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ANTIBACTERIAL ACTIVITY OF CINNAMON ESSENTIAL OIL (Cinnamomum cassia) AND CINNAMALDEHYDE ON AVIAN ESCHERICHIA COLI STRAINS

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ABSTRACT

The development of bacterial resistance to common antimicrobial agents is a major challenge for actors in the field of human and animal health. This has prompted the search for new antimicrobial agents from a variety of sources, including plants. The present study investigates the antibacterial activity of cinnamon essential oil and its major constituent, the cinnamaldehyde. This activity was evaluated using standard aromatogram technique and the liquid macrodilution technique for MIC and MBC determination using a reference germ test (E. coli ATCC25922) and forty antibiotic resistant E. coli strains isolated from broiler chickens. In a second part of the study, cinnamaldehyde was combined to certain antibiotics and tested for possible synergies. The obtained results have showed, cinnamon essential oil was more active than cinnamaldehyde. The average MIC values obtained with cinnamaldehyde were 0.031% and 0.04% for reference and avian E. coli respectively, and the average MBC value was 0.031% for the reference strain. The bactericidal activity of Cinnamaldehyde against E. coli is close to the time-dependent kind antibiotics against E. coli bacteria. Combinations of Cinnamaldehyde-antibiotics were indifferent against the reference E. coli strain.

Keywords: Essential oil, cinnamon, cinnamaldehyde, Resistance, E. coli, Aromatogram

1. INTRODUCTION

Despite advances in understanding microbial pathogenesis, infectious diseases continue to pose serious health problems with the emergence of antibiotic resistance. Indeed, a few years after the use of penicillin for the treatment of bacterial infections, more than 50% of isolated *S. aureus* strains were no longer susceptible to this antibiotic (Alanis, 2005). Since then, there has been a progressive extension of bacterial resistance, which represents a particular aspect of the extraordinary capacity for natural evolution selected by the use of antibacterial products. In humans, excessive use of antibiotics and poor monitoring of treatments (Li and Wang, 2005) are the major causes of this resistance. The use of antibiotics in animal husbandry is also partly responsible for the general development of resistance (Wegener, 2003). Antibiotic resistance is a

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natural and predictable mechanism that refers to a situation where an antibiotic that would normally have stopped the development of a certain type of bacteria is no longer able to do so (Acar and*al.*, 2006). Today, when the resistance of germs to antibiotics becomes more and more worrying, essential oils (HE) show their effectiveness. Essential oils are complex products, mostly containing more than a hundred constituents (phenols, alcohols, aldehydes, esters, terpenes, ketones). They come from so-called aromatic and medicinal plants (PAM) (Maihebiau, 1994; Thell and *al.*, 2015). The antimicrobial properties of essential oils, known since antiquity, have been the subject of a good number of publications which confirmed, by in vitro studies, their inhibitory action against varieties of bacteria (Gram+ and Gram-) belonging to the pathogenic or incriminated groups in the process of alteration of food products. Several molecules present in essential oils are endowed with antimicrobial properties and particularly phenols (such as carvacrol, thymol and eugenol), alcohols (such as linalool) and aldehydes (such as cinnamaldehyde). It is usually the essential oils rich in such molecules that have the highest antimicrobial efficacy. Certain essential oils (such as eucalyptus, chamomile, mugwort and verbena) have the potential to inhibit the germination of the spores of *Clostridium botulinum* and *Bacillus cereus* (Chaibi and *al.,* 1997). Others are able to alter the pathogenicity of bacteria; clove stem essential oils (*Syzygiumaromaticum*), cinnamon leaves (*Cinnamomum verum*), nutmeg (*Myristicafragrans*) and thyme (*Thymus vulgaris*) reduce Listeria's production of *L-listeria monocytogenes* (Smith-Palmerand and *al.,* 2002). The antibacterial activity of an essential oil appears to be influenced by the chemical structure of aromatic molecules, their proportions and their combined actions at several levels of the bacterial structure (Dorman and Deans 2000, Delaquisand *al.,* 2002).

2. METHODOLOGY

MATERIAL

Natural products of cinnamon

The essential oil of cinnamon was extracted by hydrodistillation at the laboratory level of the Department of Food and Nutrition Sciences (IAV Hassan II), and cinnamaldehyde natural (>95%) Sigmawere evaluated *in-vitro*.

Bacterial strains

The antibacterial activity of the essential oils and cinnamaldehyde was evaluated on a reference *E.coli (ATCC25922)* and 40 *Escherichia coli* strains of avian origin. The isolation of *Escherichia coli* was done from the lung, liver, heart, bone marrow and caecas.

METHODS

Essential oil extraction

The apparatus used for the hydro distillation of essential oils is of Clevenger type (Clevenger, 1928), it consists of a balloon heater, a Pyrex glass flask where the dried plate is placed and distilled water, a condensation column of the vapor (refrigerant) and a Pyrex glass collector which also receives the distillation extracts. The essential oils obtained are stored in a refrigerator in a brown glass bottle sealed at 4 ° C and in the shade.

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Essential oil chemical analysis

The essential oils have by gas chromatography (GC) according to the following conditions: Chromatograph: PEKRIN ELMER; Detector: FID; Column: PE-5; Injector temperature: 235°C; Detector temperature: 340°C; Temperature program: 50°C (4min), 5°/min, 230 ° (20 min); Injection volume: 0.02ml using a syringe; Carrier gas: N2 (nitrogen); Flow rate of the carrier gas: 1 ml / min.

Aromatogram

It is an *in-vitro* method, inspired by the antibiogram which makes it possible to determine the antibiotic activity of essential oils, by measuring the diameter of growth inhibition around an agar disk impregnated with essential oil. This method therefore makes it possible to study sensitivity of microorganisms to essential oils and measure their antimicrobial potency. This is the same material as for an antibiogram except that the antibiotics are replaced by essential oils. Discs (6 mm filter paper) soaked of Ceylon cinnamon essential oil or the cinnamaldehyde are placed on a medium of nutritious agar, where are grown bacteria. The inhibition diameters are read after 24 hours at 37 ° C. This method is also called radial diffusion test (Unlu and *al.*, 2010). The inhibition zone is measured in millimeters around the disc. Plus the diameter of the area induced inhibition is important, more essential oils inhibit bacterial growth

Dilution in a liquid medium (macrodilution)

Minimum inhibitory concentrations (MICs) of EO/ PA were determined according to the method reported by (Remmal and *al.*, 1993, Satrani and *al.*, 2001). The dilution method consists in preparing a series of tubes containing Tryptic Soy Broth (TSB), 10 test tubes are needed. The first tube is filled with 2 ml of TSB and the other 9 tubes with 1 ml. Then, 20 µl of a solution of EO/ PA in the first tube is added, followed by a dilution to obtain a concentration range ranging from 1% to 0.0039%. Afterwards, each tube is inoculated with 10 µl of the bacterial suspension (final concentration: 10^7 CFU / ml). A control without EO/PA is prepared and the tubes are incubated for 18 h at 37 ° C (Bouhdid and *al.*, 2008). The lowest concentration of the essential oil inhibiting any growth visible to the naked eye after 18 to 24 hours of incubation at 37 ° C is the minimum inhibitory concentration noted as MIC.

3. RESULTS AND DISCSSION

Chemical analysis of cinnamon EO

Cinnamaldehyde is the main component of cinnamon EO, accounting for almost 90%, followed by beta-caryophyllen (6.1%), and cinnamyl acetate and eugenol with 2.75%) and (0.46%) respectively.

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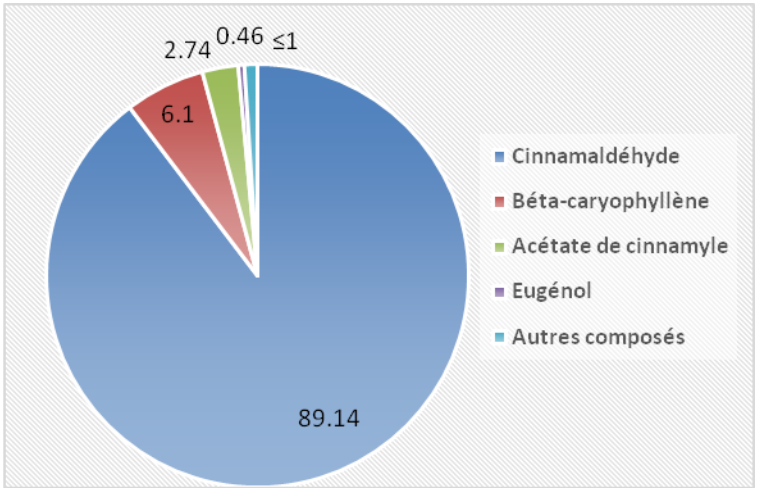

Figure 1: Chemical Composition of Cinnamon EO by CPG

The first studies on the chemical composition of this essential oil date from 1833. Two years later, it is discovered that cinnamic aldehyde is the main constituent. Others constituents will be characterized at the beginning of the 20th century. The precise composition is known only since about thirty years (Senanayake and *al.*, 1978). Its main constituent is aldehyde cinnamic, with eugenol, traces of other aldehydes and terpene carbides, our results are in agreement with these studies. The chemical composition of the essential oil of C. cassia has been widely studied (Lockwood, 1979; Qiu and *al.*, 2000). According to the European Pharmacopoeia (2011), the major component of cinnamon HE is cinnamaldehyde (up to 75%), also known as cinnamal or cinnamal aldehyde. Then come eugenol (up to 7.5%), β-caryophyllen (1-4%), cinnamyl acetate (1-8%), linalool (1-6%), 1, 8-cineole (3% maximum), the benzyl benzoate (maximum 1%), coumarin and safrole (maximum 0.5%).

The chemical composition of the essential oil of C. cassia, varies according to the season of harvest and the geographical situation, because, it has been proved that there was a variation in the chemical composition of C. cassia essential oil collected in different areas and extracted from different parts of plants and also including the degree of maturity of the plant at harvest. For example, the essential oil of C. cassia extracted at different stages displayed a different chemical profile (Geng and *al.*, 2010).

Antibacterial activity of cinnamon products

The aromatogram results, expressed as diameter of the zone of inhibition, are given in Table 1 and figure 2

Reference strain

The table 1 presents the results of the antibacterial activity of cinnamon EO and cinnamaldehyde on the reference strain (*E. coli ATCC25922*)

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Table 1: Diameters of the zones of inhibition obtained with the reference strain

Essai	Cinnamon EO	Cinnamaldehyde
1	**26**	**29**
2	**32**	**24**
Moyenne	**29±3**	**26,5±2,5**

For the reference strain, the activity of cinnamon EO was, on average, more potent than cinnamaldehyde.

Antibacterial activity on wild strains of avian origin

The figure 2 shows the results of the antibacterial activity of cinnamon and cinnamaldehyde on 40 *E. coli* strains of avian origin.

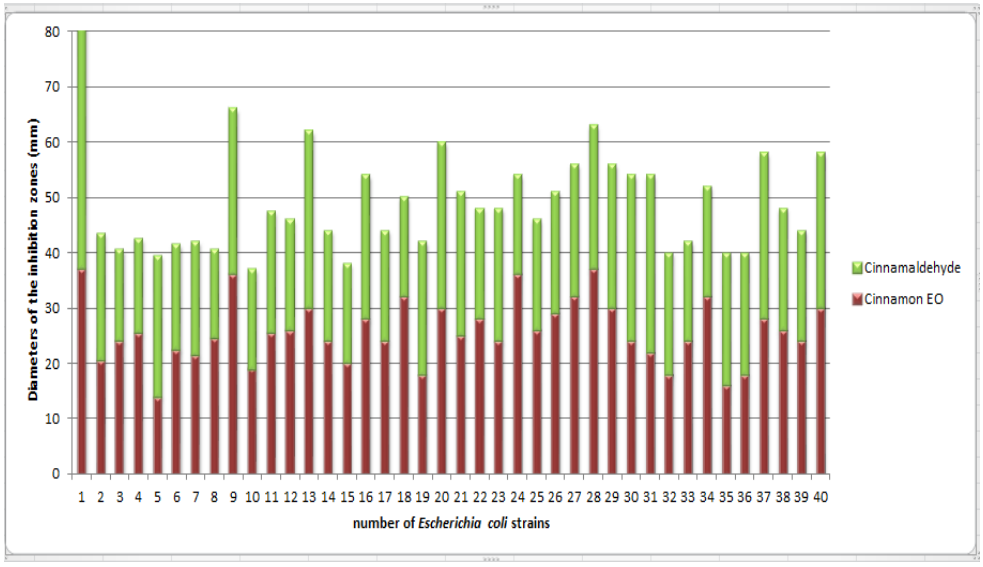

Figure 2: Inhibition zones produced by EO and cinnamaldehyde

The antibacterial activity of cinnamon EO and its active component cinnamaldehyde was

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evaluated by calculating the diameters of the inhibition zones (mm), for EO, these diameters ranged from 14 to 37 mm while cinnamaldehyde was given inhibition zones with diameters ranging from 16 to 44 mm. however, EO of cinnamon was on average more active than cinnamaldehyde with 25.8 ±4.5 mm and 23.3±4.2 respectively. This suggests that the minority compounds present in cinnamon EO have a significant effect. Referring to the results obtained, it will be easy to conclude that the antibacterial activity of essential oils is directly influenced by the nature and the proportion of their constituents. The majority compounds are often responsible for the observed antibacterial activity (Dormans and Deans 2000, Kalemba and Kunicka 2003). But according to several authors (Chorianopoulosand *al.*, 2004; Sokmen and *al.*, 2004; Penalver and *al.*, 2005; Bounatirou and *al.*, 2007; Cao and *al.*, 2009), in addition to major compounds, secondary components interact with each other to give an antimicrobial effect to EO. Some studies have concluded that minor components play a role in activity and may have an effect or influence on EO (Gill and *al.*, 2002, Rota and *al.*, 2008). According to Kalemba and *al.*, (2003), the sensitivity of a microorganism to EOs depends on the properties of these microorganisms and the microorganism itself. In general, bacteria Gram- are more resistant than Gram+ due to the structure of their outer membrane. Indeed, the outer membrane of Gram- is riche in lipopolysaccharides and proteins which make it more hydrophilic, and thus prevents hydrophobic terpenes from adhering to it.

In addition, the activity of cinnamon essential oil has been described as a bactericide on *Escherichia coli*. Indeed, on various strains of *Escherichia coli* (some of which are pathogens and cause symptoms of diarrhea), the essential oil has shown an antibacterial activity higher than streptomycin, used as a control (Senhaji and *al.*, 2007). Cinnamon essential oil has very powerful antibacterial properties to very broad spectrum, antiviral, antifungal and antiparasitic. Cinnamaldehyde seems inhibit enzyme production by bacteria and/or cause cell wall damage bacteria (Di Pasqua and *al.*, 2007). Cinnamaldehyde is one of the most active aldehydes against Gram- and Gram+ bacteria, including *Clostridium, Pseudomonas*, yeasts and fungi (Inouye S. and *al.*, 2001). The high antimicrobial activity of the oil essential cinnamon seems to be correlated with the large amount of cinnamaldehyde (Unlu and *al.*, 2010). Also, the anti-infectious activity is due in part to the family of phenols eugenol, present in the essential oil of cinnamon (Giraud-Robert and *al.*, 2004)

MIC and MBC of cinnamaldehyde

The results of the action of cinnamaldehyde on the *E. coli* strain in a liquid medium are expressed in minimum inhibitory concentration, is the lowest concentration inhibiting bacterial growth.

Reference strain

The values of the MBCs and CMIs values are equal which means that the effect of cinnamaldehyde is bactericidal on *E. coli* (Table 2).

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Table 2: CMI and CMB of cinnamaldehyde on the reference *E.coli* strain

Essai	CMI	CMB
1	**0,031**	**0,031**
2	**0,031**	-
3	**0,031**	-
Moyenne	**0,031**	**0,031**

Strains of avian origin

The MIC of cinnamaldehyde was determined using 10 *E. coli* strains of avian origin, the mean value was 0.04%. The results are presented in figure (N°3).

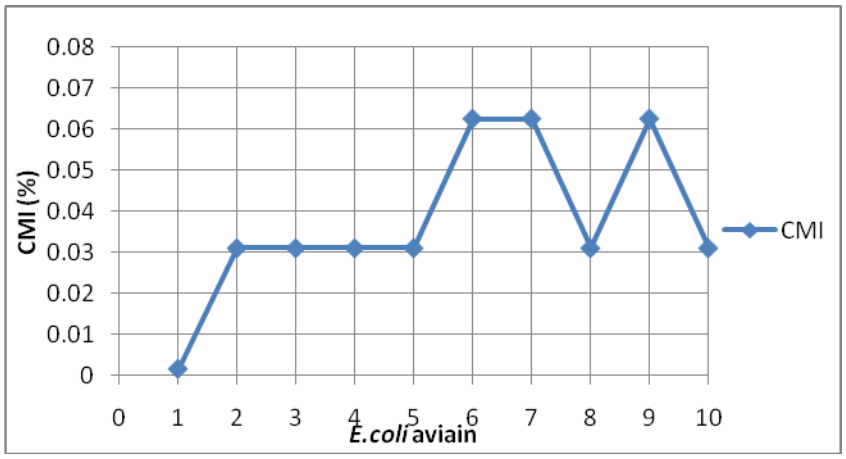

Figure 3: Distribution of MICs of cinnamaldehyde according to strains avian *E.coli*

Overall, the results obtained further confirm the greater activity of cinnamaldehyde, which also increases a lower variation of MICs according to the strain. The effect of cinnamaldehyde was tested on the growth of *L. monocytogenes, E. coli and S. enterica*. The results obtained showed that cinnamaldehyde had a MIC of 0.25% against the three pathogenic strains (Savannah, 2014).Cinnamaldehyde has previously been tested against strains of *E. coli* to determine theminimum inhibitory concentration, the MIC of cinnamaldehyde was 0.25% (Kim and *al.*, 2008). Treatment with 0.25% cinnamaldehyde caused severe damage to the cell structure inhibiting the growth of thepathogens after 2 h.

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PA-Antibiotic Interaction

Using reference *E. coli* as test germ, the interaction of Cinnamaldehyde with Enrofloxacin or Florfenicol is indifferente (Photo 1)

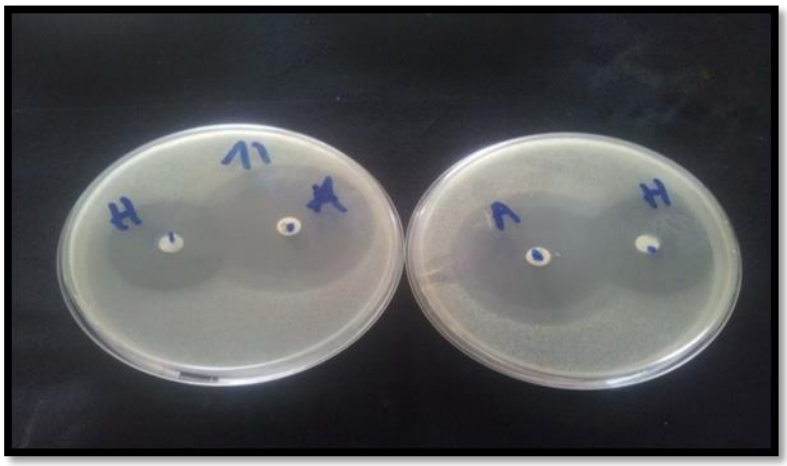

Photo 1: Enrofloxacin-Cinnamaldehyde Interaction

The indifference can be explained by the moderate susceptibility of Gram-negative bacteria to EO in terms of diffusion of the compounds through their hydrophilic outer membrane. Although this barrier is not completely impermeable, it impedes the transport of macromolecules and hydrophobic components (Pierozanand *al.*, 2009). In addition the mode of action of the EO on bacterial cells is not yet clarified (Burt, 2004). In one study, the interaction between cinnamaldehyde and certain antibiotics was tested using the checkerboard method. Cinnamaldehyde has been shown to synergistically increase the sensitivity of *E. coli* strains to erythromycin (ICF $\leq$ 0.5), another synergistic effect between tetracycline and cinnamaldehyde was observed against *E. coli ATCC 23739* (FIC = 0.3). Cinnamaldehyde synergistically and additively reduced the MIC of novobiocin when tested against *ATCC 23739*. Findings of this study suggest that cinnamaldehyde may be used in combination with several antibiotics to enhance susceptibility of drug resistant *E. coli* (Jeyachchandran and *al.*, 2017).

Time-kill curve of cinnamaldehyde

Time-killing curve simulates the effect of changes in antibacterial concentrations as a function of time in the organism to which it is administered. This approach makes it possible to more accurately determine the effect of an antibacterial agent. In the case of cinnamaldehyde, a bactericidal effect is observed from the concentrations of 1 x MIC, but this effect seems to vary with the bacterial strain (figure N°4).

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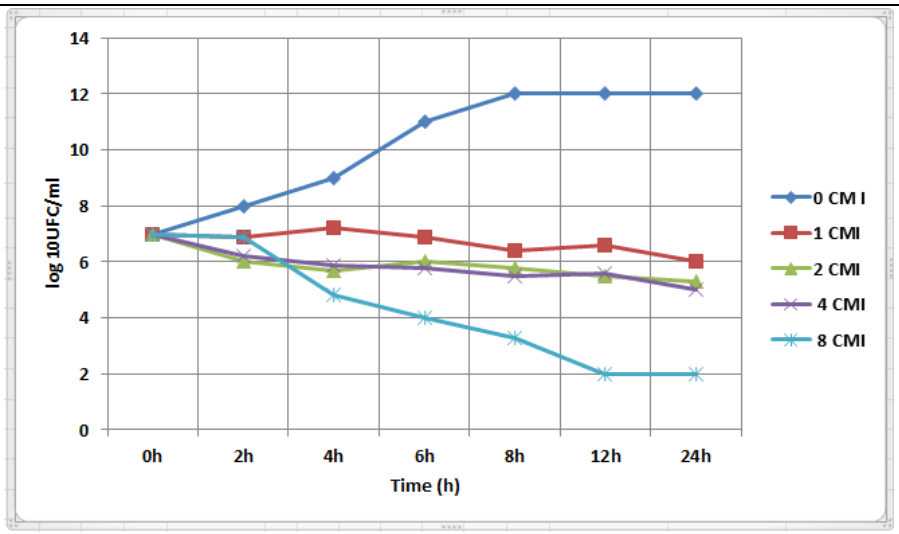

Figure 4: Time-kill curves of cinnamaldehyde against *E. coli*

The figure above shows that cinnamaldehyde action on *E. coli* varies with concentration. At concentrations of 1 x MIC, 2 x MIC and 4 x MIC, cinnamaldehyde causes visible and proportional bacteremia, but is of short duration. At the 8 x MIC concentration, cinnamaldehyde results in a 99.99% reduction of the inoculum ($(10^7-10^2)/10^7$) x100) from the 12th after the start of incubation. This data confirms the bactericidal effect of this product. However, at such a concentration, the effect obtained was not fast. This could be achieved with concentrations of 16 to 32 x MIC. It can therefore be concluded that cinnamaldehyde is a bactericidal molecule whose bactericidal activity depends on the concentration but whose effect is relatively slow, at least in the range of the concentrations tested. Note also, the shape of the curve recalls that of enrofloxacin also on a sensitive strain of *E. coli* (*O78/H12*) isolated from chicken by Haritova&Russenova (2010). In vitro studies show that essential oils containing aromatic aldehydes, such as cinnamaldehyde, and phenols are the most active against bacteria that are involved in respiratory infections. These oils target the cell membrane and destabilize it (Inouye S. and *al.*, 2001). On the other hand, there is very little data on the kinetics of essential oils once absorbed. The molecules of the essential oils would be rapidly diffused in the blood and eliminated by the respiratory route (Derbré S. and *al.*, 2013).

CONCLUSIONS

At the end of this work, several conclusions can be drawn:

- Cinnamon EO is largely composed of cinnamaldehyde with more than 90% of its chemical composition, followed by beta-caryophyllen (6.1%), and cinnamyl acetate and eugenol with 2.75%) and (0.46%) respectively.
- The most important activity was obtained with Cinnamon EO, and the activity of EO differs most often from that of the active component;
- The combination of cinnamaldehyde with florfenicol or enrofloxacin, the interaction tends towards indifference;

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- The average values of CMB and cinnamaldehyde MICs were very close, indicating that the product is bactericidal;
- The cinnamaldehyde bactericidal kinetics has shown that this product has a more bactericidal action on gram-positive germs than on gram-negative germs on the one hand and a slow approaching more time-dependent antibiotics.

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REFERENCES

Acar J.F., Moulin G., (2006). Rev. Sci. Tech. Off. Int. Epiz. 25 (2006) 775-792.

Alanis A.J., (2005). Resistance to antibiotics : are we in the post-antibiotic era ? Arch Med Res 2005 ; 36 : 697-705.

Bouhdid S., Skali S.N., Idaomar M., Zhiri A., Baudoux D., Amensour M. &Abrini J. (2008). Antibacterial and antioxidant activities of Origanum compactum essential oil. Afican Journal of Biotechnology, 7 (10), 1563-1570.

Bounatirou S., Smiti S., Miguel M.G., Rejeb M.N., Nefati M., Costa M.M., Faleiro L., Figueiredo A.C., Baroso J.G., et Pedro L.G., (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link. Food Chemistry, 105, 146 Ŕ 155.

Burt S., (2004). Essential oils: their antibacterial properties and potential applications in foods, 94(3): 223-253.

Cao L., Yong Si J., Liu Y., Sun H., Jin W., LiZ., Hong ZhaoX., and Le Pan R., (2009). Essential oil composition, antimicrobial and antioxidant properties of Moslachinensis(Maxim). Food Chem., 115, 3801 Ŕ 805.

Chaibi A, Ababouch LH, Belasri K, et al., (1997). Inhibition of germination and vegetative growth of Bacillus cereus T and Clostridium botulinum 62A spores by essential oils. Food Microbiol1997 ; 14 : 161-74.

Chorianopoulos N., Kalpoutzakis E., Aligiannis N., Mitaku S., Nychas G.J., et Haroutounian S.A., (2004). Essential oils of Satureja, Origanum, and Thymus species: Chemical composition and antibacterial activities against foodborne pathogens. J. of Agric. and Food Chem., 52, 8261 Ŕ 8267.

Delaquis PJ, Stanich K, Girard B, et al., (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol2002 ; 74 : 101-9.

Derbre S., Licznar-fajardo P., Sfeir J. (2013). Intérêt des huiles essentielles dans les angines à Stretococcuspyogenes. Actualitéspharmaceutiques n° 530.

DI Pasqua R., Betts G., Hoskins N., Edwards M., Ercolini D., Mauriello G., (2007). Membrane toxicity of antimicrobial compounds from essential oils, J.Agr. Food Chem., Volume 55, issue 12, p. 4863-4870.

Dorman H.J. et Deans S.G., (2000). Antimicrobial agents from plants: antibacterial activity of volatile oils. J. Appl. Microbiol., 88(2), pp. 308-316.

Geng S.L., Cui Z.X., Huang X.C., Chen Y.Y., Xu D., Xiong P., (2010). Variations in essential oil yield and composition during Cinnamomum cassia bark growth. Ind. Crops Prod. 2010; 33: 248-252.

Gill A.O., Delaquis P., Russo P., et Holley R.A., (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int. J. of Food Microbiology, 73, 83 Ŕ 92.

Giraud-robert AM., Myon E., Chenaie P., Albano PO., Martin N., Taieb C. (2004). Efficience du traitement des affections bronchiques aiguës par des gouttes aux essences®, Phytothérapie, volume 6, p. 175-179.

Haritova A.M. et Russenova N.V., (2010). in vitro antibacterial effect of Enrofloxacin determined by time killing curves analysis BJVM 13, No 4,1218-226.

Inouye S., Takizawa T., yamaguchi H., (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact, Antimicrobial Chemother, volume 47, p. 565-573.

Jeyachchandran V., Kavitha P., Richard A., Holley., (2017). In vitro enhancement of antibiotic susceptibility of drug resistant *Escherichia coli* by cinnamaldehyde. ScienceDirect, Food ControlVolume 79, September 2017, Pages 288-29. https://doi.org/10.1016/j.foodcont.2017.04.011.

Kalemba D. et Kunicka A., (2003). Antibacterial and antifungal properties of essential oils. Curr. Med. Chem. 10: 813-829.

KalembaD ., et *al.*, (2003). Antibacterial and Antifungal Properties of Essential Oils. Current Medicinal Chemistry, 10: 813829.

Kim, H, S Park, and H Park. (2008). Inactivation of Escherichia coli O157:H7 by cinnamic aldehyde purified from Cinnamomum cassia shoot. Food Microbiology 21: 105-110.

Li L.J, Wang P.S., (2005). Self-medication with antibiotics : a possible cause of bacterial resistance. Med Hypotheses 2005 ; 65 : 1000-1.

Lockwood G.B., (1979). The major constituents of the essential oils of Cinnamomum cassia Blume growing in Nigeria. Planta Med. 1979; 36: 380-381.

Maihebiau P., (1994). La nouvelle aromathérapie: biochimie aromatique et influence psychosensorielle des odeurs. JAKIN., Lausanne.

Mourey A., Canillac N., (2002). Anti-Listeria monocytogenes activity of essential oils components of conifers. Food Control 2002.13 : 289-92.

Peñalver P., Huerta B., Borge C., Astorga R., Romero R., et Perea A., (2005). Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. ActaPathologica, Microbiologica, et ImmunologicaScandinavica, 113, 1 Ŕ 6.

PharmacopéeEuropéenne., (2011). Version 7.1 (avril 2011).

Pieroza K.M. et *al.*, (2009).Chemical characterization and antimicrobial activity of essential oils of salvia L. species; Ciênc. Tecnol. Aliment. Campinas, 29(4): 764-770.

Qiu Q., Liu T.L., Cui Z.J., Shen Y.M., Zhao Y., (2000). Study on chemical component of essential oil of Cinnamomum cassia Presl. by GC- MS. Chin. J. Pharm. Anal. 2000; 20: 248-251.

Remmal A., TantaouiElaraki A., Bouchikhi T., Rhayour K &Ettayebi M. (1993). Improved method for the determinatin of antimicrobial activity of essential oils in Agar medium. J. Essent. Oil. Res. 1993a, 5 : 179-184.

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Rota M.C., Herrera A., Martínez R.M., Sotomayor J.A., et Jordán M.J., (2008). Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. Food Control, 19, 681 Ŕ 687.

Satrani B., Farah A., Fechtal M., Talbi M., Blaghen M. & Chaouch A. (2001). Composition chimique et activité antibactérienne des huiles essentielles de Saturejacalamintha etSaturejaalpina du Maroc. Ann. Fals. Exp. Chim. 94 (956), p.241-250.

Savannah Hawkins G., (2014). Antimicrobial Activity of Cinnamic Acid, Citric Acid, Cinnamaldehyde, and Levulinic Acid Against Foodborne Pathogens 2014. University of Tennessee, Knoxville Trace: Tennessee Research and Creative Exchange.

Senanayake U., lee T., wills R., (1978). Volatile constituants of cinnamomumzeylanicum oils. j. agric. food. chem. volume 26.

Senhaji O., Faid M., Kalalou I., (2007). Inactivation of E. coli 0157:H7 by essential oil from Cinnamomum zeylanicum. Brazilian Journal of infection disease, volume 11, issue 2, p. 234-236.

Smith-Palmer A., Stewart J., Fyfe L., (2002). Inhibition of listeriolysin O and phosphatidylcholine-specific production in Listeria monocytogenes by subinhibitory concentrations of plant essential oils. J Med Microbiol2002 ; 51 : 567-74.

Sokmen A., Gulluce M., Akpulat H. A., Daferera D., Tepe B., Polissiou M., Sokmen M. et Sahin F., (2004). The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic Thymus spathulifolius. Food Control, 15, 627 Ŕ 634.

Thell., Ksouri A., Dob T., Belkebir A., Krimat S., Chelghoum C., Mater J. , (2015). Environ. Sci. 6 (3) (2015) 784- 791.

Ultee A, Slump RA, Steging G, et al., (2000). Antimicrobial activity of carvacrol toward Bacillus cereus on rice. J Food Prot2000 ; 63 : 620-4.

Unlu M., Ergene E., Unlu G., Zeytinoglu H., Vural N., (2010). Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (lauraceae). Food and chemical toxicology, volume 48, p. 3274-3280.

Wegener H.C., (2003). Antibiotics in animal feed and their role in resistance development. CurrOpinMicrobiol2003 ; 6 : 439-45.