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Exploring the Effectiveness of Nanoemulsion Formulations Containing Essential Oils Against Various Bacterial Species

Mustafa Kamil Othman Alchalabi[1], Dina Hussein Hatif Al Mansoori[1], Firas Shawkat Al Bayati[2], Bashaer J Kahdum[1]
[1]Geomatics Technology Center, University of Kufa, Najaf, Iraq
[2]Department of Ecology, Faculty of Science, University of Kufa, Najaf, Iraq.

ABSTRACT
The investigation of alternative antimicrobial agents has been increased by the emergence of antibiotic-resistant bacteria. Although essential oils (EOs) are recognized for their broad-spectrum antibacterial properties, their hydrophobicity and instability limit their application. This study examines the potential of nanoemulsion formulations to improve the antibacterial properties of rosemary essential oil. A green method was employed to synthesize silver nanoparticles, which included rosemary oil as a reducing agent. The disk diffusion method was employed to assess the antibacterial activity of the resulting formulation against Pseudomonas aeruginosa and Staphylococcus aureus at varying concentrations. S. aureus exhibited a greater sensitivity than P. aeruginosa, which is likely due to differences in their cell wall structures, as evidenced by the dose-dependent inhibition. These results illustrate the significance of nanoemulsified essential oils in the development of innovative antimicrobial strategies and emphasize their potential as effective antibacterial agents, particularly against Gram-positive bacteria.

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KEYWORDS: Nanotechnology, Essential oils, Pseudomonas aeruginosa, Staphylococcus aureus

Dina Hussein Hatif Al Mansoori

INTRODUCTION

Antibiotic-resistant bacteria emergence is increasingly becoming a global health threat and antimicrobials alternative to antibiotics are urgently needed (World Health Organization, 2020). One of the reasons for strong antibacterial activity reported for plant essential oils (EOs) is due to their complexation with bioactive molecules such as terpenes and phenolic compounds (Burt 2004). However, they were limited into their practical use due to their hydrophobic properties, volatility and degradation in the environ ment. (Sharifi-Rad et al., 2017).

The technology of nanoemulsion has come out as a viable approach to address these shortcomings. Nanoemulsions are thermodynamically/ kinetically stable water-oil dispersions that are stabilized by surfactants, and the dimensions of the droplets are mostly between 20 and 200 nm (Solans & Sole, 2012). This nano formulation increases the solubility, stability and bioavailability of essential oils, which results in increased antimicrobial effectiveness, against a broad spectrum of bacterial species (McClements and Rao, 2011). Moreover, Nanoemulsions have been demonstrated to promote penetration of essential oils into cell membranes of bacteria better, therefore increasing their effectiveness even in antibiotic-resistant bacteria (Donsì et al., 2011).

This study aims to evaluate the antibacterial effectiveness of Nanoemulsion formulations containing essential oils against various bacterial species. This study seeks to provide insight into the potential of these formulations as alternative antimicrobial agents through an examination of their physicochemical properties and antimicrobial mechanisms. The findings may contribute to the development of innovative strategies for combating bacterial diseases, particularly in considering the rising prevalence of antibiotic resistance. Antimicrobial agents are crucial in the treatment of bacterial infections. This study measures the inhibition zone widths at various doses to assess a tested substance's antibacterial effectiveness against Pseudomonas aeruginosa and Staphylococcus aureus.

2. MATERIALS AND METHODS

Materials

The tools and equipment utilized. Petri dishes, syringes, test tubes, digital rulers, conical flasks, burners, hoods, sensitive balances, autoclaves, incubators, cylinders, swabs, alcohol for sterilization, and glassware. Essential oils: A commercially available essential oil, specifically rosemary oil, was employed in this study. The culture medium: Muller Hinton agar (MH agar) is utilized for antibiotic sensitivity testing of microorganisms. The diluent utilized is DMSO.

Methods

2.1 Green Synthesis of Silver Nanoparticles

A single-step approach was developed to synthesize silver nanoparticles. The samples were prepared and designated as A1. The beaker was utilized: A1 contained 1 g of $AgNO_3$ dissolved in 100 mL of deionized water, along with 2 mL of rosemary oil serving as a reducing agent; this was combined with the same amount of salt and water. The beaker was subsequently stirred continuously and maintained at a temperature of 80 °C for a duration of 1 hour. After 1 hour, the solution in the beaker transitioned from a transparent state to a dark brown hue. The observed color change signifies the creation of silver nanoparticles. The nanoparticles had a final wash with ether to eliminate the oils, followed by a rinse with distilled water, and were subsequently dried in an oven at 80 °C.

2.2 Preparation of Bacterial Suspension

A suspended bacterial culture of Pseudomonas aeruginosa was created by transferring around one to four colonies into five milliliters of sterile saline solution. This was done with a cotton swab that had been sterilized. Through the process of continual mixing, a turbidity level that was consistent with a standard reference was achieved. The similar procedure was followed by the bacterium of S. aureus. Alcohol was used to thoroughly disinfect all of the instruments that were utilized during the process.

2.3 Preparation of the Culture Medium

MH agar, the culture medium, was produced following the manufacturer's instructions. Twelve Petri plates were created using 240 mL of distilled water; 9.12 g of MH agar. An autoclave at 121°C for 15 minutes sterilized the culture media.

2.4 Evaluation of Antibacterial Activity

The disk diffusion technique, in which various concentrations—100%, 75%, 50%, and 25%—of the substance were applied to bacterial cultures, evaluated the antimicrobial activity. The inhibition zones were measured in nanometers (mm), with a control group included for comparison. The essential oils' ability to eradicate pathogens was tested in a systematic manner. A syringe was filled with around 3 mL of the essential oil that had been synthesized and 3 mL of dimethyl sulfoxide. The diffusion technique was used to cultivate Pseudomonas aeruginosa and Staphylococcus aureus on pre-prepared culture medium. Five tiny holes, one in the middle and four on each side of the plate, were punched into it. Each of the four outer wells received two droplets of rosemary oil, and the center well received two drops of dimethyl sulfoxide. Covering and incubating the plates at 37°C for 24 hours followed. Using Microsoft Excel, the data was recorded and examined.

3.RESULT AND DISCUSSION

The inhibition zone diameters recorded for each concentration are summarized in Table 1 and figure 1.

Table 1: Inhibition Zone Diameters (mm) of the Tested Substance

Concentration	***Pseudomonas* aeruginosa (mm)**	***Staphylococcus aureus* (mm)**
100%	21.8	26.0
75%	17.7	26.0
50%	14.0	19.7
25%	11.7	16.6
Control	0.00	0.00

Inhibition zone of Pseudomonas aeruginosa varies in terms of dose as the maximum in 100% concentration (21.8 mm) and the minimum in 25% concentration (11.7 mm). The lack of control inhibition proves the presence of the antimicrobial effect on the tested substance. This trend indicates that Pseudomonas has moderate susceptibility and that it takes a greater concentration before the best inhibition. S. aureus has an inhibition zone of 26.0 mm at 100 percent and 75 percent, which remains the same and means that the bacteria is susceptible to low concentrations. At lower concentrations of 50% and 25%, the inhibition zones decrease to 19.7 mm and 16.6 mm, respectively. This indicates that S. aureus exhibits greater sensitivity to the antimicrobial agent than Pseudomonas aeruginosa.

The variation in susceptibility is attributable to the structures of bacterial cell walls. Pseudomonas, a Gram-negative bacterium, possesses an outer membrane that restricts permeability to antimicrobial agents. In contrast, S. aureus, a Gram-positive bacterium, does not have this barrier and is typically more susceptible (Silhavy et al., 2010). The findings are consistent with earlier research, suggesting that Gram-positive bacteria exhibit greater responsiveness to antimicrobial treatments compared to Gram-negative bacteria (Andrews, 2001).

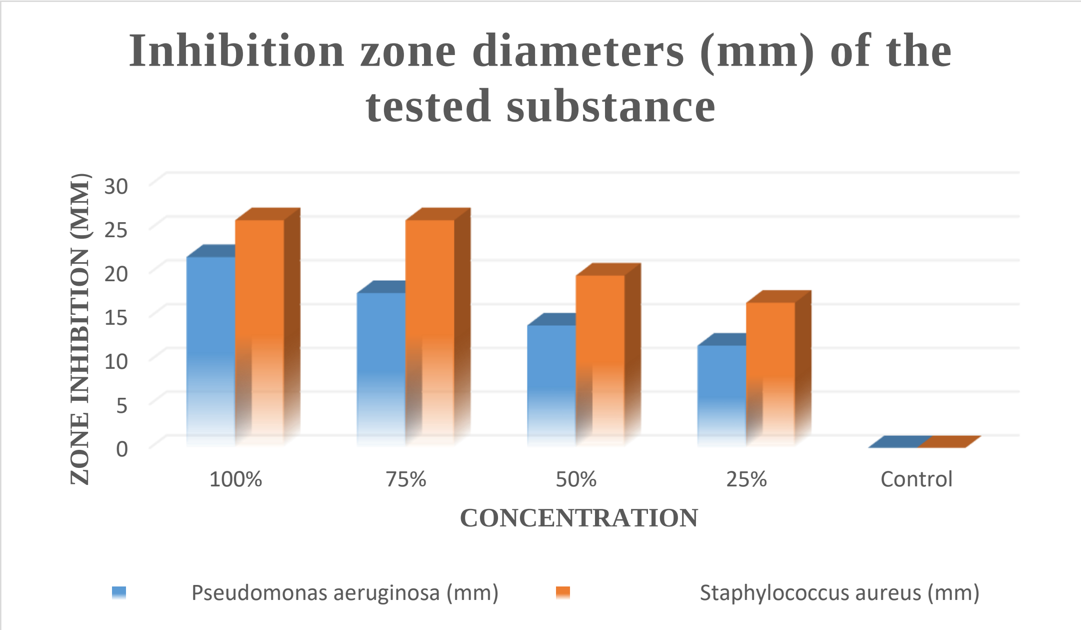

Figure 1: **Inhibition Zone Diameters (mm) of the Tested Substance**

4. IMPLICATIONS

The results indicate that the screened material exhibits very good antibacterial activity especially against S. aureus. However, it is concentration-dependent in its activity and more work will need to be done to get a handle on how it works and where its place in (industrial or clinical) practice is. Additionally MIC and MBC testing would provide additional information on its potency.

5. CONCLUSION

The sample that was examined showed antibacterial properties against Staphylococcus aureus and Pseudomonas aeruginosa. At increasing doses, the antibacterial action is greatest in Staphylococcus aureus. In addition to elucidating the structural differences between gram-positive and gram-negative bacteria in terms of sensitivity, this study provides more evidence that the antibacterial activity of this substance is dosage dependant.

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