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Evaluation of The Anti-Adherent Activity of *Thymus vulgaris* Essential Oil Against *Enterococcus faecalis* Strain

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ABSTRACT

The failures of endodontic treatments are consolidated, in part, by the inability to neutralize the existing microbiota in the canal system. *E. faecalis* can survive in harsh environmental conditions, which gives it advantages over other species. Thus, there is a great concern in the scientific sphere to solve this gap, which aims to find an effective method to combat these microorganisms. Thus, the present work aims to evaluate the anti-adherent activity of *Thymus vulgaris* essential oil against *Enterococcus faecalis* strain. A methodological assay was carried out employing the inclined tube technique to determine the Minimum Inhibitory Concentration of Adherence (MICA) to glass, in the presence of 5% sucrose. Thus, after performing the experiment, no MICA value of the essential oil against the tested representative strain was found at the concentrations tested in this methodology, compared to the MICA of 1:1 shown by 0.12% chlorhexidine digluconate, used as a positive control of the research. Thus, it was possible to conclude that the essential oil of *Thymus vulgaris* showed no anti-adherent activity against the representative strain tested, in the methodology applied.

INTRODUCTION

Endodontic treatment aims to significantly reduce microorganisms and their by-products from the interior of the root canal system and its branches, in order to prevent subsequent reinfection. The chemical-mechanical preparation and the use of intracanal medications substantially reduce the number of microorganisms inside the root canals, however, an effective elimination is practically impossible in all cases (1,2).

The infection of the root canal system occurs as a result of the multiple activities of microorganisms, among which Gram-positive, Gram-negative, facultative aerobic, or strict anaerobic bacteria (3). Fungi and viruses can also be found (4). Thus, *Enterococcus faecalis* is one of the most frequent microorganisms in endodontic alterations, especially regarding secondary infections and the appearance of periradicular lesions (3,4).

E. faecalis is an opportunistic pathogen and a major cause of nosocomial infections, it is also frequently found in roots where retreatment has failed (5,6). It presents in the form of Gram-positive, facultatively anaerobic coccus, usually detectable in persistent asymptomatic endodontic infections (5-7).

E. faecalis can survive in harsh environmental circumstances, which gives it advantages over other species. This fact may explain its persistence in endodontic infections where nutrient sources are scarce. This microorganism assumes a prominent place in cases of failure of endodontic therapy (8).

Besides *E. faecalis* being resistant to chemical and mechanical endodontic treatment, it has the ability to form biofilms (9, 10) which reduce the penetration of drugs, decreasing their effectiveness. Biofilms are complex three-dimensional structures of microorganisms embedded in an extracellular matrix, composed of glycoproteins and polysaccharides, adhered to a solid surface. These constitute an important bacterial adhesion system with great clinical relevance due to their high resistance to antimicrobials (11).

Albuquerque *et al*. (2015) (12) demonstrated that *E. faecalis* has the ability to organize into biofilm on the root dentin of human teeth. After five days of inducing biofilm formation, the microorganisms formed a thick biofilm layer and were able to penetrate the dentinal tubules.

These data suggest that biofilm formation in root dentin may play a significant role in endodontic infections.

The failures of endodontic treatments are consolidated, in part, by the inability to neutralize the existing microbiota in the canal system (13,14). Thus, there is a great concern in the scientific sphere to solve this gap, aiming to find an effective method to combat these microorganisms present in the canal system, since to achieve successful treatment, it is necessary for the satisfactory elimination of microorganisms within the canal or at least a significant reduction of this microbiota (15,16,10).

Nowadays, science recommends the development of new treatments that are less cytotoxic and more effective in smaller and smaller doses, in order to avoid the emergence of super-resistant microorganisms and reduce the probable damage of the medications in use (17-19).

Thus, the increasing use of compounds from vegetable species has been shown as a viable alternative for popular use, for its low cost, rare side effects, if correctly used, and for the appropriate antimicrobial and anti-inflammatory effect (20-22).

Natural products present greater antimicrobial activity when in the formulation of essential oils, justified by the higher concentration of active ingredients and the lipidic nature of the substance. Essential oils are mixtures of liposoluble and volatile compounds that can easily diffuse through the cell membranes of microorganisms, resulting in increased permeability of the membranes, and generating electrolyte imbalance and cell death (23,24). Furthermore, they can interfere with the membrane's electron transport function, nutrient uptake, enzyme activity, protein, and nucleic acid synthesis (25).

Aromatic medicinal plants possess essential oils, which have in their constitution a mixture of lipophilic, odorous, and volatile metabolites produced by special cells found in the various parts of plants, such as leaves, flowers, seeds, stems, and roots. The constituents of essential oils are produced by the secondary metabolism of plants and among the major classes of compounds are monoterpenes, sesquiterpenes, and phenylpropanoids (26). Monoterpenes are the most representative constituents of essential oils, constituting 90% of them, and have several described pharmacological activities, such as analgesic, anti-inflammatory, antidepressant, and anticonvulsant, among others (27).

Thymus vulgaris L., popularly known as thyme, is a plant belonging to the Lamiaceae family, native to Europe and cultivated in southern and southeastern Brazil. The leaves and flowers are used for consumption as a condiment and are also used in the process of essential oil extraction (28). Thyme essential oil has more than 60 compounds, and many of them have antimicrobial and antioxidant properties for a wide group of bacteria, Gram-positive or Gram-negative (29).

Boruga *et al*. (2014) (30) postulated that the antimicrobial activity of essential oils depends on their chemical constituents. Apparently, the antimicrobial activity of thyme essential oil is related to the presence of phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene), respectively.

The terpinene gamma (1-methyl-4-isopropylcyclohexadiene-1,4) is a monoterpene present in several pharmacologically active plant species, for example, in the essential oils of *Thymus vulgaris* L., *Origanum onites* L., among others. In γ-terpinene, the presence of unsaturation in its cyclic chain structure confers the olefin characteristic, which allows easy absorption across biological membranes, in view of its liposoluble characteristic (31).

Thus, this plant extract's biological properties should be analyzed to investigate new possibilities for constituting irrigating solutions. The general objective of this work is to evaluate the antibacterial and anti-adherent activity of *Thymus vulgaris* essential oil against *Enterococcus faecalis* strain; having a specific objective to determine the Minimum Inhibitory Adherence Concentration of *Thymus vulgaris* essential oil against *Enterococcus faecalis* strain.

METHODOLOGY

YEAR AND PLACE OF THE STUDY

The laboratory tests were performed in the Microbiology and Biochemistry laboratories of the Federal University of Campina Grande, Patos campus (CSTR), Paraíba state - Brazil, between the years 2021-2022.

TEST SUBSTANCE AND MICROORGANISMS

The essential oil of Thymus vulgaris was purchased from Indústria Quinari ® (Ponta Grossa - PARANÁ). To perform the pharmacological tests, the substance was solubilized in DMSO and diluted in distilled water. The concentration of DMSO (dimethylsulfoxide) used is less than 0.1% v/v. The project is following the rules of CGEN-Conselho de Gestão do Patrimônio Genético, registered in the SISGEN platform under protocol number A9BE23A. The following strain of Enterococcus faecalis (ATCC-29212) was used and maintained on Muller-Hinton Agar (MHA) at 4°C. Bacterial inocula were obtained from overnight cultures on MH (Muller Hinton) at 37°C and diluted in sterile saline to obtain the final concentration of approximately 1.5 x 10^8 colony forming units per ml (CFU/ml), adjusted for turbidity by comparing with the 0.5 tube of the McFarland scale (32).

CULTURE MEDIA

The culture media used in the assays to evaluate the antimicrobial activity were Muller Hinton Liquid Medium (MH) and Muller Hinton Agar Solid Medium (MHA). The culture medium was purchased from Difco® and prepared according to the manufacturer's instructions.

DETERMINATION OF THE MICA (MINIMUM INHIBITORY CONCENTRATION OF ADHERENCE)

The Minimum Inhibitory Concentration of Adherence (MICA) of thyme essential oil was determined in the presence of 5% sucrose according to Albuquerque *et al*. (2010) (33) with modifications, using concentrations corresponding to the compound up to 1:1024 dilution. Starting with bacterial growth, the *Enterococcus faecalis* strain was grown at 37°C in Mueller Hinton broth (DIFCO, Michigan, United States), then 0.9 ml of the subculture was dispensed into test tubes and then 0.1 ml of the solution corresponding to the essential oil dilutions was added. Incubation was performed at 35-37°C for 24 hours with tubes tilted at 30°. The reading was performed by visual observation of the bacteria adherence to the walls of the tube, after shaking it. The assay was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). The MICA was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

RESULTS AND DISCUSSION

In the literature, two possible mechanisms of action have been indicated to explain the biological properties of essential oils. All methods are associated with the hydrophobicity of monoterpenes and sesquiterpenes, which are their main chemical constituents. The hydrophobicity of terpenoids would allow these compounds to penetrate cell membranes easily, causing the death of microorganisms by damaging their metabolic organelles. However, considering the large number of chemical constituents and synergistic or antagonistic interactions between these compounds, essential oils can also act on specific targets of cell membranes, such as lipids and proteins (24).

According to some authors, Gram-positive bacteria, such as *E. faecalis*, are more sensitive to the action of essential oils because their cell membrane favors the penetration of hydrophobic compounds (34,35).

With regard to the anti-adherent activity, with results shown in table 1, it was observed that with the methodology used the essential oil of *Thymus vulgaris* was not efficient in inhibiting the adherence of the representative strain of *Enterococcus faecalis* ATCC-29212 to glass in any of the concentrations tested, when compared to the results of the positive control of 0.12% chlorhexidine digluconate, whose concentration of up to 1:1 was able to inhibit bacterial adherence.

Table 1. Minimum Inhibitory Concentration of Adherence (MICA) of *Thymus vulgaris* essential oil and 0.12% Chlorhexidine Digluconate against *Enterococcus faecalis* bacterial strain.

Concentration in µg/mL	*Thymus vulgaris*	0,12% chlorhexidine digluconate
1:1	-	+
1:2	-	-
1:4	-	-
1:8	-	-
1:16	-	-
1:32	-	-
1:64	-	-
1:128	-	-
1:512	-	-
1:1024	-	-

Legend: (+) Without adhesion to the tube wall (-) With adhesion to the tube wall

Source: Research data.

In contrast, although it was not possible to find MICA values in the literature against *Enterococcus faecalis* strains tested using this same methodology, another study such as that of Mohsenipour and Hassanshahian (2015) (36) found that the ethanolic extract of thyme was able to destroy the biofilm and inhibit the metabolic activity of its bacteria at different concentrations. The greatest inhibition of biofilm formation was observed against *Streptococcus pneumoniae*; when it came to the decrease in metabolic activity of biofilm bacteria, the greatest reduction was seen in *Staphylococcus aureus*.

In another research conducted by Kavanaugh and Ribbeck (2012) (37), it was reported that discs impregnated with *Thymus vulgaris* essential oil showed remarkable antibiofilm potential against bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It also confirmed that the inhibitory effect of this oil on the planktonic form of these bacteria was more efficient than some antibiotics such as ampicillin and gentamicin.

Regarding MICA, despite not obtaining promising results with the methodology applied here, it is worth pointing out that this unsatisfactory result does not reduce the importance of this research for the scientific community. Once it can be used as a reference base for future academic work.

CONCLUSION

The essential oil of *Thymus vulgaris* proved ineffective in inhibiting the adherence of the tested representative strain of this pathogen on the test tube wall by means of the methodology used. However, further studies are essential to emphasize its efficacy against different types of microorganisms and, subsequently, in vivo research to verify its behavior in the human organism.

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