ANTIBIOFILM ACTIVITY OF ORIGANUM VULGARE

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Abstract. *Oreganum vulgare, or oregano, is widely known for its antimicrobial properties. A growth in microbial biofilm resistance in recent years has presented significant issues for the food business in terms of contamination and shelf-life loss, as well as for the medical community in terms of treating diseases. This paper evaluates the antibacterial efficiency against the biofilm generated by Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922), as well as the Minimum Biofilm Inhibitory Concentration (MBIC) of the essential oil of Origanum vulgare. The evaluation was performed by measuring the microbial biofilm using the microdilution assay, crystal violet staining, and reading of OD by spectrophotometry per ISO 20776-1:2019. Confocal microscopy was used to capture the images of S. aureus biofilm before and after treatment with the tested essential oil. The statistically evaluated results suggest a very good efficacy against S. aureus bacteria and a more limited activity against E. coli. The multifaceted potential of oregano's antimicrobial properties prompts the need for continued exploration and validation in various fields, ultimately contributing to sustainable and effective practices in healthcare, agriculture, and food preservation. With growing concerns about synthetic additives and preservatives, natural alternatives like oregano oil present an exciting opportunity for the food and pharmaceutical sectors.*

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Keywords*: biofilm, Staphylococcus aureus, Escherichia coli, Origanum vulgare.*

INTRODUCTION

Oreganum vulgare, or oregano, is widely known for its antimicrobial properties. These properties have been attributed to certain compounds in oregano, such as carvacrol and thymol. These compounds have strong antimicrobial activity against various bacteria, fungi, and parasites. Some studies have even found oregano effective against antibiotic-resistant strains of bacteria, making it a potential natural alternative for treating infections (COCCIMIGLIO ET AL., 2016; LEYVA-LÓPEZ ET AL., 2017). Oregano's antimicrobial properties make it a promising candidate for use in natural remedies and as a food preservative. Its effectiveness against antibiotic-resistant bacteria suggests its potential in combating the increasing problem of antibiotic resistance. Oregano can be used in various forms, such as essential oil, dried herb, or as an extract in herbal supplements. Further research and clinical studies are needed to fully understand its antimicrobial activity's scope and potential applications in the medicine and food industry.

Microorganism assemblage comprises extracellular polymeric substance (EPS) and other biological elements representing the bacterial biofilm. Even though biofilm formations might help with some soil and wastewater treatment procedures, they might also put patients at risk for long-term infections and poor personal hygiene in both clinical and industrial settings. It has been noted that adding certain essential oils or extracts directly to various goods has an antibacterial and antioxidant impact (KOGA ET AL., 2015; MUHAMMAD ET AL., 2020).

Biofilms, multicellular communities of bacteria in the environment, are typically observed near surfaces and interfaces. Although they usually live in multicellular communities, bacterial cells are similar to many other living cells in that they can survive as single cells (TAHRIC et al., 2023).

MATERIAL AND METHODS

Minimum Biofilm Inhibitory Concentration (MBIC) represent the smallest concentration of the product tested to inhibit the initial formation of biofilm, detectable through microorganism growth analysis. Our previous research has described the method as a microbial mass loss by measuring OD by spectrophotometry following ISO 20776-1:2019 (OBIȘTIOIU ET AL., 2023, HULEA ET AL., 2022).

The crystal violet staining method determined the total biomass content, including extracellular structures of cells adhering to the surface of the entire microtiter plate.

Microbiological method

The bacterial and fungal strains were revived by growing overnight in the Brain Heart Infusion (BHI) broth (Oxoid, CM1135) at 37°C and subsequently switched to BHI Agar (Oxoid, CM1136) for 24 and 48 hours at 37°C, respectively. The strains were diluted to an optical density (OD) of 0.5 McFarland standard (1.5 × 10^8CFU×mL) using BHI broth and evaluated with a McFarland densimeter (Grand-Bio, England). The dilutions were pipetted 100 µL into each well of the 96-well microdilution plate using a Calibra 852 digital multichannel pipette. The tested OVEO was added at 2%, 4%, 8%, 16% and 32%. The plates were covered and left for 24 hours at 37°C (FLOARES ET AL., 2023).

The biofilm evaluation was done according to the method described by Obiștioiu (OBIȘTIOIU ET AL., 2022). After incubation, the broth was removed, and the wells were washed twice with 160 µl 0.9% saline solution to remove planktonic cells. Crystal violet staining was performed by adding 160 µl crystal violet 0.1% solution in each well and incubating the plates for 10 minutes at room temperature. Then, the stain was removed, and the wells were washed twice with 170 µl of saline solution 0.9%. Ethanol 96% was added (170 µl) to each well for distaining for 30 minutes. Subsequently, the DO was read at 540 nm with an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Tripled tests were performed for all samples. Strain suspensions and BHI were used as positive controls. For the values obtained consecutive to the reading, the formulas for inhibited biomass content rate (IBCR%) and biomass content growth rate (BCGR%) were used, rates calculated using the formulas (1), (2):

$$BBGR\% = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%) \quad (1)$$

$$IBCR\% = 100\text{-}BCGR\ (\%)\ (2)$$

RESULTS AND DISCUSSIONS

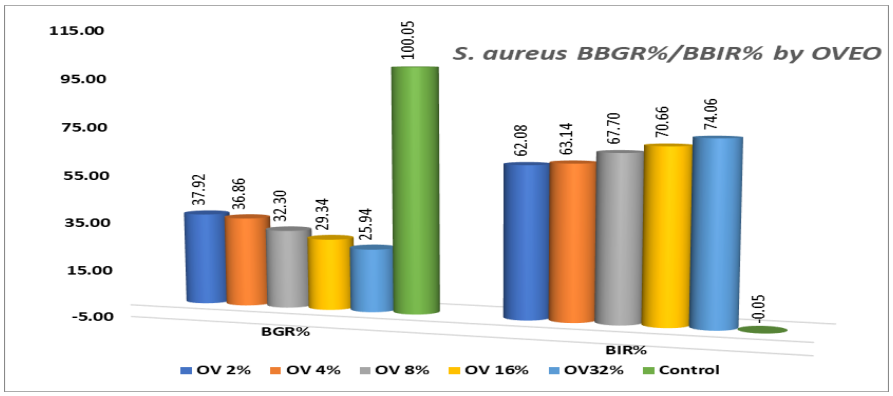

Figure 1. BBGR%/BBIR% of OVEO against *S. aureus*

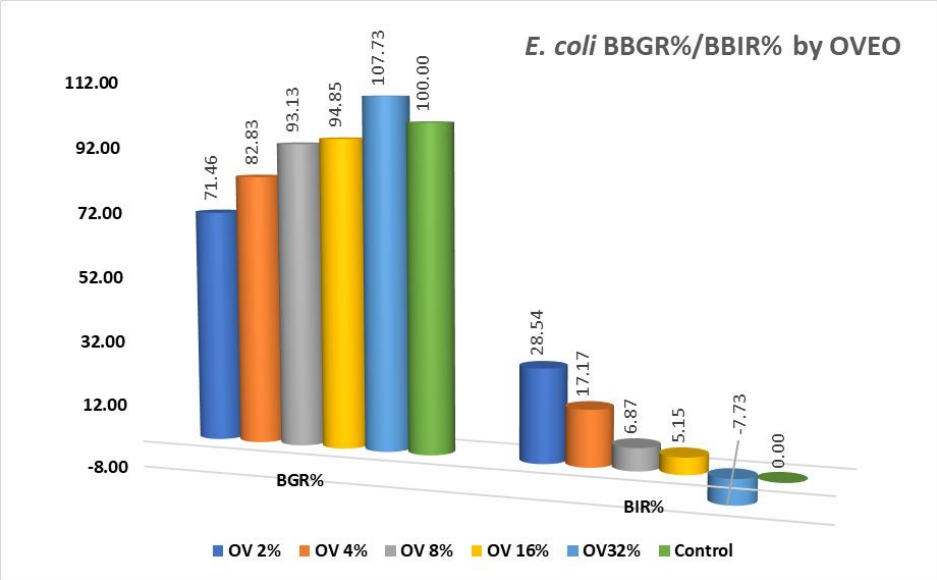

Figure 2. BBGR%/BBIR% of OVEO against *E.coli*

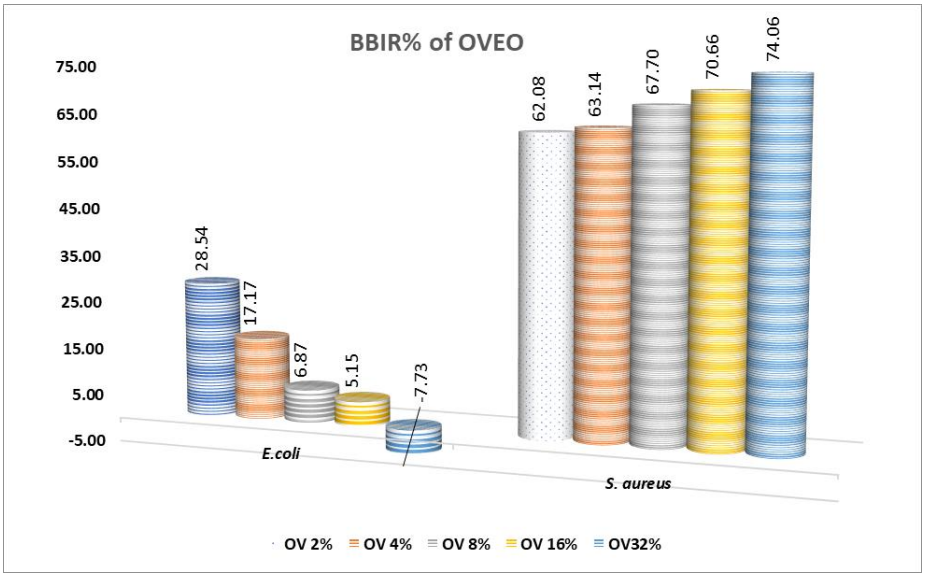

Figure 3. BBIR% of OVEO against *S. aureus* and *E.coli*

Figure 1 presents BBGR% for *S. aureus*, a bacterial biofilm rate influenced by the increase in concentration, with values ranging from 37.92% to 25.94% at the highest concentration tested. Regarding the BBIR% of *E. coli* (Fig.3), the biofilm formation inhibition presented upward dynamics in a positive correlation with the increase in concentration, the values starting at 62.08% BBIR% at 2% and reaching 74.06% in the case of the highest concentration tested (32%).

Concerning the antibiofilm activity of OVEO against *E. coli*, Fig. 2 presents the BBGR% with values that increased in tandem with the increase in concentration, ranging from 71.46% to 107.73%. The BBIR% demonstrated downward dynamics, negatively correlated with the increase in concentration. Therefore, if the first concentration tested reached a biofilm formation inhibition rate of 28.54%, the 32% concentration reached a BBIR% of -7.73%, demonstrating a strain-boosting effect.

The results of BBIR% (Fig.3) indicate a clear concentration-dependent effect of OVEO on inhibiting *E. coli* and *S. aureus* biofilm formation. The increase in concentration led to a proportional increase in the biofilm inhibition rate for *S. aureus*, demonstrating the potent antimicrobial activity. Concerning *E.coli*, the effect was strain-boosting, leading to an increase in the bacterial biofilm mass. The results obtained against *S. aureus* are sustained by the images obtained through confocal microscopy (Fig. 4 a,b) using a Leica TCS SPE- Leica Microsystem and interpreted using Image J software.

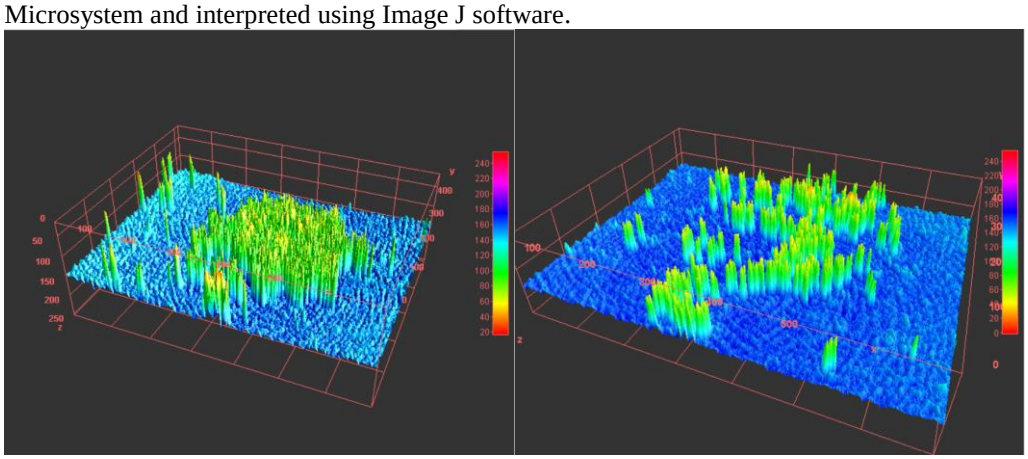

Figure 4. Surface plot of confocal images of *S. aureus* before (a) and after (b) OVEO

The concentration-dependent effects observed in this study highlight the potential of oregano essential oil as a promising antimicrobial agent for combating biofilm formation by *S. aureus*. These findings warrant further exploration and validation of the antimicrobial properties of oregano vulgare essential oil for potential therapeutic applications.

Our findings are not correlated with the results obtained by TAHRIC et al., 2023, which demonstrated that a decrease in oil concentration in the specific case of *E. coli* ATCC 14169 resulted in a reduction in the capacity to inhibit the growth of this bacteria. Another study demonstrated the weak effect against *S. aureus*. Rosato (ROSATO et al., 2010) and Scandorieiro (SCANDORIEIRO et al., 2016) also concluded that OEO has an action against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains. In our study, Gram-positive bacteria showed higher sensitivity to OEO than Gram-negative, corroborating with results from studies of Lambert and Stojković (LAMBERT et al., 2001; STOJKOVIĆ et al.,2013).

CONCLUSIONS

OVEO proved *in vitro* anti-biofilm activity against *S. aureus* starting at 2% with evolution in a positive correlation to the increase in concentration compared to *E.coli* in which OVEO demonstrated a strain-boosting effect, with a negative evolution correlated to the increase in concentration.

Using oregano essential oil as a natural antimicrobial agent in animal feed has shown promising results in controlling and preventing the spread of pathogens in livestock and poultry. This could be a significant development in reducing the need for antibiotics in animal husbandry, a growing concern due to the rise of antibiotic-resistant bacteria in animals.

The multifaceted potential of oregano's antimicrobial properties prompts the need for continued exploration and validation in various fields, ultimately contributing to sustainable and effective practices in healthcare, agriculture, and food preservation.

With growing concerns about synthetic additives and preservatives, natural alternatives like oregano oil present an exciting opportunity for the food and pharmaceutical sectors. In conclusion, the studies discussed above highlight the promising antimicrobial activity of oregano oil and its potential role in food preservation, pharmaceuticals, and medical applications. These findings underscore the importance of continued research into natural antimicrobial agents and their potential benefits for various industries.

Acknowledgement: Support was also received by the project Horizon Europe (HORIZON) 101071300 - Sustainable Horizons -European Universities designing the horizons of sustainability (SHEs).

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