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Investigating the Efficacy of Nanoemulsion Formulations Containing Essential Oils Against a Variety of Bacterial Species

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ABSTRACT

The increasing threat of antibiotic resistance necessitates alternative antimicrobial solutions. Essential oils (EOs), particularly rosemary oil, possess potent antibacterial properties but face limitations in solubility and stability. This study investigates the efficacy of nanoemulsions containing rosemary essential oil against *Escherichia coli* and *Klebsiella pneumoniae*. Silver nanoparticles were synthesized using green synthesis methods, and the antibacterial activity was assessed via diffusion assays on Mueller Hinton agar. Results indicated a concentration-dependent inhibition, with higher EO concentrations exhibiting stronger antibacterial effects. *Klebsiella pneumoniae* showed greater susceptibility than *E. coli*, suggesting potential differences in resistance mechanisms. These findings highlight the promise of EO-based nanoemulsions as alternative antibacterial agents, warranting further research into their mechanisms and applications in antimicrobial therapies.

KEYWORDS: Nanotechnology, Essential oils, Klebsiella pneumoniae, E. coli.

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INTRODUCTION

Growing awareness of antimicrobial resistance has led to the frantic search for other antibacterial agents. As EOs exhibit extensive antibacterial effects, and individuals have an open research attitude towards EOs for related antimicrobial properties, the solubility of the low volatile and water insoluble EOs has received more attention in recent years. Another interesting approach is related to nanoemulsions preparations, composed by oil-in-water or water-in-oil dispersed systems on a nanometric scale that has the potential to enhance stability, bioavailability and antimicrobial activity of EOs. Increasing attention has also been focused on nanoemulsions systems with antimicrobial activity. Nanoemulsions, an approach that favors the development of new delivery systems, is one such method. An emulsion of a mixture of water, surfactant and oil is recently in the focus of interest for the medicinal area and among the classical applications are antibacterial as well as antiviral therapeutic systems. (S. Doghish et al., 2023). Nanodiespertions of essential oils have been commonly employed at large scale in different sectors including pharmaceutics, food preservation, textile dressing, agribusiness, aroma therapy, and penetration enhancement of synthetic articles. (Baldassarre et al., 2023).

The potential use of nanoemulsions based on essential oil, as an alternative antimicrobial agent was evaluated by using the agent against a variety of bacteria in this paper. We have investigated the antimicrobial potential of Rosemary essential oils (EOs) in emulsions against Escherichia coli and Klebsiella pneumoniae in the current study. The study may bring insights on the development of new antimicrobial strategies to address bacterial infections. The major objective of the work is developing EO containing nanoemulsions, which are active against a wide range of potential susceptible bacterial species like K. pneumonia and E coli.

2. MATERIALS & METHODS

Materials

Rosemary essential oil was supplied and used. The microorganisms' antibiotic susceptibility was tested on a culture medium called Muller Hinton Agar (MH agar), with dimethyl sulfoxide (DMSO) serving as the diluent. Laboratory glassware, sterile swabs, autoclave, burner, fume hood, analytical balance, Petri dishes, test tubes, syringes, digital calipers, conical flasks, autoclave, incubator, and standard laboratory equipment were all utilized.

Methods

2.1Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using a one-pot technique. The samples were labeled as A1 after preparation. This experiment was conducted in a beaker. A1 was prepared by dissolving 1 g of $AgNO_3$ in 100 mL of deionized water and adding 2 mL of rosemary oil as a reducing agent. The other two components were the same amount of salt and water. After that, the beaker was heated to 80 °C and swirled constantly for an hour. A transformation from clear to dark brown occurred in the beaker's contents after 1 hour. The presence of silver nanoparticles is indicated by this shift in hue. After removing the oils, the nanoparticles were rinsed with distilled water and ether before being dried in an oven set at 80 °C.

2.2 Preparing the bacterial suspension

To create a suspension of E. coli bacteria, use a sterile cotton swab to mix one to four colonies with five milliliters of sterile saline solution. Stir constantly until the solution reaches a concentration identical to the standard turbidity constant solution. Follow the same procedure for Klebsiella pneumoniae bacteria. (The instruments utilized have undergone extensive alcohol sterilization).

2.3 Preparing the cultural medium

The culture medium (MH agar) was prepared in accordance with the manufacturer's guidelines. A total of 240 mL of distilled water was mixed with 9.12 g of MH agar to yield 12 Petri dishes. The medium was subsequently sterilized in an autoclave at 121°C for a duration of 15 minutes.

2.4 Determination of Antibacterial Activity

Evaluating the antibacterial efficacy of essential oils through a sequence of sequential steps: Using a syringe, remove approximately 3 milliliters of the essential oil that has been produced and 3 milliliters of DMSO. The diffusion technique was employed to cultivate *Klebsiella pneumoniae* and *E. coli* on the culture media. Create five small holes, with a single hole located in the center and the remaining four evenly spaced along the margins of each plate. In each of the four openings of the dish, add two droplets of rosemary oil. Then, add approximately two droplets of DMSO to the central opening. Cover the containers and place them in an incubator that is maintained at a temperature of 37°C for a period of 24 hours. Microsoft Excel was employed to acquire and analyze the data.

3. RESULTS AND DISCUSSION

The table displays data regarding the impact of varying concentrations of a substance on the inhibition zones (measured in nanometers) for E. coli and Klebsiella pneumoniae bacteria. The concentrations varied from 100% to 25%, with a control group included for comparison. After 24 hours of incubation, the dishes were removed, and the inhibitory zone for each oil was measured using a digital ruler, as presented in Table 1 and Figure 1.

Table 1: Evaluation of the antimicrobial efficacy of essential oils against bacterial species.

Concentration	E.coli **(mm)**	Klebsiella pneumonia **(mm)**
%100	20	21.8
%75	19.5	18.2
%50	18.4	17.9
%25	16.7	10.00
Control	0.00	0.00

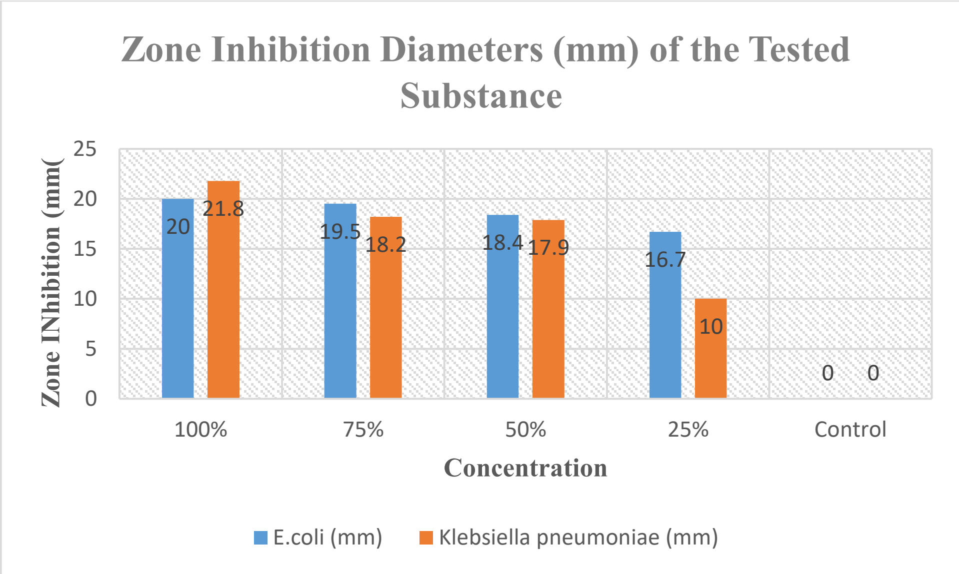

Figure 1: **Inhibition Zone Diameters (mm) of the Tested Substance**

The results indicate that the tested substance exhibits antibacterial activity against both *E. coli* and *Klebsiella pneumoniae*, as demonstrated by the formation of inhibition zones. The size of the inhibition zones decreases as the concentration of the substance decreases, showing a clear dose-dependent effect, the results consistent with previous studies on antimicrobial efficacy (Zhou et al., 2021).

At 100% strength, the same drugs exhibited the greatest diameter of inhibition zones against both bacteria, displaying greater sensitivity against K. pneumoniae (21.8 nm) than E. coli (20 nm). This indicates that the antibacterial sensitivity is stronger when acting on Klebsiella pneumoniae in a concentration of 5% STR. When the concentration was lowered, the inhibition zones formed for both bacteria region decreased, which indicated that when the concentration was reduced, the antibacterial activity attenuated. Similar(Ahmed et al., 2020).

Notably, the zone of inhibition for K. pneumonia significantly reduced to 10.0 mm at 25 percent concentration mean whiles that of E. coli still remained high as it recorded a 16.7 mm zone diameter above (Table IV). The Klebsiella pneumoniae is more sensitive to less concentrations, whereas in E. coli there was a potentially resistant or better can endure lower concentration of the substance. This could be due to the ability that Klebsiella pneumoniae has in exhibiting resistance through the production of b-lactamase and efflux pump, which have been widely documented and reported in antimicrobial resistant literature. (Liu et al., 2019; Pitout 2020).

There was no inhibition in the control group (0% concentration), and this fact confirms that the observed antibacterial effects were caused by the applied substance and not some other factors, as it is known that there are several mechanisms through which bacteria can be inhibited (Martinez et al., 2018). Diffusion of the antibacterial agent in agar might depend on the concentration, which will influence the inhibition zones (Singh et al., 2022). The effect may be reduced in low concentration because of diminished accessibility of active components like has been observed in research of natural antimicrobials. (Gupta & Sharma, 2021).

These findings indicate that the material tested is a good antibacterial agent especially at high concentrations. The difference in sensitivity of E. coli and Klebsiella pneumoniae might be due to the variations in the structure of their cell wall. Klebsiella pneumoniae is a Gram-negative bacterium that is susceptible to an outer membrane and can be easily penetrated by the antibacterial agent at high concentrations but at low concentrations has some level of protection.

More research may be done on how the substance acts, its efficacy compared with the rest of the bacterial strains and how it may be used practically, e.g., in medical or industrial context. Also, the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) would be more indicative of its strength.

4.CONCLUSION

Generally, the obtained results indicate that the tested substance is effective in the inhibition of the growth of both E. coli and Klebsiella pneumoniae with stronger action at higher concentrations. Nevertheless, the fact that the two bacteria differ in their ability to be inhibited at lower concentrations underscores the need to take into account the mechanisms of bacterial resistance in the process of establishing the effective dosages. Additional studies, including Minimum Inhibitory Concentration (MIC) testing and time-kill, can assist in gaining a better understanding of the antibacterial effect of this substance and its possible use..

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