

## Differences in antimicrobial activity of natural compounds by drop diffusion or dilution methods on agar

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### Introduction and objective

The interest for natural antimicrobials as an application in food preservation has been increasing due to the growing interest of the population for a healthy lifestyle. Natural compounds have interesting characteristics, such as biodegradability and biocompatibility [1], making them an alternative to chemical compounds in conservation. Thus, it is important to carry out screening methods to identify the antimicrobial activity of these compounds. The *in vitro* determination of the antimicrobial activity of natural compounds requires determining their minimum inhibitory concentrations to assess microbial susceptibility. This study aimed to evaluate the minimum inhibitory concentrations of three antimicrobial potential natural compounds – chitosan, ethanolic propolis extract, and nisin – against 37 microorganisms by agar dilution and drop diffusion on agar methods.

# Methodology



### Results

33 30

24

21

18



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Agar dilution methodDrop agar diffusion method

Agar dilution method
Drop agar diffusion method

**Figure 1.** Comparison of the total number of inhibited microorganisms by the chitosan, by agar dilution method (blue) and drop agar diffusion method (orange), at different pH values.

**Figure 2.** Comparison of the total number of inhibited microorganisms by the ethanolic propolis extract, by agar dilution method (blue) and drop agar diffusion method (orange), at different pH values.

**Figure 3.** Comparison of the total number of inhibited microorganisms by the nisin, by agar dilution method (blue) and drop agar diffusion method (orange), at different pH values.



Total Gram+ Gram- Yeast Total Gram+ Gram- Yeast Total Gram+ Gram- Yeast pH5 pH6 pH7

Agar dilution method
Drop agar diffusion method

#### Conclusions

Most Gram-positive bacteria were inhibited at 25 µg/mL of nisin, and most of the microorganisms were inhibited by chitosan at 0.5% (w/v) and propolis at 10 mg/mL. The inhibitory action of the compounds was influenced by the evaluation methods and pH values. In this study, it was concluded that, in general, lower minimum inhibitory concentrations were observed at lower pH values and for the agar dilution method. Some microorganisms inhibited by the compounds on the agar dilution method were not inhibited by the same compounds and at the same concentrations on the drop diffusion technique. Therefore, this study reinforces the need for using a defined standard method for the *in vitro* determination of minimum inhibitory concentrations of natural compounds because it is crucial to compare results obtained in different studies and matrices.

#### References

[1] Food Preservation. A. Grumezescu (Ed.), Cambridge, Academic Press, 2017.



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