

## Research Article

# Chemical Composition and Antimicrobial Activity of the Essential Oils of Two Aromatic Plants Cultivated in Morocco (*Cinnamomum cassia* and *Origanum compactum*)

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The present study aims to evaluate the antibacterial properties of natural products according to a pharmacodynamic approach in order to propose them as alternatives to synthetic products. Two essential oils (*Cinnamomum cassia* and *Origanum compactum*) were the subject of the chemical and biological study. First, we evaluated the sensitivity of the strains of avian *Salmonella* to the main antibiotics used and then to the chromatographic analysis of the composition of the two essential oils (EO); finally, we proceeded to the *in vitro* evaluation of the antibacterial activities of these EO (alone and in combination with antibiotics). The results obtained showed that carvacrol (35.2%), followed by  $\gamma$ -terpinene (20.1%), was the main constituent of the essential oil of *O. compactum* while cinnamaldehyde (69.1%) represents the major component of the essential oil of *C. cassia*. The antibioresistance profile of the *Salmonella* tested showed resistance to ampicillin (35%) and oxytetracycline (41.3%). Active products extracted from the essential oils studied showed antibacterial activity against *Salmonella* strains. *C. cassia* products were shown to be more active for *Salmonella enteritidis* (average inhibition diameter: 16.3 mm) and for *Salmonella gallinarum* (average inhibition diameter: 27.7 mm). The best synergistic activity with antibiotics has been obtained with the essential oil of *C. cassia* and its active product cinnamaldehyde. The minimum inhibitory concentration (MIC) of cinnamaldehyde is the lowest (0.05%). The results prove the presence of an antibacterial activity and a synergistic effect of two essential oils studied with the main antibiotics.

## 1. Background

Salmonellosis is the most common foodborne bacterial disease worldwide. The bacteria responsible belongs to the family of Enterobacteriaceae and the genus *Salmonella* and is a Gram negative, optional anaerobic, and most often mobile [1–3].

Currently, there are approximately 2610 *Salmonella* serotypes determined by their *O* and *H* antigenic structures

and classified according to the Kauffmann–White classification updated annually [4].

*Salmonella* is a predominant cause of food infections. One study estimated that they were responsible for 93.8 million human cases of gastroenteritis and 155,000 deaths worldwide every year [5].

Furthermore, according to the European Food Safety Authority (EFSA) and the European Center for Disease

Prevention and Control (ECDC), salmonellosis was the second most frequently reported gastrointestinal infection for people in the European Union in 2018 (91,857 cases), after campylobacteriosis (246,571 cases). <https://www.efsa.europa.eu/fr/news/salmonella-most-common-cause-foodborne-outbreaks-european-union>.

The misuse of antimicrobial agents has led to the emergence of resistant bacteria. This resistance to antibiotics represents a serious problem to human health [6]. The majority of antibiotic resistance is due to the genes that could be expressed in any bacteria. This notion is illustrated by the term “resistome” which defines the set of antibiotic resistance genes that can be found in environmental bacteria as well as in pathogenic bacteria [7].

*Salmonella* resistance to commonly used antimicrobials (such as tetracyclines, sulfonamides, ampicillin, and fluoroquinolones) has been detected in humans and also in poultry [8].

The *kentucky* and *infantis* *Salmonella* serotypes are of concern because they show a high level of resistance to ciprofloxacin as well as multiresistance to several other drugs [9]. Multiresistance is particularly observed for the strains of the *Salmonella typhimurium* serotype [10].

In Morocco, studies conducted in the Tetouan region have shown that 39.6% of *Salmonella* strains tested are resistant to at least one antibiotic and 22.9% are multi-resistant to at least three antibiotics [11]. In the Rabat-Casablanca region, a study showed relatively low resistance rates for colistin (10.5%) and gentamicin (8.8%). The trimethoprim-sulfamethoxazole combination has been shown to be active on most strains (resistance rate = 3.5%) [12]. In the Casablanca region, antibiotic resistance of *Salmonella kentucky* showed slightly high resistance rates: tetracycline (99.5%), nalidixic acid and flumequine (26.9%), ciprofloxacin (24.6%), amoxicillin (19.4%), gentamicin (16.4%), sulfonamides-trimethoprim (13.4%), cefquinome (8.2%), and colistin (1.5%) [13].

Alongside prevention measures, scientific research has oriented towards the development of new molecules not or hardly subject to the phenomenon of resistance and towards the discovery of products capable, by synergistic effect, of restoring sensitivity to already existing antibiotics.

In addition, in order to preserve the health of the consumer and to delay the wear of antibacterials without compromising the production performance of the chicken, chemicals based on extracts of aromatic plants can constitute a complete or partial alternative to the usual antibiotics [14, 15].

However, as with all products used for human or animal health, rational use of natural products must be done on the basis of pharmacological, toxicological, and clinical studies.

This work aims, on the one hand, to contribute to the study of the resistance to antibiotics of *Salmonella* of the animal origin, to evaluate the antibacterial properties of essential oils of cinnamon (*C. cassia*) and oregano (*O. compactum*), as well as their main active components (cinnamaldehyde and carvacrol), and, on the other hand, to study their possible interactions with usual antibiotics.

## 2. Materials and Methods

### 2.1. Biological Materials

**2.1.1. Aromatic Plants.** The aromatic plants *C. cassia* and *O. compactum* (Figures 1(a) and 1(b)) were collected in 2018, at the Rabat region. The samples obtained were kept at 4°C until the time of analysis. The extraction of essential oils from the samples was carried out at the laboratory of the Department of Food Sciences of the Agronomic and Veterinary Institute (IAV Hassan II). The reference cinnamaldehyde (Sigma-Aldrich, 99%) and carvacrol (Sigma-Aldrich, >98%) were provided by the same department.

**2.1.2. Salmonella.** The bacterial strains tested were isolated from autopsies of laying hens from different regions of Morocco. The samples were taken and identified at the Avian Pathology Unit of IAV Hassan II by Nassik et al. [18].

Isolated bacteria were conserved at +4°C and kept alive by continuous subcultures on the agar trypticase soya (TSA) medium. Twenty one (21) strains were tested: ten (10) strains of *Salmonella enteritidis*, ten (10) strains of *Salmonella gallinarum* (sensitive to florfenicol and resistant to oxytetracycline and amoxicillin), and one reference strain of *Salmonella* (CTP 8059) provided by the Pasteur Institute of Casablanca/Morocco.

**2.2. Extraction of Essential Oils.** The essential oils were extracted from the aerial parts of *O. compactum* and the bark of *C. cassia*, by hydrodistillation for three hours using the Clevenger-type apparatus. The distilled essential oils had been dehydrated with anhydrous sodium sulphate and stored at 4°C in dark, airtight bottles [19].

The oil yield was expressed as a percentage and calculated according to the following formula:

$$\text{Rdt}_{\text{EO}}: \left( \frac{M_{\text{EO}}}{M_{\text{V}}} \right) * 100, \quad (1)$$

where  $\text{Rdt}_{\text{EO}}$  is the yield in the essential oil (%),  $M_{\text{EO}}$  is the mass of the essential oil (g), and  $M_{\text{V}}$  is the mass of the dry plant material (g).

**2.3. Chemical Analysis of Essential Oils.** A gas chromatograph (CLARUS 580) mounted on a mass spectrometer (Clarus SQ 8S) and operating in the positive electronic impact mode (70 eV) and a VB-5 column (5% phenyl and 95% methyl polysiloxane), 30 m long, 0.25 mm internal diameter, and 0.25 mm thick, were used.

The temperatures of the injector and the detector were 250 and 300°C. The gas used is helium at a flow rate of 1 ml/min. The program temperature is from 50 to 280°C with a gradient of 8.1°C/min. Component identification was done using the combination of the NIST-MS Research Program and the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>).



FIGURE 1: (a) *O. compactum* and (b) *C. cassia* [16, 17].

**2.4. Antibiogram.** The antibiogram was carried out by the method of diffusion on the Mueller–Hinton agar medium. Some colonies are taken and diluted in sterile physiological water (dilution of 1/10 on a scale of 0.5 MacFarland). The antibiotic discs are deposited separately, and then the dishes are incubated at 37°C for 24 hours.

Eight antibiotics are used: florfenicol, thiamphenicol, enrofloxacin, amoxicillin, oxytetracycline, sulfamethoxazole-trimethoprim, erythromycin, and colistin.

**2.5. Evaluation of Antibacterial Activity.** The antibacterial activity of the essential oil was determined by the diffusion method on agar (Muller–Hinton) [20]. Two controls were performed: a negative control with 10  $\mu\text{l}$  of sterile distilled water in the presence of 10% DMSO and a disk representing one of the antibiotics as a positive control. The dishes are incubated at 37°C for 18 to 24 hours. Next, the diameter of inhibition is measured in millimeters.

**2.6. Determination of Minimum Inhibitory Concentration.** The minimum inhibitory concentration (MIC) was determined by the microdilution technique on a 96-well round-bottomed microplate [21], using resazurin as an indicator of viability [22]. A series of half dilutions of the essential oil (1/2, 1/4, 1/8, and up to 1/512) were prepared at a concentration range from 1% to 0.00195%, with the concentration of the stock solution of each oil being 1  $\mu\text{l}$ /100  $\mu\text{l}$ . The dilution method consists of preparing a series of wells containing tryptic soy broth (TSB). The wells are filled with 69  $\mu\text{l}$  of TSB. Then, 1  $\mu\text{l}$  of essential oil is added to the first one and then a dilution is made. Then, each well is inoculated with 25  $\mu\text{l}$  of the bacterial suspension. The wells used as a negative control do not contain essential oil, and those used as a positive control contain essential oil without bacterial suspension. The microplates were incubated at 37°C for 18–24 hours. For MIC revelation, 5  $\mu\text{l}$  of resazurin 0.01% (w/v) was added to each well. The MIC was defined as the lowest concentration of oil that did not produce a color change in the resazurin and corresponds to the absence of bacterial growth.

**2.7. Determination of Minimum Bactericidal Concentration (MBC).** The MBC is calculated using 10  $\mu\text{l}$  of wells, which have not shown bacterial growth on a solid medium after seeding and incubation at 37°C for 24–48 h. The MBC is

considered the minimum concentration of essential oil for which no bacterial development is observed.

**2.8. Interactions between Essential Oils, Their Majority Compounds, and Antibiotics.** The association of essential oils and their majority compounds with antibiotics was evaluated by the method of diffusion by discs in the solid medium recommended by Halawani and Toroglu [23, 24]. This involves comparing the inhibitory activity on a strain of *Salmonella* using three discs: the first is an antibiotic disc (ATB), the second is a sterile neutral disc soaked in 5  $\mu\text{l}$  of essential oil (EO) or the majority compound (PA) to be tested, and the third is an antibiotic disc soaked with 5  $\mu\text{l}$  of the same essential oil or of its majority compound to be tested.

After incubation, the reading of three inhibition diameters in mm is carried out using a ruler. The data were analyzed according to the following three possibilities:

A: indifference: the zones of inhibition of EO/PA alone, ATB alone, and the association EO + ATB remain unchanged

B: antagonism: the inhibition zone of the EO + ATB association is less important than that of the EO/PA alone and the ATB alone

C: synergy: the inhibition zone of the EO + ATB association is more important than that of the EO/PA alone and the ATB alone

**2.9. Data Analysis.** The method of analysis of variance (ANOVA) was used to test the effects of factors on the variables measured (diameter and percentage %). If we reject the null hypothesis of equality of means, we use Duncan's multiple comparison tests to form homogeneous groups of essential oils and active ingredients that do not differ between them. For the variables in percentage (MIC and MBC), we transformed them with the angular transformation before performing the ANOVA. These analyzes were carried out using the SAS software (SAS, 2006).

### 3. Results

**3.1. Essential Oil Yield.** The essential oil of the bark of the cinnamon of China (*C. cassia*) is a yellowish liquid with a strong and characteristic odor with a yield of 1.18% (w/w).

The essential oil of *O. compactum* was calculated based on the dry plant matter of the aerial part of the plant and had a yield of 1.6%.

**3.2. Chemical Composition.** For the essential oil of *O. compactum*, ten (10) constituents have been identified, the majority of which are carvacrol (35.17%), followed by  $\gamma$ -terpinene (20.09%), thymol (17.5%), and o-cymene (13.7%), representing 86.48% of the oil (Table 1 and Figure 2(a)).

For the essential oil of *C. cassia*, four components have been identified. Cinnamaldehyde is the main compound (69.15%), followed by methoxycinnamic acid (21.18%), benzyl alcohol (6.14%), and benzyl benzoate (3.53%) (Table 2 and Figure 2(b)).

The number of components identified in the essential oil of *C. cassia* in our study is much smaller than that reported in some previous studies [25].

**3.3. Antibiotyptyp.** Figure 3 represents the percentages of resistance of the strains of *Salmonella* tested to antibiotics. Erythromycin has shown the maximum resistance (100%), which shows that *Salmonella* are naturally resistant to this antibiotic [26].

The resistance of the strains studied to oxytetracycline was 41.28% and 35% to amoxicillin, while enrofloxacin and trimethoprim-sulfamethoxazole had a resistance percentage of ,approximately, 23 and 13%, respectively. Thiamphenicol showed 9% resistance. However, colistin (25 and 50  $\mu$ g) showed a resistance of 5% as well as florfenicol (5%).

**3.4. Evaluation of Antibacterial Activity by the Disc Diffusion Method.** Aromatograms were performed first on a reference strain (*Salmonella* CTP 8059) and then on strains of *Salmonella enteritidis* and *Salmonella gallinarum* of the avian origin.

The results are expressed by the diameters of the inhibition zones. According to Meena and Sethi and Ela et al. [27, 28], susceptibility to an essential oil was classified according to inhibition zone diameters as follows:

- (i) EO strongly inhibitory: diameter of the inhibition zone >28 mm
- (ii) EO moderately inhibitory: diameter of the zone between 16 and 28 mm
- (iii) EO slightly inhibitory: diameter of the zone varies between 10 and 16 mm
- (iv) EO noninhibiting: inhibition diameter is less than 10 mm

**3.4.1. Reference Strain.** The results of the antibacterial activity of essential oils and majority compounds were studied on the reference strain (Table 3). The null hypothesis ( $H_0$ ) assumes that the mean inhibition diameters of the essential oils and majority compounds are equal. Since the probability

TABLE 1: Chemical composition of the *O. compactum* essential oil.

Retention time (min)	Constituents	Percentage
6.792	3-Methyl-apopinene	0.848
7.922	Myrcene	1.953
8.476	4-Carene	2.267
8.647	o-Cymene	13.669
9.360	$\gamma$ -Terpinene	20.089
10.102	Linalool	4.146
13.675	Thymol	17.553
13.896	Carvacrol	35.173
15.976	Caryophyllene	3.111
18.519	Caryophyllene oxide	1.193
Total		100

TABLE 2: Chemical composition of the *C. cassia* essential oil.

Retention time (min)	Constituents	Percentage
8.814	Benzyl alcohol	6.137
13.508	Cinnamaldehyde	<b>69.146</b>
15.550	Methoxycinnamic acid	<b>21.188</b>
20.974	Benzyl benzoate	3.529
Total		100

value ( $p$  value) is less than 0.05,  $H_0$  is rejected and the mean inhibition diameters are different.

Based on the results of Duncan's test, three different groups of essential oils were identified:

- (i) The 1<sup>st</sup> group composed only of cinnamaldehyde, which gave the best average diameter of inhibition (30.3 mm)
- (ii) The 2<sup>nd</sup> group is composed of the essential oil of *C. cassia* and carvacrol with average inhibition diameters of 25 and 24.7 mm
- (iii) The 3<sup>rd</sup> group is composed only of the *O. compactum* which gave the smallest diameter (21 mm)

The essential oil of *C. cassia* (mean inhibition diameter  $25 \pm 2$  mm) and cinnamaldehyde (mean inhibition diameter  $30.0 \pm 1.5$  mm) ( $p < 0.005$ ) showed a higher antibacterial activity than the essential oil of *O. compactum* (mean inhibition diameter  $21 \pm 2$  mm) ( $p < 0.005$ ) and carvacrol (mean inhibition diameter  $24.6 \pm 1.5$  mm). This indicates that the bacterial strain is more sensitive to *C. cassia* essential oil and cinnamaldehyde than to *O. compactum* essential oil and carvacrol.

**3.4.2. Wild Strains of Avian Origin.** The null hypothesis  $H_0$  means that the average diameters of inhibition of essential oils and majority compounds are equal and there is no interaction between strains and essential oils. If the probability value ( $p$  value) is less than 0.05,  $H_0$  is rejected and the mean inhibition diameters are different.

Initial qualitative tests of essential oils and their majority compounds on the strains studied, using the agar diffusion method, resulted in zones of inhibition (Table 4).

The ANOVA indicates that there are highly significant differences between the two strains ( $F$  value = 265.73 and  $p$

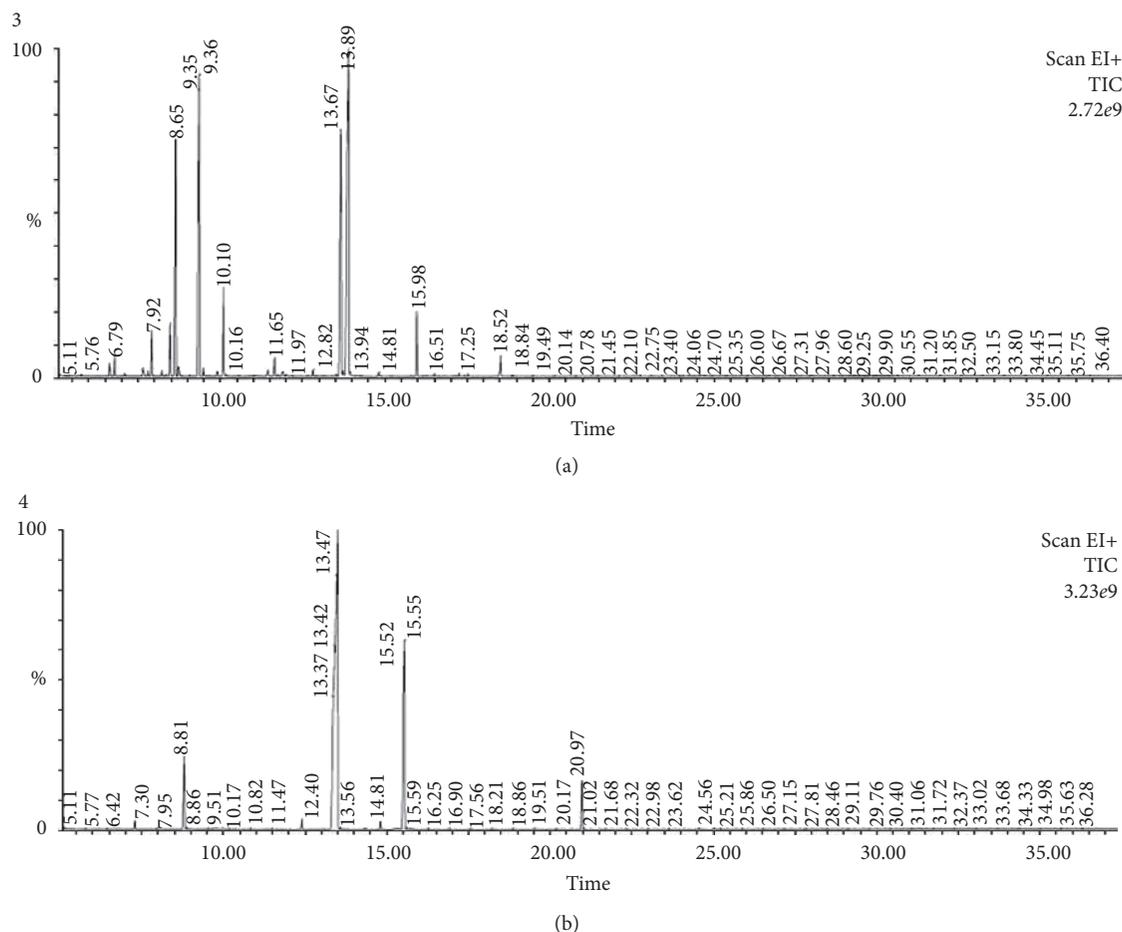


FIGURE 2: Qualitative report of the essential oil of (a) oregano and (b) cinnamon.

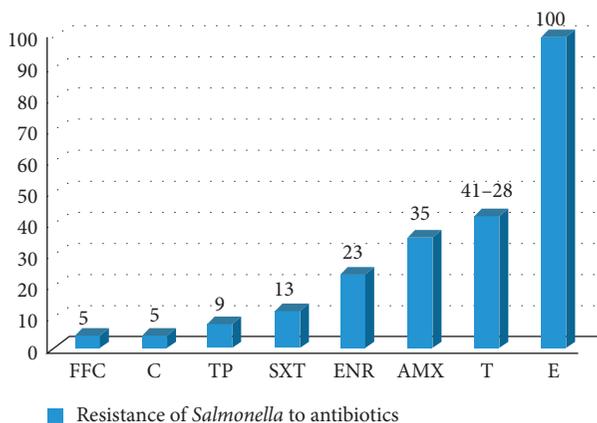


FIGURE 3: Percentage of *Salmonella* resistant to antibiotics. E, erythromycin; T, oxytetracycline; AMX, amoxicillin; ENR, enrofloxacin; SXT, trimethoprim-sulfamethoxazole; TP, trimethoprim; C, colistin; FFC, florfenicol.

value  $<0.0001$ ), between the four majority compounds ( $F$  value = 23.02 and  $p$  value  $<0.0001$ ), and a significant interaction between these two factors ( $F$  value = 32.11 and  $p$  value  $<0.0001$ ).

The essential oil of *C. cassia* presents, on average, the highest inhibitory activity on both strains (average inhibition diameter 22 mm) compared to the essential oil of *O. compactum* and carvacrol (average inhibition diameter 17.7 and 17.3 mm). However, this activity varies from strain to strain. Indeed, *Salmonella gallinarum* is more sensitive to the essential oil of *C. cassia* (average diameter of inhibition 27.7 mm) than the strain of *Salmonella enteritidis* (average diameter of inhibition 16.3 mm).

The activity of the essential oil of *C. cassia* (average inhibition diameter 22 mm) is, on average, more powerful than that of cinnamaldehyde (average inhibition diameter 18.9 mm).

However, carvacrol seems to be more active (inhibition diameter 21.4 mm) than the essential oil of *O. compactum* (inhibition diameter 17.7 mm) for *Salmonella gallinarum* which can be justified by its low content in the composition of the essential oil (35.2%) or the presence of carvacrol antagonistic compounds in the essential oil of *O. compactum* [29].

3.5. Determination of Minimum Inhibitory and Bactericidal Concentrations. Table 5 shows the results of the minimum inhibitory concentrations (MIC) and minimum bactericidal

TABLE 3: Diameters (mm) of the zones of inhibition obtained with the reference strain *Salmonella* CTP 8059.

Agents	EO of <i>O. compactum</i>	Carvacrol	EO of <i>C. cassia</i>	Cinnamaldehyde	<i>F</i>	<i>p</i> value
Mean	21.0 c	24.7 b	25.0 b	30.3 a	13.99	0.0015
SD	2.0	1.5	2.0	1.5		

TABLE 4: Diameters (mm) of the zones of inhibition obtained with wild strains of *Salmonella enteritidis* and *Salmonella gallinarum* of the avian origin.

Strains	EO					<i>F</i> value ( <i>p</i> value)
	<i>C. cassia</i>	Cinnamaldehyde	<i>O. compactum</i>	Carvacrol	Mean	
<i>S. enteritidis</i>	16.3 b a	14.4 b c	17.7 a	13.3 c	15.4	265.73 (<0001)
<i>S. gallinarum</i>	27.7 a	23.5 b	17.7 d	21.4 c	22.5	
Mean	22.0 a	18.9 b	17.7 c b	17.3 c	19.0	
<i>F</i> value ( <i>p</i> value)	23.02 (<0.0001)					

concentrations (MBC) of the two essential oils and their majority compounds on the two bacterial strains.

According to the ANOVA results, there are significant differences only between the essential oils for the two parameters measured (MIC and MBC) (*F* value = 10.45 and *p* value = 0.0005; *F* value = 22.55 and *p* value < 0.0001), whereas there is no significant difference between the two strains and no significant interaction between the strains and the essential oils.

In a liquid medium, the essential oil of *C. cassia* was, on average, the most effective, and its effect was also very stable (MIC 0.018% and MBC 0.026%). The essential oil of *O. compactum* was less effective than that of *C. cassia* (MIC 0.114% and MBC 0.167%). Similarly, cinnamaldehyde presented MICs of 0.04 and 0.05%, thus showing a strong antibacterial activity on the strains studied, thus translating a better antibacterial activity of the essential oil of *C. cassia*, which can be explained by the combined effect of cinnamaldehyde (69.15%) and other minority compounds [30].

The results obtained for the MICs are for the majority in concordance with the diameters of the inhibition zones observed in the agar diffusion test. On the other hand, all the products tested showed bactericidal activity, and the values of MBCs were similar or almost similar to those of MIC (with mean MIC of 0.076% and mean MBC of 0.10%). *C. cassia* exhibited the highest inhibitory and bactericidal activity against both strains.

**3.6. Interaction of Essential Oils-Antibiotics.** We have associated each essential oil and each active principle with different antibiotics to which the *Salmonella* strain exhibits resistance, namely, oxytetracycline and amoxicillin, to qualitatively determine the possible interactions between essential oils as well as their active principles and antibiotics (Table 6).

According to the ANOVA results, there are significant differences between bacterial strains (*F* value = 976.82 and *p* value < 0.0001), products (*F* value = 223.45 and *p* value < 0.0001), and products used (*F* value = 76.05 and *p* value < 0.0001). Similarly, all interactions are significant except

that between the strains and the products used (*F* value = 0.60 and *p* value = 0.5787).

The analysis of the data obtained shows that the association of the essential oil of *O. compactum* with oxytetracycline gave synergistic interactions (18 mm) compared to that of the essential oil alone (14.5 mm) and the antibiotic alone on both strains of *Salmonella*, while a slight antagonistic effect was obtained with amoxicillin (9 mm) compared to the essential oil alone (14.5 and 22 mm).

Table 6 shows an antagonistic interaction when combining carvacrol with amoxicillin (15.75 mm); however, synergy is achieved with oxytetracycline (20 mm).

The results show a synergistic interaction of the essential oil of *C. cassia* (23 and 22.75 mm) and cinnamaldehyde (21.5 and 20 mm) with all the antibiotics tested.

From the results cited above, we found that the essential oil of *C. cassia* and cinnamaldehyde were the most active in combination with antibiotics, and this result shows a synergistic effect.

## 4. Discussion

The study of the chemical composition of essential oils has shown that the majority constituents of the essential oil of *O. compactum* are carvacrol (35.17%), followed by  $\gamma$ -terpinene (20.09%), thymol (17.55%), and *o*-cymene (13.67%), accounting for 86.48% of the oil. These results are in agreement with those obtained in the study by Ben-Hammou et al. which showed that the most abundant compound in the chemical composition of this essential oil is carvacrol (36.31%), followed by thymol (16.88%) and *p*-cymene (9.21%) [31].

Furthermore, the analysis of essential oils of *O. compactum* from different regions of Morocco revealed the presence of three main components at variable rates: thymol (0 to 43.4%), carvacrol (3.8 to 71%), and *p*-cymene (0 to 25.4%) [32, 33].

For the essential oil of *C. cassia*, the study showed that cinnamaldehyde is the main compound (69.15%), followed by methoxycinnamic acid (21.19%), benzyl alcohol (6.14%), and benzyl benzoate (3.53%). These results are consistent with those reported in previous studies [34, 35]. Tao et al.

TABLE 5: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) (%) (v/v) means of the essential oils tested and their majority compounds according to the *Salmonella* strain.

Variable	Agent/strain	<i>C. cassia</i>	Cinnamaldehyde	<i>O. compactum</i>	Carvacrol	Mean	F value (p value)
MIC	<i>S. enteritidis</i>	0.02 b	0.05 b a	0.10 b a	0.14 a	0.08 a	0.16 (0.6920)
	<i>S. gallinarum</i>	0.02 b	0.04 b	0.13 a	0.10 a	0.07 a	
	Mean	0.02 b	0.05 b	0.11 a	0.13 a	0.076	
	F value (p value)	10.45 (0.0005)					
MBC	<i>S. enteritidis</i>	0.03 b	0.03 b	0.20 a	0.16 a	0.11 a	2.97 (0.1040)
	<i>S. gallinarum</i>	0.05 c	0.05 b	0.13 a	0.13 a	0.08 a	
	Mean	0.03 b	0.04 b	0.17 a	0.15 a	0.10	
	F value (p value)	22.55 (<0.0001)					

TABLE 6: Evaluation, by the disc method, of the effects of essential oils, active products, and antibiotics on the inhibition diameter (mm).

Bacterial strains	Products used	EO of <i>O. compactum</i>	EO of <i>C. cassia</i>	Carvacrol	Cinnamaldehyde	Oxytetracycline	Amoxicillin	Mean
<i>S. gallinarum</i>	Oxytetracycline	22	29	25	26	—	—	22.1 A
	Amoxicillin	16	29	19.5	25	—	—	
	Without antibiotic (nothing)	18	28	21	23	—	—	
	Antibiotic alone	—	—	—	—	19	9	
<i>S. enteritidis</i>	Oxytetracycline	14	17	16	17	—	—	14.2 B
	Amoxicillin	10	16.5	12	15	—	—	
	Without antibiotic (nothing)	11	16	13	14	—	—	
	Antibiotic alone	—	—	—	—	18	9	
Mean	Oxytetracycline	18**	23**	20.5**	21.5**	—	—	18.1
	Amoxicillin	13	22.8**	15.8	20**	—	—	
	Without antibiotic (nothing)	14.5*	22*	17*	18.5*	—	—	
	Antibiotic alone	—	—	—	—	18.5*	9*	

(\*): presence of a synergistic interaction; (\*\*): presence of a good synergistic interaction; (—): no test.

and Wang et al. identified, respectively, 38 and 27 components in *C. cassia* essential oil and found that cinnamaldehyde (30.67–90.74%), copaene (27.71%), and a-hexahydro-4 and 7-dimethyl-1-(1-methylethyl)-(1S-cis)-naphthalene (13.55%) were the main components of this essential oil [36, 37].

The results of the antibiogram in our study showed a resistance of 41.28% of the strains of *Salmonella* towards oxytetracycline, which is high when compared to the percentage found by Kassimi et al. (28.58%) and that obtained by Ziyate et al. which was 25% [38–40].

With regard to amoxicillin, 35% of the strains were resistant, which is a high result when compared to the percentage found by Ziyate et al. [38] which was 21%.

Almost 23% of the strains are resistant to enrofloxacin which is lower than the percentage found by Antunes et al. who reported that 75% of the strains isolated in Porto (Portugal) are resistant to one antibiotic or more and in particular to enrofloxacin [41].

Approximately, 13% of strains were resistant to trimethoprim-sulfamethoxazole, a result which remains low when compared to the rates reported by Gad and Hafez which is 59% in Germany [42]. For thiamphenicol, 9% of the

strains are resistant which is due to the fact that it is no longer allowed in poultry.

A resistance of 5% to colistin (25 and 50 µg) was found. According to Humbert and Salvat, colistin remains among the sufficient number of antibiotics active on *Salmonella* [43]. And, a 5% resistance to florfenicol could be explained by the fact that this molecule is not commonly used in poultry.

The aromatogram study showed that the essential oils and active products tested have antibacterial activity against the reference strain and the bacterial strains studied. *C. cassia*'s products have been shown as the most active.

This study revealed that *S. gallinarum* is more sensitive to the essential oil of *C. cassia* (27.65 mm) than the strain of *S. enteritidis* (16.25 mm). However, carvacrol seems to be more active (21.4 mm) than the essential oil of *O. compactum* (17.7 mm).

The majority compounds are most often responsible for the antibacterial activity observed [44, 45]. Nevertheless, other studies show that in addition to major compounds, minority compounds play an important role in the antibacterial activity of the essential oil [46]. On the contrary, the sensitivity of a bacterium to essential oils depends on the properties of the microorganism itself [44, 47].

The results of the minimum inhibitory concentration showed that the MIC values vary depending on the strain and on the natural product used, and the MIC of cinnamaldehyde was the lowest when compared to the strains tested (0.04 and 0.05%). These results are in agreement with those obtained in a previous work on Gram bacteria [48]. Nevertheless, the MIC values found remain higher than those obtained in our study, reflecting a better antibacterial activity of the essential oil of *C. cassia* [49, 50].

The results of the interaction of essential oils and antibiotics also demonstrated the potentiating effect of essential oils and their majority compounds on the activity of the tested antibiotics. The best synergistic activity with antibiotics was obtained with the essential oil of *C. cassia* (23 and 22.75 mm) and its active product cinnamaldehyde (21.5 and 20 mm). Our results are, on the whole, comparable to those reported for *O. compactum*, *C. cassia*, and their active principles [51].

The association of essential oils with antibiotics can be used to increase the antimicrobial spectrum and reduce and minimize the side effects of the antibiotic [52].

## 5. Conclusions

The essential oil of oregano and its majority component, carvacrol, and, above all, the essential oil of cinnamon and its majority component, cinnamaldehyde, are active *in vitro* against the reference strain (*Salmonella* CTP 8059) and the strains of *Salmonella enteritidis* and *Salmonella gallinarum*.

The lowest minimum inhibitory concentration (MIC) was obtained with cinnamaldehyde (0.05% (v/v)) against *Salmonella enteritidis* and (0.04% (v/v)) against *Salmonella gallinarum* compared with that of carvacrol (0.014% and 0.01% (v)) respectively, for the two bacterial strains.

The minimum bactericidal concentrations (MBCs) of the four products were very close to their respective MICs, indicating that the products studied are rather bactericidal.

The association of essential oils with antibiotics used in avian medicine indicates synergies, with the exception of the essential oil of *O. compactum* and carvacrol, which lend themselves much less to such interaction with amoxicillin. It should be noted that the resistance profile of strains isolated from poultry varies according to the antibiotic family and is comparable to the rates reported in other countries.

## Data Availability

All the data generated or analyzed during this study are included within the article.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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