SUMMARY of the PhD THESIS

The Influence of Magnetic and Electric Fields, and of LASSER Radiation on the Physico-chemical Parameters and Bioactivities of Essential Oils

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CONTENTS

CONTENTS **2**
INTRODUCTION **3**

1. ESSENTIAL OILS 4
2. PHYSICAL FIELDS 4
3. OBJECTIVES 4
4. MATERIALS AND METHODS 5
5. RESULTS 5
5.1. Evaluation of Physico-chemical Parameters 5
5.1.1. GC-MS Determination of Essential Oils 5
5.1.2. Analysis of FT-IR Spectra of Essential Oils 6
5.1.3. Analysis of ESR Spectra of Essential Oils 6
5.1.4. Determination of Thermal Diffusivity 6
5.2. Determination of Bioactivities of Essential Oils 7
5.2.1. Determination of Antimicrobian Activity 7
5.2.2. Determination of Antioxidant Activity 7
5.2.3. Determination of Citotoxic Activity 8
6. GENERAL CONCLUSIONS AND RECOMMENDATIONS 8
7. ORIGINALITY AND INOVATIVE CONTRIBUTIONS 9

BIBLIOGRAPHY **10**

INTRODUCTION

Recently, there has been growing interest from both the scientific community and consumers in investigating and utilizing essential oils (EOs), which have been increasingly appreciated and recognized for their diverse and remarkable effects on the human body. Numerous studies have revealed more and more of the myriad of biological effects of EOs, the including antiseptic, antimicrobial, antifungal, antiviral, cytotoxic, anxiolytic, immunomodulatory, anti-inflammatory, and antioxidant activities (OSAILI *et al*., 2023).

Essential oils have been recognized and used for their healing benefits since ancient times, both as therapeutic remedies and in spiritual and religious practices, and they continue to be rediscovered and treasured for their true value (SHARIFI-RAD *et al*., 2017). Therefore, the EOs market has seen an upward trend, reaching a value of USD 23.74 billion in 2023, with forcasts estimating a value of USD 40.12 billion by 2030 (GRAND VIEW RESEARCH, 2024).

This is particularly significant given the increasing global incidence of certain conditions such as cardiovascular, respiratory, and digestive diseases, diabetes, cancer, and various psycho-emotional disorders like anxiety, insomnia, and depression (OUR WORLD IN DATA, 2024). Furthermore, the inappropriate use of antibiotics has led to the development of antimicrobial resistance, which was been directly associated with 1.27 million deaths and indirectly with another 4.95 million deaths in 2019, according to WHO data (WHO AMR, 2023). Therefore, the current situation calls for solutions to the challenges that we are facing.

However, obtaining high-quality EOs requires the use of vast areas of land, as large quantities of plants are needed to produce small amounts of EOs, given the relatively low and often costly extraction yields (EODR, 2016), (FRANCHOMME, 2001). As a result, new ways to improve the extraction processes and the quality of the obtained EOs are being investigated. Several studies have shown that exposing seeds, seedlings, or plants to various physical fields (magnetic and electric) or LASER radiation has, in some cases, led not only to improved mineral absorption and secondary metabolite composition, but also to increased extraction yields of EOs and even optimized their composition and antimicrobial effects (OKLA *et. al*., 2022), (MAFFEI, 2014).

Inspired by these studies, the current research aimed to bridge these two areas of investigation. This approach is relatively unexplored in the existing scientific literature, and through our experimental research, we aimed to contribute with valuable information on the potential of such treatments to modify and potentially enhance the physico-chemical properties and bioactivities of EOs, thereby optimizing the ways and doses in which they could be used.

1. ESSENTIAL OILS

The first chapter of this work provides a comprehensive overview of essential oils (EOs) from a historical perspective, including their methods of extraction, composition, and bioactivities. EOs are complex mixtures of secondary metabolites that are soluble in organic solvents and are extracted from various parts of aromatic plants where they are produced and stored, such as roots, rhizomes, leaves, buds, flowers, fruits, or seeds (ALI *et al*., 2015; KHAYYAT & ROSELIN, 2018). To date, there are over 300 EOs available on the market, and more than 3,000 substances in their composition have been studied (SHARIFI-RAD *et al*., 2017). Their numerous biological qualities and applications make EOs among the most important natural products derived from plants (ELSHAFIE & CAMELE, 2017).

Due to their lipidic nature, consisting of very small and volatile molecules, which are primarily monoterpenes, sesquiterpenes, or phenylpropanoids, EOs have the ability to cross the blod brain barrier and to be transported to all levels of the body, where they exert a wide range of biological effects. These effects include antimicrobial, anti-inflammatory, immunomodulatory, antioxidant, cytotoxic, microbiome-regulating activitie, as well as the regulation of cognitive and emotional moods. Therefore, the study of EOs and their activities can help us understand the multiple roles they can play in contributing to our health and inner well-being, leading to an improved quality of life (BAPTISTA-SILVA, 2020), (OSAILI *et al*., 2023).

2. PHYSICAL FIELDS

The following section presents the physical fields that have been used to energize the EOs selected for the experiments discussed in this paper, focusing on their effects on plant germination and development, as well as on improving the yield of EOs extraction, composition, and even their biological effects. The encouraging results suggest that exposing various plants to magnetic and electric fields, and LASER radiation could be an innovative method of agricultural bioengineering, used specifically to obtain certain biocompounds (BERNARD *et al*., 2024). However, the mechanisms by which these fields influence living organisms have not yet been fully understood and require further in-depth research (MAFFEI, 2014).

3. OBJECTIVES

The purpose of this work is, on the one hand, to investigate certain physicochemical properties and bioactivities of the selected EOs, and on the other hand, to identify potential influences of subtle physical fields (magnetic, electric, and electromagnetic fields— LASER radiation) on their physico-chemical properties and biological activities.

4. MATERIALS AND METHODS

To develop this PhD thesis, six commercially available EOs with antimicrobial properties have been selected. These include EOs of: cinnamon, patchouli, geranium, basil, sacred frankincense, and lemon. The EO of cinnamon bark (*Cinnamomum zeylanicum* Blume) originates from Sri Lanka, the patchouli EO (*Pogostemon cablin* (Blanco) Benth.) is extracted from plants grown in Indonesia, the geranium EO (*Pelargonium graveolens* L'Hér) originates from subtropical regions of south Africa and Madagascar, the basil EO (*Ocimum basilicum* L.) comes from Asia, the sacred frankincense EO (*Boswellia sacra* Flueck.) is extracted from frankincense trees in Oman, and the lemon EO (*Citrus limon* (L.) Osbeck) is cold-pressed from lemons grown in Argentina and South Africa.

These EOs were divided into 1 ml samples, which were then exposed for 20 minutes to an magnetic field and an electric field, and for 10 minutes to a LASER radiation with a wavelength of 532 nm. Subsequently, the resulting samples (1 – untreated EO and 3 – energized EOs, for each studied EO) were investigated using methods to determine their physico-chemical properties. These methods included the analysis of composition through gas chromatography-mass spectrometry (GC-MS), vibrational modes determination through Fourier-transform infrared spectroscopy (FT-IR), electron spin resonance spectra through ESR, and thermal diffusivity using the photopyroelectric method.

To investigate the bioactivity of the EOs, the antibacterial, antifungal, antioxidant, and cytotoxic activities were determined for the six selected EOs. For testing the antimicrobial activity, the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were analyzed.

The antioxidant activity was determined by testing the capacity of EOs to reduce two free radicals: DPPH and ABTS+. The cytotoxic activity was determined throgh using the MTT assay on a human ovarian cancer cell line, A2780.

5. RESULTS

5.1. Evaluation of Physico-chemical Parameters

5.1.1. GC-MS Determination of Essential Oils

A. Untreated EOs: Following this analysis, it was observed that cinnamon EO (UES) and basil EO (UEB) exhibit a phenylpropanoid profile with a total concentration of 81.27% and 85.85%, respectively. Patchouli EO (UEP) presents a sesquiterpene profile with a concentration of 97.36%, while geranium EO (UEG), sacred frankincense EO (UETS), and lemon EO (UEL) contain monoterpenes in proportions of 95.07%, 96.91%, and 98.56%, respectively. The main compounds in UES were cinnamaldehyde, eugenol, and cinnamyl acetate; in UEP: patchoulol, α-bulnesene, α-guaiene, and seychellene; in UEG: citronellol, geraniol, and citronellyl acetate; in UEB: methyl chavicol, eucalyptol, and α-bergamotene; in UETS: α-pinene, limonene, and 3-carene; and in UEL: limonene, β-pinene, and γ-terpinene.

B. Treatead EOs: In general, the three treatments applied did not significantly influence the composition of the EOs. The main compounds remained unchanged; however, there were some slight variations in the percentages of certain molecules, either increasing or decreasing. Additionally, new constituents appeared in very small percentages that were not present in the initial samples, as observed in the cases of cinnamon EO (UES), geranium EO (UEG), and lemon EO (UEL).

5.1.2. Analysis of FT-IR Spectra of Essential Oils

A. Untreated EOs: The analysis of the FT-IR spectra revealed that the EOs maintained a common profile typical of natural products, with peaks in regions specific to EOs. However, each spectrum was distinct from the others, thereby highlighting their unique composition. This method is very valuable when identifying EOs, particularly in detecting the adulterated samples.

B. Treatead EOs: Through this analytical method, no significant changes were observed in the FT-IR spectra of the EOs following the three treatments. The exposure to physical fields did not cause any shifts in the peaks of these spectra for any of the EOs, indicating that the molecular profile remained largely stable. However, certain spectra exhibited slight changes in the amplitude of these peaks, suggesting that some alterations in vibrational modes were induced by these treatments, as seen particularly in the case of cinnamon EO (UES), especially in the spectrum of the sample exposed to LASER radiation. Additionally, this method was not sufficiently sensitive to accurately determine possible changes in the vibrational modes induced by the applied treatments.

5.1.3. Analysis of ESR Spectra of Essential Oils

A. Untreated EOs: The ESR spectra exhibit a characteristic profile for each EO, with all samples showing unresolved spectra featuring a complex signal in the 3300 – 3700 G region, which is typical for natural extracts, such as EOs.

B. Treatead EOs: As it is a much more subtle and sensitive analytical method, the electron spin resonance (ESR) spectroscopy revealed that there were differences between the spectra of the untreated EOs and those exposed to physical fields, that depended on the specific EO tested and the type of treatment applied. Among all the treatments, the exposure to the electric field stood out, as it induced noticeable changes in the ESR spectrum for all the tested EOs.

5.1.4. Determination of Thermal Diffusivity

A. Untreated EOs: The PPE analysis revealed differences in the thermal behavior of the investigated untreated EOs.

B. Treatead EOs: Slight changes in thermal diffusivity were observed between the values induced by the exposure to electric and magnetic fields compared to those of the untreated EOs. However, this method is not sensitive enough to provide more detailed information regarding changes in the molecular architecture due to the influence of physical fields.

5.2. Determination of Bioactivities of Essential Oils

Bioactivity evaluation of EOs was performed by analyzing their antimicrobial, antioxidant and cytotoxic activity.

5.2.1. Determination of Antimicrobian Activity

The antimicrobial activity of EOs was evaluated for the following bacterial strains: Gram-positive: *S. aureus, B. cereus* and *L. monocytogenes*, Gram-negative bacteria: *E. coli, S. enteritidis* and *P. aeruginosa*, and a fungal culture of *C. albicans*. All tested EOs inhibited and stopped the growth of these microorganisms. However, the intensity of their activity varied from one oil to another, with EO of cinnamon (UES) being the strongest of all, against most of the bacterial strains.

A. Untreated EOs: Cinnamon EO (UES) and geranium EO (UEG) exhibited strong antimicrobial activity against all tested microbial cultures. Additionally, patchouli EO (UEP) showed high antifungal and antibacterial activity against all cultures except for S. *enteritidis* and *P. aeruginosa,* where its antibacterial effect was more moderate. Basil EO (UEB), sacred frankincense EO (UETS), and lemon EO (UEL) demonstrated very strong antifungal activity and increased antibacterial activity against *L. monocytogenes* and *P. aeruginosa* cultures. Additionally, UEB showed good activity against bacterial cultures of *B. cereus, S. enteritidis*, and especially *E. coli*.

B. Treatead EOs: The influences of physical fields on the studied EOs varied, depending on the type of EO, the applied treatment, and the type of microbial culture tested. In many cases, the applied exposures resulted in the enhancement of the antimicrobial effect of the EOs. However, there were also instances where this effect was somewhat more or less diminished. Basil EO, the estragole chemotype, was the most affected by the treatments which led to a reduction in its antibacterial and antifungal effects. The exposure to magnetic and electric fields increased the activity of all EOs against *S. aureus* cultures, while LASER energization particularly enhanced the antimicrobial activity of geranium EO (UEG-L) against all microbial cultures, except for *S. enteritidis*.

5.2.2. Determination of Antioxidant Activity

The antioxidant activity of EU was tested by two assays: DPPH and ABTS+.

A. Untreated EOs: All the EOs demonstrated antioxidant activity in these tests. The highest capacity to reduce the DPPH free radical was exhibited by cinnamon EO (UES), followed by patchouli EO (UEP), geranium EO (UEG), and basil EO (UEB). For the ABTS+ cation, the highest reduction capacity was observed in sacred frankincense EO (UETS), followed by cinnamon EO (UES), patchouli EO (UEP), and geranium EO (UEG).

B. Treatead EOs: The applied treatments did not affect the antioxidant activity of the EOs at all, indicating that these treatments can be used to modify certain activities (such as the antimicrobial and cytotoxic effects) while still preserving their extraordinary antioxidant benefits, which also support other positive effects in the body.

5.2.3. Determination of Citotoxic Activity

The cytotoxic action of EU was evaluated on the ovarian cancer cell line (A2780).

A. EO untreated At a concentration of 0.005%, the strongest antiproliferative activity was observed in cinnamon EO (UES), followed by patchouli EO (UEP), geranium EO (UEG), and lemon EO (UEL). Sacred frankincense EO (UETS) and basil EO (UEB) exhibited a slight proliferative effect at 0.005%, which shifted to an antiproliferative effect, similar to that of UEL at concentrations of 0.01% and 0.02%.

B. Treatead EOs: The magnetic field enhanced the cytotoxic activity of cinnamon EO (UES-M) at 0.01%, patchouli EO (UEP-M) at 0.01-0.02%, basil EO (UEB-M) at all concentrations, especially at 0.01%, and sacred frankincense EO (UETS-M) at 0.02%. **The electric field** improved the antiproliferative activity of cinnamon EO (UES-E) at 0.01-0.02%, geranium EO (UEG-E) at 0.005% and 0.02%, basil EO (UEB-E) at 0.02%, and lemon EO (UEL-E) at 0.01% and 0.02%. This treatment had an exceptionally strong influence on patchouli EO (UEP-E) at all concentrations, especially at 0.01%, where the antiproliferative capacity increased 35-fold.

Regarding the influence of LASER radiation, it dramatically increased the cytotoxic activity of all EOs, regardless of their previous potency. Following this treatment, all energized EO samples reduced the viability of tumor cells to approximately 1-2%, regardless of concentration. Moreover, due to this extraordinary effect, patchouli EO (UEP) and geranium EO (UEG) were also tested at lower concentrations, and the results showed that these EOs retained their antiproliferative activity even at concentrations of 0.00125% and 0.000625%.

6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

The results generated through this research project confirm that the studied EOs represent an extraordinary resource due to their biological effects, which deserve further investigation and exploration. Moreover, their exposure to physical fields has led to significant and even remarkable changes in the bioactivity of certain EOs, particularly in their antimicrobial and cytotoxic actions, without compromising their initial antioxidant capacity.

This work has opened a new path for EO research and supports the continuation of studies in this fascinating and largely unexplored area. One possible direction for future exploration, which was not addressed in this study, involves investigating the mechanisms underlying the influence of the exposure of EOs to physical fields, particularly those concerning the enhancement of antimicrobial and cytotoxic effects.

Another research direction could involve studying how varying different parameters related to the characteristics of physical fields (such as the intensity of magnetic and electric fields, the wavelength and frequency of LASER radiation, and the duration of exposure) might influence the parameters and bioactivity of these EOs. This could help determine the most suitable treatment to optimize their biological effects.

Furthermore, research can be extended to study the influence of physical fields on other EOs, pursuing the same objectives as in this present work or even expanding the range of analyses conducted through UV-VIS, Raman, and NMR spectroscopy, etc. These methods could provide additional insights into the subtle effects of treatments on EOs. Additionally, other bioactivities of EOs, such as anti-inflammatory, immunomodulatory, and antiviral effects, could be explored.

In the field of microbiology, the spectrum of bacterial and fungal strains investigated could be broadened to include strains resistant to conventional treatments, a critical focus in current global research. This would provide a more comprehensive understanding of the antimicrobial effects of energized EOs. Regarding the study of the cytotoxic activity of EOs, it is suggested to include more tumor cell lines and to identify the mechanisms underlying these enhanced effects, particularly those observed following LASER irradiation, which demonstrated the most remarkable influences.

To take the research even further, it would be interesting to test whether these optimized "in vitro" effects can also be maintained "in vivo," both in terms of antibacterial and cytotoxic activities. A promising initial application could be in dermatological practice, where the topical application of EOs is more straightforward and does not involve ingestion, that could lead to their metabolism and the potential alteration of the acquired effects. As investigations expand, new opportunities may arise to better understand and harness the exceptional effects that EOs have on the human body, while also gaining validation from the scientific community.

7. ORIGINALITY AND INOVATIVE CONTRIBUTIONS

This PhD thesis aimed to investigate the influences of magnetic and electric fields, as well as LASER radiation, on essential oils—an innovative topic in itself that has not been explored to the extent of this research before. The literature review revealed that there are only two studies by KIEŁBASA and his team (2022, 2023), which investigated the influences of magnetic fields on tea tree and cedarwood EOs by evaluating their antiseptic effects and optical properties.

Furthermore, the results obtained, especially those related to the enhancement of antimicrobial and cytotoxic activities of certain EOs under specific treatment conditions, open the door to a completely new research direction. This direction could potentially provide answers and solutions to global challenges faced by humanity, such as the increasing incidence of antimicrobial resistance and oncological pathologies.

Additionally, shifting the perspective through which we investigate and analyze the effects of EOs can help us better understand how they act and interact with pathogenic microorganisms and the human body. This understanding supports our evolutionary journey and encourages us to reconsider our relationship with the wonderful and mysterious gifts offered by the plant kingdom.

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