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Comparative In Vitro Antibacterial Activity ofDecaffeinated and Non-Decaffeinated Powders Against *Escherichia coli*

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Abstract

This study aims to evaluate the antibacterial activity of two types of powders, namely Powder I (dekaf) and Powder II (non-dekaf), against Escherichia coli bacteria using the disc diffusion method. Tests were conducted at three different concentrations: 15,5%; 31%, and 46,5%. Results showed that Powder I (dekaf) had higher antibacterial effectiveness than Powder II (non-dekaf) at all concentrations tested. At the highest concentration (46,5%), Powder I produced the largest zoneof inhibition with an average diameter of 13,6 mm, while Powder II only reached 12,1 mm. This suggests that the decaffeination process can enhance the antibacterial activity. Therefore, further research is needed to understand the mechanism underlying this enhancement and its potential application in the clinical field.

Keywords: *coffea, decaffeinated, Escgerichia colii*

INTRODUCTION

The growing prevalence of bacterial infections, coupled with the increasing resistance of bacteria to conventional antibiotics, has created an urgent need for alternative antimicrobial agents. *Escherichia coli* (E. coli) is one of the most common bacterial pathogens associated with both hospital-acquired and community-acquired infections, such as urinary tract infections, gastroenteritis, and sepsis (1). These infections pose significant public health challenges, particularly due to the rapid spread of multidrug-resistant (MDR) strains of *E. coli*, which limit the effectiveness of many current antibiotic treatments (2).

The search for new antimicrobial agents has led researchers to explore various natural and synthetic compoundswith potential antibacterial properties. One area of interest is the study of plant-based materials, which are known to contain a wide range of bioactive compounds, such as polyphenols, flavonoids, and alkaloids, that exhibit antibacterial effects (3). Among these, coffee has gained attention not only for its widespread consumption but also for its bioactive compounds that might offer health benefits beyond its stimulant effects (4).

Coffee, a globally consumed beverage, is rich in various biologically active compounds, including chlorogenic acids, diterpenes, and caffeine, which have been studied for their potential health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties (5) (6). However, caffeine, while known for its stimulating effects, has also been suggested to possess antimicrobial properties that could contribute to the overall antimicrobial effect of coffee extracts (7). The process of decaffeination, which removes caffeine from coffee, is believed to alter the chemical composition of coffee beans, potentially affecting their antibacterial activity (8).

Despite the common perception that caffeine might be a key factor in the antimicrobial properties of coffee, studies have shown that decaffeinated coffee extracts can also exhibit significant antibacterial activity. For example, research by (6) demonstrated that both caffeinated and decaffeinated coffee extracts were effective against several bacterial strains, although the specific mechanisms by which decaffeination might alter antibacterial activity were not fully elucidated. This raises intriguing questions about the role of caffeine and other compounds in the antimicrobial properties of coffee.

To better understand the effects of decaffeination on the antimicrobial properties of coffee, it is essential to investigate how the removal of caffeine and the resulting chemical changes in coffee beans affect their ability to inhibit bacterial growth. Some studies suggest that the decaffeination process might enhance the bioavailability of certain antibacterial compounds by removing caffeine, which might otherwise compete for binding sites or inhibit the activity of these compounds (9). Furthermore, the interaction between various bioactive compounds in coffee could be altered during decaffeination, potentially leading to synergistic effects that enhance antibacterial activity (10).

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Given these considerations, this study aims to evaluate the comparative antibacterial activity of decaffeinated (Powder I) and non-decaffeinated (Powder II) powders against *E. coli* using the disk diffusion method. By testing these powders at different concentrations, this research seeks to determine whether decaffeination affects the antimicrobial efficacy of coffee extracts and to what extent these changes might be leveraged to develop novel antimicrobial agents. The results of this study could provide valuable insights into the development of coffee-based antimicrobials and contribute to a better understanding of how natural products can be optimized for therapeutic purposes.

OBJECTIVES

The objectives of this study were to evaluate and compare the antibacterial effectiveness of Powder I (decaf) and Powder II (non-decaf) against Escherichia coli bacteria at various concentrations, and to understand the effect of decaffeination on enhancing antibacterial activity.

METHODOLOGY

The study was conducted using the disc diffusion method, where Powder I (decaf) and Powder II (non-decaf) were prepared in three different concentrations: 31%, 155%, and 465%. Each powder was dissolved in 25 mL of solvent with different concentrations, then the diffusion disks that had been soaked in the powder solution were placed on agar media that had been inoculated with Escherichia coli. After incubation, the diameter of the inhibition zone around the disk was measured to determine the antibacterial effectiveness of the powder.

RESULTS

This study tested the antibacterial activity of two types of powders, namely Powder I (decaf) and Powder II (non-decaf), against *Escherichia coli* bacteria using the disc diffusion method. These powders were tested at three different concentrations: 15,5%; 31% and 46,5%. The results obtained from measuring the diameter of the inhibition zone showed significant differences between the two powders, especially at higher concentrations.

At a concentration of 15,5%, the difference between the two powders became more pronounced. Powder I (dekaf) showed increased antibacterial effectiveness with an average inhibition zone diameter of 10,4 mm, while Powder II (non-dekaf) only reached 96 mm. This indicates that at intermediate concentrations, Powder I (decaf) is more effective in inhibiting E. coli growth than Powder II (non-decaf). This difference indicates that the decaffeination process may play a role in enhancing antibacterial activity, albeit at not very high concentrations.

At 31% concentration, Powder I (decaf) produced an average inhibition zone diameter of 11,4 mm, while Powder II (non-decaf) produced an average inhibition zone diameter of 11,3 mm. These results show that at low concentrations, both powders have almost the same antibacterial effectiveness, with a slight edge on Powder I. This indicates that at low concentrations, both powders have similar antibacterial effectiveness. This indicates that at low concentrations, the difference between decaf and non-decaf powders is not very significant in inhibiting E. coli growth.

At the highest concentration tested 46,5%, the difference in effectiveness between the two powders became more significant. Powder I (decaf) produced an average inhibition zone diameter of 13,6 mm, which was the largest inhibition zone observed in this study. In contrast, Powder II (non-decaf) only produced an average inhibition zone diameter of 12,1 mm. These results indicate that at high concentrations, Powder I (dekaf) has a much stronger antibacterial effectiveness compared to Powder II (non-dekaf). This may be due to the changes in chemical composition that occur during the decaffeination process, leading to an increase in antibacterial activity.

The positive control results using chloramphenicol discs showed a very large zone of inhibition, with an average diameter of 52,0 mm, far exceeding the zones of inhibition produced by both powders. This confirms that chloramphenicol has very high antibacterial effectiveness against E. coli, which serves as a strong comparator to evaluate the effectiveness of the tested powders. In contrast, the negative control showed no zone of inhibition at all, confirming that no antibacterial activity occurred in the absence of the antibacterial agent.

These results overall indicate that Powder I (decaf) generally has stronger antibacterial activity compared to Powder II (non-decaf), especially at higher concentrations. The increase in antibacterial effectiveness with increasing concentration suggests that concentration is a key factor in determining the antibacterial ability of these powders. Further research is needed to identify the specific components in the powders that contribute to this antibacterial activity and to explore their clinical potential.

DISCUSSION

This discussion focuses on the interpretation of the results obtained from the in vitro study of the antibacterial activity of Powder I (dekaf) and Powder II (non-dekaf) against Escherichia coli. Based on the results, it was found that Powder I (decaf) showed higher antibacterial activity compared to Powder II (non-decaf) at all concentrations tested. This finding suggests that the decaffeination process may play an important role in enhancing the antibacterial effectiveness of the powders.

At 31% concentration, the difference between Powder I and Powder II was not significant, indicating that at low concentrations, the antibacterial activities of both powders were relatively similar. This may be due to the presence of similar active components in both powders at this concentration, or that the changes caused by decaffeination have not sufficiently affected the antibacterial activity at this level.

However, at a concentration of 15,5%, it was seen that Powder I (decaf) started to show a clearer advantage over Powder II (non-decaf). This suggests that decaffeination might

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increase the concentration or availability of active components

At the highest concentration tested (46,5%), the difference in effectiveness between Powder I and Powder II became very apparent. Powder I showed much higher antibacterial activity than Powder II. This may be due to the synergistic effect of the higher concentration of active components in Powder I after decaffeination. Several studies have shown that increasing the concentration of active components can enhance the antibacterial effect through a synergy mechanism, where multiple components work together to inhibit bacterial growth more effectively (12). This may explain why Powder I had higher activity at this concentration.

The difference in antibacterial activity between Powder I (decaf) and Powder II (non-decaf) may also be explained by the change in chemical composition during the decaffeination process. Decaffeination can remove or reduce the amount of certain compounds that might inhibit antibacterial activity or increase the stability and availability of active antibacterial components (13). This may explain why Powder I showed higher effectiveness than PowderII at higher concentrations.

The positive control using chloramphenicol showed a very large zone of inhibition, which confirms that E. coli is highly susceptible to this antibiotic. This result is in accordance with the existing literature which shows that chloramphenicol is a highly effective antibiotic against various types of bacteria, including E. coli. In contrast, the absence of an inhibition zone in the negative control indicated that there was no antibacterial activity caused by other materials besides the tested powder, confirming the validity ofthe test results.

Overall, the results of this study indicate that Powder I (decaf) has potential as a stronger antibacterial agent compared to Powder II (non-decaf), especially at higher concentrations. These findings highlight the importance of the decaffeination process in enhancing antibacterial activity and open up new possibilities for the development of decaffeinated-based antibacterial agents. Further research is needed to explore the specific mechanisms behind this enhancement and to identify the active components that contribute to the observed antibacterial activity.

CONCLUSION

This study showed that Powder I (decaffeinated) had stronger antibacterial potential than Powder II (non-decaffeinated), especially at higher concentrations. This suggests that the decaffeination process can enhance antibacterial activity, which has implications for the potential development of new antibacterial agents.

RECOMMENDATION

Further research is suggested to identify the active components in the powder that contribute to the antibacterial activity, as well as to explore the effect of this powder against various other types of bacteria. In vivo, studies are also needed to confirm the clinical potential of this powder as an antibacterial agent.

LIMITATION

This study was limited to in vitro testing against one type of bacteria, Escherichia coli. These results may not be directly applicable to in vivo conditions or against other bacteria. In addition, the powder concentration used may have affected the results which cannot be directly applied to clinical situations without additional studies.

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