VOLATILE ORGANIC COMPOUNDS AND ESSENTIAL OILS: DISTRIBUTION IN CONIFERS AND THEIR APPLICATIONS IN PAIN MANAGEMENT

Ph.D. Thesis

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“But those who hope in the Lord

will renew their strength.

They will soar on wings like eagles;

they will run and not grow weary,

they will walk and not be faint.”

Isaiah 40, 31

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1 LIST OF ABBREVATIONS

CAR/PDMS/DVB	carboxene/polydimethylsiloxane/divinylbenzene
CTS	carpal tunnel syndrome
CVA	canonical variates analysis
DMARD	disease-modifying antirheumatic drug
EMA	European Medicines Agency
EO	essential oil
GC/MS	gas chromatography-mass spectrometry
LBP	low back pain
MD	mean difference
MSDs	musculoskeletal disorders
MSD	mass selective detector
NDI	neck disability index
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
PCA	principal component analysis
PICO	participants, interventions, comparisons, outcomes
PPT	pressure pain threshold
PRISMA	preferred reporting items for systematic reviews and meta-Analyses
RCT	randomized controlled trial
RRT	relative retention time
QoL	quality of life
sHS-SPME	static headspace solid-phase microextraction
TPS	terpene synthase
VAS	visual analogue scale
WHO	World Health Organization
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
VOCs	volatile organic compounds

2 STUDENT PROFILE

2.1 Vision and mission statement, specific goals

My vision is that the best pharmaceutical products are developed based on scientific evidence. Essential oils are often used for medical purposes without appropriate scientific knowledge. Therefore, my mission is to provide more evidence in plant science by analyzing the composition of essential oils and studying their effects on the musculoskeletal system and pain relief. My specific goal is to contribute to the development of effective and safe plant-based products for the treatment of musculoskeletal disorders.

2.2 Scientometrics

Number of all publications:	4
Cumulative IF:	18.4
Av IF/publication:	4.6
Ranking (SCImago):	D1:1, Q1:3
Number of publications related to the subject of the thesis:	2
Cumulative IF:	9.1
Av IF/publication:	4.55
Ranking (SCIMago):	D1:0, Q1:2
Number of citations on Google Scholar:	26
Number of citations on MTMT (independent):	16
H-index:	2

The detailed bibliography of the student can be found on page 52.

2.3 Future plans

In the future, I aim to translate the findings from my PhD research to market, developing marketable formulations that contain essential oils as active ingredients. Further research is needed to focus on how conifer essential oils affect musculoskeletal system, pain, and microcirculation. To this end, I intend to collaborate with experts from different fields to improve product safety and effectiveness.

3 SUMMARY OF THE THESIS

Volatile organic compounds (VOCs) and essential oils (EOs) are important natural products known for their various pharmaceutical applications. This PhD thesis is based on two comprehensive studies that investigate the therapeutic potential of these natural products in pain management of musculoskeletal disorders (MSDs) and analyze the chemical profiles of conifers, which are commonly used in commercially available pain-relieving topical products. In our first study, a systematic review and meta-analysis was conducted to evaluate the effectiveness of topical EOs. The aim was to provide evidence for the hypothesis that topical EO therapy is beneficial as an add-on treatment for MSDs. In the second study, we determined the chemical composition of conifer samples by gas chromatography and then performed a chemometric analysis of monoterpenes and sesquiterpenes.

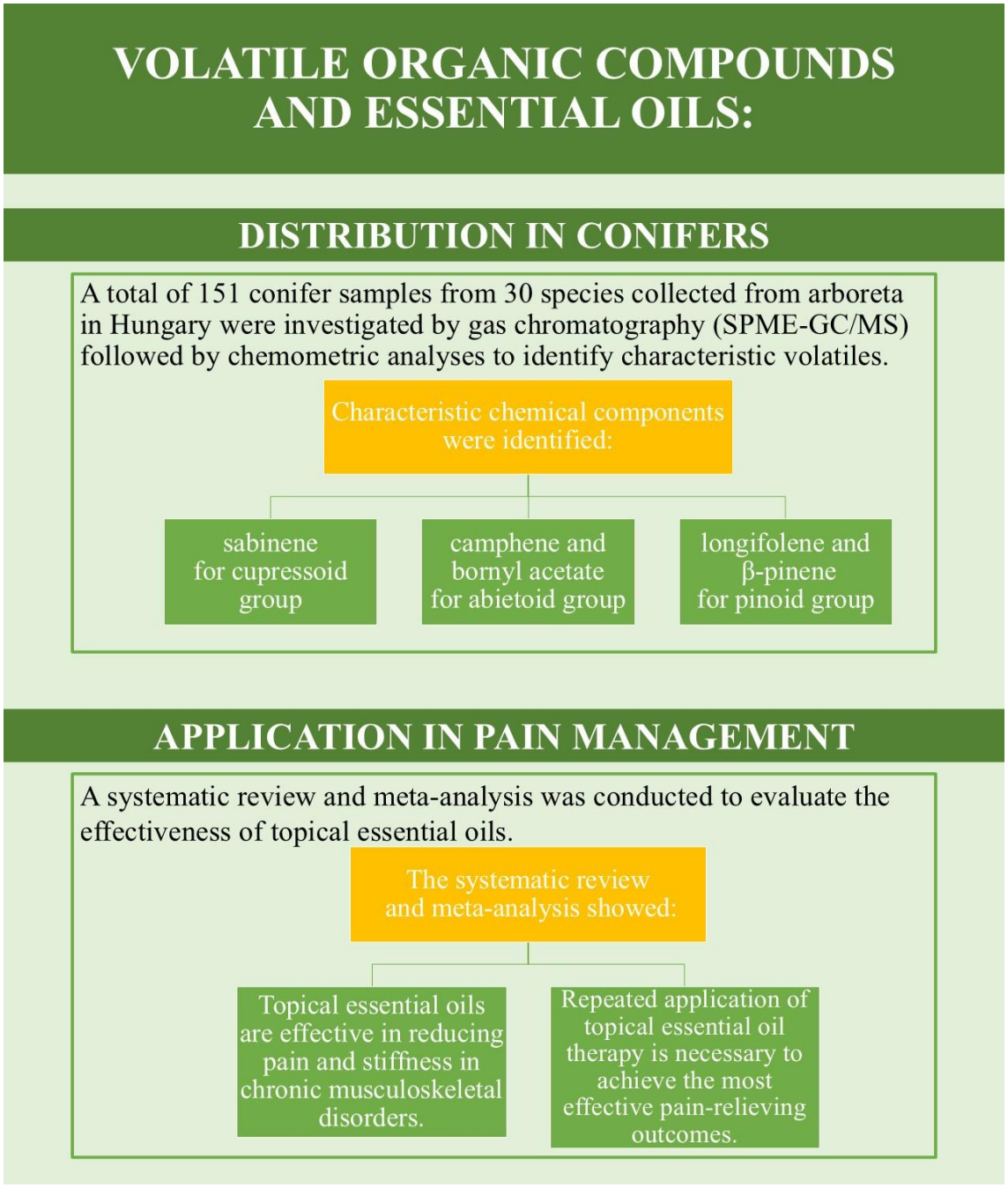
MSDs are a major public health issue, causing long-term pain and disabilities and reduced work capacity. Treatments aim to relieve pain and improve physical conditions and include various pharmacological options such as NSAIDs, paracetamol, opioids, and various other drugs (Loveless & Fry, 2016). Painkillers may have major side effects, particularly when used for extended periods of time, for chronic conditions, or in large doses (Bindu et al., 2020). Evidence-based phytotherapy could be advantageous in pain management as it may reduce the need for analgesics or prolong treatment effectiveness in managing pain (Morrone et al., 2017).

We performed a meta-analysis that included randomized controlled trials (RCTs). The findings show that EO therapy significantly reduces pain intensity, the primary outcome, compared to a placebo. The most substantial pain relief was recorded immediately after the intervention, with a mean difference of -0.87 (p=0.014). One week after the intervention, EO therapy still showed a slight analgesic advantage over the placebo (-0.58, p=0.077), and maintained efficacy at the four-week follow-up (-0.52, p=0.049). Additionally, EO therapy improved stiffness (secondary outcome) compared to the no intervention group (-0.77, p=0.061). This systematic review and meta-analysis indicates that topical essential oils are an effective add-on treatment for reducing pain and stiffness in various MSDs, offering a promising option for patient care.

VOCs and essential oils from conifers are widely used in the pharmaceutical industry, especially for pain management in topical formulations (Barizien et al., 2025; Dakowicz et al., 2022). We analyzed the VOCs of 30 conifer species from the Pinaceae and Cupressaceae families of samples collected from arboreta in Hungary. During the scientific work, chemical composition was determined by gas chromatography (SPME-GC/MS) followed by chemometric analyses to identify characteristic VOCs. The study revealed abundant compounds including α-pinene, bornyl acetate, limonene, β-pinene, among others. Specific volatiles were linked to different conifer groups: sabinene for the cupressoid group, longifolene and β-pinene for the pinoid group, and camphene and bornyl acetate for the abietoid group. By identifying the most abundant and characteristic volatile compounds in these species, this research supports the development of effective pain-relieving and anti-inflammatory products, expanding therapeutic options for MSD management.

Although the two studies differ in their methodology, both aimed to promote the evidence-based use of essential oils by providing data on their efficacy and chemical composition.

4 GRAPHICAL ABSTRACT



5 INTRODUCTION

5.1 Overview of the topic

5.1.1 What is the topic?

This PhD thesis evaluates the therapeutic potential of essential oils in the management of musculoskeletal disorders. The chemical composition of conifers was also investigated to understand their chemotaxonomic relationships, as their EOs are commonly used in pain-relieving topical products used for MSDs.

5.1.2 What is the problem to solve?

The limited scientific data on the effectiveness of essential oils for musculoskeletal diseases is a major issue. Although conifer essential oils are widely used in topical pain relief formulations, their chemical compositions and biological activities have not been thoroughly analyzed. The therapeutic efficacy of essential oils is closely linked to their chemical constituents. Comprehensive chemometric comparisons of essential oils from different conifer species are lacking. This gap in knowledge limits the ability to optimize and standardize conifer essential oil-containing treatments for MSDs.

5.1.3 What is the importance of the topic?

MSDs place a significant burden on healthcare systems and negatively impact quality of life. This research provides more evidence on the use of EOs in managing MSDs, a condition affecting millions of people worldwide. Additionally, our results help to identify conifers species with potential pharmaceutical uses in the treatment of MSDs.

5.1.4 What would be the impact of our research results?

This research highlights the potential benefits of essential oils. First, establishing evidence-based data on the effectiveness of essential oils as a complementary treatment for MSDs may lead to improved patient outcomes, reduced dependency on synthetic drugs, and moderate side effect profile. Additionally, by investigating the chemical profiles of conifers through chemometric analysis, the study provides additional information for the selection of the most promising bioactive conifer species for pain-relieving formulations.

6 OBJECTIVES

6.1 Study I. – Efficacy of topical essential oils in musculoskeletal disorders: systematic review and meta-analysis of randomized controlled trials

The purpose of this study was to conduct a systematic review and meta-analysis to evaluate the efficacy of topical EOs as an add-on treatment in MSDs based on the randomized controlled trials reported in the literature.

6.2 Study II. – Chemometric analysis of monoterpenes and sesquiterpenes of conifers

The aim of this study was to investigate the monoterpene and sesquiterpene profiles of conifer species collected from arboreta in Hungary by gas chromatography method and to explore their chemotaxonomic relationships by chemometric analysis.

7 METHODS AND MATERIALS

7.1 Study I. - Efficacy of topical essential oils in musculoskeletal disorders: systematic review and meta-analysis of randomized controlled trials

We conducted a systematic review and meta-analysis of randomized clinical trials from the literature to investigate the efficacy of topical EOs as an add-on treatment in MSDs. We followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guideline (Page et al., 2021) and the recommendations of Cochrane Handbook (*Cochrane Handbook for Systematic Reviews of Interventions*). The prestudy protocol was registered on PROSPERO (registration number CRD42021282201) (*National Institute for Health Research. International Prospective register of systematic reviews.*).

7.1.1 Literature search and eligibility criteria

The systematic search was performed in five databases on 17th November 2021: Web of Science, EMBASE, PubMed, Central Cochrane Library and Scopus. The following search key was applied: (essential oil OR aromatherapy) AND (musculoskeletal disease OR muscle OR bone OR joint) AND (topical OR cutaneous OR external OR dermal OR massage). Only randomized controlled trials (RCTs) that met the established PICO were considered. The following PICO framework was applied: Participants: adults with MSDs; Intervention: EOs applied by massage or EOs applied without massage; Comparisons: placebo product (with or without massage), or no intervention; Outcomes: pain intensity (primary outcome), Quality of Life (QoL) and functional state (secondary outcomes). The following articles were excluded: animal studies, EOs administered by inhalation, no available full texts, patients suffering from acute pain (trauma, injuries), pain associated with diabetes or dysmenorrhea, or the use of inappropriate placebo.

7.1.2 Study selection and data extraction

After duplicates were removed by using EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA), the selection process was carried out by two authors. The articles were selected based on title, abstract and full text, according to the predetermined inclusion and exclusion criteria. Inter-rater reliability was assessed by the calculation of Cohen's kappa, which indicates a strong degree of consensus. Any disagreements were resolved by a third author.

Data extraction was performed by the author and an additional researcher, with any disagreements resolved by a third reviewer. Extracted data were obtained directly from published values, except in one case (Shirazi et al., 2017), where a web plot digitizer was used. Although the studies reported outcomes at various time points, only clinically relevant time points were considered. These data were extracted as temporal thresholds: week zero (i.e., immediately after intervention), one-, and four weeks after the intervention. Pain intensity was measured using two scales: Visual Analogue Scale (VAS), ranging from 0 to 10, and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), with a 20-point range. Stiffness was similarly assessed by VAS (0-10) and the WOMAC questionnaire (0-8). To ensure comparability, results from both scales were standardized for statistical analysis.

7.1.3 Quality assessment

The available data in the literature were insufficient to conduct a quantitative evaluation of quality of life (QoL) as a secondary outcome. Based on the inclusion criteria, only studies involving adult patients were eligible for the meta-analysis. However, in the trial by Kong et al. (2012), the age criterion for inclusion ranged from 15 to 35 years. For this study, participants under 18 were classified as young adults. Disagreements were resolved by a third researcher. Risk of bias was independently assessed by two researchers using the Cochrane risk-of-bias tool for RCTs (RoB 2) (Sterne et al., 2019), with any disagreements resolved by a third reviewer. GRADE (Grading of Recommendations, Assessment, Development and Evaluations) approach was used to evaluate the levels of evidence of the included trials (Balshem et al., 2011).

7.1.4 Data synthesis and analysis

Mean before/after change difference in pain intensity measured on VAS as the primary outcome was pooled using multilevel mixed effect models (Konstantopoulos, 2011; Trikalinos & Olkin, 2012). A multilevel approach was necessary because some studies reported VAS scores across multiple time periods. Pooling mean change differences requires the knowledge of the standard deviation (Hovelstad et al., 2006) of within-group difference between time points or the correlation of within-group changes. However, most studies did not report these values. In such cases, we used the sum of the reported before and after treatment group SDs as a conservative (Ayache et al., 2020) estimate of

variability. This approach allows us to conclude that if a result is significant with the sum of group SDs, it would certainly be significant, had we used the true SDs of within group changes. To calculate the I^2 statistic we followed Jackson's methodology (Jackson et al., 2012). Results are presented in rainforest plots (Zhang et al., 2017), where uncertainty is visualized by the height of the raindrops for each individual estimate while the width of the raindrop corresponds to the estimated CI. All analyses were performed in R version 4.1 (Team, 2021) using the following packages: *tidyverse* (Wickham et al., 2019), *meta* (Balduzzi et al., 2019), *dmetar* (Harrer et al., 2021) *metafor* (Viechtbauer, 2010) and *metaviz* (Kossmeier et al., 2020).

7.2 Study II. – Chemometric Analysis of Monoterpenes and Sesquiterpenes of Conifers

7.2.1 Plant material

Conifer samples were collected in the Jeli Arboretum in 2020 and 2021 and in the Folly Arboretum in 2021, Hungary. Needles (needles and branch tips), resin, cones, and bark were collected. The species are listed in Table 1.

Table 1. The collected Pinaceae and Cupressaceae species and the place and date of the collection (Bakó et al., 2024)

Jeli Arboretum, (9841 Kám, Hungary) October 2020	**Folly Arboretum (8257 Badacsonyörs, Hungary) May 2021**	**Jeli Arboretum (9841 Kám, Hungary) June 2021**
Abies concolor (Gordon & Glend.) Lindl. ex Hildebr.	*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.	*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.
Abies firma Siebold & Zucc.	*Abies firma* Siebold & Zucc.	*Abies firma* Siebold & Zucc.
Abies grandis (Douglas ex D.Don) Lindl.	*Calocedrus decurrens* (Torr.) Florin	*Abies grandis* (Douglas ex D.Don) Lindl.
Abies holophylla Maxim.	*Cedrus atlantica* (Endl.) G.Manetti ex Carrière	*Calocedrus decurrens* (Torr.) Florin

Cedrus atlantica (Endl.) G.Manetti ex Carrière	*Cupressus macnabiana* A.Murray bis	*Cedrus atlantica* (Endl.) G.Manetti ex Carrière
Chamaecyparis pisifera (Siebold & Zucc.) Endl.	*Juniperus chinensis* L.	*Cryptomeria japonica* (Thunb. ex L.f.) D.Don
Calocedrus decurrens (Torr.) Florin	*Juniperus drupacea* Labill.	*Juniperus chinensis* L.
Cryptomeria japonica (Thunb. ex L.f.) D.Don	*Juniperus rigida* Siebold & Zucc.	*Juniperus communis* L.
Picea omorika (Pančić) Purk.	*Pinus coulteri* D.Don	*Juniperus sabina* L.
Picea sitchensis (Bong.) Carrière	*Pinus heldreichii* Christ	*Juniperus virginiana* L.
Pinus aristata Engelm.	*Pinus nigra* J.F.Arnold	*Picea sitchensis* (Bong.) Carrière
Pinus cembra L.	*Pinus pinaster* Aiton	*Pinus aristata* Engelm.
Pinus heldreichii Christ	*Pinus strobus* L.	*Pinus cembra* L.
Pinus peuce Griseb.	*Pseudotsuga menziesii* (Mirb.) Franco	*Pinus heldreichii* Christ
Pinus strobus L.	*Sequoia sempervirens* (D.Don) Endl.	*Pinus peuce* Griseb.
Pseudotsuga menziesii (Mirb.) Franco	*Tsuga canadensis* Carrière	*Pinus strobus* L.
Sequoia sempervirens (D.Don) Endl.	*Tsuga heterophylla* Sarg.	*Pseudotsuga menziesii* (Mirb.) Franco
Thuja koraiensis Nakai		*Sequoia sempervirens* (D.Don) Endl.
Tsuga heterophylla Sarg.		*Thuja koraiensis* Nakai
Tsuga canadensis Carrière		*Tsuga canadensis* Carrière
		Tsuga heterophylla Sarg.

18 species were collected from the Pinaceae family: *Abies concolor, Abies firma, Abies grandis, Abies holophylla, Cedrus atlantica, Picea omorika, Picea sitchensis, Pinus aristata, Pinus cembra, Pinus coulteri, Pinus heldreichii, Pinus nigra, Pinus peuce, Pinus pinaster, Pinus strobus, Pseudotsuga menziesii, Tsuga canadensis,* and *Tsuga*

heterophylla. 12 species were collected from the Cupressaceae family: *Calocedrus decurrens, Chamaecyparis pisifera, Cryptomeria japonica, Cupressus macnabiana, Juniperus chinensis, Juniperus communis, Juniperus drupacea, Juniperus rigida, Juniperus sabina, Juniperus virginiana, Sequoia sempervirens,* and *Thuja koraiensis*.

7.2.2 SPME-GC/MS Measurement and Evaluation

The chemical composition of the samples was measured using static headspace solid-phase microextraction (sHS-SPME) combined with gas chromatography-mass spectrometry (GC/MS). The plant material was chopped and placed into individual 20-mL headspace vials, sealed with silicon/PTFE septum, and loaded into the GC/MS autosampler tray.

SPME was performed using an automatic CTC Combi PAL multipurpose sampler (CTC Analytics AG, Zwingen, Switzerland). After a 5-minute incubation period at 100°C, a 65 μM carboxene/polydimethylsiloxane/divinylbenzene fiber (CAR/PDMS/DVB, StableFlex, Supelco, Bellefonte, PA, USA) was immersed into the headspace vial by the autosampler. The volatile components on the surface of the fiber were absorbed; the extraction was performed at 100°C for 20 minutes. Then, the fiber was transferred to the injector port of the GC/MS and desorbed (250°C, 1 minute). The injections were made in splitless mode. Then, the fiber was cleaned and conditioned in pure nitrogen (in Fiber Bakeout Station, 250°C, 15 min). Analyses were performed using an Agilent 6890N/5973N GC/MSD (Santa Clara, CA, USA) system with a 30 m × 250 μm × 0.25 μm SLB-5MS capillary column (Supelco, Sigma-Aldrich, Philadelphia, PA, USA). The GC oven temperature was set to increase from 60 °C (3 minutes isothermal) to 250 °C at a rate of 8 °C/min (1 minute isothermal). High-purity helium (6.0) (Messer) was used as the carrier gas at a 1.0 mL/min (37 cm/s) flow rate in constant flow mode. The mass selective detector (MSD) was equipped with a quadrupole mass analyzer and operated in electron ionization mode (41–500 atomic mass units (amu) at 3.2 scan/s, at 70 eV, full scan mode). (Bakó et al., 2024)

The GC/MS measurement data were evaluated using MSD ChemStation D.02.00.275 software (Agilent, Santa Clara, CA, USA). Volatile components was identified by comparing the calculated Kovats indexes to those available in the literature (Adams, 2007/2007), and the NIST (National Institute of Standards and Technology) Chemistry

WebBook (Linstrom) was also considered for identification of spectra. Percentage calculation was performed by area normalization. This article presents the findings and measurement results for monoterpenes, sesquiterpenes, and their derivatives. It is important to note that our GC/MS method is not suitable for the accurate identification of diterpenes.

7.2.3 Statistical Analysis and Chemometric Analyses

The statistical analysis aimed to explore the correlation between the chemical profiles of conifers and various attributes (such as species and plant organs). Calculations were performed by the SYN-TAX 2000 package (Podani, 2001).

Principal Component Analysis (PCA) is a dimensionality reduction technique that effectively visualizes data structure across fewer dimensions while preserving the original information. This method generates new artificial variables, known as components, and displays object positions along with the correlations between variables and components in a coordinate system called a biplot. This approach enhances the visualization of the results (Podani, 1997).

Canonical Variate Analysis (CVA) is another multivariate technique that assesses the separation of observations into two or more groups and evaluates the contribution of each variable to this grouping (Podani, 2000). We investigated whether the three taxonomic groups - pinoid, abietoid, cupressoid - or plant organs, including needles, cones, resin, and bark, were separated. Additionally, we aimed to identify which volatile components could best explain this separation. Based on the PCA results, we selected the VOCs that exhibited the highest correlations with the first and second principal components for our analyses. (Bakó et al., 2024)

8 RESULTS

8.1 Study I: Efficacy of topical essential oils in musculoskeletal disorders: systematic review and meta-analysis of randomized controlled trials

With the initial searching, 752 articles were identified. After duplication removal, the title and abstract selection phase, and full text screening, altogether 12 studies were included in the systematic review. More details on the search and selection process are presented in the PRISMA flowchart in Figure 1.

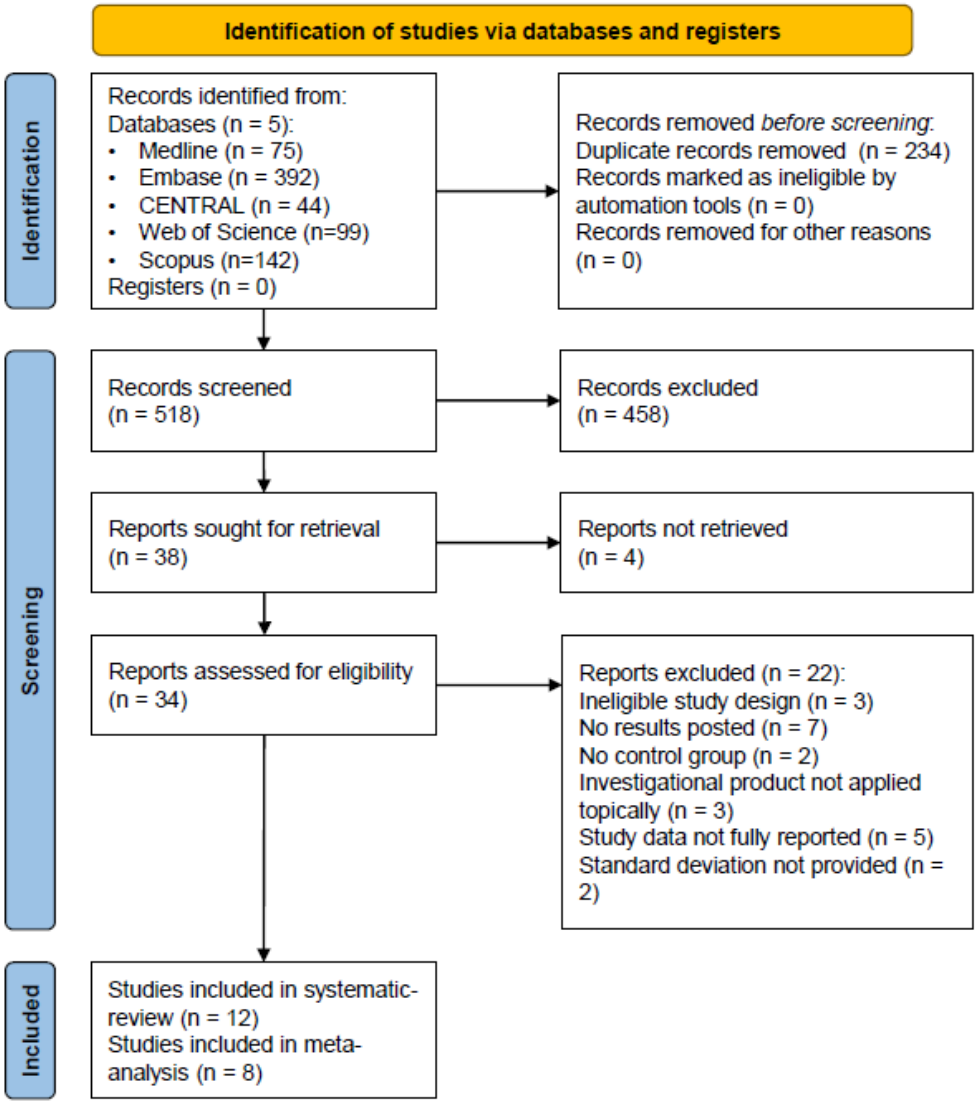


Figure 1. *PRISMA flowchart representing the study selection process (Bako et al., 2023)*

8.1.1 Basic characteristics of included studies

Baseline characteristics of the RCTs included in this study (Bahr et al., 2018; Eftekharsadat et al., 2018; El Sayed et al., 2020; Gok Metin & Ozdemir, 2016; Kong et al., 2012; Nasiri et al., 2016; Ou et al., 2014; Pehlivan & Karadakovan, 2019; Shirazi et al., 2017; Yip & Tam, 2008; Yip & Tse, 2004; Yip & Tse, 2006) are presented in Table 2. The trials were conducted between 2004 and 2020. A total of 817 patients with musculoskeletal disorders (MSD) were included across all studies. Three trials were conducted in Iran, four in China, two in Turkey, and one in Taiwan, USA, and Egypt, respectively.

Table 2. Basic characteristics of the included studies (Bako et al., 2023)

Study	Patients	Study design	Country	Number of patients	Applied essential oils	Intervention	Placebo	No intervention	Outcomes
(Nasiri et al., 2016)	patients with knee OA	RCT	Iran	90	3% lavender oil	aromatherapy massage with lavender EO	placebo massage with sweet almond oil	no massage	pain intensity (qualitative and quantitative analysis)
(Kong et al., 2012)	athletes with nonspecific low back pain	RCT	China	110	herbal ointment containing 20% of EOs (extracted from Dang Gui, Chuan Xiong, Xi Xin, and Rou Gui)	Chinese massage combined with herbal ointment	massage therapy with placebo ointment	n/a	pain intensity (qualitative and quantitative analysis)
(Eftekharsadat et al., 2018)	patients with mild to moderate CTS	RCT	Iran	48	1.5% lavender EO	night wrist orthotic and topical lavender oil ointment	night wrist orthotic and a placebo ointment	n/a	pain intensity (qualitative and quantitative analysis)
(Pehlivan & Karadakovan, 2019)	elderly individuals with knee osteoarthritis	RCT	Turkey	90	two EOs (2.5% ginger and 2.5% rosemary) were added to the black seed oil	aromatherapy massage	massage group (sunflower oil)	control group (no aromatherapy or massage)	pain intensity, stiffness (qualitative and quantitative analysis)
(Shirazi et al., 2017)	women with pregnancy-related low back pain	RCT	Iran	120	rose oil (in the carrier of almond oil)	EO applied topically	almond oil	no intervention (no EO, no massage)	pain intensity (qualitative and quantitative analysis)
(Yip & Tam, 2008)	moderate-to-severe knee pain among the elderly	RCT	China	59	1% ginger and 0.5% orange EO	massage with ginger and orange oil	massage intervention with olive oil only	no massage	pain intensity, stiffness (qualitative and quantitative analysis)

(Ou et al., 2014)	patients with neck pain	RCT	Taiwan	60	3 % cream containing marjoram, black pepper, lavender and peppermint EOs	the cream was applied on the neck and upper trapezius muscles	placebo ointment	n/a	pain intensity (qualitative and quantitative analysis)
(Yip & Tse, 2006)	sub-acute, non-specific neck pain	RCT	China	32	3% lavender oil with olive seed oil	manual acupressure massage with natural aromatic lavender oil	n/a	conventional treatment	stiffness (qualitative and quantitative analysis)
(Yip & Tse, 2004)	non-specific low back pain	RCT	China	61	3% lavender oil with grape seed oil	acupressure massage with natural aromatic lavender oil	n/a	conventional treatment	pain intensity (qualitative analysis)
(Bahr et al., 2018)	hand arthritis	RCT	USA	36	mixture of EOs (main components: 16% methyl salicylate, 6% menthol, 27% beta-caryophyllene)	hand massage	coconut oil	n/a	pain intensity (qualitative analysis)
(El Sayed et al., 2020)	knee osteoarthritis	RCT	Egypt	60	3% lavender EO	aromatherapy massage	n/a	conventional treatment	pain intensity (qualitative analysis)
(Gok Metin & Ozdemir, 2016)	rheumatoid arthritis	RCT	Turkey	51	mixture of EOs (5%) in coconut oil (lavender, juniper, Cananga odorata and rosemary)	aromatherapy massage	reflexology	conventional treatment	pain intensity (qualitative analysis)

EO: essential oil; RCT: randomized controlled trial; n/a: not applicable

8.1.2 Qualitative synthesis of results

In addition to the conventional therapy of MSDs, the EOs were applied topically as an add-on treatment in the EO therapy group. A placebo product (a vegetable carrier oil or an ointment without any EOs) was used as a complementary treatment in the "Placebo group". Patients in the "No intervention" group only received conventional therapy (no EO therapy nor other intervention). In most of the trials, the EO-containing products and placebo products were applied by massage. In the trials, the duration of the interventions varied, although they were typically conducted for three or four weeks.

Various EOs were used in the trials. Seven studies were conducted using lavender essential oil, with doses ranging from 1.5% to 3%. In one instance (Kong et al., 2012), an ointment contained 20% EO, while the applied concentrations for other EOs ranged from 0.5% to 2.5%.

According to the primary outcomes of all the trials that were examined, EO therapy may be a useful treatment for the severity of pain. Knee osteoarthritis (OA), hand OA, rheumatoid arthritis, low back pain, carpal tunnel syndrome (CTS) and neck pain were investigated in the trials. Only stiffness was included in the quantitative analysis because other secondary outcomes and measurements relating to the functional state were highly heterogeneous. Two publications measured quality of life. Yip and Tam (2008) investigated how ginger and orange EOs affected QoL. The findings showed that EO therapy did not enhance quality of life (Yip & Tam, 2008). Aromatherapy massage enhances quality of life according to Pehlivan and Karadakovan (Pehlivan & Karadakovan, 2019).

8.1.3 Quantitative synthesis of results

Primary outcome

Seven articles (Eftekharsadat et al., 2018; Kong et al., 2012; Nasiri et al., 2016; Ou et al., 2014; Pehlivan & Karadakovan, 2019; Shirazi et al., 2017; Yip & Tam, 2008) with 577 patients participating in the trials were taken into consideration for the study of pain intensity. Only the results of EO therapy groups and the Placebo groups were taken into account in the quantitative analyses of pain intensity to avoid unnecessarily introduced bias.

Figure 2 shows the calculated mean differences (MDs), within-group I^2 statistics, and CIs.

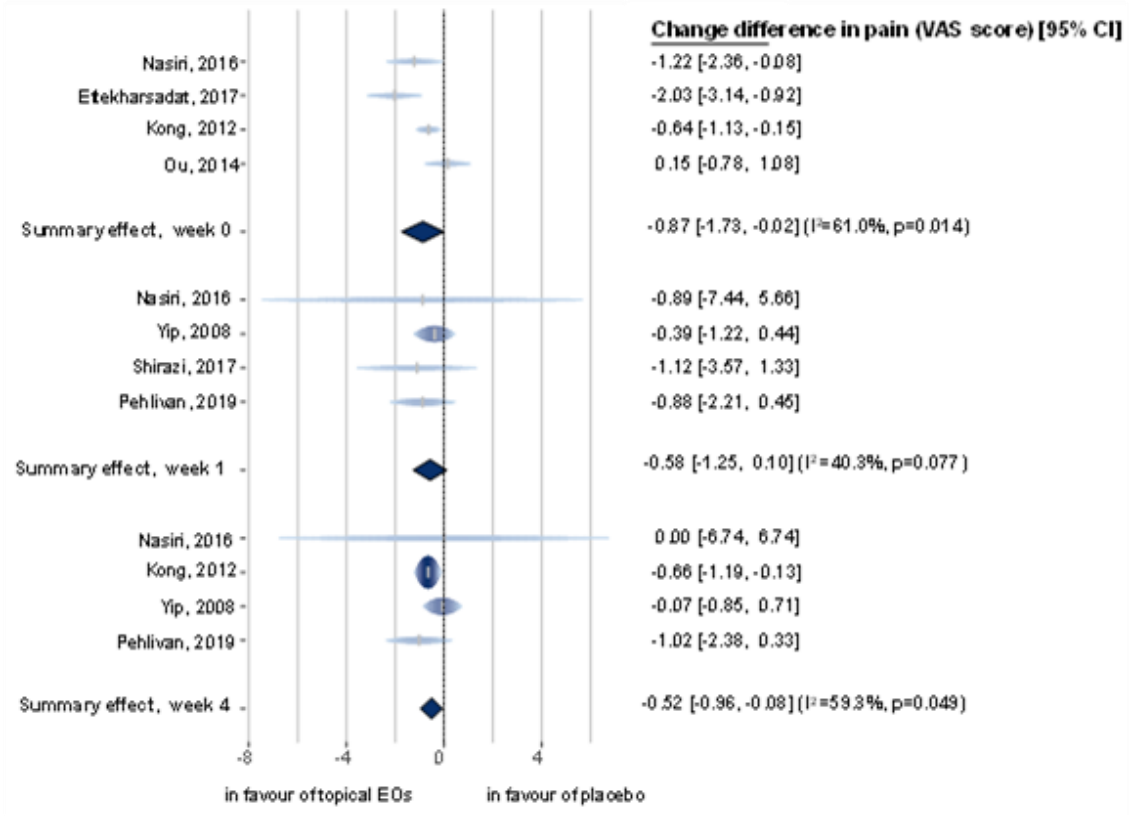


Figure 2. *Rainforest plot of the mean difference of the changes of pain intensity. Mean difference is presented between the EO therapy group and the Placebo group at different time points. The height and color intensity of individual studies correspond to the relative importance of the study in the model. The width of the raindrop-like structure corresponds with their respective confidence intervals. VAS: visual analogue scale; EO: essential oil; I^2: level of heterogeneity; p: probability of obtaining the observed effect. (Bako et al., 2023)*

Subgroups were created according to the measurement time points of the trials (i.e. immediately after the intervention or one week or four weeks after the intervention). The overall test of moderators was significant (QM = 9.98, df =3, *p*-value = 0.0465) indicating that the time-points had an overall effect on the outcomes. The test of residual heterogeneity of the overall model was not significant (QE = 12.24, df =9, *p* =0.2). Model results indicate that the application of EOs was beneficial at all time-points compared to placebo treatments with significant results on week zero (i.e., immediately after the application) and week four.

a) **Pain intensity measured immediately after the intervention (subgroup analysis):** Four trials (Eftekharsadat et al., 2018; Kong et al., 2012; Nasiri et al., 2016; Ou et al., 2014) were included in the analysis. MD of the change between the two groups indicates that topical EOs decreased the VAS scores significantly better than the placebo group (MD of pain intensity = -0.87 [95% CI, -1.73 to -0.02; I^2=61%; *p*=0.014]). The difference is statistically significant between the EO group and the Placebo group.

b) **Pain intensity measured one week after the intervention (subgroup analysis):** The results of four trials (Nasiri et al., 2016; Pehlivan & Karadakovan, 2019; Shirazi et al., 2017; Yip & Tam, 2008) were included for the one-week after the intervention subgroup. Our results indicate a non-significant slight effect of EOs one week after the intervention (MD of pain intensity = -0.58 [95% CI, -1.25 to 0.10; I^2=40.3%; *p*=0.077]).

c) **Pain intensity measured four weeks after the intervention (subgroup analysis):** This analysis was performed on four trials (Kong et al., 2012; Nasiri et al., 2016; Pehlivan & Karadakovan, 2019; Yip & Tam, 2008). Baseline data and data measured four weeks after the intervention were used to calculate MD between the two groups. The difference is statistically significant between the two groups (MD of pain intensity = -0.52 [95% CI, -0.96 to -0.08; I^2=59.3%; *p*=0.049]). (Bako et al., 2023)

Secondary outcomes (stiffness)

Three articles with 124 patients participating in the trials were taken into consideration for the stiffness analysis (Pehlivan & Karadakovan, 2019; Yip & Tam, 2008; Yip & Tse, 2004). In the rainforest plot (Figure 3) changes in stiffness are shown one week after the intervention.

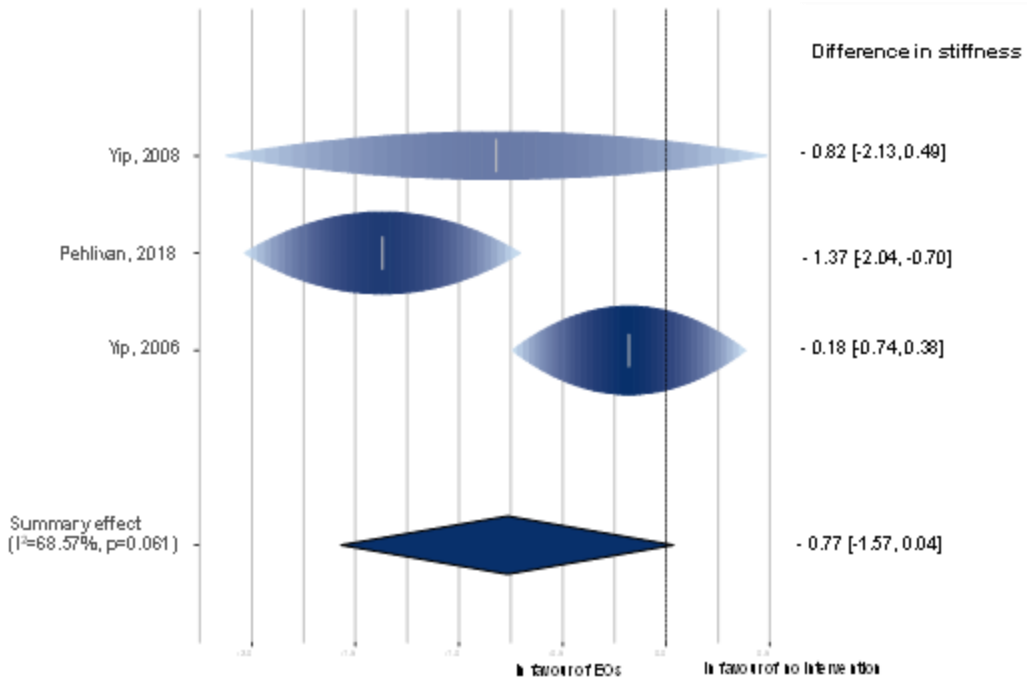


Figure 3. *Rainforest plot of the mean difference of stiffness. Mean differences are presented between the EO therapy group and the No intervention group. The height and color intensity of individual studies correspond to the relative importance of the study in the model. The width of the raindrop-like structure corresponds with their respective confidence intervals. I^2: level of heterogeneity; p: probability of obtaining the observed effect. (Bako et al., 2023)*

When compared to no intervention, the result (MD = –0.77 [95% CI, -1.57 to 0.04; I^2=72%; CI: 6%-96%; τ2=0.3312; *p*=0.061]) shows a minor improvement in the functional state of the MSD. The result is nearly significant.

8.1.4 Risk of bias assessment and GRADE assessment

Risk of bias assessment was performed, and all studies were evaluated to have “high risk of bias” or “some concerns”. A short summary of the performed assessment is presented in Figure 4 (intention-to-treat) and in Figure 5 (per protocol).

GRADE assessment was performed, and the overall certainty of evidence is very low in the case of both outcomes, the reason for this may be the lack of blinding and heterogeneity.

Figure 4. *Risk of bias graphs that illustrate the proportions of studies (intention to treat).*

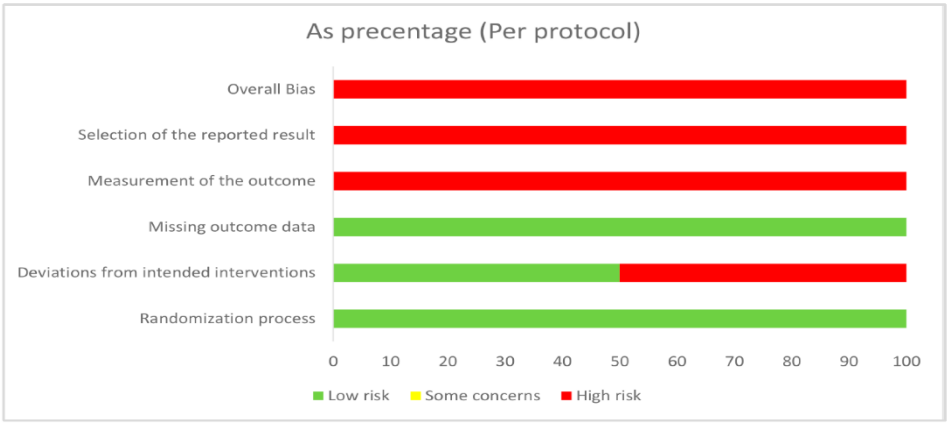


Figure 5. *Risk of bias graphs that illustrate the proportions of studies (per protocol).*

8.2 Study II. –Chemometric Analysis of Monoterpenes and Sesquiterpenes of Conifers

8.2.1 GC/MS Measurement Results

SPME-GC/MS method was used to examine 151 conifer samples from 30 species that were collected from arboreta in Hungary. We found the highest concentrations of the following volatiles, taking into account the data from all 151 samples: α-pinene, bornyl acetate, limonene, β-pinene, β-caryophyllene, β-myrcene, δ-3-carene, β-phellandrene, longifolene, and germacrene D. Terpenes are abundant compounds in conifers, as the SPME-GC/MS results clearly show. The resin of *Cupressus macnabiana* contained the highest concentration of α-pinene, which was the most prevalent component of all the VOCs, at almost 61%.

Pinaceae family

The most prevalent components (mean value) were: α-pinene (15%), β-pinene (7%), bornyl acetate (7%), limonene (5%), β-caryophyllene (4%), β-phellandrene (4%), δ-3-carene (3%), longifolene (3%), β-myrcene (3%), and camphene (2%), according to our GC/MS analysis of 103 samples from the Pinaceae family.

In each genus, α-pinene was abundant (8.4%-20.7%). The genera *Abies* (9.5%), *Pinus* (7.8%) and *Pseudotsuga* (9.7%) contain high concentration of β-pinene. β-Phellandrene concentration was high in *Picea* (9.0%) and in *Tsuga* (5.8%), and β-myrcene content was considerable in *Cedrus* (7.3%) and in *Picea* (9.5%) genera. Bornyl acetate levels were above 10% in the *Abies*, *Tsuga*, and *Pseudotsuga* genera (11.2%, 15.5%, and 11.3%, respectively). β-Caryophyllene was detected in large amounts in *Cedrus* (13.2%) and *Pinus* (6.7%), and limonene was measured to be around 7% in *Picea*, *Pinus*, and *Pseudotsuga* genera (7.0%, 7.3%, and 6.8%, respectively). (Bakó et al., 2024) For the respective bar plot, see Figure 6.

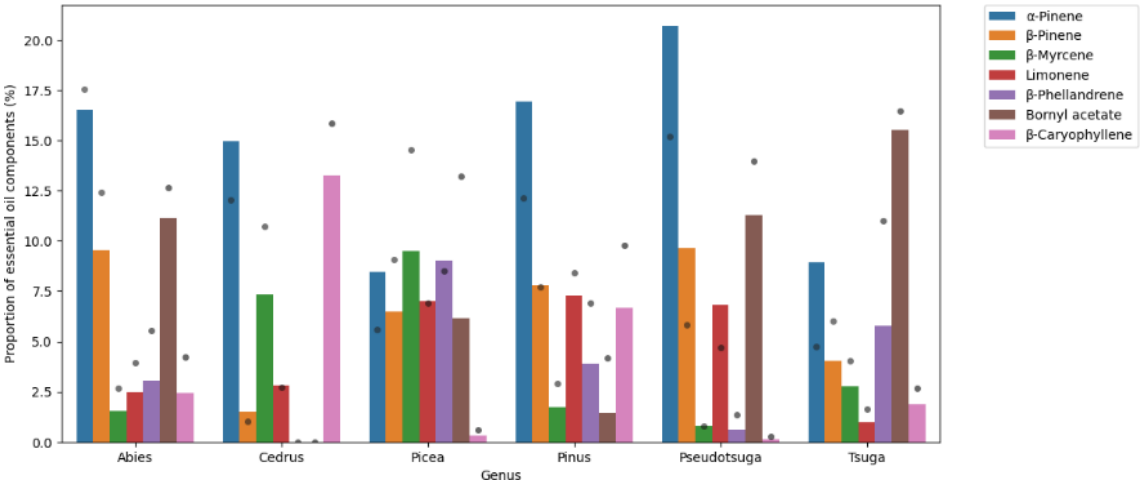


Figure 6. *Bar plot showing the main volatile organic compounds of Pinaceae family. Results are mean area% measured by GC/MS. Grey dots represent standard deviation. (Bakó et al., 2024)*

47 needle samples from the species of Pinaceae family were collected and measured. Figure 7 shows the results on a heat map alongside the phylogeny of the species to better illustrate the differences.

Figure 7. *Heat map showing GC/MS results of all needle samples from the Pinaceae family together with the phylogeny of the species. Results are mean area% measured by GC/MS. (Bakó et al., 2024)*

α-Pinene was detected in all samples, and its amount was considerable in all species (4%-35%). *P. nigra* (34.8%), *P. cembra* (24.9%), *P. peuce* (24.8%), *P. coulteri* (21.9%), and *C. atlantica* (20.8%) contained the highest percentage of α-pinene. Camphene was abundant in the species of *Picea omorika* (12%) and *Pseudotsuga menziesii* (15%). β-Pinene was also present in all species (1%-31%), with the highest percentage in *Abies*

grandis and *Abies concolor* (30.8% and 24.7%, respectively). All species contained β-myrcene. *C. atlantica* and *P. sitchensis* contained the highest percentage (18% and 17%, respectively); however, the β-myrcene content remained below 8% in other species (0.5%-8%). Not all species contained δ-3-carene; only four species contained more than 10% of this VOC (*Abies holophylla* 19%, *P. aristata* 42%, *P. peuce* 15%, and *P. strobus* 11%). β-phellandrene was the major VOC of *P. sitchensis*, *P. cembra*, and *Tsuga heterophylla* (16%, 16%, and 17%, respectively). γ-Terpinene, terpinene-4-ol, terpinolene, and α-terpineol could not be detected in substantial amounts. Bornyl acetate was abundant in species of *Abies* (13%-16%), in *P. omorika* (33%), and in species of *Tsuga* and *Pseudotsuga* (15%-36%). Other species contained relatively low amounts of this VOC (0-3%). Almost all species contained β-caryophyllene; *C. atlantica* and *P. pinaster* had the highest levels of this VOC (30% and 34%, respectively). Germacrene D was measured to be above 10% in *P. nigra*, *P. heldreichii*, *P. cembra*, and *P. coulteri* (21%, 30%, 14%, and 13%, respectively), while other species contained lower amounts (0-9%). α-Muurolene, γ-cadinene, and δ-cadinene were not detected in high amounts (usually below 5%) but were present in most species. (Bakó et al., 2024)

Cupressaceae family

Based on the results of 48 samples, the most abundant components in the Cupressaceae family were (mean value): α-pinene (17%), limonene (7%), sabinene (5%), β-myrcene (4%), δ-3-carene (3%), bornyl acetate (3%), germacrene D (2%), terpinolene (2%), β-eudesmol (2%), and terpinene-4-ol (2%).

Cupressus and *Calocedrus* genera contain high amounts of α-pinene (41.2% and 26.1%, respectively) and the other genera contain this VOC in large amounts as well (8.3%-12.2%). Sabinene was measured at around 10% in *Cryptomeria* and *Juniperus* genera (9.5% and 7.9%, respectively). δ-3-carene is abundant in *Calocedrus* (9.6%) and in *Chamaecyparis* (10.8%) genera. The amount of α-cadinol was high in the *Cupressus* genus (11.6%), and bornyl acetate was above 10% in the *Chamaecyparis* genus only (11.7%). (Bakó et al., 2024). For the bar plot, see Figure 8.

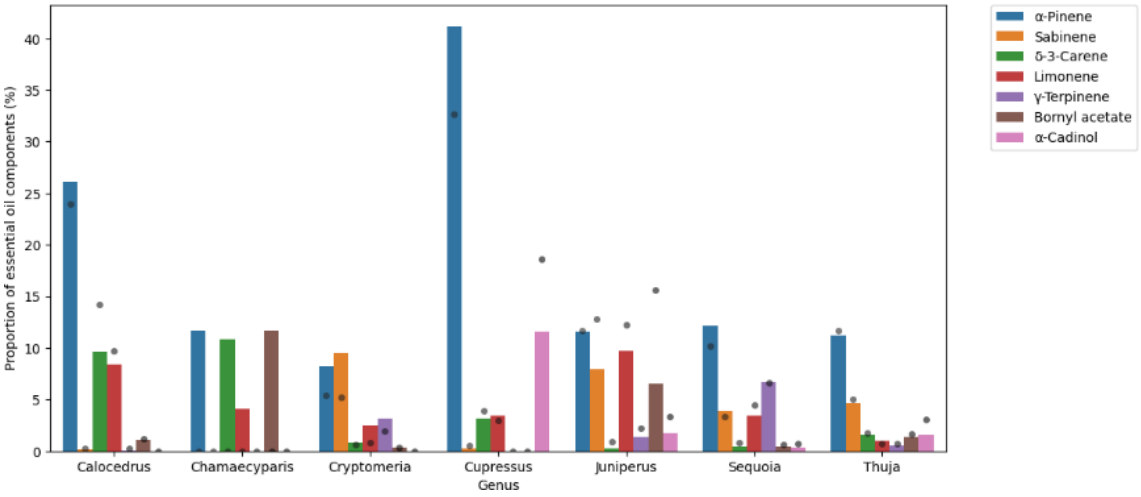


Figure 8. *Bar plot showing the main volatiles of Cupressaceae family. Results are mean area% measured by GC/MS. Grey dots represent standard deviation. (Bakó et al., 2024)*

8.2.2 Investigation of the Relationships between Volatiles and Species by PCA

The GC/MS data of the conifer samples were subjected to PCA. The PCA biplot revealed that two compounds were characteristic of the samples and thus represent the most considerable variance: α-pinene (RRT=5.1) and bornyl acetate (RRT=12.6). The substance with the highest correlation to axis 1 was α-pinene which was found in the highest concentrations in samples near it. Samples around axis 2 have the highest concentration of bornyl acetate. A large group of samples were surrounded around the origin, and the above mentioned two compounds were mostly absent from these samples. The total variance of the first two axes was 38%. See Figure 9.

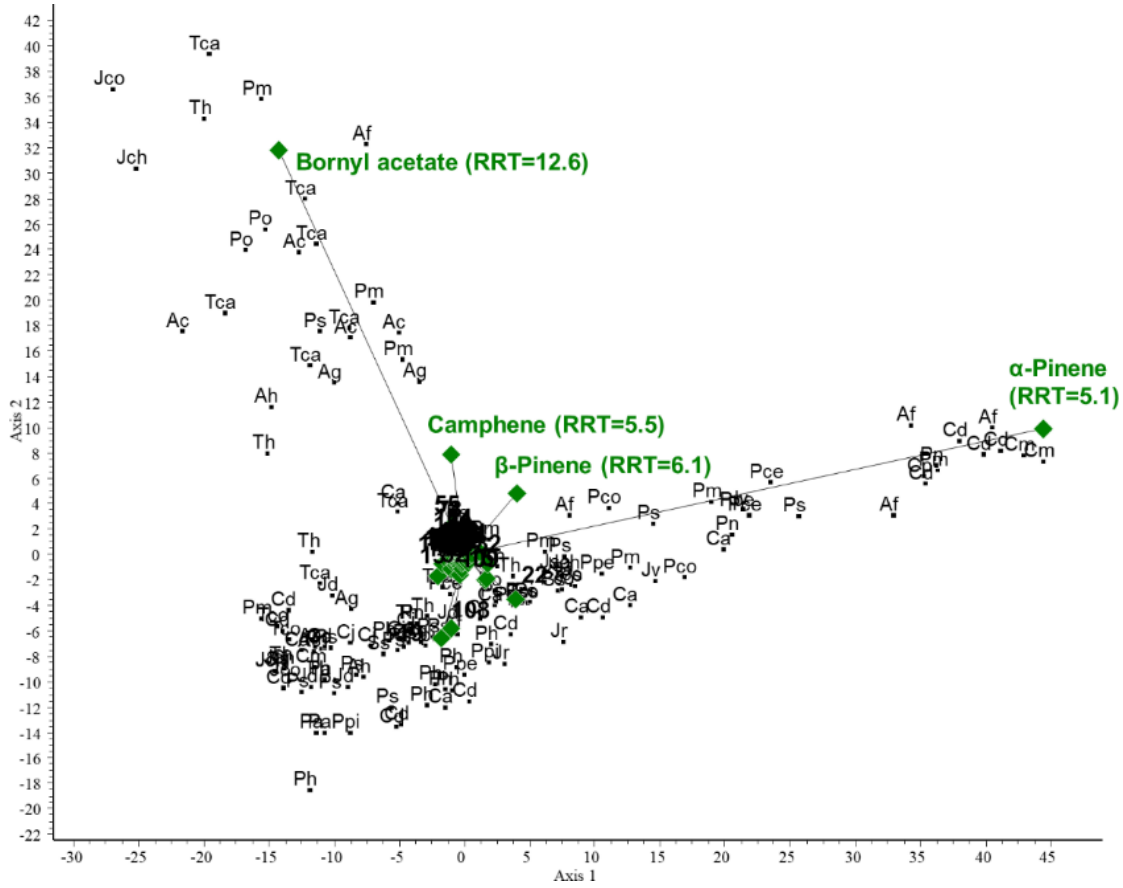


Figure 9. *PCA biplot for investigating the relationships between volatile organic compounds and plant species. Green diamonds are volatile components (RRT: relative retention time) and black dots are species. Ac=Abies concolor, Af=Abies firma, Ag=Abies grandis, Ah=Abies holophylla, Ca=Cedrus atlantica, Po=Picea omorika, Ps=Picea sitchensis, Pa=Pinus aristata, Pce=Pinus cembra, Pco=Pinus coulteri, Ph=Pinus heldreichii, Pn=Pinus nigra, Ppe=Pinus peuce, Ppi=Pinus pinaster, Ps=Pinus strobus, Pm=Pseudotsuga menziesii, Tca=Tsuga canadensis, Th=Tsuga heterophylla, Cd=Calocedrus decurrens, Cp=Chamaecyparis pisifera, Cj=Cryptomeria japonica, Cm=Cupressus macnabiana, JCh=Juniperus chinensis, Jco=Juniperus communis, Jd=Juniperus drupacea, Jr=Juniperus rigida, Jsa=Juniperus sabina, Jv=Juniperus virginiana, Ss=Sequoia sempervirens, and Tco=Thuja koraiensis. (Bakó et al., 2024)*

Then, according to the PCA results, we selected the variables (VOCs) with the highest variance. We applied CVA to investigate the correlation between these VOCs and the three conifer groups. Based on the classification of the species, three groups were established: abietoid (containing *Abies*, *Cedrus*, and *Tsuga* species), pinoid (containing

Picea, *Pinus* and *Pseudotsuga* species), and cupressoid groups (containing the *Cupressaceae* species). For the CVA plot, see Figure 10.

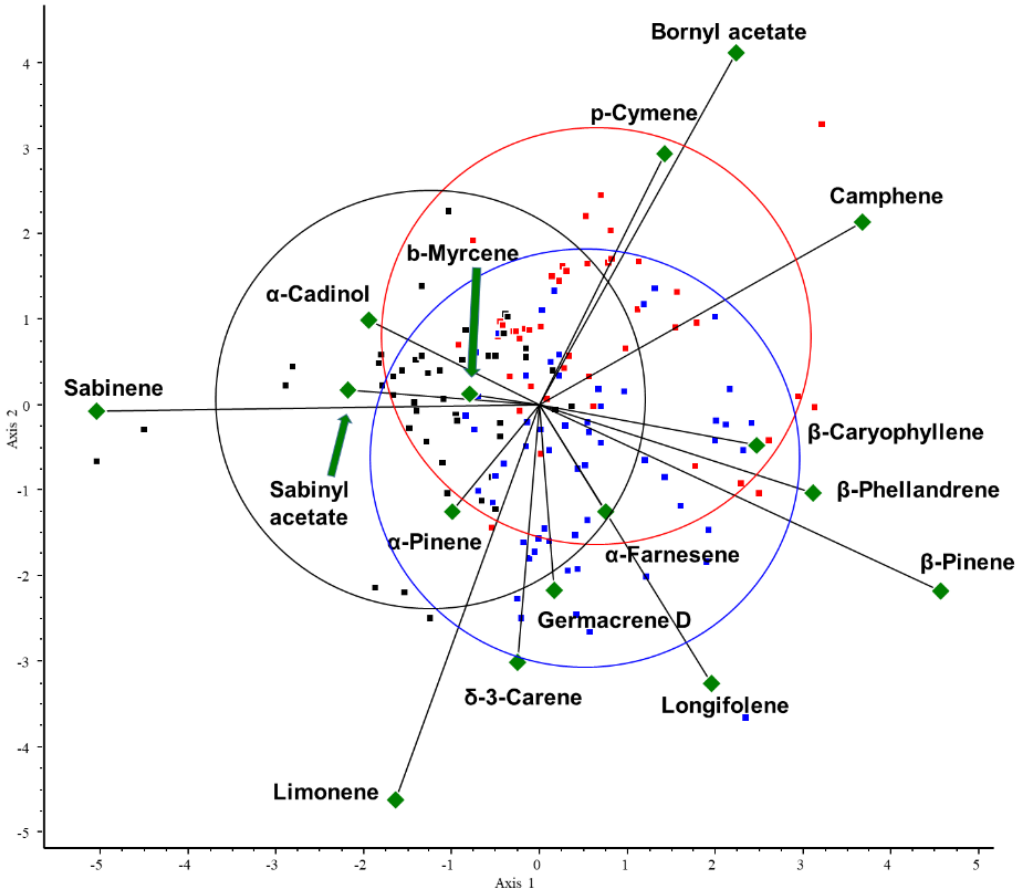


Figure 10. *CVA biplot for investigating the relationships between volatiles and species. CVA analysis was performed on all samples. Different groups are shown in different colors. Green triangles: volatile components. Red dots: abietoid group (containing Abies, Cedrus, and Tsuga species); blue dots: pinoid group (containing Picea, Pinus, and Pseudotsuga species); and black dots: cupressoid group (containing the Cupressaceae species).*

The results show that the following volatiles are characteristic of certain groups: the cupressoid group is characterized by sabinene (RRT=6.0), the pinoid group by longifolene (RRT=15.0) and β-pinene (RRT=6.1), and the abietoid group by camphene (RRT=5.5) and bornyl acetate (RRT=12.6). The results show that the amount of bornyl acetate and limonene are negatively correlated (see Figure 11).

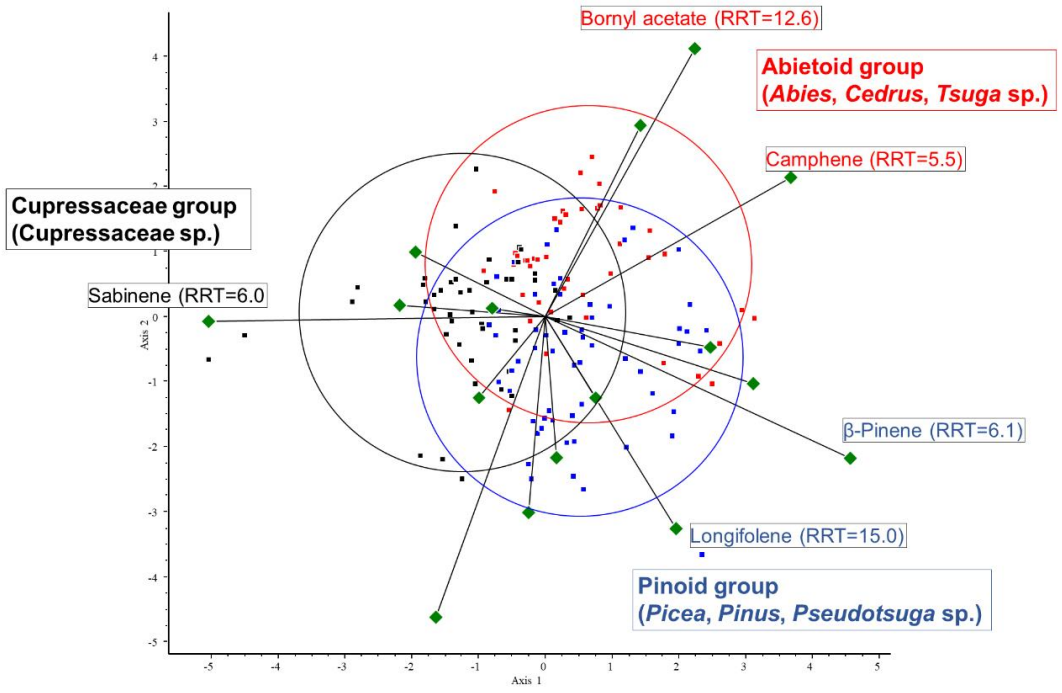


Figure 11. Main results of canonical variate analysis (CVA). Sabinene (RRT=6.0) is characteristic of the cupressoid group, longifolene (RRT=15.0) and β-pinene (RRT=6.1) are characteristic of the pinoid group, and camphene (RRT=5.5) and bornyl acetate (RRT=12.6) are characteristic of the abietoid group. RRT: relative retention time. (Bakó et al., 2024)

8.2.3 Investigation of the Relationships between Volatiles and Collected Plant Organs of the Pinaceae Family by PCA

To examine the relationships between VOCs and collected plant organs of the Pinaceae family, we first applied centered PCA and then CVA. The following plant parts were taken into consideration: needles, cones, bark, and resin. PCA was conducted on all gas chromatography data of the Pinaceae family. 40% of the total variance is explained by the first two ordination axes. α-Pinene (RRT=5.1) and bornyl acetate (RRT=12.6) were responsible for a large portion of the variance. α-Pinene was abundant in the samples. Large concentrations of the corresponding compound were found in samples close to the arrowheads. Camphene and β-pinene are positively correlated, whereas longifolene is negatively correlated with axis 2. The results show a negative correlation between the amount of bornyl acetate and limonene. For the PCA biplot, see Figure 12.

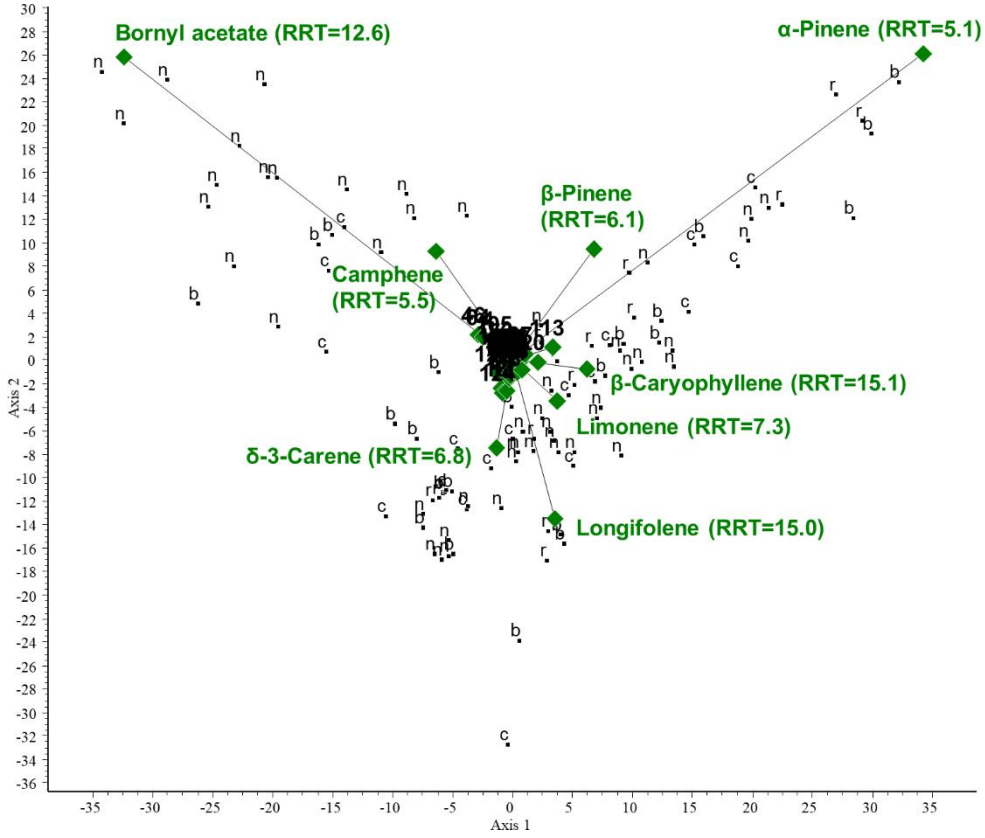


Figure 12. *PCA biplot for investigating the relationships between volatiles and collected plant organs of the Pinaceae family. Green diamonds are volatile components (RRT: relative retention time) and black dots are plant organs. n=needles, c=cones, r=resin, b=bark. (Bakó et al., 2024)*

The data was then subjected to CVA to examine the correlation between the plant organs and the VOCs with the highest variance. The results show that β-caryophyllene (RRT=15.1) and germacrene D (RRT=16.2) are characteristic of needles, and their amounts are negatively correlated with α-pinene. Longifolene and α-pinene are characteristic of bark samples. There is also a negative correlation between the amounts of p-cymene and β-phellandrene or β-myrcene. For the CVA plot, see Figure 13.

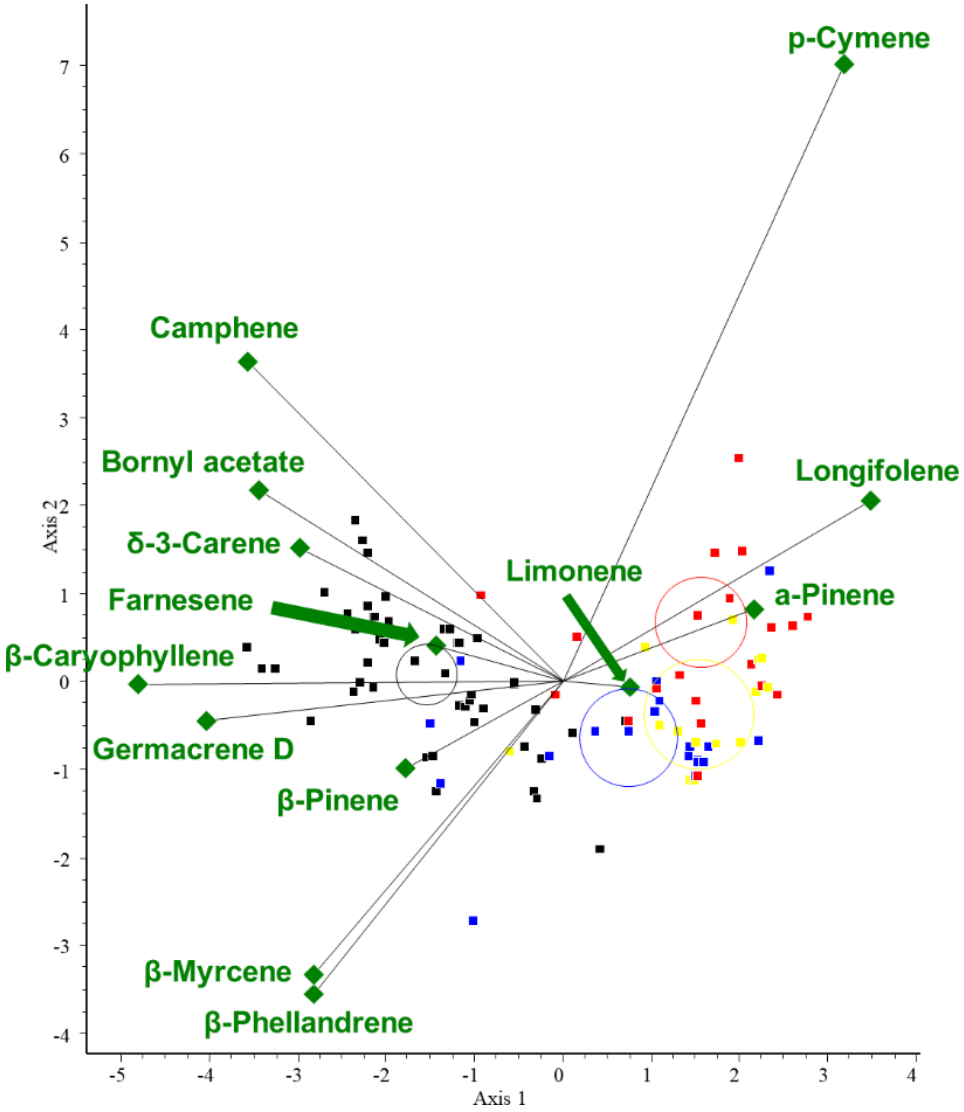


Figure 13. *CVA biplot for investigating the relationships between volatiles and collected plant organs of the Pinaceae family. Different groups are shown in different colors. Green diamonds: volatile components. Red dots: bark; blue dots: cones; black dots: needles; yellow dots: resin. (Bakó et al., 2024)*

Table 3 shows the chemical composition (mean area%) of samples of the Pinaceae family categorized by plant organs (only the highest values are shown).

Table 3. Main volatile organic compounds expressed in the mean area% of samples measured by the SPME-GC/MS method in the Pinaceae family and categorized by plant organs. (Bakó et al., 2024)

Name of the compound (only the relevant components are listed)	***KI***	**RRT**	**Chemical composition of samples investigated of the Pinaceae family (mean, area%)**			
			resin	**bark**	**cones**	**needles**
			n=15	**n=22**	**n=19**	**n=47**
α-Pinene	923	5.1	19.8	17.2	13.1	12.6
Camphene	941	5.5	1.2	1.9	0.5	3.8
β-Pinene	968	6.1	6.2	5.1	6.6	8.3
β-Myrcene	977	6.3	1.0	0.3	4.4	4.5
δ-3-Carene	1003	6.8	0.6	1.7	1.3	5.6
p-Cymene	1018	7.2	0.8	4.7	0.4	0.1
Limonene	1023	7.3	7.5	4.9	3.7	4.6
β-Phellandrene	1023	7.3	3.4	0.4	4.5	5.7
α-Terpineol	1190	10.9	1.7	2.6	0.2	0.3
Verbenone	1200	11.1	0.4	2.1	1.4	0.0
Bornyl acetate	1279	12.6	1.4	5.0	4.1	10.6
Longifolene	1412	15.0	3.6	7.7	4.8	0.4
β-Caryophyllene	1418	15.1	0.5	0.8	2.8	7.9
α-Humulene	1453	15.7	0.1	0.1	0.9	2.1
Germacrene D	1482	16.2	0.1	0.1	1.6	4.3
δ-Cadinene	1519	16.8	0.8	1.0	1.0	2.1

KI: calculated Kovats index; n: number of the measured samples; RRT: relative retention times

9 DISCUSSION

9.1 Summary of findings, contextual perspective

9.1.1 Efficacy of topical essential oils in musculoskeletal disorders: systematic review and meta-analysis of randomized controlled trials

Based on qualitative and quantitative analysis of RCTs included, we can conclude that EO therapy has a beneficial effect on pain intensity in MSD, and the most favorable effect was observed immediately after their usage compared to placebo. The treatment has a modest favorable effect on pain in MSDs one week after the intervention and four weeks after the intervention. This seeming contradiction in results is presumably due to sample size issues, as the mean value of the effect is similar to week one compared to week four, and the p-value is also near significant. Nonetheless, the decrease in effect compared to week zero (i.e., immediately after the application) is apparent. The reduction of about 1 VAS score means about 10% difference in pain intensity which is a non-negligible effect. For stiffness, the results are noteworthy, albeit only marginally significant. All three involved RCTs point in the direction of the same effect and considering that our applied methodology of conservatively estimating change SDs results in a highly robust approach, we are confident that involving further analyses will yield statistically significant results.

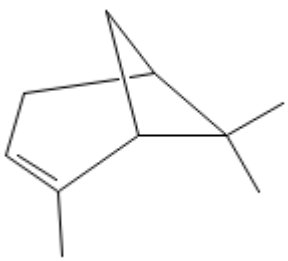
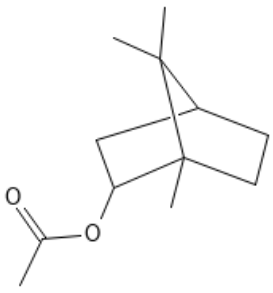
There is a previous meta-analysis in the literature (Lakhan et al., 2016), in which pain-relieving effect of aromatherapy was evaluated in all types of pain (e.g. postoperative pain, menstrual pain, knee pain, etc.). Lakhan et al. concluded that aromatherapy as add-on treatment is effective in reducing pain.

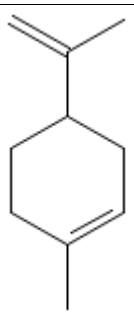
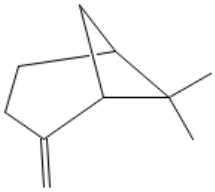
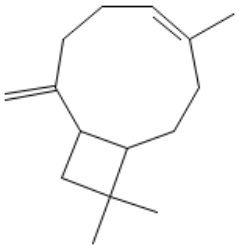
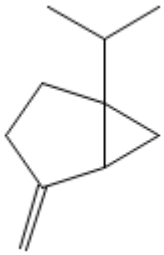
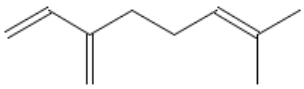
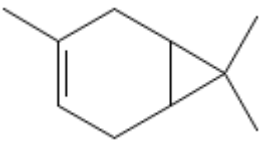
It is known that massage therapy alone could be beneficial in MSDs via a multimodal mechanism (Bervoets et al., 2015; Bhoi et al., 2021). Our summarized data suggest that the pain-relieving effect is more pronounced when massage is combined with an EO-containing product. The choice of EOs in the studies was based on scientific data or traditional use, with lavender being the most frequently investigated. Linalool and linalyl acetate, the two major constituents of lavender essential oil, showed analgesic and anti-inflammatory effects (Nasiri & Mahmodi, 2018). To reveal the differences between the effects of different EOs, more studies are needed in the future, but the tendency is obvious. EOs have beneficial effects on MSD pain and stiffness compared to placebo.

9.1.2 Chemometric Analysis of Monoterpenes and Sesquiterpenes of Conifers

Our samples were abundant in the following components: α-pinene, bornyl acetate, limonene, β-pinene, β-caryophyllene, sabinene, β-myrcene, and δ-3-carene that are the subject of medicinal and pharmaceutical research. Table 4 shows the chemical structure of these components, the chemical classification, and biological activities.

Table 4. Chemical structure, chemical classification and biological activity of the most abundant volatile organic components measured in the samples. (Bakó et al., 2024)

The most abundant volatile components (VOCs) in conifer samples measured	**Chemical structure of VOC**	**Chemical classification of VOC**	**Biological activities**	**References**
α-pinene		hydrocarbons, monoterpenes	antibacterial, antifungal, anti-inflammatory, antioxidative, neuroprotective, antitumor, etc.	(Allenspach & Steuer, 2021)
bornyl acetate		monoterpene esters	anti-inflammatory, immune-modulatory, sedative, etc.	(Q. Zhao et al., 2023; Z. J. Zhao et al., 2023)

limonene		hydrocarbons, monoterpenes	antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, immune-modulatory, etc.	(Anandakumar et al., 2021)
β-pinene		hydrocarbons, monoterpenes	antimicrobial, anticancer, anti-inflammatory, antiallergic, etc.	(Salehi et al., 2019)
β-caryophyllene		hydrocarbons, sesquiterpenes	antioxidant, anticancer, cardioprotective, immunomodulatory, antimicrobial activities, etc.	(Gyrdymova & Rubtsova, 2022)
sabinene		hydrocarbons, monoterpenes	anti-fungal, anti-inflammatory, etc.	(Cao et al., 2018)
β-myrcene		hydrocarbons, monoterpenes	anxiolytic, antioxidant, anti-aging, anti-inflammatory, analgesic, etc.	(Surendran et al., 2021)
δ-3-carene		hydrocarbons, monoterpenes	anti-inflammatory	(Ocete et al., 1989)

Our findings confirm that sabinene is a typical component of the Cupressaceae family (Foster et al., 2013). Samples from the Pinaceae family did not contain α-thujone or β-thujone, but they were found in the Cupressaceae family. Sabinol is an intermediate product in the biosynthesis of thujone, which is derived from sabinene (Nemeth &

Nguyen, 2020). Sabinol was also present in most cases when thujone was detected in our samples. All thujone-containing samples are listed in Table 5. Since thujone is neurotoxic, preparations containing α- and β-thujone should be used with caution (Nemeth & Nguyen, 2020).

Table 5. Presence of thujone and sabinol in relevant samples

Sample (species, plant organ)	**Volatile component (relative amount, %)**			
	Sabinene (RRT=6.0)	**α-Thujone (RRT=9.0)**	**β-Thujone (RRT=9.2)**	**Sabinol (RRT=9.7)**
Juniperus chinensis, needles	5.1	0.0	1.0	0.7
Juniperus sabina, needles	16.4	0.0	1.3	2.9
Juniperus chinensis, needles	8.1	0.7	4.8	0.0
Thuja koraiensis, needles	8.7	1.0	7.9	0.4
Thuja koraiensis, needles	9.4	1.1	7.6	1.0

RRT: relative retention times

In accordance with the literature, our study shows variations in the terpene profiles of the plant organs of Pinaceae species (see Figure 13) (Butnaru et al., 2022; Chizzola & Müllner, 2021; Dormont et al., 1998). Previous research has shown that the proportions of various terpenes in conifers exhibit strong positive correlations across various plant parts, or that the same primary VOCs can be found in them (Dormont et al., 1998; Manninen et al., 2002). Furthermore, environmental conditions or the development of plant organs may potentially influence terpenoid production in addition to genetics (Bai, 2022; de Simon et al., 2021). Despite the fact that certain studies have found notable variations in diterpenes (Chizzola & Müllner, 2021; de Simon et al., 2021; Nantongo et al., 2022), they were not examined in our study.

Terpenes are important plant metabolites in conifers. While terpenoids with higher molecular weight, e.g. diterpenes are often not volatile, monoterpenes and sesquiterpenes

are constituents of essential oils. The terpene synthase (TPS) family mainly determines the volatile profiles of species (Alicandri et al., 2020); however, the precise concentrations of volatiles vary depending on many circumstances. These include developmental factors, stress, and other stimuli e.g. temperature, biotic stress, pollution, or season (Kopaczyk et al., 2020). Certain terpene synthase enzymes in plants produce terpenoid metabolites. Our data can offer important scientific information for future studies in this field.

9.2 Strengths (including all studies)

Regarding the strengths, we followed our protocol registered in PROSPERO. Rigorous methodology was applied, and we included only RCTs in the meta-analysis. We investigated the time-dependency of the effect of EOs.

In the phytochemical study, a large number of conifer samples were analyzed by GC-MS method providing robust and comprehensive data on the chemical profiles and bioactive compounds relevant to pain relief and musculoskeletal health. A total of 151 conifer samples from 30 species were measured, and a total of 183 VOCs were identified, with an average of 18 per sample.

9.3 Limitations (including all studies)

Limitations of the meta-analysis are as follows: low number of trials involving few patients were available in the literature, and the low-quality studies that were characterized by high risk of bias. The definition of randomization process differed among the studies, or it was even missing. Blinding was problematic in all studies because hiding the smell of EOs was not possible completely, and it might influence the staff and the patients. High heterogeneity was identified. The reason for heterogeneity may be that different EOs were used in the studies. MSDs include several conditions, consequently the EOs were applied in different areas of the body. Also, the length of interventions and the follow-up periods were different.

The limitation of the phytochemical study was that the collection of conifer samples was not completely consistent (i.e., we did not always collect and analyze the same plant parts and did not consider the developmental stages of the plant organ).

10 CONCLUSIONS

This comprehensive and interdisciplinary work provides valuable information on the chemical diversity of conifers and therapeutic benefits of EOs in pain management, highlighting their potential in pharmaceutical applications.

The systematic review and meta-analysis showed that topical EOs are effective in reducing pain and stiffness in chronic MSDs. Based on our results, we suggest that repeated application of topical EO therapy is necessary to achieve the most effective pain-relieving outcomes.

The GC/MS study on conifers is important because it can complement the findings of the meta-analysis by providing detailed chemical profiles of essential oils (EOs) from the Pinaceae and Cupressaceae families. Our gas chromatographic analyses in the Pinaceae family reveal that the most abundant components are α-pinene, β-pinene, bornyl acetate, limonene, and β-caryophyllene. In the Cupressaceae family, the most important components are α-pinene, limonene, sabinene, β-myrcene, and δ-3-carene. Characteristic chemical components were identified, such as sabinene for cupressoid group, camphene and bornyl acetate for abietoid group, and longifolene and β-pinene for pinoid group.

Conifer EOs and VOCs can be used as ingredients in topical products for treating musculoskeletal disorders. By identifying the most abundant and characteristic volatile organic compounds in these species, this research supports the quality control products based on conifer EO, including pain-relieving and anti-inflammatory topical formulations, thereby promoting the rational use of EO.

11 IMPLEMENTATIONS FOR PRACTICE

The main conclusion of our meta-analysis is that EOs exert a positive effect on the symptoms of MSDs. No interactions were reported with the conventional therapy during the studies, and in clinical practice, the dose of painkillers might be decreased due to the pain-relieving effect of EOs. Based on the statistical analysis, repeated application of EOs is recommended at least within a week because the effect decreases after a week. It is safe, cost-effective and easy to reach for the public.

The major VOCs detected in conifer samples, such as α-pinene, bornyl acetate, limonene, β-pinene, β-caryophyllene, sabinene, β-myrcene, and δ-3-carene have known anti-inflammatory and pain-relieving properties. Essential oils containing these components may be suitable for developing pain-relieving topical products to treat the main symptoms of MSDs. Our results support the quality control of products based on conifer EOs.

12 IMPLEMENTATION FOR RESEARCH

Methodology issues

MSDs are long-term conditions; therefore, the length of the intervention and the follow-up periods should be determined carefully. Improving methodological quality and reducing heterogeneity are important tasks in further trials.

Study design

It would be advisable to uniform inclusion and exclusion criteria for each disorder (e.g. severity of the disease should be considered), to improve blinding and to provide comparable results, i.e., to reach a consensus on measurement tools intended to be used.

New aspects

Further investigations are needed to determine the most potent EOs and to understand the mode of actions. For future trials, we recommend using standardized and more comprehensive sample collections to improve the reliability of the data and reflect a more accurate composition of VOCs.

13 IMPLEMENTATION FOR POLICYMAKERS

Most people use essential oils primarily in cosmetics, not as medicines. The consumers use these products to alleviate their symptoms regardless of the product category (cosmetics are marketed for skin care purposes). However, the evaluation of efficacy and safety, and quality assurance of these cosmetic products are currently insufficient.

It would be advisable to establish standards that ensure the consistent quality and effectiveness of essential oil-containing cosmetic products. This would not necessarily require clinical testing for each product; if a reference product has undergone an appropriate evaluation and the essential oil composition of the new cosmetic product remains within defined limits, this could serve as acceptable evidence of safety and efficacy. While this requirement would be less stringent than that for medicines, it would be significantly more rigorous for cosmetic products than the current regulations.

14 FUTURE PERSPECTIVES

Collaboration among pharmacologists, botanists, and clinicians could facilitate the translation of this research into practical healthcare applications. As interest in natural and holistic therapies grows, advancing this field could lead to safer, more effective, and sustainable treatment options for musculoskeletal disorders.

Further high-quality RCTs with more homogeneous study designs are necessary to support the findings of this work and to answer further questions. The most important questions are which EOs or EO constituents have the most beneficial effect on reducing pain and stiffness and which type of MSDs can be most effectively treated with EOs. Future research should focus on expanding the scope of chemometric analyses to include a broader range of conifer species and essential oils. By developing robust methodologies to compare and classify essential oils, researchers can identify the most potent formulations for musculoskeletal treatments.

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16.1 Publications related to the thesis

Bakó E, Böszörményi A, Vargáné Szabó B, Engh MA, Hegyi P, Ványolós A, Csupor D. Chemometric analysis of monoterpenes and sesquiterpenes of conifers. **Front Plant Sci**. 2024 Sep 4;15:1392539. doi: 10.3389/fpls.2024.1392539.

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Q1, IF: 4.3

16.2 Publications not related to the thesis

Schneider, G., Schweitzer, B., Steinbach, A. S., Hodován, Á. S., Horváth, M., Bakó, E., Mayer, A., & Pál, S. (2025). The Therapeutic Potential of West Indian Lemongrass (*Cymbopogon citratus*) Essential Oil-Based Ointment in the Treatment of Pitted Keratolysis. **Antibiotics**, 14(3), 241. https://doi.org/10.3390/antibiotics14030241

Q1, IF: 4.6

Meznerics FA, Kemény LV, Gunther E, Bakó E, Dembrovszky F, Szabó B, Ascsillán A, Lutz E, Csupor D, Hegyi P, Bánvölgyi A, Nagy G. Multibiomarker disease activity score: an objective tool for monitoring rheumatoid arthritis? A systematic review and meta-analysis. **Rheumatology (Oxford)**. 2023 Jun 1;62(6):2048-2059. doi: 10.1093/rheumatology/keac715.

D1, IF: 4.7

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