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**STUDIES REGARDING THE BIOLOGICAL
ACTIVITY OF NATURAL PREPARATIONS
BASED ON ESSENTIAL OILS IN ORAL PATHOLOGY**

ABSTRACT

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SUMMARY

Oral diseases such as dental caries and periodontal disease are still major public health problems in most countries. Current scientific concerns are still looking for solutions to improve oral health by preventing the aetiological factors that lead to oral diseases. Although herbal medicine has existed for thousands of years, in recent decades with the advancement of biotechnology in the medical and pharmaceutical fields research into how plants and plant extracts act on the human body has taken a real step forward.

Phytotherapy, also called phytomedicine or botanical medicine, refers to plants, herbal preparations and finished herbal products that include plants as active components. One type of plant extract that has been utilized for centuries to treat a variety of medical and dental issues is represented by essential oils (EOs). These are secondary metabolites that have antibacterial, antifungal, and antioxidant properties and are produced by a variety of plants. Over the years some of these essential oils have proven to be effective in dental medicine presenting biological activities such as antibacterial, antioxidant, analgesic, sedative, anti-inflammatory, spasmolytic, or anesthetic properties.

In recent years however, there has been a growing attempt to revive the use of plants for medical purposes. Some of the reasons for the rise of this trend could be certain side effects of chemical drugs, the lack of effective curative treatment methods for several types of

chronic diseases, microbial resistance, and an extraordinary increase in the investment in pharmaceutical research and development. Numerous medicinal plants are used today in different forms, either prescribed by a doctor or pharmacist, or on one's own initiative. They can be used as complementary medicine, by combining with synthetic medications or independently.

Considering the fact that the use of medicinal plants, especially in the form of essential oil extracts, has gained great momentum in recent years in all medical fields and that dental caries and periodontal disease is still the most prevalent chronic disease, even though they are largely preventable it is important to know the exact implications essential oils have on the oral health as well as to use them following specific medical guidelines according to scientific evidence.

In this thesis the aim was to study the antibacterial potential of essential oils and natural nanoemulsions on bacteria involved in the proliferation of oral diseases, as well as to examine the cytotoxic and/or antiproliferative role of natural compounds on oral and skin cell lines. An other objective was to test natural preparations as a possible preventive or complementary treatment to eliminate biofilm adhesion and formation on fixed orthodontic appliances. The importance of this subject to the research team's international, national, and regional interests stems from the widespread occurrence of dental caries and periodontal diseases. These conditions are prevalent chronic illnesses across various geographical areas and are the primary contributors to dental pain and tooth loss. Consequently, discovering alternative or supplementary approaches for prevention and treatment is of worldwide significance.

The studies covered in this thesis were conducted to investigate the synergistic/antagonistic antibacterial activity of EOs and natural preparation against *Streptococcus mutans* developed in the oral cavity, to examine the main active chemical compounds from natural preparations based on essential oils such as bergamot, orange and clove. Also the studies in this thesis examine the antimicrobial, antifungal, antiproliferative and cytotoxic potential of essential oils in the field of oral health, as well as testing their potential preventive and curative activity on orthodontic archwires.

Hence, data from these studies could pave the way for enhancing strategies in preventive dentistry. This could involve incorporating natural preparations as supplementary oral hygiene products to enhance oral health and overall quality of life, particularly among individuals with fixed orthodontic devices. This is particularly significant given the challenges these patients face in maintaining good oral health.

The thesis will be divided into two main sections.

The general part presents the current state of knowledge. This section additionally furnishes a summary detailing the healing properties of essential oils derived from three plant varieties: bergamot, orange, and cloves. It includes a brief overview describing the role of biofilm in dental caries, periodontal disease, and fungal infections, which are among the most commonly encountered oral diseases. Furthermore, it explores their impact on overall quality of life and underscores the global imperative to discover more effective treatment approaches to reduce them.

The special section will investigate the possible advantages of

employing essential oils derived from clove, bergamot, and orange as potential preventive or therapeutic agents in the field of oral health. This section includes four studies whose results have been published in Clarivate Web of Science indexed journals.

Study 1 covered in chapter 1 focuses on an area of significant interest in preventive dental medicine, specifically exploring the potential use of essential oils (EOs) as a natural alternative to chemical products for inhibiting the growth of *Streptococcus mutans* in the oral cavity to promote oral health. To achieve this goal, we conducted chemical characterization of four essential oils (cinnamon, clove, bergamot, and orange) using gas chromatography coupled with mass spectrometry (GC-MS). Subsequently, we conducted in vitro tests to evaluate their impact on *S. mutans* (ATCC 25175).

The findings from our study demonstrated the antibacterial effects of clove essential oil (CLEO), bergamot essential oil (BEO), and orange essential oil (OEO) on *S. mutans*. These essential oils were then incorporated into natural emulsion-type preparations for potential use in dental medicine. To further understand the synergistic or antagonistic effects arising from the chemical constituents of these essential oils, we prepared binary and tertiary emulsions and tested them in a saliva-enhanced medium against *S. mutans*.

The results from our saliva tests revealed a synergistic effect when using tertiary emulsions containing the active components of the essential oils, leading to the inhibition of *S. mutans*.

Study 2 seeks to investigate the antimicrobial properties of a natural preparation containing clove, orange, and bergamot essential oils against a broad spectrum of human infection-causing microorganisms,

including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Shigella flexneri*, *Candida parapsilosis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, and *Haemophilus influenza*. The research involved the creation, characterization, and assessment of three types of natural preparations: single-component emulsions (clove - ECEO, bergamot - EBEO, and orange - EOEO), three binary combinations (E(BEO/CEO), E(BEO/OEO), E(CEO/OEO)), and a tertiary emulsion E(OEO/BEO/CEO). The aim was to understand the antimicrobial effects and potential synergistic or antagonistic interactions between the essential oil compounds to recommend an optimal antimicrobial preparation.

The findings revealed that the single-component emulsion ECEO exhibited antimicrobial activity, whereas EOEO and EBEO did not inhibit the growth of the tested strains. In the case of binary or tertiary emulsions (E(BEO/CEO), E(CEO/OEO), E(OEO/BEO/CEO)), the antimicrobial effect of clove oil was enhanced due to the synergistic interactions among the chemical components of the essential oils.

In **study number 3**, the primary focus is on examining the impact of natural preparations in the form of emulsions derived from essential oils (EOs) extracted from *Citrus bergamia* (bergamot-BEO), *Citrus sinensis* (orange-OEO), and *Syzygium aromaticum* (clove-CEO) on various types of cells. This investigation encompasses both healthy cells, namely human immortalized keratinocytes (HaCaT) and primary human gingival fibroblasts (HGF), as well as human tumor cell lines, specifically human melanoma (A375) and oral squamous carcinoma (SCC-4) cells. The assessment includes the examination of cell

viability and cellular morphology.

The findings from this research reveal that the CEO emulsion (ECEO) exhibited a dose-dependent cytotoxic effect on both healthy (HaCaT and HGF) and tumor (A375 and SCC-4) cells. The OEO emulsion (EOEO) increased cell viability percentages for both HaCaT and A375 cells, while it had an antiproliferative impact at the highest concentration on HGF and SCC-4 cells. The BEO emulsion (EBEO) reduced the viability percentage of SCC-4 tumor cells. When combining OEO with CEO in a binary mixture within an emulsified formulation, there was an enhanced inhibition of tumor cell viability. The E(BEO/OEO) binary emulsion demonstrated an antiproliferative effect on oral health and tumor cells, with minimal impact on skin cells.

Furthermore, non-invasive tests conducted to assess the safety of these emulsions on the skin indicated that these compounds do not significantly alter the skin's natural parameters and can be considered safe for human skin.

The final **study number 4**, the objective was to investigate the impact of natural preparations containing essential oils (EOs) on preventing the adhesion of biofilm on orthodontic devices. This research involves the use of five distinct orthodontic archwire materials, each possessing identical dimensional characteristics: aesthetic tooth-colored epoxy coated stainless steel, copper nickel alloy (Cu-NiTi), titanium molybdenum alloy (TMA), stainless steel (SS) and nickel-titanium alloy (NiTi).

The experiment had two distinct phases using essential oils (EOs):

In the preventive approach, orthodontic archwires were immersed in natural preparations created as outlined in subchapter 1.2.5. These wires

were then placed into a culture medium containing *Staphylococcus* or *Streptococcus* strains to observe their preventive effects.

In the therapeutic approach, orthodontic archwires contaminated with bacteria were treated with emulsions containing EOs, and the outcomes were monitored over various time intervals.

The study's second objective aimed to investigate the interaction between essential oil compounds and crucial proteins found in pathogenic bacteria like *S. aureus* and *S. mutans*, which are known to develop in the oral cavity. To achieve this, molecular docking was employed as a rational, computer-based tool. Its purpose was to confirm findings from traditional laboratory experiments and expedite the comprehension of potential interactions between the identified oil compounds and the proteins within these bacteria, particularly the key proteins responsible for their pathogenicity.

The findings from study 4 indicate that orthodontic appliances made from aesthetic coated steel are the most effective materials in terms of antimicrobial protection against *Streptococcus* strains, with a maximum inhibition rate of 28.82% observed on SS aesthetic appliances. However, when it comes to preventive measures against *Staphylococcus*, only Titanium molybdenum alloy (TMA) orthodontic archwires displayed inhibition effects for all tested emulsions, with a maximum inhibition rate of 29.44%. On the other hand, CuNiTi-based alloys did not show suitability for preventive treatments using essential oils, as neither *Staphylococcus* nor *Streptococcus* bacterial growth was inhibited for any of the EO mixture combinations. Alloys based on stainless steel (SS) exhibited a good protective effect against *Streptococcus*, with positive inhibition rates observed for both single-component preparations and binary and tertiary

mixtures. However, they were not found to be effective for preventing contamination with *Staphylococcus*.

In terms of treating orthodontic archwires after they were contaminated with *Streptococcus* and *Staphylococcus* strains, the tertiary emulsion demonstrated the highest effectiveness among four out of the five tested archwire materials. The exception was the CuNiTi-based archwire, which still exhibited low inhibition rates for both strains (*Streptococcus* inhibition rates ranged from 4.24% to 7.69%, and *Staphylococcus* inhibition rates ranged from 6.50% to 12.93%).

The study confirmed that combining various chemical components from the three analyzed essential oils leads to synergistic antimicrobial effects, which collectively enhance their ability to inhibit bacterial growth in the oral cavity.

Taking into account the outcomes observed for both bacterial strains and the five different orthodontic arch materials examined, our recommendation is to opt for either aesthetic stainless steel (SS) or Titanium molybdenum alloy (TMA) archwires when taking into account plaque accumulation during orthodontic treatment. As preventive measure during orthodontic treatment, we suggest utilizing the tertiary mixture of bergamot (BEO), clove (CEO), and orange (OEO) essential oils, as it offers the optimal choice for achieving the highest level of antibacterial protection.

In summary, the results of this thesis affirm the antibacterial capabilities of natural preparations containing bergamot, clove, and orange essential oils, along with their individual chemical components, against certain microorganisms relevant to oral pathology. Moreover, their effectiveness becomes even more pronounced when these natural

preparations are used together, exhibiting a synergistic impact in preventing plaque accumulation on various types of orthodontic archwires.

Ultimately, this doctoral thesis serves as a launching point for exploring new research avenues in the evaluation of additional essential oils (EOs) and their combinations, along with the development of other natural preparations designed for both preventive and therapeutic roles in addressing periodontal diseases and dental caries.

Given the established antimicrobial and antiproliferative properties of the examined essential oils and emulsions against specific microorganisms and various cell lines, future investigations should be directed towards understanding their inhibitory potential against other microorganisms involved in caries development and the proliferation of oral infections. This research should also encompass the exploration of their effects on different cell lines relevant to oral pathologies.

Furthermore, the focus should shift towards identifying optimal solutions for excipients and methods of marketing essential oils in oral hygiene products such as mouthwashes and toothpaste, making them available to the general population for use according to specific medical guidelines in line with current scientific evidence.