Volatile organic compounds and essential oils: distribution in conifers and their applications in pain management

Ph.D. Thesis Booklet

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1.Introduction

1.1. What is the topic?

This PhD thesis evaluates the therapeutic potential of essential oils (EOs) in the management of musculoskeletal disorders (MSDs). 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.

1.2. What is the problem to solve?

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

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 evidence-based data regarding the application of EOs in the management of MSDs, a condition impacting millions globally. Furthermore, the chemometric analysis of conifers may support the identification of conifer species with the most promising therapeutic potential for incorporation into pain-relieving formulations.

1.4. What would be the impact of our research results?

This research highlights the potential benefits of EOs. First, establishing evidence-based data on the effectiveness of EOs as a complementary treatment for MSDs may lead to improved patient outcomes, reduced dependency on synthetic drugs, and moderate side effect profile. Furthermore, the findings of this research may contribute to the formulation of more potent analgesic and anti-inflammatory preparations.

2. Objectives

2.1. 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 randomized controlled trials (RCTs) reported in the literature.

2.2. 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-mass spectrometry (GC/MS) method and to explore their chemotaxonomic relationships by chemometric analysis.

3.Methods

3.1. Study I.

We conducted a systematic review and meta-analysis of RCTs 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 and the recommendations of Cochrane Handbook (Cochrane Handbook for Systematic Reviews of Interventions). 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 RCTs involving adult participants suffering from musculoskeletal disorders were considered. The intervention involved of essential oils applied either with or without massage, and these treatments were compared to placebo products (also

applied with or without massage) or a no-intervention control group. The primary outcome measured was pain intensity, while secondary outcomes included quality of life and functional status. The following studies 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.

3.2. Study II.

Conifer samples were collected in the Jeli Arboretum in 2020 and 2021 and in the Folly Arboretum in 2021, Hungary. Needles (including branch tips), resin, cones, and bark were obtained. Samples were collected from 18 species of 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*) and 12 species of 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*).

The chemical composition of the collected samples was initially characterized by static headspace solid-phase microextraction (sHS-SPME) using gas chromatography-mass spectrometry (GC/MS). GC/MS measurement data were evaluated, and volatile components were identified. Subsequently, chemometric analyses employing multivariate methods were conducted on the obtained data to explore the correlation between the chemical profiles of conifers and various attributes (such as species and plant organs) and identify characteristic volatile organic compounds (VOCs) specific to conifer species.

4.Results

4.1. Study I.

After the systematic literature search and study selection process, 12 RCTs were included in the systematic review. 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, and one in Turkey, Taiwan, USA, and Egypt, respectively.

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, 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, hand osteoarthritis, rheumatoid arthritis, low back pain, carpal tunnel syndrome 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: one found that essential oil therapy had no effect, while the other showed that aromatherapy massage improved it.

Quantitative synthesis of results– primary outcome

Seven RCTs 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. 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.

The following subgroup analyses were performed:

Pain intensity measured immediately after the intervention: mean difference (MD) of the change indicates that topical EOs decreased the Visual Analogue Scale (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.

Pain intensity measured one week after the intervention: 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]).

Pain intensity measured four weeks after the intervention: 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]).

Quantitative synthesis of results– secondary outcome

Three articles with 124 patients participating in the trials were taken into consideration for the stiffness analysis. 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 status of the MSD. The result is nearly significant.

4.2. Study II

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%.

The most prevalent components (mean value) were: α-pinene (15%), β-pinene (7%), bornyl acetate (7%), limonene (5%), according to our GC/MS analysis of 103 samples from the Pinaceae 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%).

Investigation of the Relationships between Volatiles and Species by PCA

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). 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).

5. 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 found that topical EOs reduce chronic MSD pain and stiffness. Our findings imply that repeated application of topical EO therapy is needed for optimal pain relief. The GC/MS investigation on conifers provides chemical profiles and characteristic chemical components of volatiles of the selected Pinaceae and Cupressaceae families.

Although the two studies differ in their methodology, both aimed to promote the evidence-based use of essential oils. By identifying the most abundant and characteristic volatile organic compounds in these species, this research supports the quality control of products based on conifer EO, including pain-relieving and anti-inflammatory topical formulations, thereby promoting the rational use of EO.

6. Bibliography

1.

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