INHIBITORY EFFECT OF *CINNAMOMUM CASSIA* ESSENTIAL OIL AND HIS COMPONENTS ON DIFFERENT MICROORGANISMS

A. ANDRIŞ[1], C. PÎNZARIU[1], V. LE-TILLY[2], A. LE GRAND[2], O. SIRE[2], R. GRUIA[1], A. MĂRCULESCU[1]

[1] Transilvania University of Brasov – Romania;
[2] University of South Brittany – France

Abstract: *The antibacterial and antifungal potentials of cinnamon essential oil and two of his compounds (cinnamaldehyde and eugenol) were investigated in this study. For the antibacterial study were used two strains of bacteria Escherichia coli and Proteus mirabilis and for the antifungal study a yeast strain Saccharomyces cerevisiae. The disk diffusion assay showed that Cinnamomum cassia oil and his major compound, cinnamaldehyde, have an important antibacterial and antifungal effect. We observed that this two have a major inhibitory effect against E. coli and S. cerevisiae while against P. mirabilis the inhibitory effect is less important. The eugenol has a low inhibitory effect against all three microorganisms. In conclusion C. cassia oil has an effectively inhibition effect over E. coli and S. cerevisiae.*

Keywords: *Cinnamomum cassia essential oil, Cinnamaldehyde, Eugenol, Inhibition, Disk diffusion assay, Bacterial growth curves;*

1. Introduction

Cinnamon is a spice used for thousands of years and nowadays is one of the most used. In Europe cinnamon has appeared during the 16th and 17th centuries when the Portuguese found cinnamon in Sri Lanka.

The genus *Cinnamomum* (family *Lauraceae*) comprises over 300 aromatic evergreen trees and shrubs, located in almost all tropical regions of Earth. The most used species are *Cinnamomum zeylanicum* Blume (*Cinnamomum verum* J. Presl, Sri Lanka cinnamon or Ceylon cinnamon) and *Cinnamomum aromaticum* Nees (*Cinnamomum cassia* (L.) J. Presl, Chinese cinnamon or false cinnamon) due their multiple uses worldwide.
Cinnamon volatile oils are obtained from bark, leaves, flowers and buds.

The oil is a sweet yellowish liquid with a strong cinnamaldehyde taste. With time darkens to brown-red. The chemical composition of cinnamon oil varies depending on the part of the plant used for distillations process.

The main constituent of cinnamon bark oil is cinnamaldehyde, whereas eugenol is the main constituent of cinnamon leaf oil.

2. Materials and methods

2.1. Chemicals

Cinnamon essential oil (*Cinnamomum cassia*) was purchased from MLW FRAGRANCE (Bonneuil sur Marne, France). Cinnamaldehyde, Eugenol were obtained from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared in absolute ethanol. Working solutions were obtained from stock solution by serial dilutions. All the culture media components were purchased from either Biokar diagnostics (Allone, France) or Carlo Erba Reagents (Val de Reuil, France). Others chemicals used in this study were analytical grade and obtained either from Carlo Erba Reagents or Sigma-Aldrich.

2.2. Bacterial and yeast strains and growth conditions

Two strains of bacteria were used in this study: *Escherichia coli* ATCC 25922 and *Proteus mirabilis* WT19 (corresponding to the clinical isolate U6450). These bacteria were grown in LB broth (Tryptone 10g/L, NaCl 10g/L, Yeast extract 5g/L, pH7) or LB agar plate (15g/L) at 37°C. The yeast *Saccharomyces cerevisiae* strain used in this study was W303.1B and was cultivated on YPD medium (Tryptone 20g/L, Yeast extract 10g/L, pH7, Glucose 20g/L) or YPD agar plate (15g/L).

2.3. Bacterial growth curves

Growth curves were performed in 96 well plates. A 7-hours pre-culture was diluted to an $OD_{600nm,l=1cm}$ = 0.02 in LB ((2,22±0,16)x10^6 CFU/mL for *E. coli* and (4,5±0,73)x10^6 CFU/mL for *P. mirabilis*). 125µL of cell suspensions were inoculated in wells containing 125µL of cinnamon essential oil or cinnamaldehyde with a final concentration ranging from 81.7 to 413.6 µg/mL or 78,8-399,9 µg/mL.

The plates were incubated in a Polarstar Omega microplate reader (BMG Labtech, Ortenberg, Germany) at 37°C. $OD_{600nm,l=1mm}$ values were recorded every 15 minutes. Three replicates were performed for each concentration.

2.4. Disc diffusion assay

E. coli and *P. mirabilis* were cultivated in LB for 16 hours at 37°C and *S. cerevisiae* in YPD at 30°C. Cultures were adjusted to an $OD_{600nm,l=1cm}$ = 0.001 ((1,11±0,08)x10^5 CFU/mL for *E. coli*, (2,25±0,36)x10^5 CFU/mL for *P. mirabilis* and (8,70±0,08)x10^7 CFU/mL for *S. cerevisiae*) with sterile tryptone-salt Broth (Tryptone 1g/L, NaCl 8.5g/L, pH 7). 100 µL of the bacterial and yeast suspensions were spread on LB agar or YPD agar. Filter paper discs (Whatman n°3, 7 mm in diameter) were incubated on the agar surface and soaked with 5 µL of each dilution tested of *Cinnamon cassia* oil, cinnamaldehyde or eugenol (0,5%, 1%; 2%, 4%, 8%, 10%, 12%, 16%, 20%, 25% which corresponds to 0,025; 0,05; 0,1; 0,2; 0,4; 0,5; 0,6; 0,8; 1; 1,25 µL of tested compounds).

After 30min at room temperature, the plates were incubated at 37°C for 24 hours and respectively at 30°C for 48 hours for yeasts.

The diameters of the inhibition zones were measured in centimeters. Values are described as mean ± SD as assays were performed in triplicates.

3. Results

3.1. Disc diffusion assay for antibacterial and antifungal activity of C. cassia oil

The antibacterial and antifungal activities (diameter of inhibition zone) of *C. cassia* oil, cinnamaldehyde and eugenol to *Escherichia coli* ATCC 25922, *Proteus mirabilis* WT19 and *Saccharomyces cerevisiae* W303.1B are summarized in Table 1, 2 and 3.

The inhibitory effect strengthened significantly with increasing amount of tested compound per disc. Discs with 5 µL of 25% (v/v) *C. cassia* oil and cinnamaldehyde solution resulted in highest inhibition zones around 3-4 cm. Among tested bacteria, *E. coli* and *S. cerevisiae* are most sensitive to *C. cassia* oil and cinnamldehyde.

Table 1. *Inhibitory zone of different concentration of C. cassia oil against microorganisms (cm)*

Cinnamon oil	Concentration (%,V/V)										
	0	0,5	1	2	4	8	10	12	16	20	25
E. coli	1,10 ±0,08	1,31 ±0,14	1,41 ±0,14	1,47 ±0,03	1,51 ±0,11	2,11 ±0,07	2,43 ±0,09	2,68 ±0,35	2,93 ±0,31	3,23 ±0,07	3,86 ±0,20
P. mirabilis	0	0	0	0	0,75 ±0,05	1,41 ±0,27	1,75 ±0,21	2,2 ±0,17	2,6 ±0,31	2,78 ±0,36	3,31 ±0,24
S. cerevisiae	1,08 ±0,12	1,21 ±0,08	1,22 ±0,10	1,23 ±0,03	1,4 ±0,32	2,34 ±0,18	2,58 ±0,10	2,64 ±0,14	3,08 ±0,17	3,43 ±0,20	4,16 ±0,15

Table 2. *Inhibitory zone of different concentration of Cinnamldehyde against microorganisms (cm)*

Cinnamaldehyde	Concentration (%,V/V)										
	0	0,5	1	2	4	8	10	12	16	20	25
E. coli	1,10 ±0,09	1,23 ±0,02	1,31 ±0,05	1,35 ±0,12	1,43 ±0,12	2,11 ±0,16	2,36 ±0,15	2,56 ±0,15	2,87 ±0,26	3,5 ±0,21	4,07 ±0,09
P. mirabilis	0	0	0	0	0,93 ±0,11	1,6 ±0,34	1,75 ±0,23	2,43 ±0,20	2,83 ±0,23	3,08 ±0,10	3,4 ±0,13
S. cerevisiae	1,08 ±0,08	1,125 ±0,02	1,17 ±0,03	1,25 ±0,05	1,33 ±0,13	2,1 ±0,16	2,56 ±0,31	2,95 ±0,07	3,53 ±0,15	3,85 ±0,07	4,05 ±0,07

Table 3. *Inhibitory zone of different concentration of Eugenol against microorganisms (cm)*

Eugenol	Concentration (%,V/V)										
	0	0,5	1	2	4	8	10	12	16	20	25
E. coli	1,08 ±0,08	1,13 ±0,02	1,21 ±0,06	1,25 ±0,15	1,43 ±0,09	1,46 ±0,11	1,49 ±0,02	1,5 ±0,1	1,78 ±0,16	2,2 ±0,2	2,4 ±0,11
P. mirabilis	0	0	0	0	0	0	0	1,48 ±0,10	1,5 ±0,1	1,6 ±0,08	1,79 ±0,18
S. cerevisiae	0,98 ±0,08	1,1 ±0,05	1,18 ±0,10	1,18 ±0,02	1,18 ±0,08	1,2 ±0,17	1,21 ±0,19	1,4 ±0,08	1,63 ±0,20	1,8 ±0,2	2,13 ±0,11

Antibacterial activity of *Cinnamonum cassia* oil and compounds against *Escherichia coli* by disc diffusion method.

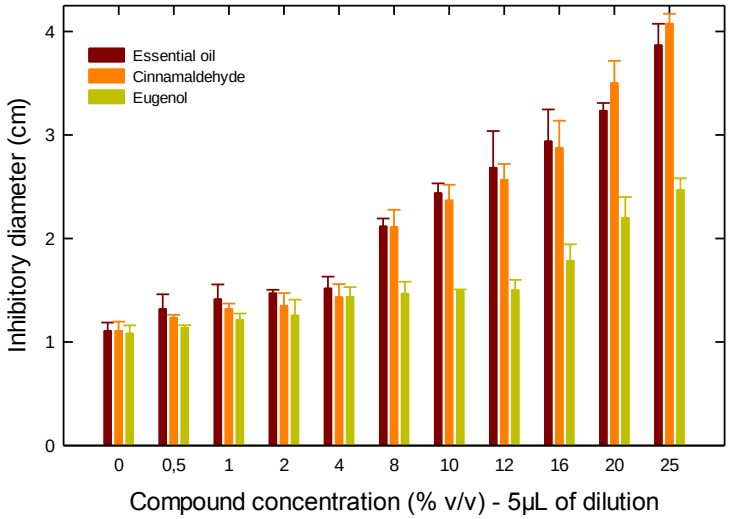


Fig. 1. *Antibacterial activity of Cinnamonum cassia oil and compounds against Escherichia coli by disc diffusion method.*

Antibacterial activity of *Cinnamonum cassia* oil and compounds against *Proteus mirabilis* by disc diffusion method.

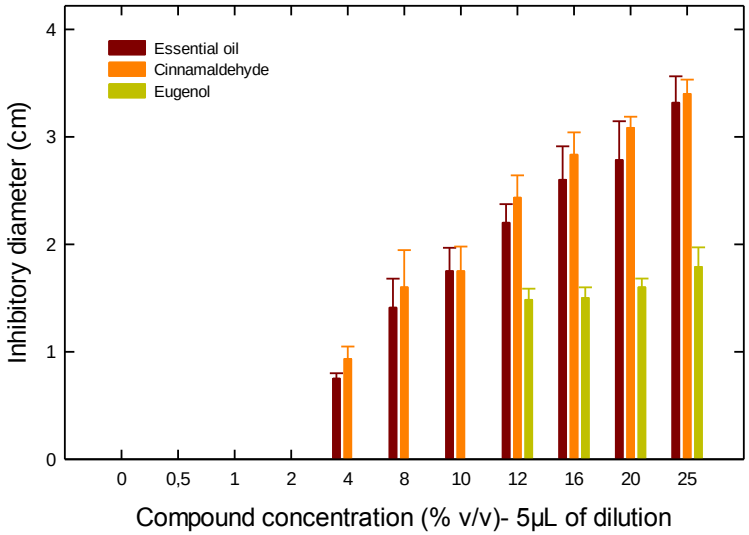


Fig. 2. *Antibacterial activity of Cinnamonum cassia oil and compounds against Proteus mirabilis by disc diffusion method.*

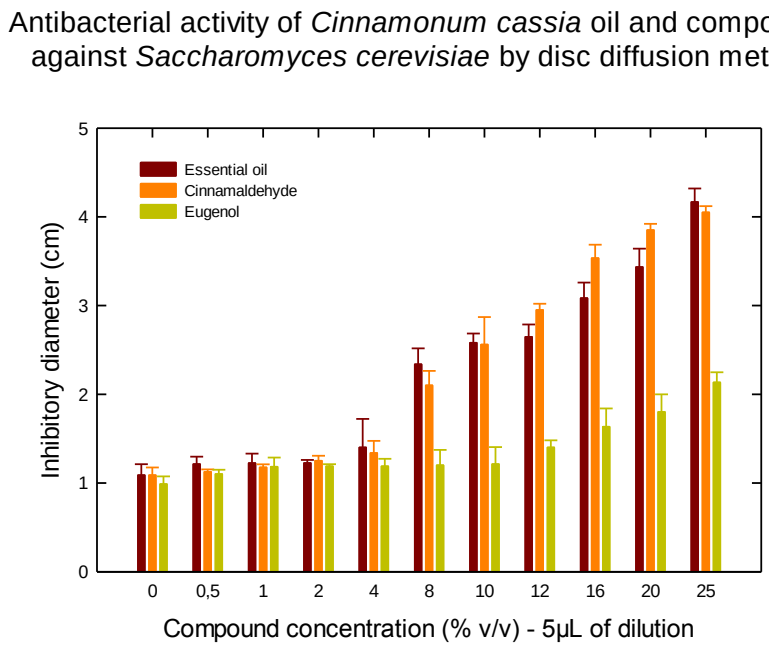
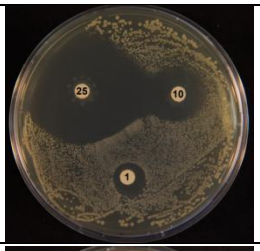
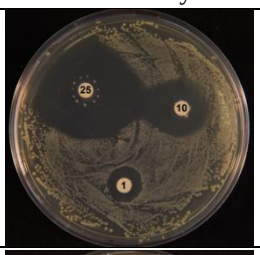
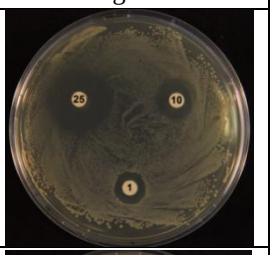
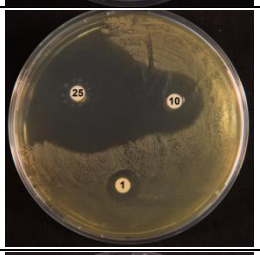
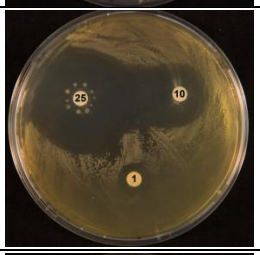
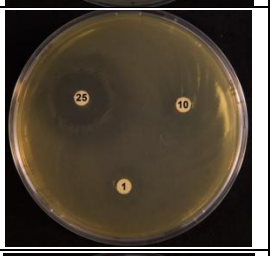
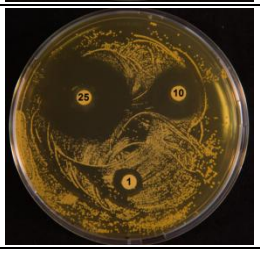
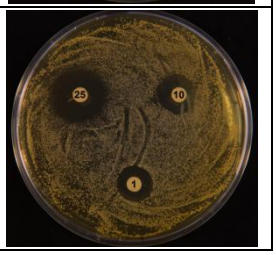


Fig. 3. *Antibacterial activity of Cinnamonum cassia oil and compounds against Saccharomyces cerevisiae by disc diffusion method.*

Table 4. *Antibacterial activity of C. cassia oil, cinnamaldehyde and eugenol against microorganisms by disc diffusion method. Representative disc diffusion image. Antimicrobial activity was determined by forming a clear zone around the disc*

	C. cassia oil	Cinnamaldehyde	Eugenol
E. coli ATCC25922			
P. mirabilis WT19			
S. cerevisiae W303-1B			

3.2. Bacterial growth curves

We observe that the lowest concentration of C. cassia oil and cinnamaldehyde, 0.0079% (v/v), increased the lag phase of all tested bacteria. At the concentration of 0.016% (v/v), C. cassia oil delayed the log phase of E. coli for about 8 h. C. cassia oil at the concentration of 0.02% (v/v) or above completely inhibited the growth of E. coli, while for P.mirabilis is 0,016(v/v) or above. The cinnamaldehyde completely inhibited the growth

of E. coli and P. mirabilis from a concentration of 0,025% (v/v).

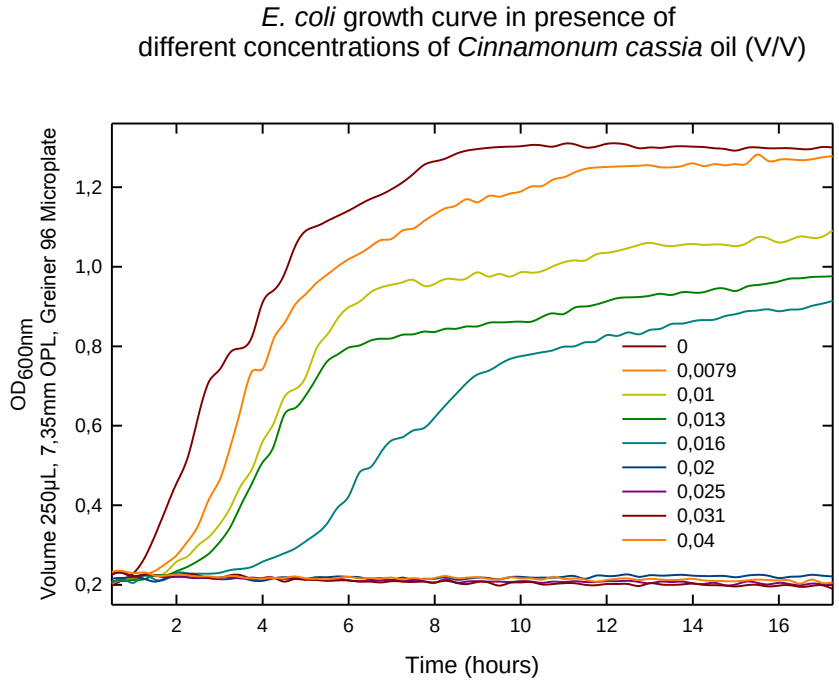


Fig. 4. *Growth curves of E. coli in LB broth containing different concentrations of C. cassia oil*

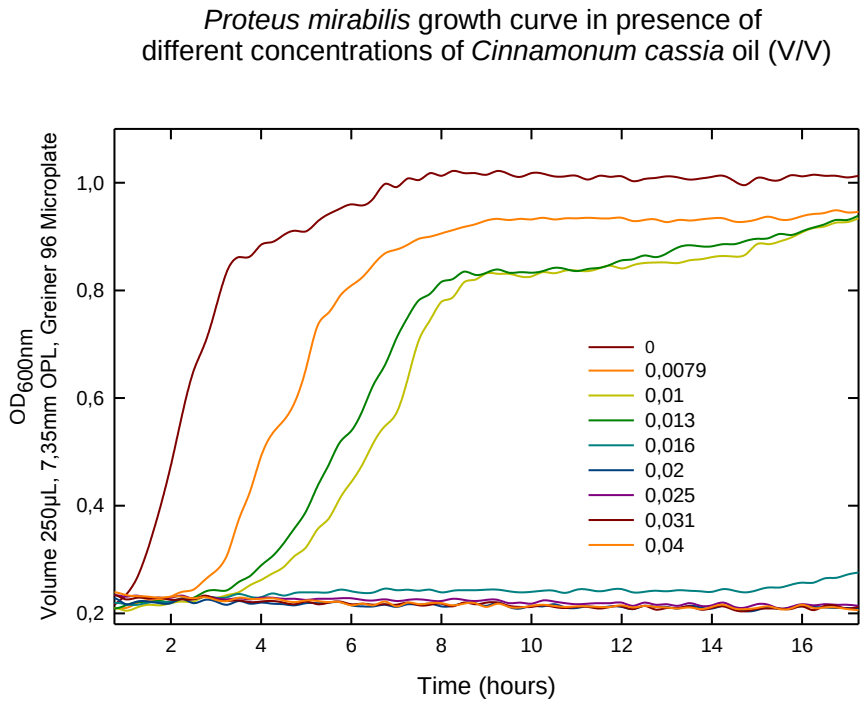


Fig. 5. *Growth curves of P. mirabilis in LB broth containing different concentrations of C. cassia oil*

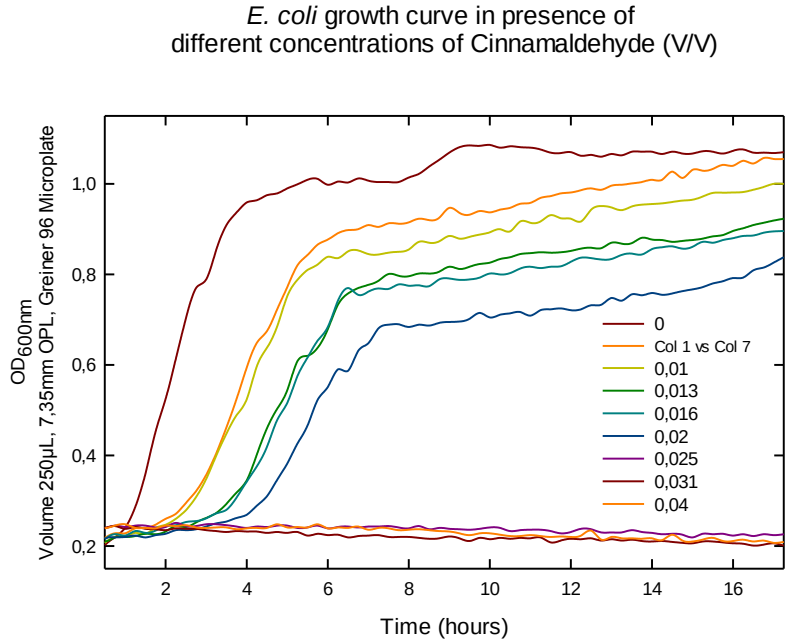


Fig. 6. *Growth curves of E. coli in LB broth containing different concentrations of cinnamaldehyde*

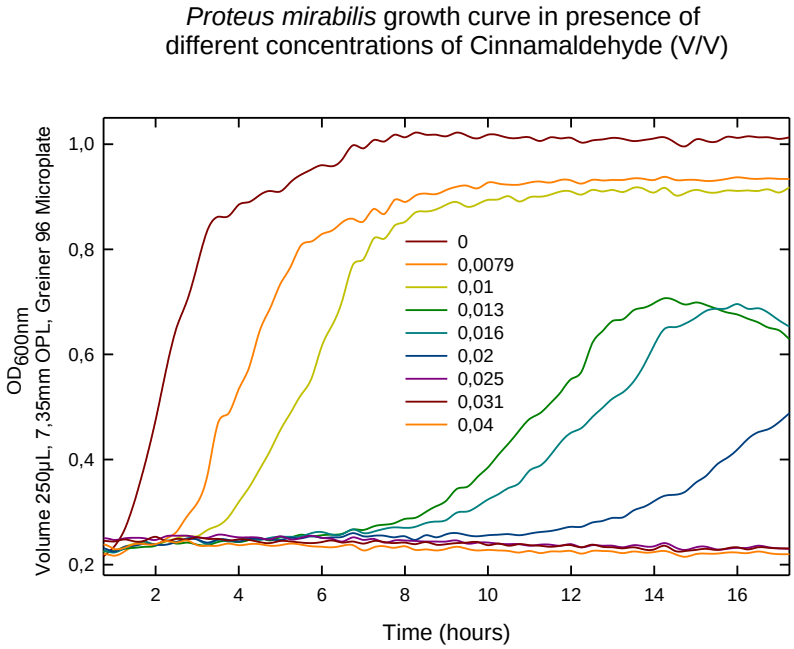


Fig. 5. *Growth curves of P. mirabilis in LB broth containing different concentrations of cinnamaldehyde*

Conclusions

The disk diffusion assay showed that Cinnamomum cassia oil and his major compound, cinnamaldehyde, have an important antibacterial and antifungal effect. We observed that this two have a major inhibitory effect against E. coli and S. cerevisiae while against P. mirabilis the inhibitory effect is less important. The eugenol has a low inhibitory effect against all three microorganisms.

From the growth curves we observe that C. cassia oil and cinnamaldehyde have an inhibitory effect using very low concentrations but is the cinnamon oil that has the most important antibacterial effect. The results suggest that C. cassia oil and cinnamaldehyde can be used as natural antimicrobial agents in food industry.

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