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TOXIC *THUJA OCCIDENTALIS* ESSENTIAL OIL

RESEARCH ARTICLE

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Intraspecific stability of the thujone-rich chemotype of *Thuja occidentalis* L. in Estonia indicates persistent toxic potential

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ABSTRACT

The essential oil (EO) yield and chemical composition of *Thuja occidentalis* L. (Cupressaceae) growing in Estonia were investigated to assess intraspecific variability, chemotypic stability, and the consistency of toxic potential associated with thujone content. Fresh leaf material was collected from several geographically distinct populations (n = 12), together with dried leaves and fruits (n = 5), and analysed by hydrodistillation and gas chromatography–mass spectrometry (GC–MS). Fresh leaf EO yields ranged from 0.73% to 1.32% (mean 1.04%), whereas dried leaves yielded up to 2.98%. In total, 105 volatile constituents were identified. Across all fresh leaf samples, the EO composition was consistently dominated by oxygenated monoterpenes, particularly fenchone (12.08%–29.99%), α-thujone (10.75%–22.74%), and β-thujone (5.13%–17.17%). Their combined proportion ranged from 28% to 70% of the total EO composition (mean 52%). The α-/β-thujone ratio ranged from 0.84 to 2.50. Fresh and dried plant materials did not differ qualitatively in their chemotypic profiles, as both retained the same fenchone–thujone dominance, although drying increased the essential oil yield quantitatively. Fruit EOs differed quantitatively from leaf oils, exhibiting markedly lower proportions of fenchone and both thujone isomers, together with substantially higher levels of monoterpene hydrocarbons, particularly sabinene. No significant geographical trends in total thujone content were observed across growing locations. Given the well-documented neurotoxicity of thujones, the observed chemical uniformity indicates that *T. occidentalis* in Estonia has geographically independent toxic potential. The absence of thujone-poor chemotypes and the consistently high proportion of thujones suggest a persistent toxicological potential, based on literature data on thujone toxicity. These findings are relevant for risk assessment and the safety evaluation of *Thuja*-derived materials.

1. Introduction

White cedar, also known as arborvitae (*Thuja occidentalis* L., Cupressaceae), is native to eastern North America and is widely cultivated in Europe as an ornamental species. Although first grown in North America, it is now also considered naturalised in parts of Europe. The tree typically reaches 15–20 m in height and has a conical, pyramidal form. Its branches and branchlets are arranged in flattened sprays within a single plane and are covered with small, scale-like leaves [1].

T. occidentalis contains essential oil (EO) with toxic monoterpenoid ketones α-thujone and β-thujone [1,2]. The 1988 European Council Directive 88/388/EEC established maximum permitted levels of thujone in alcoholic beverages, with the resulting daily intake estimated to range between 0.2 and 2.4 mg [3]. Despite its toxicity, *Thuja* has been identified to have several biological and pharmacological activities. It has been reported to possess a broad spectrum of biological activities, including anti-inflammatory [4], antitumoral [5,6], antibacterial and antifungal [7], as well as antioxidant, antidiabetic, hypolipidemic, atheroprotective, gastroprotective, antiviral, and immunostimulatory effects [8]. The monoterpene α-thujone has demonstrated efficacy against malignant glioblastoma multiforme cells and shows promise as an active agent against polycystic ovary syndrome [9].

Thujone at 25 mg/kg caused behavioural changes, weight loss, reduced organ weight, impaired liver and kidney function, and mortality in 6–8-week-old mice. The median lethal dose (LD_{50}) was 45 mg/kg via intraperitoneal (i.p.) injection and 230 mg/kg via oral administration. Long-term use of subconvulsive doses may result in chronic toxicity. The estimated maximum daily human intake from food or herbal products is 3–7 mg. In addition to neurotoxicity, thujone may have genotoxic and carcinogenic potential, although antimutagenic and anticarcinogenic effects have also

been reported [10]. Fenchone exhibits low toxicity, enhances gastric healing, and prevents duodenal ulcers [11]. Sabinene shows no significant genotoxicity or other major toxic effects at typical consumer exposure levels [12]. Acetylation of sabinol, which is formed by oxidation of sabinene, produces the toxic sabinyl acetate, which is an abortifacient [13].

In Estonia, as well as in neighbouring countries, *T. occidentalis*, as an evergreen plant and a symbol of eternal life, has been widely distributed in cemeteries and, to a lesser extent, in parks. In recent decades, it has become increasingly popular near residential buildings as an ornamental plant [14]. Due to its possible deadly toxic effect on humans and domestic animals [10], it is important to study its poisonous substance content and possible chemotypes.

Although the fenchone–thujone chemotype of *T. occidentalis* has been described previously [15–18], most available studies are based on single-site collections or limited sample sets. Systematic evaluation of intraspecific chemical stability across multiple geographically distinct populations, particularly under northern European climatic conditions, remains limited. The aim of this study was therefore to evaluate the intraspecific variability and chemotypic stability of the EO of *T. occidentalis* across multiple Estonian populations and to assess compositional differences between fresh and dried leaves and fruits in relation to toxicologically relevant constituents.

2. Materials and methods

2.1. Plant material

The *T. occidentalis* research material (n = 17) was collected in September 2025 from different growing locations in Estonia. Branch tips without fruits, 20 cm long, were removed from a single bush with scissors. Branch tips with leaves and fruits, as well as separate fruits, were also collected for comparison (Table 1). Fresh research material was stored in a freezer at –18 ºC before analysis. Dried plant material was left at room temperature for 10 days. Before analysis, samples were stored in closed paper bags. Plant material was identified and collected by Prof. A. Raal. The voucher specimen is kept in the Institute of Pharmacy of the University of Tartu (Cupressaceae/Thu1).

2.2. Hydrodistillation of essential oils

Before distillation, the leaves were separated from the tops and cut without the wooden parts. EOs were obtained by hydrodistillation using the European Pharmacopoeia apparatus [19]. The plant materials (50 g of fresh or 20 g of dried material) were hydrodistilled with 300 mL of purified water in a 1000 mL round-bottom flask for 2 hours (2–3 mL/min). Hexane (0.5 mL) was added to a graduated tube to remove the distilled oil. The yield of volatile fractions was determined according to the European Pharmacopoeia method, using xylene as a measuring solvent [19].

2.3. GC–MS analysis of essential oil

The EO samples were analysed on an Agilent 6890/5973 gas chromatography–mass spectrometry (GC–MS) system using MSD ChemStation. 1 µL of the sample was introduced into an Agilent HP-5ms UI column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) using split mode (150:1). The injector temperature was 280 ºC, and the carrier gas (He) flow was kept constant at 1 mL/min throughout the whole analysis. The oven was held at 50 °C for 2 minutes, followed by a ramp of 4 °C/min to a final temperature of 280 °C, which was maintained for 5 minutes.

The MSD was operated in electron ionisation (EI) mode at 70 eV, scanning across the mass range of 29–400 m/z with a delay time of 4 minutes and a scan speed of 3.8 scans per second. The data were analysed using the Agilent MassHunter software package, applying a deconvolution algorithm at different window size factors. Resulting compounds were identified by using the NIST23 library with a Match Factor ≥ 90 and by retention indices (relative to n-alkanes C8–C30). The area percentages of each peak were calculated from the total areas in the chromatograms without using correction

Table 1. *Thuja occidentalis* samples studied: origin and yield of essential oil

Sample	Plant part	Origin	Geographical coordinates	Yield of EO, v/m%
1	Fresh leaves	Tammelinn, Tartu	58.362956° N, 26.704613° E	1.32
2	Fresh leaves	Jõgeva	58.747113° N, 26.398665° E	0.93
3	Fresh leaves	Väike-Maarja settlement	59.130531° N, 26.258091° E	1.04
4	Fresh leaves	Tõrva	58.002545° N, 25.923938° E	0.96
5	Fresh leaves	Luua village, Jõgeva parish, Jõgeva county	58.645453° N, 26.598401° E	1.17
6	Fresh leaves	Endla Nature Reserve, Tooma village, Jõgeva parish, Jõgeva county	58.873394° N, 26.277245° E	1.30
7	Fresh leaves	Äksi settlement, Tartu parish, Tartu county	58.529726° N, 26.638756° E	0.92
8	Fresh leaves	Priipalu village, Valga parish, Valga county	57.984819° N, 26.180835° E	0.91
9	Fresh leaves	Kärde village, Jõgeva parish, Jõgeva county	58.850047° N, 26.280170° E	0.73
10	Fresh leaves	Nõo settlement, Nõo parish, Tartu county	58.279714° N, 26.535497° E	1.30
11	Fresh leaves	Tapa	59.261271° N, 25.964627° E	1.01
12	Fresh leaves	Lohkva village, Luunja parish, Tartu county	58.370237° N, 26.793363° E	0.85
13	Dried leaves	Lohkva village, Luunja parish, Tartu county	58.370237° N, 26.793363° E	2.98
14	Fresh fruits	Lohkva village, Luunja parish, Tartu county	58.370237° N, 26.793363° E	0.51
15	Dried fruits	Lohkva village, Luunja parish, Tartu county	58.370237° N, 26.793363° E	0.40
16	Fresh leaves with fruits	Luke village, Nõo parish, Tartu county	58.242097° N, 26.575289° E	0.84
17	Dried leaves with fruits	Luke village, Nõo parish, Tartu county	58.242097° N, 26.575289° E	1.98

factors. The analysis was performed as the mean of three parallel determinations, and the corresponding standard deviation values were added. The same GC–MS method has previously been successfully used to analyse other EOs [20,21].

2.4. Statistical analysis

For each sample, GC–MS analyses were performed in triplicate, and results are expressed as mean ± standard deviation. Fresh leaf samples (1–12) served as the reference group and were compared with selected samples using one-sample t-tests ($p < 0.05$). Principal component analysis (PCA) was carried out on standardised relative abundance data to assess chemotypic differentiation. In addition, Pearson and Spearman correlation analyses were performed to evaluate potential relationships between geographical coordinates (latitude and longitude) and the relative contents of major constituents (fenchone, α-thujone, β-thujone, and total thujone). Correlation coefficients (r or ρ) and corresponding p-values were calculated; $p < 0.05$ was considered statistically significant. Ratios of α- to β-thujone were calculated to evaluate isomeric variation. All statistical analyses were performed using Python.

3. Results

3.1. Yield of essential oil

EO yields of fresh leaf samples (1–12) ranged from 0.73% to 1.32%, with an average value of 1.04%. Despite the geographic distribution of sampling sites, yield variation remained moderate, with most samples ranging from 0.9% to 1.3% (Table 1). No statistically significant differences were observed between EO content in fresh or dried leaves, fresh or dried fruits, as well as in the fresh or dried mixture of leaves and fruits. Although fresh fruits showed numerically higher values than dried fruits, the difference was not statistically significant, and the remaining paired comparisons likewise revealed no significant differences. Tsiri et al. [16] found that in dried *Thuja* leaves, the EO yield varied from 0.58% v/w to 0.87%. Araújo et al. [22] mentioned the EO yield of 0.15%–0.63% in dried leaves. The higher EO yields observed in this study may be explained by differences in plant material (fresh vs dried) and by geographical, seasonal, and methodological factors [23].

However, the EO yield from dried leaves was approximately three times higher (2.98%) than from the average of fresh leaves (1.02%). In contrast, the EO yield in the mixture of dried leaves and fruits was only twice as high (1.98%) as in the fresh material (0.84%), which probably results from the lower EO content in the fruits. Regardless of their drying, the EO content in fruits was lower (0.40%–0.51%) than in leaves. Also, according to the literature, the EO content in *T. occidentalis* fresh leaves ranges from 0.35% to 0.82%. Twigs with leaves of the cultivar 'Brabant' contain 2.5 times more oil than the 'Smaragd' variety (1.20% and 0.48%, respectively) [18]. According to other studies, the fresh plant contains approximately 0.6% EO [17] and dried twigs 1.4%–4% EO [1].

3.2. Content of essential oils from different parts of *Thuja occidentalis*

A total of 105 volatile compounds were identified in the fresh and dried parts of *T. occidentalis*, and their percentages of the total area were determined (Tables 2, S1, S2; Fig. 1).

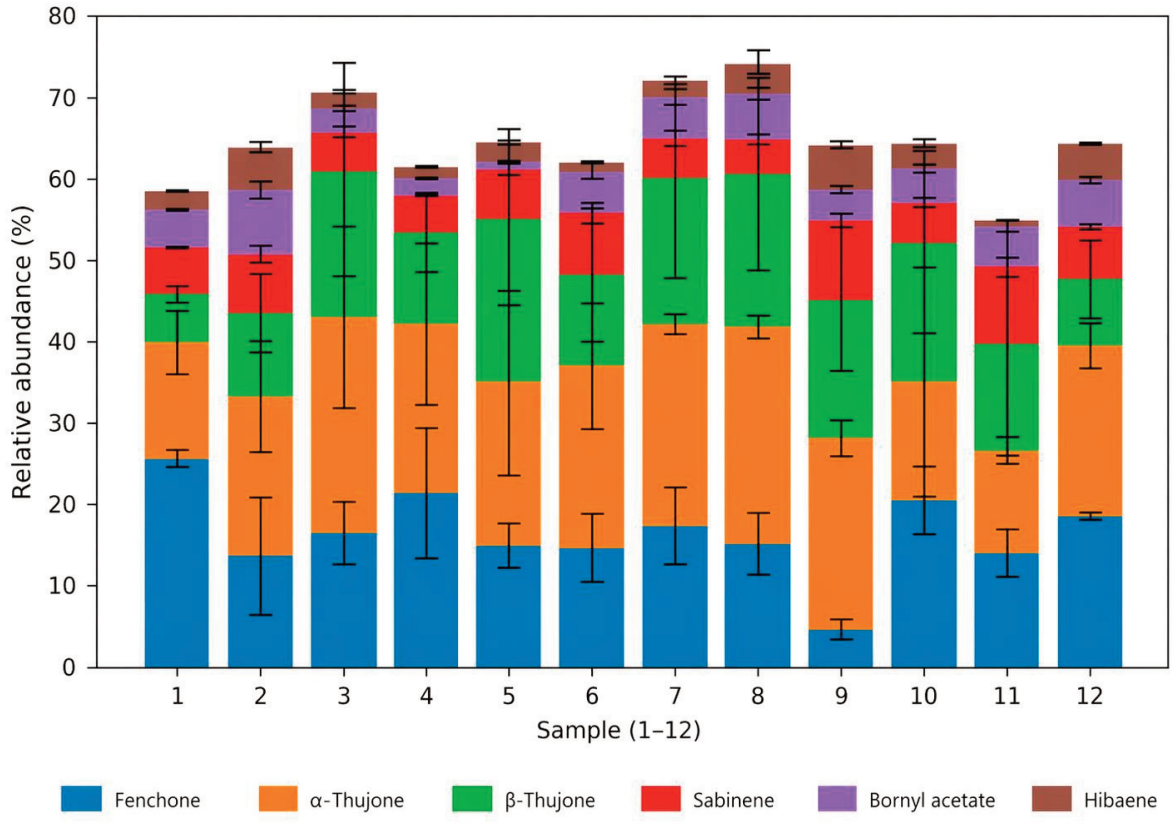

Fig. 1. Stacked bar chart showing the relative abundances (mean ± SD) of the six most abundant constituents across the essential oils of *Thuja occidentalis* fresh leaves (samples 1–12).

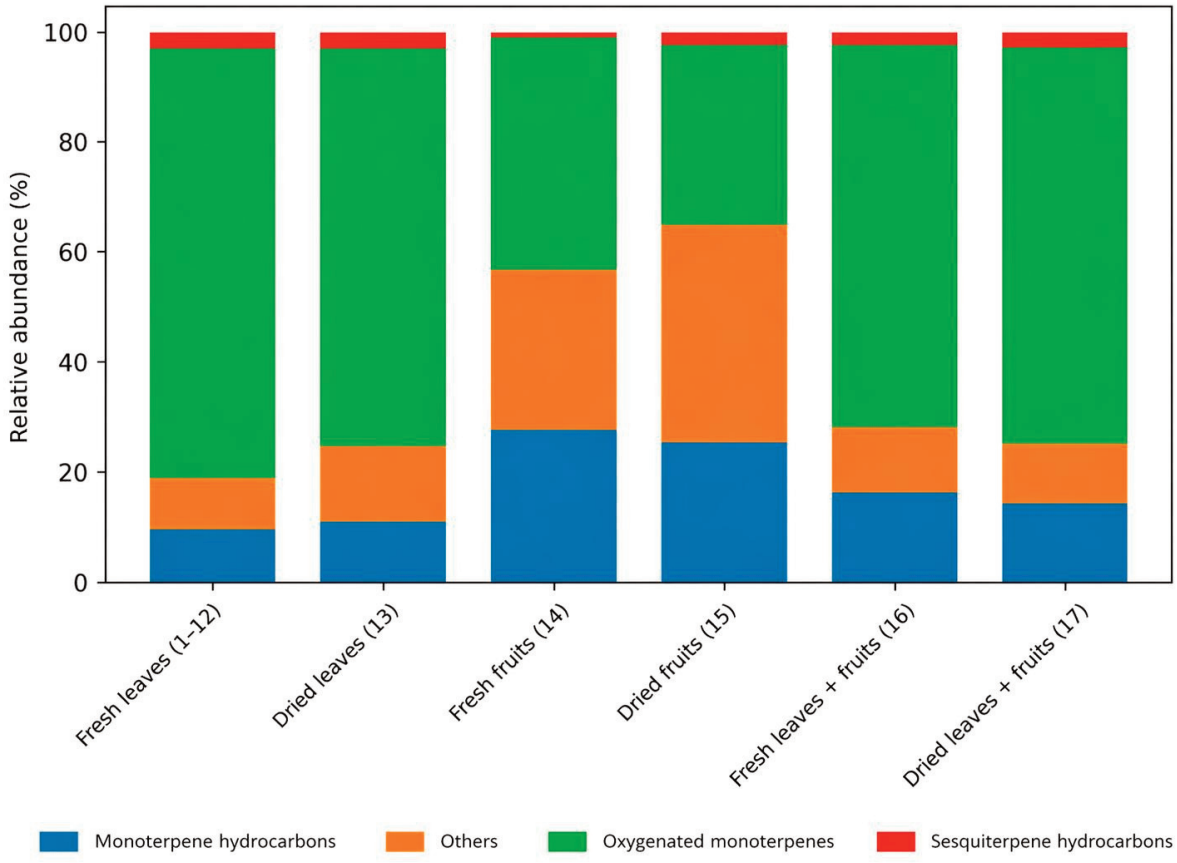

Fig. 2. Classification of compounds in the essential oils of *Thuja occidentalis*.

The analysis of EOs from fresh leaves (samples 1–12) showed that the composition is clearly dominated by three major constituents: fenchone (12.08%–29.99%), α-thujone (10.75%–22.74%), and β-thujone (5.13%–17.17%), followed by sabinene, bornyl acetate, and hibaene. Together, fenchone and the two thujone isomers account for more than half (mean 52%) of the total composition.

The EOs of *T. occidentalis* were dominated by oxygenated monoterpenes in all sample types, particularly in fresh and dried leaves, where they constituted the major fraction of the total identified compounds (Table S1; Fig. 2). In fresh leaves (samples 1–12), oxygenated monoterpenes accounted for approximately 78% of the total identified constituents, whereas fruit samples contained a substantially higher proportion of monoterpene hydrocarbons. In contrast, fruit samples showed a relatively higher proportion of monoterpene hydrocarbons, while sesquiterpenes and other constituents remained minor components across all groups (Fig. 2).

α-Thujone is more toxic than β-thujone [24]. Thujone exhibits neurotoxic effects at relatively low doses, with toxic effects reported in the tens of mg/kg range and lethal doses in the low hundreds of mg/kg in animal studies [10]. Regulatory limits for thujone have been established in the European Union, restricting its content in food and beverages to low mg/kg levels depending on the product category [3].

The α- and β-thujone ratio ranged from 0.84 to 2.50, with a mean value of approximately 1.47, indicating that α-thujone was typically present at roughly one and a half times the level of the β-isomer (Tables 2, S1, S2; Fig. 1). Thus, all fresh leaf samples represented a fenchone–thujone chemotype, dominated by fenchone, α-thujone, and β-thujone. The characteristic fenchone–thujone chemotype remained stable also in dried leaves (sample 13), and differences were confined to minor constituents. The fresh and dried fruit samples (14 and 15) differed clearly from fresh leaf samples (1–12), showing markedly lower levels of fenchone and both thujone isomers but substantially higher levels of sabinene (Table S1; Fig. 3). Secondary components such as sabinene, bornyl acetate, and hibaene remained subordinate and did not alter the overall chemotypic identity.

Correlation analysis revealed a moderate negative association between latitude (Tables 1 and S1) and fenchone content (Spearman $\rho = -0.664$, $p = 0.018$), suggesting a slight decrease in fenchone proportion towards northern sampling sites. However, no statistically significant correlations were observed between latitude and α- or β-thujone contents, nor between longitude and any of the major constituents ($p > 0.05$).

Table 2. Content of selected toxic compounds in the essential oils of fresh *Thuja occidentalis* leaves

Compound	Content in essential oils (%)		
	Minimum	Maximum	Mean
Fenchone	12.08	29.99	22.01
α-Thujone	10.75	22.74	17.65
β-Thujone	5.13	17.17	12.08
Sabinene	3.63	15.80	5.40
α-Thujene	0.0	0.51	0.11
4-Thujanol	0.17	0.38	0.25
3-Thujanol	0.03	0.22	0.09

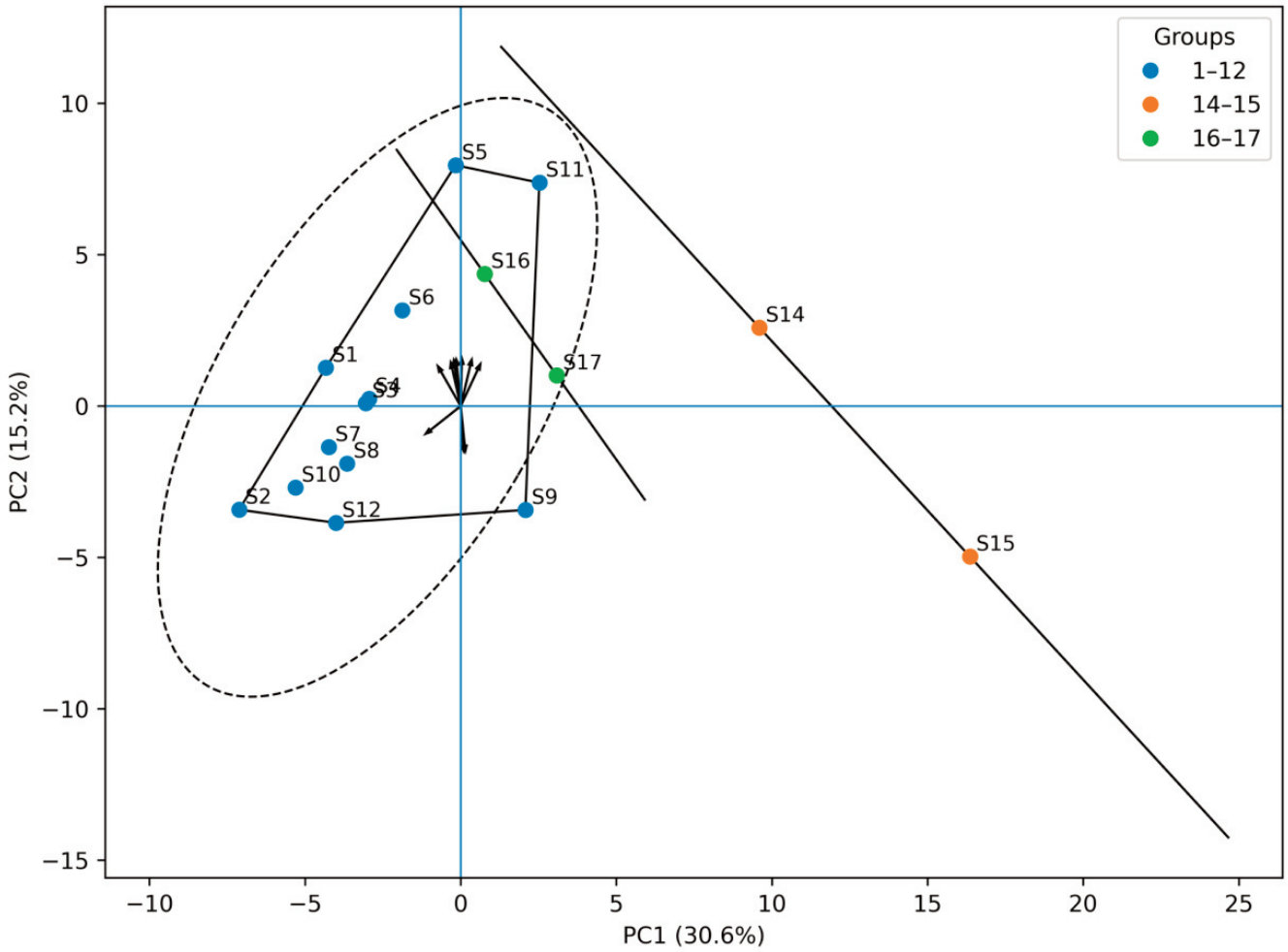

Fig. 3. Principal component analysis (PCA) biplot of *T. occidentalis* samples 1–17, based on the relative abundances of identified constituents.

These findings indicate relative chemotypic stability of thujone components across the studied geographic gradient.

4. Discussion

It is noteworthy that the EO yield varied only approximately twofold in fresh leaves (samples 1–12). Our previous studies have shown that for many plants collected from different growing sites, the difference between maximum and minimum EO yields is much larger: *Achillea millefolium* (27 times), *Coriandrum sativum* (17), *Levisticum officinale* (16), *Thymus serpyllum* and *Valeriana officinalis* (14), *Chamomilla recutita* (10), and *Pimpinella anisum* (5 times) [25]. Thus, the EO yield in *T. occidentalis* fresh leaves is surprisingly consistent, indicating its toxic potential regardless of the growing location.

Fenchone and either α- and β-thujone or only α-thujone have been mentioned as the principal compounds of *T. occidentalis* EOs in practically every previous study [15–18, 22,26–31]. In several studies, sabinene [15–17,27] and beyerene [15,18,32] were also reported as among the main compounds. This confirms our data that thujone isomers, together with fenchone, are the main components of *T. occidentalis* leaf EO, and chemotypes with other dominant compounds are not observed.

Although a higher number of volatile compounds (105) was identified in the present study compared to most previous reports, the overall composition and dominant constituents remain consistent with the established chemotypic profile of *T. occidentalis*. Numerous additional minor constituents were detected; however, due to their low abundance and limited relevance to the overall profile, they are not discussed individually.

In our study, the α-thujone content was as low as 11%–23%, whereas in other studies, the maximum content has been reported to range from 30%–65% [10,15,17,18, 27,29,30], even up to 85% [1,2]. A possible reason is that we identified a large number of *T. occidentalis* EO components in our work (105 compounds), but in most cases, only a few dozen have been identified. On the other hand, low concentrations of α-thujone, such as 1.2% [22], 17% [31], or 20% [15] in the EOs of *T. occidentalis*, indicate the presence of thujone-poor chemotypes. They were not found in our study.

Limitations of the study. Hydrodistillation was performed once for each sample, whereas GC–MS analyses were conducted in triplicate. Although replicate distillations could further refine yield variability estimates, the relatively low analytical variation (3%–4%) observed and the consistency of compositional profiles across samples suggest that the main chemotypic conclusions are robust. Future studies may

include replicated distillation experiments to further evaluate intra-sample yield variability.

5. Conclusion

The essential oils of *Thuja occidentalis* cultivated in Estonia are characterised by a stable fenchone–thujone chemotype. Despite sampling across geographically distinct populations, fresh leaf oils showed remarkable compositional uniformity, and no thujone-poor chemotypes were detected. Drying increased oil yield without altering the qualitative profile, while fruit oils differed quantitatively but retained detectable thujone levels.

The demonstrated chemical stability indicates a geographically independent and persistent toxic potential of *T. occidentalis* in Estonia. These findings are relevant for chemotaxonomic characterisation and safety assessment of *Thuja*-derived materials.

Data availability statement

The raw, unprocessed data obtained during phytochemical analyses are available from the corresponding author upon request.

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Supplementary material

Tables S1 and S2, available at https://doi.org/10.3176/proc.2026.4.S01, display the composition of essential oils from *Thuja occidentalis* from Estonia.

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Tujoonirikka *Thuja occidentalis* L. kemotüübi liigisisene stabiilsus Eestis viitab püsivale toksilisele potentsiaalile

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Uuriti Eestis kasvava hariliku elupuu *Thuja occidentalis* L. (*Cupressaceae*) eeterliku õli saagist ja keemilist koostist, et hinnata selle liigisisest varieeruvust, kemotüübilist stabiilsust ja tujoonisisaldusega seotud toksilisuse potentsiaali. Värske lehematerjal koguti geograafiliselt erinevatest populatsioonidest (n = 12) koos kuivatatud lehtede ja viljadega (n = 5) ning analüüsiti hüdrodestillatsiooni ja GC–MS-meetodi abil. Värskete lehtede eeterliku õli saagis oli 0,73–1,32% (keskmiselt 1,04%), samas kui kuivatatud lehtede saagis ulatus 2,98%ni. Kokku tuvastati 105 lenduvat koostisosa. Kõigis värskete lehtede proovides domineerisid eeterliku õli koostises järjepidevalt hapnikuga rikastatud monoterpeenid, eriti fenkoon (12,08–29,99%), α-tujoon (10,75–22,74%) ja β-tujoon (5,13–17,17%). Nende ühine osakaal oli 28–70% (keskmiselt 52%) kogu eeterliku õli koostisest. α-/β-tujooni suhe oli 0,84–2,50. Värsked ja kuivatatud taimeosad ei erinenud kemotüübilise profiili poolest kvalitatiivselt, kuna mõlemas domineerisid fenkoon ja tujoonid, kuigi kuivatamine suurendas eeterliku õli saagist. Viljade eeterlikud õlid erinesid kvantitatiivselt lehtede omadest: neis oli märksa väiksem fenkooni ja mõlema tujooni isomeeri osakaal ning märksa suurem monoterpeensete süsivesinike, eriti sabineeni sisaldus. Tujooni kogusisalduse osas ei täheldatud kasvukohast sõltuvaid olulisi geograafilisi suundumusi. Arvestades tujoonide hästi dokumenteeritud neurotoksilisust, näitab täheldatud keemiline ühtlus, et hariliku elupuu toksiline potentsiaal Eestis ei sõltu geograafilisest kasvukohast. Tujoonivaeste kemotüüpide puudumine viitab sellele, et Eestis kasvavaid hariliku elupuu populatsioone ei saa pidada ohutuks. Need leiud on olulised elupuust saadud materjalide riski ja ohutuse hindamiseks.