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Evaluation of The Anti-Adherent Activity of *Thymus vulgaris* Essential Oil Against *Escherichia coli* Strain

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ABSTRACT

The microbial diversity in the oral cavity is notorious, several microorganisms, mainly bacteria, integrate a complex structure called biofilm. The development of this complex matrix promotes the emergence of odontogenic infections, such as, for example, endodontic infections. Among the microorganisms involved in this process is *Escherichia coli* (*E. coli*), a gram-negative bacterium present especially in cases of secondary endodontic infections. In endodontic treatments, the Chemical Mechanical Preparation (CMP) is responsible for sanitizing the Root Canal System (RCS), however, the microbial resistance exhibited by *E. coli* and its products to these protocols potentiate the host's immune system reactions, worsening its overall health status. Thus, the present study aims to evaluate the anti-adherent activity, from the determination of the Minimum Adhesion Inhibitory Concentration (MICA). The methodology used investigates the anti-adherent potential by determining the MICA, carried out according to the sowing technique in inclined glass tubes, in the presence of 5% sucrose, using concentrations corresponding to the pure *Thymus vulgaris* essential oil up to dilution 1:1024. The MICA was considered the lowest concentration of essential oil capable of preventing the adherence of the E. coli biofilm formed in the glass tube. All experiments were performed in duplicate. Therefore, it was found that the MICA of *Thymus vulgaris* essential oil against the representative strain of *E. coli* 110 was not identified at the concentrations tested in this methodology, compared to the MICA of 1:8 shown by 0.12% chlorhexidine digluconate, used as a positive control. Taking this into account, it is concluded that the essential oil of *Thymus vulgaris* has a strong antibacterial activity and no anti-adherent activity against *E. coli* strain tested in this methodology.

INTRODUCTION

The term "human microbiome" was adopted by the Human Microbiome Project (HMP) initiative, which began 2008 and concluded in 2016, and is currently used to designate the wide variety of microbial species that inhabit our body[1]. In the oral cavity, specifically, we have the "oral microbiome" that consists of an agglomeration of microorganisms, such as viruses, fungi and bacteria, integrating a complex ecological environment with more than 700 recognized species[2].

Soon after birth, the process of oral colonization begins through the adherence of pioneer microorganisms to oral surfaces. This primary colonization gives rise to changes in the oral environment due to the production and excretion of metabolites, enhancing the growth of other subsequent strains. Thus, as age advances, microbiological diversity and complexity increases, giving rise to highly diverse ecosystems in adulthood[2]. The literature reports that among the main bacterial genera present in the oral cavity of healthy individuals are: *Streptococcus*, *Actinomyces*, *Veillonella*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Treponema*, among others[3].

The colonization of oral microflora occurs mainly on dental elements, the multi-species cell aggregates, immersed in a polymeric matrix, adhered on a surface, are called biofilm. The biofilm development occurs through a dynamic coaggregation, initially there is primary microbial adhesion and subsequently secondary bacterial multiplication. As a result of this process occurs its maturation, playing relevant roles in the maintenance of oral and systemic health and disease[4,5].

For biofilm control, mechanical techniques are used, such as tooth brushing and flossing, associated with chemical control methods such as the use of fluoride dentifrices and mouth rinses, the combination of these resources is widespread and efficient in the prevention and control of dental caries, periodontal disease, endodontic infections, among other oral pathologies, when properly guided by the dentist and well performed by patients[6].

Pulpal pathologies, generally, come from the ingression and microbial proliferation in the pulp cavity arising from carious processes, recent research estimates that more than 600 species are involved in the process. Endodontic infections can be classified as primary, caused by microorganisms commonly found in the oral cavity (*Porphyromonas*, *Streptococcus*); secondary, inserted into the root canal after endodontic intervention during treatment sessions (*Escherichia coli*, *Enterococcus faecalis*); or persistent, resulting from endodontic treatment failures (*Enterococcus faecium*, *Staphylococcus epidermidis*)[7,8,9,10,11,12].

Escherichia coli (*E. coli*) is a bacillus coccus-shaped bacterium from the Enterobacteriacea family, characterized as gram-negative, facultative anaerobic, non-sporulated, glucose fermenting and widely found in the human gastrointestinal tract, with high adhesion capacity and colonization of surfaces, responsible for numerous systemic diseases. It can be transmitted through drinking water, contaminated food and feces, and is capable of killing more than 2 million humans per year through intraintestinal and extraintestinal diseases[13,14,15,16,17,18].

In dentistry, it has been found to be present in secondary endodontic infections that originate after professional intervention and with access to root canals during treatment due to breaks in the aseptic chain, or in root canals with periapical lesions. The most worrying factor arising from the presence of these bacteria is their characteristic of having an endotoxin in the outer cell membrane with high virulence potential, whose lipid structure is responsible for the toxicity of lipopolysaccharide (LPS), a major motivator of excessive immune and inflammatory responses in patients [14,15,16, 11,19].

According to Brito (2010)[20] and Marinho (2012)[21], the antimicrobial chemicals (sodium hypochlorite and chlorhexidine digluconate) and instruments used in the chemical mechanical preparation (PQM) of the root canal system (RCC) have shown some effectiveness in endodontic treatment. However, hypochlorite has shown low or no effectiveness *in vitro* and *in vivo* in killing endotoxins (LPS), as well as causing periapical tissue toxicity and allergic reactions in patients. Chlorhexidine has shown inability to dissolve organic matter, dental staining, and toxicity at high concentrations. Regarding antibiotic therapy, sometimes associated with PQM, *E. coli* has shown to be resistant to ciprofloxacin, sulfamethoxazole, norfloxacin and ampicillin[22,23,24,25,26,27].

The habit of using flora-derived components as a treatment method dates back to ancient times. As stated by the World Health Organization (WHO), three quarters of the global public make use of plants as a preventive and therapeutic resource for numerous pathologies[28,29]. Among the most commonly used substances for medicinal purposes are essential oils, included on the GRAS (Generally Recognized as Safe) list of the Food Drug Administration (FDA), that is, there are no complications involving toxicity associated with these substances. Added to this, they are accessible, biocompatible compounds with antimicrobial, anti-inflammatory and antioxidant properties, which makes them promising sources for the generation of new drugs[30,31,32,33].

Thymus vulgaris, popular thyme, is a species native to Europe, widely cultivated in the south and southeast regions of Brazil. The *Thymus vulgaris* widely used in gastronomy and traditional medicine, its essential oil has antimicrobial, anti-inflammatory, antioxidant actions, among others[34,31,35,36,37]. Among its main components are thymol, p-cymene and carvacrol, the major responsible for its antimicrobial potential against several gram-positive and gram-negative bacterial species[38].

Therefore, the general objective of this work is to evaluate the anti-adherent activity of *Thymus vulgaris* essential oil against *Escherichia coli* strains; and as specific objectives: to determine the minimum adherence inhibitory concentration of *Thymus vulgaris* essential oil against the representative strain of *Escherichia coli* 110.

METHODOLOGY

YEAR AND PLACE OF THE STUDY

The laboratory tests were performed at 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

The essential oil of *Thymus vulgaris* was purchased from Indústria Quinari ® (Ponta Grossa - PR). For the pharmacological tests, the essential oil of *Thymus vulgaris* was solubilized in DMSO and diluted in distilled water. The concentration of DMSO (dimethylsulfoxide) used was less than 0.1% v/v. The project followed the rules of CGEN - Genetic Heritage Management Council, registered in the SISGEN platform under protocol number A7A6B17.

MICROORGANISM

The bacterial strain of *Escherichia coli* EC 110, was maintained on MuellerHinton (MH) agar at 4°C. The inoculum was obtained from overnight MH cultures at 37°C and diluted in sterile saline to obtain a final concentration of approximately 1.5 x 10^8 colony forming units per mL (CFU/mL), adjusted for turbidity against 0.5 McFarland tube scale[39].

DETERMINATION OF MICA (MINIMUM ADHESION INHIBITORY CONCENTRATION)

The Minimum Adhesion Inhibitory Concentration (MICA) of the compound was determined in the presence of 5% sucrose, according to Albuquerque *et al* (2010)[40] with modifications, using concentrations corresponding to the essential oil of *Thymus vulgaris* up to dilution 1:1024. After bacterial growth, the *Escherichia coli* strain was grown at 37°C in Mueller Hinton broth (DIFCO, Michigan, USA), then 0.9 mL of the subculture was dispensed into test tubes, and then 0.1 mL of the solution corresponding to the dilutions of *Thymus vulgaris* essential oil was added. The tubes were incubated at 35° - 37ºC for 24 hours with the tubes inclined at 30º. The reading was done by visual observation of the adherence of *Escherichia coli* strain to the tube walls, after shaking the tube. The test was performed in duplicate. The same procedure was performed for the positive control, with 0.12% chlorhexidine digluconate (Periodefense TM Oral-B). The MICA was considered the lowest concentration of *Thymus vulgaris* essential oil in contact with sucrose that prevented the adherence of *Escherichia coli* strain to the glass tube.

RESULTS AND DISCUSSION

Regarding the anti-adherent activity, with results shown in table 1, it was observed that the essential oil of *Thymus vulgaris* was not efficient in inhibiting the adherence of the representative clinical strain of *Escherichia coli* (110) to glass in any of the concentrations tested in the methodology used, when compared to the results of the positive control of 0.12% chlorhexidine digluconate, whose concentration of up to 1:8 was able to inhibit bacterial adherence to glass.

Table No. 1: The Minimum Adhesion Inhibitory Concentration (MICA) in µg/ml of ***Thymus vulgaris*** **essential oil and 0.12% Chlorhexidine Digluconate against the representative clinical strain of** ***Escherichia coli*** **110.**

Concentration in µg/mL	*Thymus vulgaris*	**Chlorhexidine Digluconate 0.12**
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 tube wall.

The anti-adherent activity of essential oils has been evaluated in the same proportion as the antimicrobial activity, mainly due to the adverse effects related to the use of chlorhexidine digluconate, the gold standard chemical agent used as an adjuvant for biofilm control. A research conducted by Caceres and Colleagues (2020)[37], where the anti-adherent activity of 17 essential oils was evaluated, including *Thymus vulgaris*, against more than one strain of *Escherichia coli* and other microorganisms, concluded that this oil has a high antibiofilm potential against these bacteria. It is worth noting that the research in question employed a different methodology than the one used in this study.

Another study by Kowalczyk *et al*. (2020)[41], obtained positive results regarding the anti-adherent activity, confirming the broad spectrum of biological and therapeutic actions of thymol (main component of *Thymus vulgaris* essential oil) and thyme essential oil (*Thymus vulgaris*) against several microorganisms, including the antibiofilm effect against *Escherichia coli*, but giving up studies with methodologies different from the one used here.

Moreover, Martinez *et al.* (2021)[42], using modified microdilution techniques, with substances and concentrations different from those used in this research, concluded that the essential oils of *Thymus vulgaris*, as well as that of L. origanoides (Thymol/carvacrol chemotype, LTC II), have antibacterial and antibiofilm activities against *E. coli* ATCC 25722 and *S. aureaus* ATCC 29213.

Regarding the MICA, this study did not obtain promising results with the methodology applied here in any of the tested concentrations of *Thymus vulgaris* essential oil against the clinical strain of *Escherichia coli* (110), compared with the 0.12% chlorhexidine digluconate, which inhibited the adherence of bacteria in a concentration of 1:8. Nevertheless, it is worth noting that the unsatisfactory MICA results do not reduce the importance and uniqueness of this research for the scientific community. Since it can be used as a reference base for future studies in the area.

CONCLUSION

Thymus vulgaris essential oil proved ineffective in inhibiting the adherence of the representative clinical strain of *Escherichia coli* 110 to the test tube glass in the methodology used. However, further studies are essential to emphasize its effectiveness against different types of microorganisms, mechanisms of action, and subsequently in vivo investigations to verify its behavior in the human body.

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