides and alkaloids. The essential oil of the fresh leaves (related to the monoterpene fraction) composed of 65% thujone, 8% isothonjone, 8% fenchone, 5% sabines and 2% –pinene as the main monoterprenes. Other monoterpenes, namely carvotanacetone, origanol, origanes, myrcen and camphen have also been described. T. orientalis leaves contain rhodoxanthin. The heartwood contains aroma-dendrin, taxifolin, widdrene, cedrol, thujopsadiene, dehydro -α-curcumene, β-isobiotol and curcumeneether. T. orientalis essential oil showed the presence of 38 compound for 100% of total oil. The major components were α-pinen (22.25%), 3-carene (20.65%), cedrol (18.71%), β –Caryophyllene (6.13%), α-humulene (5.68%), terpinolene (4.53%), and limonene (3.35%). Twenty-one compounds were identified; quantitative differences, mainly, between cone and needle oils by Ismile et al. Both oils were rich in monoterpene hydrocarbons and the major components were α-pinen (64.2 and 49.3%, respectively, in cones and needles), β-phellandrene (6.7–9.6%) and α-cedrol (3.9 and 8.2%).

**Pharmacological activity of T. orientalis:**
Various extracts of T. orientalis are used in the treatment of hemorrhages, coughs, excessive menstruation, bronchitis, asthma, skin infections, mumps, bacterial dysentery, arthritic pains and premature bladderness. It is used as a medicinal plant in various forms of traditional medicines like folk medicine, homeopathy, etc. for treatment of bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism. Traditionally it is used in the treatment of cough. The plant has been exhibited extensively biological activities like hair growth-promoting, antiviral, anti-allergic, anti-epileptic, anti-inflammatory, antibacterial, antioxidant, and antifungal activities. The root bark is used in the treatment of burns and scalds. The stems are used in the treatment of coughs, colds, dysentery and parasitic skin-diseases. The wood of Thuja is commonly used for guitar sound boards. A yellow dye is obtained from the young branches. The root bark is used in the treatment of burns and scalds.

**Antibacterial activity:**
Essential oils are useful sources of antimicrobial compounds. The presence of antibacterial components in plants are well established as they have provided a source of inspiration for novel drug compounds as herbal medicine for human health. T. orientalis contain large amounts of alpha, beta and gamma thujaplicin that in low concentration would serve as chelators for Solmonella typhimurium. Chen et al. reported the antibacterial activity of T. orientalis against Streptococcus mutans. It was very effective in inhibiting the growth of serotypes c and d of Solmonella mutans (MIC less than or equal to 2.0-7.8 mg/ml). Manimegalai et al. studied the antibacterial activity of chloroform extract of T. orientalis against two gram positive pathogenic bacteria, Staphylococcus aureus and Bacillus thuringiensis infecting mulberry silkworm, Bombyx mori L.. GCMS revealed the presence of phenanthrene carboxylic acid in the bark extract of T. orientalis. Duhan et al. explored methanol, acetone and ethyl acetate extracts prepared from leaves of T. orientalis against selected bacterial strains like S. aureus, B. subtilis, P. aeruginosa, A. faecalis and K. pneumoniae. Among the tested strains of bacteria, B. subtilis (i.e. causal organism of dysentery) was found most sensitive against acetone extract of leaf with maximum zone of

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inhibition (15.55 mm) followed by A. faecalis (15.50 mm). These results were even better than synthetic antibiotics i.e penicillin and ampicillin. Jasuja et al.² also established the antibacterial properties of leaves of T. orientalis of methanol; distilled water (70:30) extract against S. aureus, B. subtilis, Escherichia coli and Agrobacterium tumefaciens. The minimum inhibitory concentrations (MICs) of the extract ranged from 0.55 to 1.15 mg/ml. Wajaht¹⁸ also studied the antibacterial activity of Thuja essential oil against two gram positive bacteria B. subtilis MTCC 441 and K. pneumoniae MTCC 19 and against three gram negative bacteria like E. coli MTCC 443, P. aeruginosa MTCC 1688 and P. vulgaris MTCC 426. This oil was mainly effective against P. vulgaris and E. coli with highest inhibition zones of 24 and 22 mm respectively. The MIC value of all tested bacteria was found between 12.8-25.6 mg/ml.

Similar research works were carried out by various workers¹⁹-²¹. Bissa et al.²² also reported antibacterial activity of petroleum ether extract of T. orientalis stem against A. tumefaciens. Jabeen²³ screened 25 medicinal plants against bacterial leaf blight. Among all tested plants, cone of T. orientalis extract showed excellent antibacterial properties. Manimegalai et al.¹⁶ identified a component phenanthrene carboxylic acid through GCMS isolated from the bark extract. This component was found to responsible for antibacterial behavior against Staphylococcus aureus and Bacillus thuringiensis infecting mulberry silkworm, Bombyx mori L.

Antibacterial potentiality of the crude extract and different solvent extracts of the strobilus of T. orientalis was evaluated on microbial strains like gram negative Pseudomonas fluorescens, Pseudomonas putida and gram positive Bacillus mycoides, B. licheniformis using agar well diffusion method. Chloroform; methanol (1:1 v/v) extracts showed the best result against the test bacteria²⁴. Khubeiz et al.¹ screened essential oil of T. orientalis leaves for their chemical compositions and antibacterial activities. Volatile oil exhibited the highest activity against the Vibrio parahaemolyticus (42.04 ± 0.18) and Staphylococcus aureus (40.12 ± 1.14). Bacillus subtilis (39.4± 0.11) and Streptococcus pyogens (33.9 ± 0.11), while it showed no activity against the Klebsiella pneumonia, Proteus vulgaris and Salmonella typhimurium and Micrococcus luteus.

Noruzi and Mousivand ²⁵ carried out green synthesis of zarovenlat nanoparticles from T. orientalis extract and screened their antibacterial activity against selected bacteria. Zerova lent iron nanoparticles showed a strong antibacterial effect on B. subtilis and E. amylovora bacteria.

**Antimicrobial activity:**

Antimicrobial activity of essential oil of the seed coats of T. orientalis against six bacterial like Bacillus subtilis, Corynebacterium diphtheriae, Staphylococcus aureus, Salmonella typhi, Shigella sp., Escherichia coli and five fungal pathogenic organism like Aspergillus niger, A. fumigatus, Rhizopus oryzae, Fusarium psidii and Curvularia lunata were carried out by Jain and Garg.²⁶ The oil showed good to moderate activity of the oil against all six test bacteria. The activity of the oil against S. typhi has been found remarkable at 1:1000 dilutions. The activity against the fungal organisms at dilutions is moderate. Tsiri et al.²⁷ studied the different cultivated species of Thuja in Poland for their chemosynthetic value and antimicrobial activity. The main constituents in all samples were the monoterpene ketones α- and β-thujone, fenchone and sabinene, as well as the diterpenes bayerene and rimuene. Antimicrobial activity of essential oil were evaluated against S. aureus, S. epidermidis, P. aeruginosa, E. cloacaæ, K. pneumoniaæ, E. coli, Candida albicans, C. tropicalis and C. glabrata. Malik and Singh ²⁸ studied the antimicrobial activity of Thuja leave essential oil against microorganism isolated from urinary tract infections. P. mirabilis and S. aureus was found to be most sensitive while E. coli was found to be resistant bacteria.

Kamal et al.²⁹ studied antimicrobial activity of callus induced from leaf explant of T. orientalis. Callus of explants were grown on
MS media supplemented with 3 mg/l PCIB +0.1 mg/l BA extract is the most active fraction against *E. coli* also it is active against *Streptococcus pneumonia* and *Bacillus subtilis*. Callus of explants were grown on MS media (3 mg/l NAA +0.1 mg/l BA) has higher activity against *Streptococcus pneumonia*. Callus of explants were grown on MS media supplemented with (3 mg/l PCIB +0.1 mg/l BA) has higher activity against *Bacillus subtilis*. Callus extracts were also found infective against *Candida albicans* and *Aspergillus* sps.

**Antinflammatory activity:**

Panthong et al. 30 used fresh leaves of *P. orientalis* as an anti-inflammatory agent. Tanveer et al. 31 conducted the study for evaluating a natural source to treat inflammation and pain, to avoid the severe side effects of currently used agents for these ailments. Carrageenan induced inflammatory model, acetic acid induced writhing test and hot plate methods were used to evaluate anti-inflammatory, peripheral and central analgesic properties of aqueous methanolic extract of *T. orientalis* fruit (To-Cr) in albino rats. Completely randomized design (CRD) was constructed for the study and one way ANOVA was applied to compare means. The results showed that TO-Cr has significant anti-inflammatory and analgesic properties. Moon et al. 32 investigated anti-vascular inflammatory activity of an aqueous extract of *T. orientalis* (ATO) and its possible mechanisms in human umbilical vein endothelial cells (HUVECs). Aqueous extract re- incubation inhibited tumor necrosis factor and U937 monocytes adhesion to HUVECs suggesting that it may inhibit the binding of monocytes to endothelium. Kim et al. 33 isolated a new labdane diterpenoid from the leaves and stem methanolic extracts of *T. orientalis* and screened it for antiinflammatory properties by the suppression of NF-κB activity and ERK phosphorylation. Si-Yang Fan et al. 34 evaluated the anti-inflammatory activities of the chloroform fraction (CHL) and pure compounds of LPO for their abilities to inhibit pro-inflammatory enzymes in vitro, and production of tumor necrosis factor-α (TNF-α) and nitric oxide in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The CHL and its components showed beneficial effects on NO and TNF-α production. Consequently, these results provided a rationale for LPO's traditional applications in the treatment of inflammatory airway diseases. Tanveer et al. 31 conducted experiment for evaluating a *T. orientalis* to treat inflammation and pain, to avoid the severe side effects of currently used agents for these ailments. The aqueous methanolic extract of *T. orientalis* exhibited dose dependant effects as compared with standard drug. The standard drug Indomethacin showed 79.70 % inhibition, whereas, TO-Cr showed 13.04 %, 34.00 % and 59.57 % inhibition at doses of 50, 100, 300 mg/kg of extract after 3 hours of carrageenan injection.

**Antioxidant Activity:** Antioxidants play an important role in protecting against damage by reactive oxygen species. Jung et al. 35 extracted, purified and examined seven compounds namely myricitrin, isoquercitrin, hypoletin-7-O-b-D-xylopyranoside, quercitrin, kaempferin, kaempferol, and amentoflavone. Among these isoquercitrin was found to be the most effective at attenuating the death of RGC-5 cells in culture caused by exposure to hydrogen peroxide (H2O2). Antioxidant effects of *T. orientalis* were studied by various workers 36–39. Nizam and Mushfiq 40 studied the water and ethanolic extract of dried and powdered leaves of *T. orientalis* as antioxidant. At a concentration of 200 mg, water and alcohol extracts of *T. orientalis* inhibited the hydrolysis of DNA by 72.859% and 65.312%, respectively. Water and alcohol extracts of *T. orientalis* also inhibited 2,2'-Azobis(2-amidinopropane) dihydrochloride induced RBC hemolysis to the extent of 69.30% and 54.55%, respectively. Wajaht 18 evaluated DPPH free radical scavenging activity by measuring the scavenging activity of the essential oil on stable 2,2-diphenyl-1-picryl hydrazyl radical. This plant oil exhibited prominent DPPH free radical scavenging activity of 49.8% in comparison to ascorbic
Acid and α-tocopherol standard which showed the activity of 67.95 and 71.2%, respectively. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This method has been used extensively to predict the antioxidant activity because of the relatively short time required for analysis. Aher et al.\textsuperscript{44} studied the antioxidant activity of bark extract of \textit{T. orientalis} by using various \textit{in vitro} models like Hydrogen peroxide scavenging method, and, Nitric oxide method and found significant antioxidant properties.

\textbf{Antipyrctic Activity:} Jaswal et.al\textsuperscript{42} reported that alcoholic extract of \textit{P. orientalis} produced significant antipyrctic activity (p < 0.05). They suggested that non-steroidal anti-inflammatory drugs produce their antipyrctic action through the inhibition of prostaglandin synthesis within the hypothalamus.

\textbf{Antiproliferative Activity:} Spacer and Breder\textsuperscript{43} investigated the antiproliferative activity of essential oils obtained from some medicinal plants and isolated the effective components. Essential oil of \textit{T. orientalis} was found to be effective against amelanotic melanoma with an IC50 of 330.04μg/mL, respectively. Three components, linalool, terpenes, β-caryophyllene and α-cedal were found to be active on tested cell lines.

\textbf{Cytotoxicological activity:} Amirghofran and Karimi \textsuperscript{44} studied the cytotoxic activity of ethanolic extract of \textit{T. orientalis} on various tumor cell line. Various concentrations of \textit{T. orientalis} showed stimulatory effects on this cell line (%inhibition range - 34.00 to -21.4). Study of the effect of two low and high concentrations of the extracts on nitrogen-induced human lymphocytes resulted in a slight increase at 50 lg/mL (Stimulation index, SI range 1.19 to 1.37, p<0.01). Kosuge et al.\textsuperscript{45} isolated deoxy podophyllotoxin (a lignane) from \textit{P. orientalis} leaves and studied its cytotoxic effect against HeLa cells.

\textbf{Antiviral Activity:} Gohla et al.\textsuperscript{46-47} described that \textit{Thuja} polysaccharides (TPS) inhibited human immunodeficiency virus (HIV)-dependent cell death at a final concentration of 625μg/ml. At this concentration, TPSg was found to be completely non-toxic for MT-4 cells, which had not been infected with HIV-1. TPS were shown to inhibit HIV-1-specific antigen expression on freshly infected MT-2 cells in a dose dependent manner. Hassan et al.\textsuperscript{48}, Monica et al.\textsuperscript{49} studied essential oils for their inhibitory activity against Sever Acute Respiratory Syndrome \textit{Coronavirus} (SARS- \textit{Coronavirus}) and \textit{Herpes Simplex Virus} Type-1 (HSV-1) replication \textit{in vitro} by visually scoring of the virus-induced cytopathogenic effect post-infection. Offergeld et al.\textsuperscript{50} also demonstrated that allographic extracts of \textit{T. orientalis} could be used as strong antiviral agents against plant and animal viruses.

\textbf{Anticancer Activity:} Cytotoxicologic studies of the extracts of Iranian \textit{Juniperus sabina} and \textit{P. orientalis} on cancer cells was carried out by Jafarian et al.\textsuperscript{51}. The cytotoxic effects of the extracts on three human tumor cell lines (Hela, KB, and MDA-MB-468) were determined. Different concentrations of extracts were added to cultured cells and incubated for 72 h. Cell survival was evaluated using MTT-based cytotoxicity assay. Elsharkawy et al.\textsuperscript{52} carried out the comparative analysis of antioxidant and anticancer activity of \textit{T. orientalis} growing in Egypt and Saudi Arabia. Saudi oil extract also possess highest cytotoxic activities against MCF7, followed by, PC3 and, Hep-G2 while the least activity was recorded against lung carcinoma cell line. The highest antioxidant and cytotoxic activity of \textit{Thuja} plant growing in Saudi Arabia were correlated with its high content of some compounds which are rich in Saudi plant and absence in Egyptian plant. Essential oil was found to be very rich in Phellandrene, Terpenyl acetat, and β - Caryophyllene with high concentrations. Sunila et al.\textsuperscript{53} reported that a long-chain sugar molecule or polysaccharide, obtained from leaves extract of \textit{T. orientalis} decreased the inflammation caused by cancer. It also prevented the cancer from spreading throughout the body.


Larvicidal Activity: Larvicidal activities of T. orientalis oil against 4th-instar larvae of Aedes aegypti and Culex pipiens pallens has been observed by Ju-Hyun et al.\textsuperscript{54}. Larvicidal activity of T. orientalis leaf oil was higher than those of stem, fruit, and seeds oils. Essential oils of leaves and fruits of T. orientalis at 400 ppm caused 100% and 71.6% mortalities against A. aegypti. Dwivedi and Shekhawat\textsuperscript{55} reported leaf extracts of T. orientalis as repellent agent against Chilo partellus. T. orientalis ether extract (68.63%), acetone extracts (67.51%) have sufficient repellent action. Anju and Sharma\textsuperscript{56} also reported foliar application of semi-solid crude extract of T. orientalis on maize very effective against Chilo partellus.

Nematicidal Activity: Plant parasitic nematodes are the most destructive group of plant pathogens worldwide and their control is extremely challenging. Parihar et al.\textsuperscript{57} conducted a experiment for the test of nematostatic potential of aqueous extracts of different plant parts viz., leaves, flowers and seeds of T. orientalis and Calotropis procera. They reported that the higher concentration of plant parts of C. procera and T. orientalis showed more potential nematostatic properties as compared to lower concentration.

Insecticidal Activity: Foliar application of semi-solid crude extract of T. orientalis on maize was very effective against Chilo partellus\textsuperscript{56}. Leaf extracts of T. orientalis shows a repellent activity against Chilo partellus. T. orientalis ether extract (68.63%), acetone extracts (67.51%) have sufficient repellent action\textsuperscript{55}. Essential oils derived from plant species of Platycladus have been evaluated for insecticidal properties by Keita et al.\textsuperscript{58}; Pavela\textsuperscript{59}; Jeon et al.\textsuperscript{60} revealed that insecticidal activity of P. orientalis leaves oils against 4th-instar larvae of Aede aegypti and Culex pipiens pallens was significantly higher than stem, fruit, and seed oils.

Hashemi and Safavi\textsuperscript{61} studied the toxicity of essential oils of leaves and fruits obtained from P. orientalis against adults of cowpea weevil (Callosobruchus maculatus Fab.), rice weevil (Sitophilus oryzae L.), and red flour beetle (Tribolium castaneum Herbst). Twenty-six compounds (92.9%) and 23 constituents (97.8%) were identified in the leaf and the fruit oils, respectively. Leaf oils were more toxic than fruit oils against three species of insects α-Pinene, a monoterpeneoid, is the major component in P. orientalis essential oil. There are numerous reports on biological activity of α-pinene. Ojimelukwe and Adler\textsuperscript{62} found α-pinene was toxic to Tribolium confusum du Val.

Hair growth promoting activity: T. orientalis has been applied to treat patients who suffer from baldness and hair loss in East Asia from decades. Zhang et al.\textsuperscript{63} observed that hot water extract of T. orientalis promoted hair growth by inducing the anagen phase in telogenic C57BL/6 N mice. Topical application of T. orientalis extract induced an earlier anagen phase and prolonged the mature anagen phase, in contrast to either the control or 1% minoxidil-treated group.

Molluscicidal Activity: The molluscicidal activity leaf powder of T. orientalis against the snail Lymnaea acuminata was studied. The molluscicidal activity of all the plant products was found to be both time and concentration dependent. The 96 h LC\textsubscript{50} of T. orientalis leaf powder against L. acuminata was 250.5 mg/l. Ethanol extracts were more toxic than other organic extracts. Thujone (24 h LC50- 08.09 mg/l) was identified as active molluscicidal component in T. orientalis\textsuperscript{64}.

Antifungal activity: Essential oil are potential source of antifungal agents. Ezzat\textsuperscript{65} studied the inhibition of Candida albican through different plant extract and essential oils. Methanolic extract of Thuja leave and male cone showed considerable inhibition of C. albicans by cut plug technique and filter paper disc assay. Mishra et al.\textsuperscript{66} studied antifungal activity of aqueous leaf extract of T. orientalis against Curvularia lunata. Rakotonirainy\textsuperscript{67} studied the use of Thuja oil in control of bio contamination in libraries and archives storage areas, and showed that this oil had little inhibitory effect on the fungal combination.
The essential oil exhibited antifungal activity in the inhibition zone against *Alternaria alternata* and *Curvularia lunata* in a direct bioautography assay by lipophilic leaf extract of *T. orientalis*. Best bioactive component (Rf = 0.80) were isolated and noted for antifungal activity. The maximum zone of inhibition 30mm was reported against *A. alternata* followed by 22mm against *C. lunata*.

**Anthelmintic activity:**
Niranjan et al. studied ethanol extract from the leaves of *P. orientalis* for their anthelmintic activity against *Pheretima posthuma*. Three concentrations (1%, 2.5% and 5%) of extract were studied in activity, which involved the determination of time of paralysis and death of the worm. The extract exhibited significant dose dependent anthelmintic activity. Jaswal et al. first time studied the anthelmintic activity of ethanolic extract of leaves of *T. orientalis* against *Pheretima posthuma*. Three concentration(1%,2.5%,5%) of extract were used which involved the determination of time of paralysis and death of worm. The extract exhibited significant dose dependent anthelmintic activity.

**CONCLUSION**
The review article discussed all the pharmacological activity of all the extract of all plant parts of *T. orientalis* till date. The major pharmacological activity of *Thuja* are antibacterial, antioxidant, antifungal, anti-inflammatory, anthelmintic, anticarcinogenic, molluscicidal, nematicidal, larvicidal, and antiviral. Hot water extract of thuja was reported as hair growth promoter. All the prominent activities of *T. orientalis* are presented and significant results have been reported regarding the various activities discussed in the review. Furthermore considering its multifaceted medicinal uses, there is wide scope for future research.

**Conflict of Interest:** Authors has no conflict of interest

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