

# Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics

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## ABSTRACT

The objective of this study was to evaluate the antibacterial activity of *Cinnamomum cassia* (cinnamon) essential oil (EO) alone and in combination with some classical antibiotics against three multidrug-resistant bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, to search a possible synergy. The antibacterial activity of all tested compounds was determined by agar disc diffusion and minimum inhibitory concentration assays. The checkerboard method was used to quantify the efficacy of cinnamon EO in combination with these antibiotics. Fractional inhibitory concentrations were calculated and interpreted as synergy, addition, indifferent, or antagonism. A synergistic interaction was shown against *S. aureus* with the combination cinnamon EO and ampicillin or chloramphenicol and against *E. coli* when cinnamon EO was combined with chloramphenicol. However, the combination of cinnamon oil and streptomycin displayed additive effects against all bacteria stains. The combinations of cinnamon EO and antibiotics can be used as an alternative therapeutic application, which can decrease the minimum effective dose of the drugs, thus reducing their possible adverse effects and the costs of treatment.

**Key words:** Antibacterial drugs, fractional inhibitory concentration index, resistant bacteria, synergy

## INTRODUCTION

Multidrug-resistant bacteria are widely known as a serious threat to global health in the 21<sup>st</sup> century.<sup>[1]</sup> The World Health Organization published in 2017 a report listing the most dangerous multidrug-resistant bacteria to which new antibiotics should be urgently discovered.<sup>[2]</sup> The discovery of new antibacterial agents was mainly based on natural products that can be obtained from different sources including plants, bacteria, algae, fungi, and animals; but, there has been an increased interest in bioactive compounds provided by the plant as an alternative to the common

antibiotics.<sup>[3]</sup> Essential oils (EOs) account for a source of very promising natural compounds for producing new antibacterial drugs. Numerous studies have reported a strong antibacterial effect for some EOs.<sup>[4,5]</sup> Among these EOs, the potential antibacterial of cinnamon has been documented frequently.<sup>[6-8]</sup> Furthermore, the combinations, either single EOs or mixtures of purified main components, would assure the exhibition of the target bacteria to many chemical compounds and usually lead to better activity.<sup>[9]</sup> Combining different EOs has been recently studied in a view to increasing their antibacterial effect without increasing their concentration.<sup>[10-12]</sup> It was argued that the combination of cinnamon and some plants' EOs showed an additive effect against bacterial species compared to their pure EOs.<sup>[12,13]</sup> Limited reports on the combinations of cinnamon

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with antibiotics demonstrated synergistic and additive effects against various microorganisms.<sup>[14-16]</sup> However, to the best of our knowledge, there are no available data about the antibacterial activity of cinnamon EO associated with antibiotics against multidrug-resistant *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. Therefore, the aim of this study was to search the possible synergistic antibacterial effect of cinnamon EO combined with certain classical antibiotics as ampicillin, streptomycin, or chloramphenicol against *E. coli*, *S. aureus*, and *P. aeruginosa*.

## MATERIALS AND METHODS

### Essential oils extraction

*Cinnamomum cassia* (cinnamon) barks were purchased from a local supermarket in Fez province (Morocco). A total of 200 g of cinnamon barks was subjected to hydrodistillation for 3 h with 700 ml of distilled water using a Clevenger-type modified apparatus. The hydrosol was collected in a separatory funnel (1 L) so that the heavy oil was decanted to the bottom of the flask, while the hydrosol water was recycled into the flask containing the boiling plant material. The EO was collected and stored at 4°C before analysis.

### Bacterial strains

In this study, the antibacterial activity of cinnamon oil alone and in combination with some antibiotics as ampicillin, streptomycin, and chloramphenicol was tested against three bacterial strains (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853) provided by the Pasteur Institute of Casablanca (Morocco). The inoculum suspension was obtained by taking colonies from 24 h cultures. The colonies were suspended in sterile 0.9% aqueous solution of NaCl. The density was adjusted to the turbidity of a 0.5 McFarland Standard ( $10^8$  colony-forming unit [CFU]/mL).<sup>[17]</sup>

### Agar disc diffusion assay

The agar disc diffusion assay was determined according to the experiment of Kirby-Bauer;<sup>[18]</sup> the suspensions of microorganisms ( $10^8$  CFU/ml) were flood inoculated onto the surface of Mueller Hinton (MH) agar plates. Sterile 6 mm diameter filter discs (Whatman paper No 3) were impregnated with 10 µg/disc of the compounds and were put onto the surface of the inoculated MH agar. The plates were incubated at 37°C for 18 h. The antibacterial effect was evaluated by measuring the inhibition zones against the tested bacterial strains. All the tests were performed in triplicate. The values of inhibition diameter are given as mean ± standard deviation. The results were analyzed by one-way ANOVA followed by Tukey's test, using GraphPad Prism 5 software. Differences at  $P < 0.05$  were considered to be significant.

### Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was performed using a microdilution assay in 96 well plates according

to the experiment of the National Committee for Clinical Laboratory Standards<sup>[19]</sup> with modification; the different concentrations of cinnamon EO and antibiotics were prepared in a suspension containing 0.2% agar in sterile distilled water.<sup>[20]</sup> They were carried out by successive dilutions 1/2 ranging from 5000 to 9 µg/ml for EO and from 200 to 0.4 µg/ml for antibiotics. Cinnamon EO or antibiotics were added at different concentrations at the corresponding well in plate. The concentrations obtained in the wells are between 1250 and 2 µg/ml for EO and between 50 and 0.1 µg/ml for antibiotics. Bacterial suspensions were prepared in the same manner described previously and diluted in MH broth and plated in 96 well plates at a density of  $10^6$  CFU/ml. Finally, the plates were incubated at 37°C for 18–24 h. Bacterial growth was visually by adding to each well 20 µl of 2,3,5-triphenyl tetrazolium chloride aqueous solution (1%). MIC was defined as the lowest concentration that does not produce red color.<sup>[17]</sup>

### Checkerboard assay

The evaluation of the interaction between cinnamon EO and antibiotics was carried out according to the method of Mody<sup>[21]</sup> with some modifications. Briefly, ten concentrations of cinnamon EO and eight concentrations of each antibiotic were prepared in sterile tubes by dilutions 1/2. Subsequently, EO at decreasing concentrations, going from MIC × 4 to MIC/128, was introduced horizontally into the 96 well plates. In the same manner, the antibiotics at decreasing concentrations, going from MIC × 4 to MIC/32, was introduced vertically. The final volume in each well was 200 µl comprising 25 µl of EO, 25 µl of one of the antibiotics' dilution, and 150 µl of MH media containing  $10^6$  CFU/ml of bacterial suspensions. The plates were then incubated at 37°C for 18 h. The analysis of the combination was obtained by calculating the fractional inhibitory concentration index (FICI) using the following formula:<sup>[22]</sup>

$$\text{FIC index (FICI)} = \text{FIC}_A + \text{FIC}_B$$

$$\text{FIC}_A = \frac{\text{MIC of (A) in combination}}{\text{MIC of (A) alone}}$$

$$\text{FIC}_B = \frac{\text{MIC of (B) in combination}}{\text{MIC of (B) alone}}$$

Where (A) is cinnamon EO and (B) is one of the antibiotics.

The FICI values were interpreted as a synergistic effect when  $\text{FICI} \leq 0.5$ ; an additive effect when  $0.5 < \text{FICI} < 1$ ; an indifferent when  $1 < \text{FICI} < 4$ ; and an antagonistic effect when  $\text{FICI} > 4$ .

## RESULTS

### Antibacterial activity

The antibacterial activity of the cinnamon EO, streptomycin, ampicillin, and chloramphenicol tested against three

bacterial strains (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853) and the MIC results are shown in Table 1. Cinnamon oil was able to inhibit all strains tested: *E. coli*, *S. aureus*, and *P. aeruginosa* with the MIC of 4.88, 4.88, and 19.53 µg/ml, respectively. However, streptomycin had the same MIC (3.13 µg/ml) against all bacteria strains. Ampicillin and chloramphenicol inhibited *E. coli* with the same MIC of 0.31 µg/ml. However, the antibacterial effect of ampicillin was higher than that of chloramphenicol against *S. aureus* with the MIC of 0.16 and 0.31 µg/ml, respectively. While *P. aeruginosa* was resistant to ampicillin and chloramphenicol [Table 1].

### Combined effects of cinnamon essential oil and antibiotics

Tables 2 and 3 show the cinnamon oil tested against three multidrug-resistant bacteria in combination with some

antibiotics. As shown in Table 3, mixing antibiotics with cinnamon EO reduces the MICs, 2 fold for *P. aeruginosa*, 2–8 fold for *S. aureus*, and 2–4 for *E. coli*. As revealed in Table 3, the synergistic effect was obtained by the combination of cinnamon EO with ampicillin or chloramphenicol against *S. aureus* and by the combination of EO and chloramphenicol against *E. coli* with the FICI of 0.5. However, an additive effect was founded when EO was combined with streptomycin against all stains tested; *E. coli*, *S. aureus*, and *P. aeruginosa*; with FICI of 1.00, 0.75, and 1.00, respectively. Moreover, the combination EO–ampicillin showed an indifferent effect against *E. coli* with the FICI of 1.25 [Table 3].

### DISCUSSION

Based on the results in Table 1, it was known that the cinnamon oil was able to inhibit all strains tested; these results are

**Table 1: Inhibition zone diameter and minimum inhibitory concentration of cinnamon essential oil and antibiotics**

	<i>Escherichia coli</i> ATCC 25922		<i>Staphylococcus aureus</i> ATCC 25923		<i>Pseudomonas aeruginosa</i> ATCC 27853	
	ID	MIC	ID	MIC	ID	MIC
Cinnamon EO	29.0±0.7 <sup>a</sup>	4.88	40±0.5 <sup>a</sup>	4.88	30.5±1.0 <sup>a</sup>	19.53
Streptomycin	15.3±0.1 <sup>d</sup>	3.13	16±0.0 <sup>d</sup>	3.13	15.2±0.6 <sup>b</sup>	3.13
Ampicillin	20±0.5 <sup>c</sup>	0.31	37.1±0.2 <sup>b</sup>	0.16	NI	NT
Chloramphenicol	25±1.0 <sup>b</sup>	0.31	25.0±0.4 <sup>c</sup>	0.31	NI	NT

Inhibition zone includes diameter of disk (6 mm). Values of inhibition diameter are given as mean±SD. In each column, different letters are significantly different by the Tukey's test ( $P<0.05$ ). ID: Inhibition zone diameter (mm), MIC: Minimum inhibitory concentration (µg/ml), NT: Not tested, NI: No inhibition, EO: Essential oil, SD: Standard deviation, ATCC: American type culture collection

**Table 2: Minimum inhibitory concentrations (µg/ml) and fractional inhibitory concentration values of the combinations of Cinnamon EO and antibiotics**

	<i>Escherichia coli</i> ATCC 25922			<i>Staphylococcus aureus</i> ATCC 25923			<i>Pseudomonas aeruginosa</i> ATCC 27853		
	MIC alone	MIC combined	FIC	MIC alone	MIC combined	FIC	MIC alone	MIC combined	FIC
EO + streptomycin									
EO	4.88	2.44	0.50	4.88	1.22	0.25	19.53	9.77	0.50
Streptomycin	3.13	1.56	0.50	3.13	1.56	0.50	3.13	1.56	0.50
EO + ampicillin									
EO	4.88	1.22	0.25	4.88	0.61	0.13	19.53	NT	-
Ampicillin	0.31	0.31	1.00	0.16	0.04	0.25			
EO + chloramphenicol									
EO	4.88	1.22	0.25	4.88	1.22	0.25	4.8	NT	-
Chloramphenicol	0.31	0.08	0.25	0.31	0.08	0.25	NT		

FIC: Fractional inhibitory concentration, MIC: Minimum inhibitory concentration, EO: Essential oil, NT: Not tested, ATCC: American type culture collection

**Table 3: Fractional inhibitory concentration indices values of the combinations of cinnamon EO and antibiotics**

	<i>Escherichia coli</i> ATCC 25922		<i>Staphylococcus aureus</i> ATCC 25923		<i>Pseudomonas aeruginosa</i> ATCC 27853	
	FICI	Interaction	FICI	Interaction	FICI	Interaction
EO + streptomycin	1.00	Additive	0.75	Additive	1	Additive
EO + ampicillin	1.25	Indifferent	0.38	Synergy	-	-
EO + chloramphenicol	0.50	Synergy	0.50	Synergy	-	-

EO: Essential oil, FICI: Fractional inhibitory concentration index, ATCC: American type culture collection

relatively in agreement with the previous reports.<sup>[7,12]</sup> The high antibacterial activity observed in cinnamon EO may be due to the action of transcinnamaldehyde, considered as its single major compound.<sup>[7,8]</sup> It has been reported that transcinnamaldehyde possesses the highest antimicrobial in comparison with other constituents of cinnamon oil.<sup>[9,23,24]</sup> On the other hand, synergistic interactions between different plant extracts are the aim of the herbal formulation in folk medicine.<sup>[25]</sup> Some authors had determined the antibacterial effect of cinnamon EO combinations; Utchariyakiat *et al.* showed that cinnamon EO combined with colistin demonstrated a synergistic action against multidrug-resistant *P. aeruginosa*.<sup>[14]</sup> Mahadlek *et al.* used the checkerboard assay to determine the activity of cinnamon oil associated with the same antimicrobial agents; they observed that cinnamon EO combinations with doxycycline hyclate, ciprofloxacin HCl, or metronidazole displayed an additive against *S. aureus* ATCC 6538P.<sup>[16]</sup> It was reported that the association of cinnamon oil and piperacillin demonstrated a synergistic interaction against *E. coli*.<sup>[15]</sup> There are some reports on the interaction between plant EOs and antibiotics, which indicated a synergistic action.<sup>[25,26]</sup> These data were confirmed by the present work, thus highlighting the efficacy of cinnamon oil when combined with ampicillin or chloramphenicol. The effect of a combination of cinnamon and other EOs was reported, for example, Clemente *et al.* demonstrated that the combination of cinnamon with mustard EOs showed an additive effect against *E. coli* ATCC25922, *P. aeruginosa* ATCC 27853, and some other bacteria species.<sup>[12]</sup> Lu *et al.* showed that cinnamon combined with thyme or clove EOs displayed in most cases an additive or indifferent action against foodborne bacteria.<sup>[13]</sup> However, the mechanism that is responsible for the antimicrobial activity of cinnamon includes its chemical composition such as Cinnamaldehyde,<sup>[6]</sup> which is an electronegative molecule that could interfere with the cellular biological process, particularly nitrogen-containing substances such as proteins and nucleic acids.<sup>[25]</sup> Furthermore, cinnamon EO has been reported to inhibit bacteria through antiquorum sensing effects; inhibiting cell division, ATPase, biofilm formation membrane porin, and mobility; altering the lipid profile<sup>[6]</sup> and thereby acting cell membrane producing lumps and autoaggregation.<sup>[12]</sup> These properties could conduct the reduction of bacterial susceptibility to antibiotics, decrease the dose of the antibiotic required for treatment, and therefore will decrease the toxic side effects of the drugs.<sup>[25]</sup> However, other studies are needed to explain the mechanism responsible for the antibacterial effect of the combination of cinnamon and antibiotics.

## CONCLUSION

The present study reported that the combinations between cinnamon EO and some classical antibiotics had synergistic and additive interactions against multidrug-resistant bacteria. These combinations can be used as an alternative

therapeutic application, which could decrease the minimum effective dose of the drugs, thus reducing their possible adverse effects and the costs of treatment.

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## Conflicts of interest

There are no conflicts of interest.

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