

## Ranking the Antimicrobial Activity of Four Principle Essential Oil Components Against Some Food Pathogenic Bacteria

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**Abstract:** Four essential oil compounds namely, cinnamaldehyde, eugenol, carvacrol and thymol were evaluated for their antimicrobial activity against some food-borne pathogenic bacteria like *Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli* and *Salmonella typhimurium*. Results showed that thymol has the highest significant antimicrobial activity against all tested bacteria. The rest of the compounds have deferential antimicrobial activity depends on the microorganism.

**Key words:** Antimicrobial activity • Cinnamaldehyde • Eugenol • Thymol

### INTRODUCTION

Essential oils (EOs) are used as natural flavorings and biologically active agents. Numerous investigations illustrated the antimicrobial activity of EOs for protection against pathogenic and food spoilage microorganisms, especially in packaging materials [1-5]. However, the chemical composition of EOs varies from one geographical region to another and also from one variety to another. These factors make the reproducibility of results of antimicrobial activity for certain EO quite doubtful. To overcome this problem, researchers use a single pure component known for its major contribution to the antimicrobial activity of its parent EO [6].

The top 4 single EO components renowned for their highest antimicrobial activity are: cinnamaldehyde, eugenol, carvacrol and thymol. This investigation is directed towards ranking the antimicrobial activity of these individual EO constituents against different pathogenic bacteria. The study is part of our trial to find a universal antimicrobial agent from plant origin that can be considered as the “Gold standard” for natural antimicrobials.

### MATERIALS AND METHODS

**Materials:** Cinnamaldehyde, eugenol, thymol and carvacrol are purchased from Sigma-Aldrich (St. Louice, USA).

### Methods:

- Antimicrobial evaluation study
- Microorganisms and cultures

The tested microorganisms were provided from the culture collections of the Microbiological Department National Research Center (NRC) Dokki, Giza, Egypt. These include two strains of Gram-positive bacteria *Staphylococcus aureus* (ATCC 43300), *Listeria monocytogenes* (ATCC 35152) and two strains of Gram-negative bacteria *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 27325).

**Antibacterial Assay:** The agar well diffusion method [7] was employed for the determination of antibacterial activities. In details: 0.1 ml of the diluted inoculums ( $10^7$  CFU/ml) of test organism was spread on tryptone soy agar (TSA) plates. Wells of 5 mm diameter were punched into the agar medium and filled with 50  $\mu$ l of 1% EO component dissolved in absolute ethanol (Control).

**Statistical Analysis:** Results are expressed as mean  $\pm$ SD. Data was analyzed using SPSS software (Version 22). Kruskal-Wallis test and Mann Whitney test were used to analyze the data in tables 1 and 2 respectively ( $p \leq 0.05$ ).

### RESULTS AND DISCUSSION

Table (1) compares the antimicrobial activity of 4 EOs components namely cinnamaldehyde, eugenol, carvacrol

Table 1: Comparison of different EOs compounds on pathogenic bacteria

Compound	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>E. coli</i>	<i>Salmonella typhimurium</i>
Cinnamaldehyde	20.00 <sup>ab</sup> ± 4.20	15.17 <sup>c</sup> ± 1.17	14.00 <sup>a</sup> ± 0.89	17.00 <sup>b</sup> ± 0.89
Eugenol	13.83 <sup>c</sup> ± 0.98	17.33 <sup>b</sup> ± 1.21	15.00 <sup>a</sup> ± 2.45	20.83 <sup>a</sup> ± 2.32
Carvacrol	16.50 <sup>b</sup> ± 0.84	21.33 <sup>a</sup> ± 1.63	12.67 <sup>b</sup> ± 0.52	19.17 <sup>a</sup> ± 1.72
Thymol	21.00 <sup>a</sup> ± 0.89	20.33 <sup>a</sup> ± 1.21	14.67 <sup>a</sup> ± 0.52	20.00 <sup>a</sup> ± 1.26
Control	7.00 <sup>d</sup> ± 0.00	10.33 <sup>d</sup> ± 0.58	6.67 <sup>c</sup> ± 0.58	6.00 <sup>c</sup> ± 0.00

- Results are expressed as mean ±SD
- Within the same column, means with different letters are significantly different at  $p \leq 0.05$

Table 2: Comparison of Thymol and a mixture of 3 components on different pathogenic bacteria

Strains	Thymol	Mix	P Value
<i>Staphylococcus aureus</i>	21.00± 0.89	15.83± 1.17	*0.002
<i>Listeria monocytogenes</i>	20.33± 1.21	17.83± 0.75	*0.004
<i>E. coli</i>	14.67± 0.52	14.83± 1.17	0.699
<i>Salmonella typhimurium</i>	20.00± 1.26	14.83± 1.60	*0.002

- Results are expressed as mean ±SD
- \*  $p \leq 0.05$ , Means within the same row are significantly different

and thymol on 4 pathogenic bacterial strains known for their deleterious effect on human health. All 4 components showed significant antibacterial effect against all 4 strains compared to control group. Among the 4 components, thymol was found to be the most effective, with the highest inhibitory activity against all tested pathogens. The largest inhibition zone for Thymol was against *S. aureus* (21 mm) while the smallest was against *E. coli* (14.67 mm).

On the other hand, the antimicrobial activity ranking of the 3 other EO components (cinnamaldehyde, eugenol and carvacrol) appears to be strain-dependent. For instance, cinnamaldehyde showed the largest inhibition zone against *S. aureus* (20 mm), among the 3 components, followed by carvacrol (16.50 mm), then eugenol (13.83 mm).

For *L. monocytogenes*: Carvacrol had the highest activity (21.33 mm), followed by eugenol (17.33 mm), while cinnamaldehyde was the least active (15.17 mm).

Regarding *E. coli*: the highest activity was found for eugenol and cinnamaldehyde (with no significant difference between them), while the least was for carvacrol.

Carvacrol and eugenol showed the highest activity against *S. typhi* among the 3 EO components (with no significant difference between them), while the least was for cinnamaldehyde (17 mm).

Due to the potent antimicrobial activity of thymol, as evident from Table (1), it was compared against the activity of a mixture of equal volumes of the 3 other components (cinnamaldehyde, eugenol and carvacrol). This was to evaluate potentials of synergism that may

exist between the 3 components and if it can compensate for the relatively low antimicrobial activity of some components compared to thymol (Table 1).

The data in Table (2) focuses on the significance of difference between thymol (the number 1 ranked antimicrobial compound) and a mixture of the 3 other components. From the table it is clear that thymol is still ranked first against all strains compared to the mixture except for *E. coli* in which there is no significant difference in activity between thymol and the mix.

## CONCLUSION

Thymol proved to be a potent antimicrobial agent among the 4 tested essential oil components. That can offer a new horizon of application in disinfection.

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