

Antibacterial Potential of Chitosan Scaffolds Incorporated with Oregano and Thyme Essential Oils against *Escherichia coli*

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ABSTRACT

The aim of this study was to evaluate the antibacterial potential of chitosan-based scaffolds associated with the essential oils of *Origanum vulgare* L. and *Thymus vulgaris* L. against a strain of *Escherichia coli*. The antibacterial potential was assessed by reading the inhibition halos formed around the chitosan-based scaffolds incorporated with thyme EO, oregano EO and thyme and oregano EO against the *Escherichia coli* strain, using the Mueller Hinton agar diffusion methodology per well. To do this, Mueller Hinton Agar was used as the culture medium in Petri dishes, and holes 6 mm in diameter were formed using a mold to create the wells. The plates were inoculated with the microorganism using a swab, then incubated in a bacteriological oven at 37 °C for 24 hours. Scaffolds, in the form of disks, were aseptically deposited in the wells of the inoculated media, which were incubated at 37 °C for 24 hours. After incubation, the plates were observed for homogeneity of bacterial growth and the diameter of the inhibitory halo was measured using a millimeter ruler in cases where inhibition of bacterial growth was verified. The tests were carried out in duplicate and the results correspond to the average values. The association of chitosan with *Origanum vulgare* L. and *Thymus vulgaris* L. EOs was found to have antibacterial activity against the *Escherichia coli* strain. For the scaffolds incorporated with *Origanum vulgare* L. EO, *Thymus vulgaris* L. EO and associated *Origanum vulgare* L. and *Thymus vulgaris* L. EOs, 16 mm inhibition halos were formed against the *Escherichia coli* strain. The association of chitosan with the EOs of *Origanum vulgare* L. and *Thymus vulgaris* L. has antibacterial activity, acting as a bactericide against the *Escherichia coli* strain tested.

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1. INTRODUCTION

The oral cavity has a wide microbial density, ranging from bacteria, fungi, viruses to protozoa, which interact with each other and with the host, performing specific functions in the health-disease process [1]. The cooperative union of bacteria results in the formation of a complex matrix with the potential to adhere to both biotic and abiotic surfaces, which can be called a biofilm, made up

of organic components originating from these microorganisms or acquired from the environment in which they are established [2].

It is common knowledge that oral infections are the main reason for dental consultations and interventions, with biofilm formation being the first stage in establishing these disorders [3]. Changes in the ecology of the bacteria that make up the biofilm, as well as in the population proportion of this microbiota, promote dysbiosis and favor



the emergence of simple or even complex pathological states [4].

Polymicrobial dysbiotic biofilms can be more tolerant to antimicrobial drug treatments and resistant to the immune responses of the host organism, allowing the survival and persistence of some bacteria, such as *Escherichia coli*, which has a potential relationship with infections in oral wounds, periodontitis, peri-implantitis and infections in the root canal system [5]. Therefore, the search for alternatives to control resistant microorganisms has aroused great scientific interest, expanding the use of biomaterials and herbal medicines in healthcare [6].

Biomaterials are devices that are compatible with organic tissues and can be of natural or synthetic origin, used in healthcare for diagnostic and/or therapeutic purposes [7]. Among the biomaterials is chitosan, a linear biopolymer with anti-inflammatory and antimicrobial action, derived from chitin and a possible candidate for application in the treatment of infectious and inflammatory oral diseases [8].

Thus, chitosan shows good prospects for dental applications in periodontal infections, endodontic treatments, adhesion of restorative materials to the dental substrate, tissue repair, hemostasis, formation of odontoblastic cells in dentin-pulp complex treatments and wound healing [9]. In addition, this biomaterial can be used to complement and improve the properties of currently commercial suture thread coatings, reducing post-operative infections and accelerating the healing process in oral and maxillofacial surgeries [10].

To the detriment of the slow and continuous biodegradation of the chitosan molecule, synthetic and plant drugs can be incorporated into this biopolymer to act in the process of tissue repair, hemostasis, analgesia, as well as other properties [11]. Among the plant drugs that can be associated with biopolymers in order to enhance their effects are essential oils, which are natural, complex, liquid and volatile compounds obtained as products of the secondary metabolism of aromatic plants, which have components with numerous pharmacological properties, including antimicrobial activity [12], [13].

Among the mechanisms of action of essential oils, most are believed to act on the cell membrane and the microbial cell wall [14]. Some compounds, such as terpenes, are capable of interacting with bacterial cells and promoting bacteriostatic or bactericidal activity due to their hydroxyl groups, as well as being responsible for antifungal, antiparasitic, antioxidant, anti-inflammatory and antitumor effects [15], [16].

Thymus vulgaris L., popularly known as thyme, belonging to the Lamiaceae family, is an aromatic medicinal herb widely used in the food industry as a spice and which has phenolic compounds such as thymol, carvacrol and p-cymene [17]. Similar to thyme, *Origanum vulgare* L., popularly known as oregano, also belongs to the Lamiaceae family and has numerous bioactive pharmacological substances with therapeutic potential [18].

Thus, the essential oils of oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) are natural substances that are attracting increasing scientific interest due to their monoterpene phenol isomers: carvacrol (which has a light

yellow color, a pungent and aromatic odor) and thymol (a white substance with an aromatic odor and an acid taste), which exert antimicrobial activity, damage lipid plasma membranes, interfere with pH homeostasis and the balance of organic ions, compromise cell division and promote dehydration in bacterial cells [1]. Therefore, in order to increase the antimicrobial action, the association of essential oils with chitosan is considered relevant, since they have numerous pharmacological properties, as well as being biocompatible and biodegradable [19].

In this context, the aim of this study was to evaluate the antibacterial potential of chitosan-based scaffolds associated with the essential oils of *Origanum vulgare* L. and *Thymus vulgaris* L. against a strain of *Escherichia coli*.

2. METHODOLOGY

2.1. Chitosan Scaffolds

The pure chitosan and chitosan scaffolds associated with the essential oils of thyme and oregano used in this work came from the study by Silva [20]. These scaffolds were made using the freeze-drying process obtained from adaptations by Cruz et al. [21].

2.2. Bacterial Species and Culture Medium

The microorganism used in this study was the *Escherichia coli* strain (CCCD-E003) from the Microbiology Laboratory of the Biological Sciences Academic Unit (UACB) of the Center for Rural Health and Technology (CSTR) of the Federal University of Campina Grande (UFCG). The strain was maintained on Muller Hinton Agar (MHA) at a temperature of 4 °C, and 24-hour replicates on MHA incubated at 35 °C were used for the tests. For the antibacterial activity study, a bacterial inoculum of approximately 1.5×10^8 CFU/mL was used, standardized according to the turbidity of the 0.5 tube of the McFarland scale [22], [23].

2.3. Evaluation of the Antibacterial Activity of Chitosan Scaffolds Incorporated With Essential Oils

Microbiological tests were carried out to determine whether chitosan scaffolds incorporated with thyme EO, oregano EO and thyme and oregano EO are capable of inhibiting the growth of *Escherichia coli* bacteria, using the agar diffusion method per well.

The test technique was developed according to Bona et al. [24], with modifications. For this purpose, Mueller Hinton Agar was used as the culture medium in Petri dishes, and holes 6 mm in diameter were formed using a mold to create the wells. The plates were inoculated on the surface by the microorganism using a swab, then incubated in a bacteriological oven at 37 °C for 24 hours.

Disc-shaped scaffolds were aseptically placed in the wells of the inoculated media and incubated at 37 °C for 24 hours. After incubation, the plates were observed for homogeneity of bacterial growth and the diameter of the inhibitory halo was measured using a millimeter ruler in cases where inhibition of bacterial growth was found. The tests were carried out in duplicate and the results correspond to the average values.

TABLE I: EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF CHITOSAN-BASED SCAFFOLDS INCORPORATED WITH THE ESSENTIAL OILS OF *ORIGANUM VULGARE* L. AND *THYMUS VULGARIS* L. AGAINST *ESCHERICHIA COLI*

Chitosan scaffolds with and without the incorporation of the essential oils of <i>Origanum vulgare</i> L. and <i>Thymus vulgaris</i> L.	Diameter of the inhibition halo against <i>Escherichia coli</i> CCCD-E003
Chitosan (control)	16 mm
QOEO 3%	16 mm
QOEO 4%	16 mm
QOEO 5%	16 mm
QOET 3%	16 mm
QOET 4%	16 mm
QOET 5%	16 mm
QOET 6%	16 mm
QOET 8%	16 mm
QOET 10%	16 mm

Key: QOEO: Chitosan incorporated with *Origanum vulgare* L. essential oil QOET: Chitosan incorporated with *Thymus vulgaris* L. essential oil QOET: Chitosan incorporated with *Origanum vulgare* L. and *Thymus vulgaris* L. essential oils. Source: Author's collection (2023).

3. RESULTS AND DISCUSSION

The increase in bacterial resistance to currently marketed drugs has encouraged the search for new therapeutic alternatives to combat bacterial infections, whether local or widespread [12]. Among the main alternatives that have shown promising results in tackling this problem are phytotherapy and the use of chitosan-based biomaterials. These options stand out because they are less expensive, more accessible, have a natural composition and fewer side effects [9], [11]. In this context, the association of natural products that have pharmacological properties with natural biopolymers that aim to replace and/or treat some component of the body is of substantial relevance to dentistry [25], [26]. This study evaluated the antibacterial activity produced by the interaction between oregano and thyme essential oils and chitosan-based scaffolds against a strain of *Escherichia coli*.

3.1. Study of the Association of Scaffolds with and without the Incorporation of Essential Oils against *Escherichia coli*

From the diffusion bioassays on Muller Hinton Agar using the diffusion per well method, it was found that both the pure biopolymer (control group) and the biopolymer incorporated with the plant drugs tested against the CCCD-E003 strain inhibited its growth, with the formation of inhibitory halos up to 16 mm in diameter around the wells where the scaffold discs were deposited. In this way, all the chitosan scaffolds evaluated, with and without the plant drug association, had an antibacterial effect against the *Escherichia coli* strain. Table I illustrates the measurements of the typical halos of inhibition of bacterial growth produced by the action of pure chitosan scaffolds, chitosan with oregano or thyme EOs at 3%, 4% and 5%, and chitosan scaffolds with oregano and thyme EOs at 6%, 8% and 10%.

For the agar diffusion test per well, halos with a diameter ≥ 6 mm were considered to have inhibitory activity [24]. In this way, it was found that all the chitosan-based scaffolds,

with and without the incorporation of plant drugs, were able to demonstrate an antibacterial effect against the strain of *Escherichia coli* evaluated in this study.

Chitosan is often known to be a biopolymer with promising activities in the production of biomaterials with biomedical properties, and it has considerable antimicrobial action [19]. One of the main mechanisms justifying chitosan's antimicrobial action is its ability to interact with microbial DNA, whereby the hydrolysis products of its molecule affect protein synthesis rather than inhibiting messenger RNA [17], [19], [24].

In the present study, it was possible to observe inhibitory activity for the pure chitosan scaffold (control group) against the *Escherichia coli* strain (CCCD-E003) using agar diffusion by the well method. In agreement with this result, studies available in the literature show that biomaterials containing chitosan without the addition of essential oils have antimicrobial potential against some strains, including *E. coli*, *S. aureus*, *C. albicans*, *P. aeruginosa*, among others [13], [25]–[27].

In relation to the scaffolds incorporated with plant drugs, it was found that the scaffolds QOEO at 3%, 4% and 5%, QOET at 3%, 4% and 5%, and QOET at 6%, 8% and 10% were able to inhibit the bacterial strain studied and showed considerable antibacterial activity, forming halos of up to 16 mm. Similarly, Busatta et al. [28] studied the antibacterial activity of OEO, using the disk diffusion method, against various pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella choleraesuis*. In their study, these researchers reported the formation of inhibition halos of 16 mm, 17 mm and 29 mm for these strains, respectively. Rota et al. [29] also obtained similar results to the present study, as they found strong antibacterial activity of OET against strains of *E. coli*, *L. monocytogenes*, *S. enterica*, *S. enteritidis* and *S. aureus*.

Although the association of chitosan with oregano and/or thyme EOs did not result in inhibition halos with diameters greater than the halo formed by the control group, it was clear that antibacterial activity was present in all the scaffolds evaluated. On the other hand, Kooshegoli et al. [30] noted in their research, using chitosan films with PEGF and thymol, that the higher the concentration of thymol, the greater the antibacterial activity. For these researchers, this ratio is directly consistent with the slow release of some amounts of thymol, which is physically trapped between cross-linked chitosan chains.

Although the antibacterial action of OEO has been reported in this work, there was no increase in the inhibitory effect between the 3%, 4% and 5% concentrations of this substance, with both concentrations remaining with the same inhibitory efficacy, unlike the results shown by the authors mentioned above. All the concentrations of the plant drug evaluated showed the same inhibitory efficacy, with no variation between the sizes of the inhibition halos, i.e., the increase in the EO content did not potentiate the antibacterial effect against the strain studied.

It is suggested that the variation in the effectiveness of antibacterial activity between studies may be related to the chemical composition of the essential oils and the biopolymer evaluated, the stability of the chitosan itself tested, the controlled release kinetics of the essential oils by the

scaffolds, the presence of interactions between the active components of these drugs, the lipophilic characteristics of the substances and their dispersion in the culture medium, as well as the variation in antibacterial potential depending on the bacterial strain evaluated [19], [26], [28].

In this study, the antibacterial action against the CCCD-E003 strain was proven for both plant drugs evaluated at their lowest concentrations. Similarly to these results, Reis et al. [31] found that OEO was capable of developing antibacterial action against *E. coli* at concentrations lower than or equal to those in this study, such as 0.5%, 1%, 1.5%, 2%, 2.5% and 3%. However, according to Sobczyk et al. [26] Gram-negative bacteria, such as *Escherichia coli*, are more resistant than Gram-positive bacteria and may require higher concentrations of drugs with antimicrobial properties to inhibit their growth.

In contrast to the present study, Millezi et al. [32] found no antibacterial activity of ETO against *E. coli*. Sobczyk et al. [26] observed the absence of antibacterial activity of ETO against *E. coli* only at concentrations below 4%, with ETO being effective at concentrations $\geq 4\%$. On the other hand, Pereira et al. [33] evaluated the antibacterial activity and the thermochemical death curve of essential oil disinfectant solutions on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Enteritidis*. These researchers concluded that the EO with the greatest bactericidal activity on all the strains was thyme, with a MIC of 0.25% and *Escherichia coli* proved to be the most sensitive bacterium to the EOs tested.

It is also important to note that, according to Ernandes and Garcia-Cruz [34], Gram-positive bacteria are more sensitive to the antimicrobial activity of essential oils, with 44.2% more halos formed than Gram-negative bacteria (36.7%). Nevertheless, in the present study it was possible to observe, by comparing the inhibitory effects shown, that the drugs with pharmacological properties tested promoted inhibitory activity with good efficacy for Gram-negative bacteria (*E. coli*).

In this work, it was possible to see the formation of inhibition halos with considerable diameters against the *E. coli* strain, as in the study by Deineka et al. [35], which showed that the formulation of chitosan aerogel was more effective against Gram-negative bacteria than Gram-positive bacteria.

4. LIMITATIONS OF THE STUDY

The scaffolds evaluated in this study have not undergone controlled drug release tests. Only one bacterial strain of *E. coli* was evaluated.

5. CONCLUSION

The use of phytotherapy in association with biomaterials such as chitosan is a viable alternative for the treatment and prevention of infections in the human body. Essential oils extracted from medicinal plants, as well as biopolymers that are biocompatible with organic tissues, have strong pharmacological activity. Chitosan-based scaffolds incorporated with *Origanum vulgare* L. and *Thymus vulgaris* L.

EOs show antibacterial action and are considered effective inhibitors for *Escherichia coli* strains, characterizing them as therapeutic options for oral infections caused by these pathogens.

6. RECOMMENDATIONS

It is essential to carry out new in vitro studies to develop controlled drug release assays in order to see if there is any interference in the release kinetics of the active compounds of the essential oils of *Origanum vulgare* L. and *Thymus vulgaris* L. by the cross-linked chains of chitosan. In addition, these studies will make it possible to emphasize the effectiveness of these devices against different types of microorganisms, including fungi, viruses, protozoa and other bacterial strains. In vivo studies are also needed to analyze the behavior of these scaffolds in the body.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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