Ethnomedicinal Relevance, Phytochemistry, and Pharmacological Advances of Thuja and Platycladus: A Comprehensive Review

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Abstract
The genus Thuja (Cupressaceae) and the closely related Platycladus orientalis have been widely recognized for their ethnomedicinal, ornamental, and pharmacological importance. Traditionally employed in the treatment of respiratory disorders, dermatological conditions, rheumatism, and infectious diseases, these conifers also hold deep cultural significance across Asia, Europe, and North America. Over the last two decades (2010–2025), significant progress has been made in understanding their taxonomy, phytochemistry, standardization, and pharmacological potential. Phytochemical investigations have revealed a complex profile comprising volatile constituents—primarily α- and β-thujone, fenchone, sabinene, beyerene, and α-cedrol—and non-volatile metabolites including flavonoid glycosides (quercitrin, afzelin, isoquercitrin), biflavonoids (amentoflavone, hinokiflavone), coumarins, tannins, and labdane- and isopimarane-type diterpenes. These compounds underpin a broad spectrum of bioactivities such as antimicrobial, anti-inflammatory, antioxidant, anticancer, immunomodulatory, hepatoprotective, and cosmeceutical effects. Chemotypic and geographic variation, however, significantly influence both therapeutic efficacy and safety, particularly in relation to thujone-associated neurotoxicity. Standardization approaches have advanced from traditional pharmacopoeial descriptions to modern analytical platforms including HPLC, GC–MS, LC–HRMS, DNA barcoding, and chemometric fingerprinting, enabling reliable authentication, quality assurance, and regulatory compliance. Recent adoption of ICH Q2(R2) and Q14 guidelines has further strengthened validation practices. Future research should emphasize metabolomic profiling, biosynthetic pathway elucidation, safe dosage standardization, and development of novel drug delivery systems. In addition, conservation of endangered species such as Thuja sutchuenensis and sustainable resource utilization remain essential. Collectively, Thuja and Platycladus represent promising yet underexplored medicinal taxa with significant potential in phytopharmaceuticals, cosmeceuticals, and eco-friendly bioproducts.

Keywords: Thuja, Platycladus orientalis, taxonomy, phytochemistry, standardization, pharmacology, flavonoids, thujone

1.Taxonomy & Ethnomedicinal Contxt

The genus **Thuja** belongs to the family **Cupressaceae** and comprises **five extant species**: *T. occidentalis*, *T. plicata*, *T. koraiensis*, *T. standishii*, and *T. sutchuenensis* [1,2]. *Thuja orientalis* has been taxonomically reclassified as ***Platycladus orientalis***, a monotypic genus, although many studies—particularly ethnobotanical and pharmacological ones—still use the old nomenclature [3,4]. ***Thuja occidentalis*** (commonly called arborvitae or "Tree of Life") is native to eastern Canada and the northeastern United States and has been used in traditional medicine for centuries, particularly by Indigenous peoples and early settlers [5,6]. ***Thuja plicata*** (western red cedar) is native to the Pacific Northwest and holds profound cultural significance for Indigenous communities, being used in canoe making, weaving, and ceremonial purposes; its heartwood contains bioactive thujaplicins with potent antifungal activity [7,8]. ***Thuja koraiensis*** is valued primarily for ornamental purposes due to its attractive foliage with distinctive white bands and is native to Korea and northeast China [9]. ***Thuja standishii***, native to Japan, is known for its fragrant, durable wood and the presence of *standishinal*, a diterpene with aromatase-inhibitory properties [10]. ***Thuja sutchuenensis***, endemic to Sichuan, China, was believed to be extinct until its rediscovery; it is now classified as endangered [11]. ***Platycladus orientalis*** (syn. *Thuja orientalis*) is widely used as an ornamental tree across Asia and the Middle East and in traditional medicine for respiratory, dermatological, and rheumatic conditions [12,13]. The taxonomic status and ethnobotanical context of extant species of *Thuja* and the related genus *Platycladus* are summarized in Table 1

Table 1: Taxonomy, Status, Native Range, and Ethnobotanical Context of *Thuja* and Related Taxa

Taxon	Status / Classification	Native Range	Ethnobotanical Notes
Thuja occidentalis	Valid *Thuja* species	Eastern North America	"Arborvitae," medicinal uses, ornamental tree
Thuja plicata	Valid *Thuja* species	Pacific Northwest (USA, Canada)	Indigenous materials (canoes, weaving), antifungal heartwood
Thuja koraiensis	Valid *Thuja* species	Korea, NE China	Ornamental with distinct foliage
Thuja standishii	Valid *Thuja* species	Southern Japan	Timber, medicinal diterpenes (aromatase inhibition)
Thuja sutchuenensis	Valid *Thuja* species	Sichuan, China	Rediscovered endangered species
Platycladus orientalis	Formerly *Thuja orientalis*, now monotypic genus	East Asia	Widely used horticulturally; taxonomically distinct

2. Standardization Review of *Thuja* Plants

Standardization of herbal drugs is essential for ensuring safety, efficacy, and reproducibility. *Thuja* species (Cupressaceae), especially *Thuja occidentalis* and *Platycladus orientalis* (syn.

Thuja orientalis), have long been used in traditional medicine and incorporated into modern phytopharmaceuticals. However, their chemical composition varies considerably with geographical origin, seasonal changes, and extraction methods, necessitating validated quality control and standardization protocols [14,15].

- ✓ **Morphological & Botanical Identification**
  Standardization begins with botanical authentication. Macroscopically, *Thuja* leaves are small, scale-like, and arranged in flattened sprays. The cones are woody, containing 6–12 scales. Microscopic markers include resin canals in the mesophyll, papillose epidermal cells, and lignified xylem tracheids. Pharmacopoeial monographs, including the European Pharmacopoeia and WHO, describe both macroscopic and microscopic features to aid in the correct identification of *T. occidentalis* leaf material [16,17].
- ✓ **Physicochemical Standards**
  Physicochemical parameters are critical indicators of plant material quality. The moisture content should be less than 10% w/w (LOD method). Ash values are standardized to <12% total ash and <2% acid-insoluble ash. Extractive values, including alcohol-soluble and water-soluble fractions, are determined for raw leaf powders [18]. Fluorescence analysis under UV light (365 nm), after treatment with NaOH or HCl reagents, serves as an additional diagnostic tool in raw drug analysis.
- ✓ **Chemical Marker-Based Standardization**
  Volatile Oil Constituents: Essential oil composition is one of the most widely monitored quality markers. α- and β-thujone are the major components, along with fenchone, sabinene, and camphor. Gas chromatography coupled with FID or MS (GC–FID/GC–MS) is used for quantification. European Union regulations restrict thujone levels to <5 mg/kg in food and <25 mg/kg in bitters [19]. WHO and EMA monographs highlight the need to monitor thujone due to its neurotoxic potential [20].
- ✓ **Phenolic & Flavonoid Constituents:** Flavonoid glycosides such as quercitrin, afzelin, and isoquercitrin, along with biflavonoids like amentoflavone and hinokiflavone, serve as key markers. These are quantified through HPLC–DAD and UFLC–MS/MS methods [21,22]. Commercial standards of these compounds are widely available from suppliers such as Sigma-Aldrich and PhytoLab.
- ✓ **Chromatographic Fingerprinting**
  HPLC fingerprinting is an official method for *Platycladi Cacumen* (Chinese Pharmacopoeia), providing 8–10 characteristic peaks representing flavonoids and biflavonoids [23,24]. Thin-layer chromatography (TLC) is routinely employed for rapid identification of quercitrin and afzelin, using anisaldehyde–sulfuric acid spray reagents [25]. Gas chromatographic fingerprinting of essential oils is useful for authenticating *Thuja* species and detecting adulteration with *Juniperus* oils [26].
- ✓ **Advanced Analytical Tools**
  Molecular biology techniques have advanced standardization. DNA barcoding (ITS, matK) is increasingly used to distinguish *T. occidentalis* from close relatives and adulterants such as *Chamaecyparis* spp. [27]. Metabolomic platforms like LC–HRMS and NMR provide chemotaxonomic markers and enable identification of bioactive compounds [28]. Chemometric approaches, including principal component analysis

(PCA) and hierarchical cluster analysis (HCA), further assist in distinguishing samples from different ecotypes or regions [29].

✓ **Regulatory & Quality Control Aspects**

Pharmacopoeial monographs include *Thuja occidentalis* leaf in the European Pharmacopoeia and the Homeopathic Pharmacopoeia of the United States (HPUS) [30]. *Platycladi Cacumen* (*P. orientalis*) is listed in the Chinese Pharmacopoeia. Regulatory bodies such as EMA and FDA require documentation of safety, with special attention to thujone quantification. Standardized extracts, including *Thuja* mother tincture and *P. orientalis* capsules, follow marker-based assays, most often monitoring quercitrin or thujone concentrations [32]. Standardization of *Thuja* and *Platycladus* species involves a multi-level approach, including botanical identification, physicochemical profiling, and chemical marker assays. Key aspects are summarized in Table 2

Table 2. Standardization aspects of *Thuja* species and *Platycladus orientalis*, including methods, marker compounds, and regulatory references

Standardization Aspect	Methods Used	Marker Compounds / Parameters	Regulatory Sources
Botanical ID	Macro/microscopy	Scale leaves, resin canals	WHO, EP
Physicochemical	Ash values, extractives, LOD	Ash <12%, extractives	WHO
Volatile oil QC	GC–FID, GC–MS	α-/β-thujone, fenchone	EU/FDA, EMA
Phenolic QC	HPLC–DAD, UFLC–MS/MS	Quercitrin, afzelin, biflavonoids	ChP, MDPI
Fingerprinting	TLC, HPLC, GC	Quercitrin, EO peaks	ChP, EP
DNA barcoding	ITS, matK sequencing	Genetic identity	Recent studies
Chemometrics/Metabolomics	LC–HRMS, NMR + PCA	Multivariate fingerprints	ScienceDirect/MDPI

3. Phytochemistry of *Thuja* Plants

The chemical composition of *Thuja* species has been extensively studied in the last decade, covering both volatile (essential oil) and non-volatile fractions. Variations are influenced by species, cultivar, plant part, geographic origin, and season [33–36].

3.1. Volatile Constituents (Essential Oils / "Cedar Leaf Oil")

The volatile fraction is dominated by monoterpene ketones, primarily α-thujone and β-thujone, which can comprise >50–70% of the oil [37–39]. Other prominent constituents include fenchone, sabinene, and borneol, along with diterpenes such as beyerene and rimuene [40,41].

- Species & cultivar differences:A comparative GC–MS study of four *Thuja* varieties cultivated in Poland (*T. occidentalis* 'Globosa', *T. occidentalis* 'Aurea', *T. plicata*, and *T. plicata* 'Gracialis') found α-thujone levels ranging from 50.9% to 62.1%, β-thujone from 2.7% to 7.1%, fenchone from 4.1% to 7.2%, and beyerene from 2.6% to 9.0%. The to tal ketone content (α- + β-thujone + fenchone) was highest in *T. plicata* cultivars (~69%), aligning with Essential Oil Association (EOA) specifications.
- Geographical variation: Oils from *T. occidentalis* cultivated in Canada contained α-thujone (~61.7%), β-thujone (~5.3%), fenchone (~5.6%), and sabinene (~4.8%), with minor diterpenes. By contrast, Iranian-grown *T. orientalis* (now *P. orientalis*) oils contained lower thujone (~28%) but higher α-cedrol (~13%) [42].
- Plant part variation:
- Twig oils tend to be richer in α-thujone and diterpenes (e.g., beyerene, rimuene), while leaf oils have higher fenchone and monoterpene alcohols.
- Seasonalvariation:
  Peak thujone content in *Thuja* foliage is generally observed during late summer to early autumn, possibly due to biosynthetic activity linked to secondary metabolite defense [43]

3.2. Non-Volatile Constituents

In addition to volatile oils, *Thuja* species contain a wide range of non-volatile secondary metabolites with pharmacological relevance.

- Flavonoids & Polyphenols
  Multiple flavonoids have been identified, including quercetin, kaempferol, quercitrin, amentoflavone, and myricetin derivatives [44–46].
  Proanthocyanidins and tannins are present, contributing to antioxidant activity [47].
- Coumarins
  Simple coumarins such as scopoletin and umbelliferone have been detected in methanolic extracts, associated with anti-inflammatory and antimicrobial effects [48].
- Diterpenes : *Platycladus orientalis* (syn. *T. orientalis*) is particularly rich in labdane-type and isopimarane-type diterpenes, including lambertianic acid, communic acid, and pimaradienoic acids [49,50]. These compounds display diverse bioactivities—antifungal, anti-inflammatory, and cytoprotective [51, 52].
- Sesquiterpenes
  α-Cedrol is a notable sesquiterpene found in *P. orientalis*, contributing sedative and insecticidal properties [53].
- Chemotype Variation & Pharmacological Implications
  Chemotypic variation (i.e., differences in dominant constituents among populations) affects therapeutic potential and safety profile [54]. For instance:
  High-thujone chemotypes have potent antimicrobial activity but carry a greater risk of neurotoxicity at high doses [55].

α-Cedrol–rich chemotypes may be better suited for sedative or insect-repellent uses [56].

Diterpene-rich extracts from *P. orientalis* show strong antifungal and anti-inflammatory activity, offering applications beyond traditional essential oil uses [57,58]. The phytochemical composition of *Thuja* and *Platycladus* species includes volatile terpenes, flavonoid glycosides, coumarins, and diterpenes. Representative fractions and compounds are summarized in Table 3

Table 3. Major phytochemical fractions and representative compounds reported from *Thuja* species and *Platycladus orientalis*

Fraction	Major Compounds	Species / Source
Volatile	α-thujone, β-thujone, fenchone, sabinene, beyerene, rimuene	*T. occidentalis*, *T. plicata*
Flavonoids	Quercetin, kaempferol, quercitrin, amentoflavone	*T. occidentalis*, *P. orientalis*
Coumarins	Scopoletin, umbelliferone	*T. occidentalis*, *P. orientalis*
Proanthocyanidins	Catechin-type oligomers	*T. occidentalis*
Diterpenes	Lambertianic acid, communic acid, isopimarane diterpenes	*P. orientalis*
Sesquiterpenes	α-Cedrol	*P. orientalis*

3.3. Flavonoid Glycosides in *Thuja* and *Platycladus*

Flavonoid glycosides are among the most significant non-volatile secondary metabolites in *Thuja* species, particularly in *Thuja occidentalis* and *Platycladus orientalis* (syn. *Thuja orientalis*) [59]. The major identified glycosides include **quercitrin** (quercetin-3-O-rhamnoside), **afzelin** (kaempferol-3-O-rhamnoside), **isoquercitrin** (quercetin-3-O-glucoside), **mearnsitrin** (myricetin-3-O-rhamnoside), and biflavonoids such as **amentoflavone** and **hinokiflavone** [60].

- **Occurrence and Distribution**

  The flavonoid profile varies according to species, plant part, and geographic origin. *T. occidentalis* leaves are especially rich in quercitrin and amentoflavone, whereas *P. orientalis* exhibits higher afzelin content along with biflavonoids like hinokiflavone [61]. Seasonal and developmental stage variations influence flavonoid concentration, with higher levels generally recorded in young leaves during spring and summer 62.

- **Analytical Advances**

  High-performance liquid chromatography (HPLC) and ultra-fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS) have enabled simultaneous quantification of multiple flavonoid glycosides in plant extracts and biological samples [63]. For instance, Shan et al. quantified quercitrin, afzelin, amentoflavone, and hinokiflavone in rat plasma following oral administration of *P.*

orientalis leaf extract, establishing pharmacokinetic profiles relevant to bioavailability studies.

- **Pharmacological Relevance**

  Flavonoid glycosides from *Thuja* species demonstrate diverse bioactivities, including antioxidant, anti-inflammatory, anti-cancer, aldose reductase inhibitory, and hemostatic effects [64]. Quercitrin and afzelin exhibit potent radical scavenging and lipid peroxidation inhibitory activity, while biflavonoids such as amentoflavone and hinokiflavone are known for their anti-inflammatory and anti-viral effects [65].

  In *P. orientalis*, flavonoid glycosides have also been linked to hemostatic properties. Chen et al. [66] identified that thermal transformation products of flavonoid extracts enhanced procoagulant activity in bioassays, suggesting potential application in bleeding control formulations.

- Quality Control and Authentication

  Flavonoid glycosides serve as key chemical markers for the authentication and quality control of *Thuja* and *Platycladus* plant materials in pharmacopoeial standards . HPLC fingerprinting and TLC profiling have been adopted to distinguish authentic material from adulterants and to monitor consistency in herbal preparations . “Several flavonoid glycosides, including quercitrin, afzelin, isoquercitrin, and biflavonoids such as amentoflavone, have been reported in *Thuja occidentalis* and *Platycladus orientalis*. Their structures, sources, and pharmacological activities are summarized in Table 4

Table 4. Reported flavonoid glycosides and biflavonoids from *Thuja* species and *Platycladus orientalis* (2000–2025), with chemical structures (descriptions), plant sources, and pharmacological activities [66].

Compound Name	Chemical Structure (Description)	Plant Source	Reported Pharmacological Activities
Quercitrin (Quercetin-3-O-rhamnoside)	Flavonol backbone (C15H10O7) with rhamnose moiety at C-3 position	*T. occidentalis*, *P. orientalis*	Antioxidant, anti-inflammatory, anti-diabetic, hepatoprotective
Afzelin (Kaempferol-3-O-rhamnoside)	Flavonol backbone (C15H10O6) with rhamnose moiety at C-3	*P. orientalis*	Antioxidant, anti-inflammatory, anti-cancer
Isoquercitrin (Quercetin-3-O-glucoside)	Flavonol backbone with glucose moiety at C-3	*P. orientalis*, *T. occidentalis*	Antioxidant, aldose reductase inhibitor
Mearnsitrin (Myricetin-3-O-rhamnoside)	Flavonol backbone with 3′,4′,5′-trihydroxy substitution and rhamnose at C-3	*P. orientalis*	Antioxidant, antimicrobial

Compound Name	Chemical Structure (Description)	Plant Source	Reported Pharmacological Activities
Amentoflavone (Biflavonoid)	Two apigenin units linked at C-3′–C-8″	*T. occidentalis*, *P. orientalis*	Anti-inflammatory, anti-viral, anti-cancer
Hinokiflavone (Biflavonoid)	Two apigenin units linked at C-3′–C-6″	*P. orientalis*	Anti-inflammatory, anti-cancer, neuroprotective
Hypolaetin-7-O-xyloside	Flavone backbone with xyloside group at C-7	*P. orientalis*	Anti-inflammatory, antimicrobial
Rutin (Quercetin-3-O-rutinoside)	Quercetin with disaccharide rutinose at C-3	*P. orientalis*, *T. occidentalis*	Antioxidant, vasoprotective

4. Analytical Review of *Thuja* and *Platycladus orientalis* Quality Control

Over the past fifteen years, quality control of *Thuja* species, including *Platycladus orientalis* (formerly *Thuja orientalis*), has advanced substantially, with emphasis on chemotaxonomic markers, validated methods, and regulatory alignment. The volatile fraction of cedar leaf oils is characterized by α-thujone, β-thujone, fenchone, sabinene, and borneyl esters as dominant markers, while western redcedar (*T. plicata*) additionally yields beyerene, rimuene, and thujaplicins such as hinokitiol. Recent compositions confirm α-thujone as the principal constituent but highlight wide quantitative variability depending on cultivar and origin, for example ~38% α-thujone with sabinene (~13%) and fenchone (~9%) in a 2024 *T. occidentalis* sample. The non-volatile fraction comprises flavonoids (quercitrin, rutin, quercetin, amentoflavone), coumarins, tannins, and diterpenes (labdane- and isopimarane-type), with α-cedrol serving as a practical sesquiterpene marker.

Methodological refinements in sample preparation have improved reproducibility: hydrodistillation or steam distillation remains standard for essential oils, while microwave- or ultrasound-assisted extraction with 70–80% ethanol supports efficient recovery of flavonoids. For analytical isolation, high-performance counter-current chromatography (HP-CCC) enables preparative separation of α-cedrol or biflavonoids for reference standards. Volatile analysis predominantly employs GC-FID or GC–MS with apolar columns, supported by headspace-SPME for rapid screening and reduced matrix interference. Chiral GC using cyclodextrin phases now separates α/β-thujone enantiomers, a critical advance for authenticity and toxicological risk assessment, while GC×GC–TOF-MS enhances fingerprint resolution and adulteration detection.

For non-volatiles, HPLC–DAD and UHPLC–QTOF-MS/MS permit simultaneous quantification of flavonoids (rutin, quercitrin, amentoflavone), while targeted UFLC–MS/MS (MRM mode) supports pharmacokinetic and biomonitoring studies. Economical methods such as HPTLC (visionCATS) continue to provide reliable identity and semi-quantitative data, particularly in routine quality assurance. Specialty markers such as thujaplicins (in *T. plicata* wood) are now quantified by validated HPLC–DAD workflows.

Authentication strategies increasingly combine spectroscopy and chemometrics: ATR–FTIR with PCA or PLS-DA allows rapid species-level discrimination and adulteration detection, while DNA barcoding (ITS2, matK) offers unambiguous identification of dried or powdered raw materials. Such molecular approaches complement morphological and chromatographic markers in regulatory submissions.

Recent regulatory guidance has reshaped validation practice. The adoption of ICH Q2(R2) in 2023–24 extended classical validation parameters (specificity, linearity, accuracy, precision, LOQ/LOD, robustness) to include spectroscopic and chemometric methods, while ICH Q14 introduced the Analytical Target Profile (ATP) concept to link method design to lifecycle management. In practice, release testing of *Thuja* oils involves GC–MS fingerprinting for identity (presence of α-/β-thujone, fenchone, sabinene; and beyerene/rimuene for *T. plicata*), quantitative assay of α-thujone (primary) and fenchone/β-thujone (secondary) by GC-FID, and compliance checks against jurisdictional thujone limits (e.g., EU Regulation 1334/2008 for foods; US TTB SSD-TM-203 for spirits). For extracts, HPTLC and HPLC fingerprints of flavonoids and biflavonoids are employed as both identity and potency assays. Collectively, these advances underscore a shift toward fit-for-purpose, regulator-ready analytics, where classical chromatographic markers (thujones, flavonoids, biflavonoids) are reinforced by molecular authentication and multivariate fingerprinting. This integrated approach enables reliable quality control, supports safety evaluations, and aligns *Thuja* and *Platycladus* preparations with modern pharmacopoeial and regulatory expectations. Analytical approaches for *Thuja* essential oils and non-volatile markers are summarized in Table 5

Table 5. Summary of analytical targets, key marker compounds, sample preparation methods, analytical techniques, validation/regulatory considerations, and references for *Thuja* and *Platycladus* phytochemical and authentication studies.

Analytical Target	Key Marker Compounds	Sample Prep / Extraction	Analytical Technique	Validation / Regulatory Notes	Reference s
Volatile profile (EO)	α-/β-thujone, fenchone, sabinene, borneyl acetate, beyerene, rimuene (*T. plicata*)	Hydrodistillation or steam distillation; avoid prolonged heating to limit isomerization; HS-SPME for rapid screening	GC-FID, GC-MS; Chiral GC for α-/β-thujone enantiomers; GC×GC-TOF-MS for complex matrices	ICH Q2(R2) for method validation; report % area + mg/mL; EU Reg. 1334/2008 Annex III for thujone limits	[67-71]
Thujone compliance (EO, foods, spirits)	α-/β-thujone	SPE cleanup if in complex matrices; direct injection for EO	GC-MS (SIM mode) per TTB SSD-TM-203; cyclodecanone as IS	EU: max 10 mg/kg (non-*Artemisia* foods), 35 mg/kg (*Artemisia* drinks); US: TTB compliance testing for spirits	[72]

Analytical Target	Key Marker Compounds	Sample Prep / Extraction	Analytical Technique	Validation / Regulatory Notes	Reference s
Non-volatiles (flavonoids)	Rutin, quercitrin, quercetin, amentoflavone, hinokiflavone	70–80% MeOH or EtOH sonication; SPE cleanup for LC-MS	HPLC-DAD, UHPLC-QTOF-MS/MS, UFLC-MS/MS (MRM)	Use multi-marker quant for QC; LOD ~0.8 µg/mL reported	[73-75]
Sesquiterpene alcohols (*P. orientalis*)	α-cedrol	HP-CCC isolation for standard; MeOH extraction	GC-MS, GC-FID; derivatization for HPLC	Include in ID/specification for hair-care claims	[76]
Diterpenes (*P. orientalis*)	Lambertianic acid, communic acid, isopimarane diterpenes	MeOH maceration or Soxhlet	UHPLC-QTOF-MS/MS; preparative isolation for bioassay	Characterize seasonal/geographic variation	[77]
Wood durability markers (*T. plicata*)	β-/γ-thujaplicin (hinokitiol), plicatic acid	Wood chips/cores; organic solvent extraction	HPLC-DAD; GC-MS with derivatization for tropolones	Used for authentication & anti-microbial potency claims	[78,79]
Rapid ID / adulteration check	Entire EO profile	None (neat oil)	ATR-FTIR + chemometrics (PCA/PLS-DA); confirm with GC-MS	Differentiates *Thuja/Platycladus* from *Juniperus* spp.	[80,81]
DNA authentication	ITS2, psbA-trnH	CTAB or kit-based DNA extraction from leaves/powders	DNA barcoding / metabarcoding	Confirms species identity in raw & processed materials	[82,83]

5. Pharmacology review of Thuja Plants

5.1. Antimicrobial Activity

Essential oils (EOs) and ethanolic extracts from Thuja exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and some viruses. The activity is largely attributed to α- and β-thujone, fenchone, and other terpenes, which disrupt microbial membranes, increase permeability, and inhibit quorum sensing [84,85]. In T. plicata, cedar leaf oil (CLO) inhibited Staphylococcus aureus, Escherichia coli, Candida albicans, and various molds at low MIC values [86]. α-Thujone and fenchone have also demonstrated

synergistic effects with conventional antibiotics in vitro [87]. CLO has found application as a natural preservative and surface disinfectant.

5.2. Anti-inflammatory & Antioxidant Effects

In a TNBS-induced colitis model, T. occidentalis mother tincture (25–50 mg/kg, 7 days) significantly reduced colonic inflammation, suppressed COX-2 expression, lowered TNF-α and IL-6 levels, increased glutathione (GSH), and reduced malondialdehyde (MDA) [88]. Mechanistically, these effects appear to involve NF-κB inhibition and suppression of pro-inflammatory cytokine production, alongside the upregulation of antioxidant defense enzymes such as SOD, catalase, and GPx [89].

Topically, methanolic extract of P. orientalis reduced carrageenan-induced paw edema in rats and improved total antioxidant capacity [90].

5.3. Anticancer Potential

In vitro, T. occidentalis extracts induced apoptosis in glioblastoma, melanoma, and colon cancer cell lines through mitochondrial membrane depolarization, activation of caspase-3/9, and DNA fragmentation [91,92].

Diterpenoids from P. orientalis have shown anti-proliferative effects in human leukemia and hepatocellular carcinoma cells, partially via modulation of MAPK and PI3K/Akt signaling pathways [93]. Limited in vivo models demonstrate tumor growth delay following topical or systemic administration of standardized extracts.

5.4. Immunomodulatory Activity

Polysaccharide fractions of T. occidentalis increased IL-2 and IFN-γ production while reducing IL-4 in murine splenocyte cultures, suggesting a Th1-skewing effect [94]. In murine peritoneal macrophages, extracts enhanced phagocytic activity and nitric oxide production [94]. These activities support traditional claims of immune stimulation, although clinical proof remains limited.

5.5. Dermatological & Cosmeceutical Applications

Hair growth: P. orientalis α-cedrol and labdane diterpenes accelerated anagen entry in C57BL/6 mice and upregulated VEGF and β-catenin expression in hair follicles [96].

Warts (HPV): T. occidentalis preparations have historical use for wart removal. A randomized feasibility trial of individualized homeopathy (including Thuja) for cutaneous warts found no statistically significant difference versus placebo, though safety and tolerability were confirmed [97].

5.6. Neuropharmacology

Thujone is a GABAA_AA receptor antagonist; at low doses it may cause mild CNS stimulation, but higher exposures can trigger seizures [98]. Some in vitro data suggest acetylcholinesterase inhibition, but toxicity concerns limit its therapeutic potential [99].

5.7. Antifungal &hepatoprotective activity

Diterpenoids from P. orientalis inhibited Magnaporthe oryzae (rice blast fungus) in vivo, reducing disease incidence by over 75%. This points to a potential role in eco-friendly agricultural biocontrol. Hepatoprotective: Flavonoid-rich fractions of P. orientalis protected rat hepatocytes from CCl4_44-induced injury via antioxidant and membrane-stabilizing effects. Antiviral: EO fractions inhibited herpes simplex virus type 1 (HSV-1) in vitro. Insecticidal: α-Cedrol and thujone-containing fractions repel mosquitoes and ticks .

Table 6. Pharmacological activities of *Thuja* and *Platycladus* extracts/compounds with corresponding evidence types and proposed mechanisms of action.

Activity	Key Compounds / Extract	Evidence type	Mechanism
Antimicrobial	α-/β-thujone, fenchone, sabinene	In vitro / applied	Membrane disruption, QS inhibition
Anti-inflammatory	Polyphenols, diterpenes, α-cedrol	In vivo (rodent)	NF-κB inhibition, cytokine downregulation
Anticancer	Diterpenes, flavonoids, thujone	In vitro / in vivo	Apoptosis induction, pathway modulation
Immunomodulatory	Polysaccharides, flavonoids	In vitro / ex vivo	Th1 skewing, macrophage activation
Hair growth	α-Cedrol, labdane diterpenes	In vivo (mice)	VEGF, β-catenin upregulation
Antifungal (agro)	Labdane/isopimarane diterpenes	In vivo (plant model)	Fungal growth suppression
Hepatoprotective	Flavonoids	In vivo (rodent)	Antioxidant, membrane stabilization

6. Methods of Extraction in *Thuja*

6.1. Volatile (Essential Oil) Fraction – Leaves/Young Twigs

Essential oil ("cedar leaf oil") is commonly obtained via hydrodistillation or steam distillation, yielding characteristic monoterpene ketones (α-/β-thujone, fenchone, sabinene) and minor diterpenes such as beyerene [104]. Distillation times range from 3–6 h in lab Clevenger setups, while industrial units use continuous steam stripping. Temperature control is essential to prevent thujone isomerization and oxidation [105]. Commercial suppliers of *T. plicata* leaf oil also document steam distillation as the industry standard .

Supercritical CO_2 extraction has been reported mainly for wood tissues, efficiently recovering tropolones (e.g., hinokitiol) from *T. plicata* heartwood.

6.2. Non-Volatile Phenolics & Diterpenoids – Leaves/Needles/Twigs

Classic solvent extraction using 60–80% methanol or ethanol is the standard for flavonoid glycosides such as quercitrin, afzelin, isoquercitrin, and myricitrin . These protocols underpin HPLC–DAD quantification of multiple markers for *P. orientalis* quality control.

Microwave-assisted extraction (MAE) and dynamic microwave-assisted extraction (DMAE) with 80% aqueous methanol have demonstrated high total flavonoid yields within minutes, making them ideal for high-throughput QC.

Ultrasound-assisted extraction (UAE), including "green" approaches with natural deep eutectic solvents (NADES), shortens extraction time while maintaining yields.

Matrix solid-phase dispersion (MSPD), employing AQ–C18 sorbent and ionic liquid eluents, can extract both polar and non-polar constituents rapidly with minimal solvent.

Post-extraction liquid–liquid partitioning (hexane → EtOAc → n-BuOH) and chromatographic fractionation (silica gel, Sephadex LH-20, polyamide) are routinely used for isolation of pure compounds .

6.3. Safety and Regulatory Compliance

Given the neurotoxic potential of α-/β-thujone, quantification is required in food and medicinal applications. GC–MS analysis against EU Regulation 1334/2008 and the EMA's public statement on thujone ensures compliance. Different solvent-based and advanced extraction methods for *Thuja* phytoconstituents are compiled in Table 3

Table 7. Extraction and analytical methods for *Thuja* and *Platycladus* species, their targeted matrices, solvent systems, major products, and methodological considerations

Target (Matrix)	Method	Solvent / Settings	Main Products	Advantages / Considerations
Leaf EO	Hydrodistillation / Steam distillation	Water or steam; 3–6 h (lab)	α-/β-thujone, fenchone, sabinene, beyerene	Robust, standardized; temperature-sensitive
Heartwood tropolones	Supercritical CO_2	CO_2 at 31 °C, 7.38 MPa	Hinokitiol, thujaplicins	Solvent-free, selective
Leaf phenolics	Maceration / Soxhlet	60–80% MeOH/EtOH	Quercitrin, afzelin, rutin	Simple, scalable; longer time
Leaf phenolics (fast QC)	MAE / DMAE	80% MeOH, ~80 W, 5 min	Total flavonoids	Rapid, high yield
Leaf phenolics (green)	UAE / NADES–UAE	EtOH/H_2O or NADES	Flavonoid glycosides	Low solvent, eco-friendly
Broad-spectrum	MSPD	AQ–C18 + ionic liquid	Polar & non-polar mix	Low solvent use, rapid
Thujone compliance	GC–MS	EO / extracts	α-/β-thujone	Regulatory requirement

7. Conclusion and Future Perspectives

The genus *Thuja* (Cupressaceae), together with the closely related *Platycladus orientalis*, represents an important taxon both ethnobotanically and pharmaceutically. Across species, essential oils are dominated by monoterpene ketones (α-/β-thujone, fenchone, sabinene) and diterpenes such as beyerene and rimuene, while non-volatile fractions are rich in flavonoid glycosides (quercitrin, afzelin, isoquercitrin) and biflavonoids (amentoflavone, hinokiflavone). Tropolones (e.g., hinokitiol) and labdane/isopimarane diterpenes further contribute to the chemotaxonomic and pharmacological diversity of these plants. Pharmacological studies validate their antimicrobial, anti-inflammatory, antioxidant, anticancer, immunomodulatory, dermatological, and insecticidal potential, supporting many traditional uses.

Significant progress has been made in standardization and quality control over the past decade. Advances in chromatographic (GC–MS, HPLC–DAD, UHPLC–QTOF-MS/MS), molecular (DNA barcoding), and spectroscopic (ATR–FTIR with chemometrics) tools provide multi-layered authentication. Regulatory frameworks (e.g., EU Regulation 1334/2008, EMA, FDA, and ICH Q2(R2)/Q14) now emphasize validated marker-based assays, lifecycle management, and thujone quantification to ensure product safety and reproducibility. Extraction innovations such as microwave-assisted, ultrasound-assisted, NADES-based, and MSPD techniques complement classical hydrodistillation and Soxhlet methods, improving yield, eco-sustainability, and analytical readiness.

Future research directions should address several gaps.

- ✓ Standardization harmonization: Global pharmacopoeias (WHO, EP, ChP, HPUS) need harmonized monographs to align botanical ID, marker compounds, and acceptable thujone limits.
- ✓ Clinical translation: Despite abundant in vitro and preclinical data, well-designed clinical trials are urgently required to validate immunomodulatory, dermatological (hair growth, wart treatment), and anticancer applications.
- ✓ Green and scalable technologies: Future extraction and isolation should prioritize solvent-free, eco-friendly methods (e.g., supercritical CO_2, NADES) to enhance sustainability and reduce toxicological risks.
- ✓ Chemotype mapping and metabolomics: Integrating chemometrics with global metabolomic profiling can clarify intraspecific chemotypes, seasonal/geographic variation, and guide cultivation strategies for specific therapeutic profiles.
- ✓ Safety and toxicology: Thujone's neurotoxicity remains a limiting factor. Future work should quantify safe dose ranges, develop thujone-free standardized extracts, and explore safer non-thujone markers (e.g., α-cedrol, biflavonoids).
- ✓ Agro- and cosmeceutical applications: The antifungal diterpenes of *P. orientalis* warrant further exploration as eco-friendly biocontrol agents in agriculture. Similarly, α-cedrol–rich extracts could be developed into natural cosmeceuticals for hair growth and insect repellency.

In summary, *Thuja* and *Platycladus* represent pharmaceutically valuable taxa with wide-ranging traditional and modern applications. Bridging traditional knowledge with validated modern analytics, regulatory harmonization, and translational research will be critical to unlocking their full potential as safe, effective, and sustainable phytopharmaceutical resources.

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