

# Antibacterial Activity of Essential Oils Extracts from Cinnamon, Thyme, Clove and Geranium Against a Gram Negative and Gram Positive Pathogenic Bacteria

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**Abstract:** Essential oils and other extracts of plants have evoked have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. In this context, the aim of this study was to investigate the antibacterial activity of the essential oils from Cinnamon, Thyme, Clove and Geranium against four strains of Gram negative bacteria and two Gram positive bacteria. The in-vitro antimicrobial effects of these essential oils was determined by the disc diffusion method. The Minimum Inhibitory Concentration (MIC) was evaluated by using the broth serial dilution method and Minimum Bactericidal Concentration values (MBC) were defined as the lowest concentration of sample which resulted in  $\geq 99.9\%$  kill of the initial inoculum. The antibacterial effect was deemed bactericidal or bacteriostatic depending on the ratio: MB/CMI. Cinnamon possesses an important antimicrobial activity against all tested microbes, with the inhibition zones ranging from 26 to 32 mm. The essential oils of thyme and Clove showed the antibacterial activity with inhibition zones at 16–22 mm and 16–20 mm, respectively. However, the Geranium essential oil failed to inhibit any of the tested strains. Both gram-positive and gram-negative bacteria were resistant to this essential oil. Results according to the MICs and MBCs revealed that the essential oil from Cinnamon showed the most remarkable bactericidal effect. The essential oil from Cinnamon might be exploited as natural antibiotic for the treatment of several infectious diseases caused by the pathogens germs and conservation agents in the food.

**Keywords:** Pathogenic Bacteria, Antibacterial Activity, Essential Oils, Cinnamon, Thyme, Clove, Geranium

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## 1. Introduction

Essential oils have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Indeed, interest in essential oils has revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine that claims

that essential oils and other aromatic compounds have curative effects.

Emerging resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad-spectrum activities [1]. The antimicrobial activity of Essential oils (EOs) has long been recognised and they have been extensively tested *in*

*vitro* against a wide range of pathogenic bacteria and fungi [2]. Some oils have been used in cancer treatment [3]. Some other oils have been used in food preservation [4], aromatherapy [5] and fragrance industries [6].

The main constituents of essential oils –mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones – are responsible for the biological activity of aromatic and medicinal plants as well as for their fragrance. The mechanism by which the essential oils exert their antimicrobial activity is poorly understood but the main target appears to be the cell membrane of bacterial cells [7].

Essential oils such as cinnamon, thyme, clove and geranium have been traditionally used by people for various purposes in different parts of the world. In this context, the aim of the current study was to investigate the *in vitro* activity of these essential oils against four strains of Gram negative bacteria and two strains of Gram positive bacteria.

## 2. Materials and Methods

### 2.1. Essential Oils

Four essential oils obtained from Morocco society phytonutrition (table 1). These oils were selected based on literature survey and their use in traditional medicine. Quality of the oils was ascertained to be more than 98% pure.

Table 1. Essential oils and their components.

Common name	Botanical name	Main components
Thyme	<i>Thymus Vulgaris</i> <i>Thymol</i>	Thymol
		Carvacrol
		Linalol
		Para-cymene
		Gamma-terpinene
Clove	<i>Syzygium aromaticum</i>	Myrcene
		Eugenol
		Beta-caryophyllene
		Acetate eugenyle
		E-cinnamaldehyde
Cinnamon	<i>Cinnamomum cassia</i>	Z- cinnamaldehyde
		Trans-2-methoxycinnamaldehyde
		Acete cinnamyle
Geranium	<i>Pelargonium asperum ssp Egypte</i>	Citronnellol
		Geraniol
		Formate citronnellyle

### 2.2. Bacterial Strains and Medias

Four strains of Gram-negative bacteria [*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonelle spp*] and two strains of Gram-positive bacteria [*Streptocoque de groupe D* and *Staphylococcus aureus* (ATCC 25923)] were used in this study. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures. Mueller–Hinton agar (MHA) and

Mueller–Hinton broth (MHB) were used in this study

## 3. Antimicrobial Activity Assay

### 3.1. Aromatogram Test

The disk diffusion method was used in the present study. An overnight culture for each microbial strain was adjusted to 0.5 McFarland standards ( $10^8$  CFU/ml). 500 µL of the suspension were spread over agar plates containing Mueller Hinton Agar (MHA).

Under aseptic conditions, empty sterilized discs (Whatman 6 mm in diameter) were impregnated with 10 µL of pure essential oil and placed on the agar surface. The inoculated plates were incubated at 37°C for 18-24h. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the test organisms. Each assay was repeated 2 times.

### 3.2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The Minimum Inhibitory Concentration (MIC) values were evaluated by using the broth serial dilution method. To obtain the highest concentration (80 mg/ml) to be tested, 400 µl of the essential oils used in the antimicrobial tests were placed in test tubes that contained 4.6 ml of MHB supplemented with Tween 80 (0.01% v/v). Serial dilutions of the high concentration were made in sterile test tubes contained (MHB + Tween 80 (0.01% v/v).to develop a concentration range from 0.3 to 80 mg/ml. After pre-culturing, microorganism suspensions ( $10^8$  CFU/ml) were inoculated into fresh broth MHB + Tween 80 (0.01% v/v) containing one EO. Following incubation at 37°C for 24 h, MIC values were determined as the lowest concentration of the essential oils at which the completely inhibited the visible growth of microorganisms in broth.

For the determination of the Minimum Bactericidal Concentration (MBC) values of the essential oils, a total of 20 µL from clear wells of the MICs test were plated on Mueller- Hinton agar (MHA). MBC values were defined as the lowest concentration of sample which resulted in  $\geq 99.9\%$  kill of the initial inoculum.

The antibacterial effect was deemed bactericidal or bacteriostatic depending on the ratio: CMB/CMI. Indeed, if CMB/MIC = 1-2, the effect is bactericidal and if CMB/CMI = 4-16, the effect is bacteriostatic [8].

## 4. Results

The antimicrobial activity of four essential oils against a range of six bacterial species is summarized in Table 2. These results relieved that the Cinnamon oil exhibit the higher activity antibacterial against all microorganisms tested, with inhibition zones ranging from 26 to 32 mm. The highest antibacterial activity showed in *Pseudomonas aeruginosa* (32 mm), and lowest inhibition effect observed in both *Streptocoque group D* and *Salmonelle spp* (26 mm).

**Table 2.** Antibacterial activity of the essential oils (10µl/disc.) against the bacterial strains tested using disc-diffusion method, inhibition zones in mm.

Bacteria	Geranium	Clove	Thyme	Cinnamon
<i>E. coli</i>	0	16	18	28
<i>Salmonelle spp</i>	0	20	18	26
<i>K. pneumoniae</i>	0	0	0	28
<i>P. aeruginosa</i>	0	18	0	32
<i>Streptocoque</i> group D	0	18	16	26
<i>S. aureus</i>	0	20	22	28

Depending on the susceptibility of the tested bacteria, Clove essential oil produced an inhibition zone varying from 16 to 20 mm, the lowest inhibitory zone was observed against

*E. coli* (16 mm) but the highest inhibitory zone was showed against *Salmonelle spp* and *Staphylocoque* group D (20 mm), of same clove has no antibacterial effect on *K. pneumoniae*.

On the other hand, inhibition zone produced by Thyme

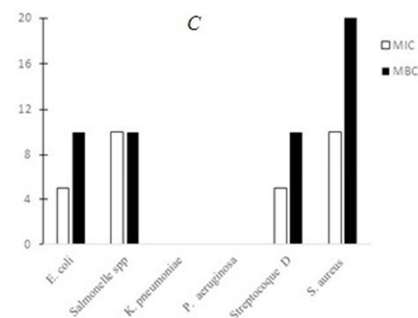
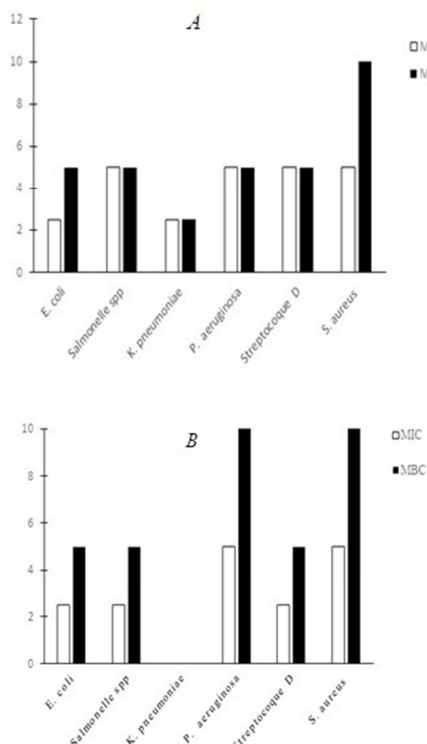
essential oil varying from 16 to 22 mm. The minimal inhibition zone was observed against *Staphylocoque* group D (16 mm) whereas the maximal inhibition zone was showed against *S. aureus* (22 mm). This oil shows that the growth of both test organisms *K. pneumoniae* and *Pseudomonas aeruginosa* were not inhibited.

Results according to the MICs and MBCs of each essential oil against microbial strains studied are showed in Tables 3 and figure 1. In this study Cinnamon oil exhibited the antibacterial activity with MIC and MBC values of 2.5-5 mg/mL and 2.5-10 mg/mL respectively. The lower MIC was showed in *Escherichia coli* and *Klebsiella pneumoniae* but the higher MIC was observed in *Salmonelle spp*, *Pseudomonas aeruginosa*, *Streptocoque* group D and *Staphylococcus aureus*. On the other hand, the lowest MBC value was observed in *Klebsiella pneumoniae* and the highest MBC value was observed in *Staphylococcus aureus*.

**Table 3.** MIC and MBC values (MIC and MBC mg/mL), of selected essential oils against the bacterial strains tested.

Bacteria	Clove			Thyme			Cinnamon		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
<i>E. coli</i>	2.5	5	2	5	10	2	2.5	5	2
<i>Salmonelle spp</i>	2.5	5	2	10	10	1	5	5	1
<i>K. pneumoniae</i>	-	-	-	-	-	-	2.5	2.5	1
<i>P. aeruginosa</i>	5	10	2	-	-	-	5	5	1
<i>Streptocoque</i> group D	2.5	5	2	5	10	2	5	5	1
<i>S. aureus</i>	5	10	2	10	20	2	5	10	2

The essential oil from Clove possessed MIC at 2.5-5 mg/ml. and MBC at 5–10 mg/ml. The lowest MIC and MBC are showed in *Escherichia coli*, *Salmonelle spp* and *Streptocoque* group D, but the higher MIC and MBC observed in *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

**Figure 1.** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC) Concentration of selected essential oils against the bacterial strains tested. A: Cinnamon B: Clove and C: Thyme.

Thyme oil possessed MIC in the range of 5-10 mg/mL, while its MBC was at 10-20 mg/ml. The maximal MIC value observed in *Salmonelle spp* and *Staphylococcus aureus*, but the minimal MIC value showed in *Escherichia coli* and *Streptocoque* group D. The lower MBC values found for Thyme essential oil showed in *Escherichia coli*, *Salmonelle spp* and *Streptocoque* group D, but the higher MBC values are showed in *Staphylococcus aureus*.

From the obtained ratio, MBC/MCI (Table 3), it can be noticed that the essential oils from Thyme and Clove showed bactericidal effect against bacterial species tested except *K. pneumoniae* and *P. aeruginosa* for Thyme. and *K. pneumoniae* for Clove. The essential oil from Cinnamon showed a bactericidal effect against both Gram-positive and Gram-negative bacteria,

## 5. Discussion

*In vitro* studies in this work showed that the essential oils from Cinnamon, Thyme and Clove exhibited the antibacterial activity against microbial strains studied, but their effectiveness varied. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food-borne pathogens [9, 10].

Our results revealed that the essential oil of Geranium did not affect all bacterial species. Both Gram-positive and Gram-negative bacteria were resistant to this essential oil. The essential oils from Thyme and Clove showed the good antibacterial activity with inhibition zones at 16–22 mm and 16–20 mm, respectively. These results are in agreement with those reported by Lopez *et al.* [11] that Clove oil was found active against foodborne Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *P. aeruginosa*). Similarly, in another study Sabahat and Perween reported that Clove oil exhibited maximum activity against *V. cholerae* with 23.75 mm mean diameter of zone of inhibition [12]. Deise *et al.* [13] reported that the essential oil of thyme had better activity against *E. coli*. The antimicrobial activity of the thyme essential oil and of thymol has been evaluated in other studies. Ivanovic *et al.* [14] reported significant activity of the extract and essential oil of thyme against *E. coli* and *Salmonella* strains. It was also reported antimicrobial activity of the essential oil of thyme against *E. coli* 5% (V/V) and other food borne bacteria [15].

The cinnamon essential oil showed the strongest antibacterial activity against all the bacterial species tested. Results of the present study are in harmony to those carried out by [16], that Cinnamon oil exhibited strong activity against the selected bacterial strains.

Minimum inhibitory and bactericidal concentrations (MIC, MBC) for the three selected oils (Thyme, Clove and Cinnamon) ranged from 2.5 to 10 and 2.5 to 20 respectively. In particular, the essential oil of Cinnamon possesses an important antibacterial effect against both Gram-positive and Gram negative bacteria with MIC and MBC values of 2.5 to 5 and 2.5 to 10 mg/ml respectively. Smith-Palmer *et al.* [17] reported the reduction of *L. monocytogenes* from 6.0 log CFU/g to less than 1.0 log CFU/g in low and high fat cheese with low concentration of Clove essential oil. In the other hand, several studies have shown that Cinnamon, Clove and rosemary oils had strong and consistent inhibitory effects against various pathogens [18].

Many studies showed that the activity of the oils could be attributed, to characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane, disturbing the cell structures and rendering them more permeable [19, 20]. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [21].

From this study it can be concluded that cinnamon oil has the most potential bactericidal properties. This essential oil might be exploited as natural antibiotic for the treatment of several infectious diseases caused by the pathogens germs. We believe that the present investigation together with previous studies provide support to the antibacterial properties of cinnamon.

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