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ANTIMICROBIAL ACTIVITY OF EUGENYL ISOBUTYRATE IN PATHOGENIC MICROORGANISM

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ABSTRACT

Eugenyl isobutyrate is an ester derived from eugenol, the major component of clove essential oil. Eugenol esters has been studied due to their antimicrobial and antioxidant properties. The aim of this work was to verify the antimicrobial activity of eugenyl isobutyrate in pathogenic microorganisms. Eugenyl isobutyrate was obtained by esterification reaction, using isobutyric anhydride and eugenol molar ratio 2:1, with the enzymatic catalyst Novozym 435 (10% in relation to the total weight of the substrate) in batch mode, for 72 hours at 70 °C. The ester presented antimicrobial activity, against the microorganisms Salmonela sp. *Salmonella enterica and Staphylococcus aureus, the concentration that showed the greatest inhibition halo in all the microorganisms tested was 0.50 mg.mL^{-1}.*

1. INTRODUCTION

Clove essential oil has eugenol as the major component. (Barbosa et al., 2012). Eugenol esters as eugenyl acetate and eugenol benzoate are known to have different properties, such as antimicrobial (Chiaradia et al., 2012) and antioxidant (Horchani et al. 2010).

Eugenyl isobutyrate is an ester derived from eugenol, which may be obtained by enzymatic catalyzed esterification reaction; as lipases. The enzymatic synthesis has advantages for example; milder

reaction conditions, low energy, selectivity and less reaction time compared to the traditional method; that uses chemical catalysts. (Freitas et al., 2010).

This work aimed to verify the antimicrobial activity of eugenyl isobutyrate in pathogenic microorganisms. Among which; *Staphylococcus aureus, Salmonella* sp. and *Salmonella enterica* are the main cause of foodborne illness (Martinovic et al., 2016).

2. MATERIALS AND METHODS

2.1. Reagents

In the esterification reaction of eugenyl isobutyrate commercial substrates were used; eugenol (Sigma- Aldrich 98% purity) and isobutyric anhydride (Acros Organics 97% purity); Novozym 435, produced from the lipase of the *Candida antarctica* B, immobilized in acrylic resin, kindly provided by Novozymes S.A. Ethyl acetate PA (Qhemis), NaOH (1M) and distilled water were used in the step of purifying the compound.

2.2. Synthesis

The enzymatic synthesis of eugenyl isobutyrate was performed in batch mode, molar ratio 2:1 being anhydride isobutyric to eugenol, Novozym 435 (10% in relation to the total weight of the substrate) for 72 hours at 70 °C with stirring. After the product was obtained, a liquid-liquid extraction of the compound was made. The sample was diluted in ethyl acetate with NaOH (1M) under stirring of 600 rpm for 2 hours, after, a funnel was used to separate the aqueous and organic phase. As the last step ethyl acetate was evaporated.

2.3. Quantification of Eugenyl Isobutyrate

The quantification of the produced ester was performed by gas chromatography (Shimadzu GC- 2010), according to the modified method of Chiaradia et al. (2012). The conversion determination was calculated by reducing the area limiting agent (eugenol), based on the reaction stoichiometry.

2.4. Antimicrobial activity

The methodology described by Costa et al. (2010) was used with some modifications. The sensitivity and resistance of *Salmonella* sp., *Salmonella enterica* and *Staphylococcus aureus* against eugenyl isobutyrate was tested by measuring the size of inhibition halo. The microorganisms were seeded in nutrient broth and incubated at 37 °C for 24 hours. After this period, the inocula were seeded on Mueller Hinton agar and standardized according to the McFarland scale ($OD_{(600\ nm)}$ =0.5). After the plates were prepared, the obtained extracts were distributed on 6 mm diameter Whatman paper disks and added 20 µL of eugenyl isobutyrate previously purified, at the following concentrations: 0.05, 0.10, 0.25 e 0.50 $mg.mL^{-1}$. As positive control 30 $\mu g.L^{-1}$ of amoxicillin and 20 µL of dimethylsulfoxide (DMSO) were used as the negative control. Petri dishes were kept at room temperature for 1 hour to pre-diffuse the substances and were subsequently incubated at 37 °C for 24 hours. After the time, the Petri dishes were analyzed. The results are expressed in millimeters and were calculated in the arithmetic mean of total halo diameter obtained after triplicates of each microorganism.

3. RESULTS AND DISCUSSION

The results of the antimicrobial activities for the different concentrations of eugenyl isobutyrate against the microorganisms *Salmonella* sp. *Salmonella enterica* and *Staphylococcus aureus*, are presented in Table 1.

Table 1: Antibacterial activity of eugenyl isobutyrate acetate by the diffusion plate.

Microorganism	**Eugenyl isobutyrate concentration ($mg.mL^{-1}$)**			
	0.05	**0.10**	**0.25**	**0.50**
	Inhibition halo (mm)			
***Salmonella* sp.**	10.0±0.00	10.3±0.50	10.0±0.00	19.0±7.23
Salmonella entérica	11.3±1.20	11.0±0.00	10.0±0.00	12.0±1.41
Staphylococcus aureus	10.7±1.20	11.0±0.80	10.0±0.00	12.0±1.00

The concentration of 0.50 $mg.mL^{-1}$ was efficient in inhibiting the growth of *Salmonella* sp. exhibiting an inhibition halo of 19.0 mm, the same concentration was also efficient for growth inhibition of *Salmonella enterica* and *Staphylococcus aureus*, whose inhibition halos were 12.0 mm.

4. CONCLUSION

From the results obtained in the test of antimicrobial activity, against the microorganisms *Salmonella* sp. *Salmonella enterica* and *Staphylococcus aureus*, the concentration that presented the greatest inhibition halo in all the tested microorganisms was 0.50 $mg.mL^{-1}$.

5. ACKNOWLEDGMENTS

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