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## Original Research Article

# Influence of Some Antibiotics and Essential Oils Used Alone or in Combination on the Vitality of Presumptive Probiotic Lactic acid Bacteria

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### ABSTRACT

**Aims:** The aim of this study was to assess the *in vitro* antibacterial activity of selected antibiotics and essential oils alone or in combination, on selected presumptive probiotic lactic acid bacteria.

**Study design:** Experimental studies.

**Place and Duration of Study:** Department of Microbiology of the University of Yaounde I between August 2017 and December 2017 (5 months).

**Methodology:** The chemical composition of five essential oils was determined by gas chromatography coupled with Solid-phase micro extraction. Then the sensitivity of four lactic acid bacteria to the essential oils and four antibiotics was assessed by the well diffusion and macrodilution method. Subsequently, two essential oils active on these bacteria and broad spectrum antibiotics were combined according to the central composite design plan.

**Results:** In general, the chemical composition of essential oils is very diverse, with the example of carvacrol found only in *Origanum compactum* at 53.24% and thymol in *Thymus*

*vulgaris* at 56.19% and in *Origanum compactum* at 15.28%. The antibacterial activity shows that the majority of antibiotics used are active on the bacteria in the study compared to the essential oils where two were active (*Origanum compactum* and *Cymbopogon winterianus*). The evaluation of the combinations of essential oils and antibiotics in terms of kinetics has given us three cases: the first case is the one with no acidity or no growth at all; the second is the one where growth is normal; the third where growth is delayed with a more pronounced latency phase.

**Conclusion:** This study suggest that the effect of essential oils and medicinal plant used alone or in combination to antibiotics on the gut microbiota have to be evaluated for validation as well as their toxicity activities before using them for human therapy.

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*Keywords: Essential oils, antibiotics, probiotics, vitality, combination*

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## 1. INTRODUCTION

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## 2. MATERIAL AND METHODS

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### 2.1 Commercial plant extracts

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The plant material used in this work consisted of five essential oils purchased from the PIERRE FABRE laboratory, Boulogne-France: *Cymbopogon winterianus* (panorome citronella, flowering tops, n° 501919), *Thymus vulgaris* L. *chemotype thymol* (thymol thyme, aerial parts, n° 403746), *Origanum compactum* (compact oregano, flowering tops, n°OF19950), *Eucalyptus globulus labill* (eucalyptus, leaf and boughs, n°402124) and *Rosmarinus officinalis* L. *chemotype 1, 8- cineole* (rosemary, boughs and flowering tops, n°K00001).

## 72 **2.2 Antibiotics and microorganisms**

73 Ampicillin (AMP), Amoxicillin (AMOX), Streptomycin (STREP) and Ciprofloxacin (CPF) from  
74 Sigma-Aldrich, St Quentin Fallavier, France were used.

75 Microorganisms included in this study for antimicrobial activity were four lactic acid bacteria  
76 amongst which *Lactobacillus casei* LBLDL (LC), *Lactobacillus plantarum* ATCC 14197 (LP),  
77 *Lactobacillus rhamnosus* C1112 (LRH1) and *Lactobacillus rhamnosus* C24 (LRH2). All  
78 these strains are Gram+ bacteria kindly offered by the Laboratory of Food Microbiology,  
79 University of Bologna (Italy). Strains stored at -80°C were subcultured at 37°C for 24 hours  
80 twice in milk broth before being used in the tests.

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## 82 **2.3 Determination of the chemical composition of essential oils**

83 The chemical composition of the essential oils was determined by using an Agilent  
84 Technology gas-chromatograph 7890N (Palo Alto, CA, US), equipped with an Agilent  
85 Network Mass Selective detector HP 5975C (Palo Alto, CA, US). The injector temperature  
86 was maintained at 250°C while the detector was at 280°C with fragmentations carried out at  
87 70 eV. The analysis was performed in conditions 1:10 split, using a capillary column SPB-5  
88 30m length, 0.25mm ID and 0.25 µm film thickness (Supelco Park. Belfonte code number  
89 24034). The following temperature programme: from 50 to 240 °C with a temperature  
90 increase of 3 °C/min, and a 1min hold at 240°C.

91 For the essential oil head space analysis, 3 ml of the same dilution as previously indicated  
92 was introduced in a 10 ml vials and hermetically sealed. After heating the sample in water  
93 bath at 30°C for 10min with a SPME-DVB-carboxen/PDMS, 50/30 µm fiber (Supelco,  
94 Bellefonte, PA, USA) was exposed in the head space for 30min for absorption. Subsequently  
95 the fiber was then immediately inserted for desorption into the injector of a GC-MS for 5min.  
96 The identification of the volatile compounds was performed using the NIST (NIST/EPA/NIH  
97 Mass spectral Library, 1998, Version 1.6, USA) and WILEY (sixth edition, 1995, USA) and  
98 with the Kovach retention index in comparison with those of authentic samples or with  
99 published data in the literature [12].

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## 101 **2.4 Evaluation of the antibacterial activity**

102 Two methods were used to evaluate the antibacterial activity of the different essential oils  
103 and antibiotics: the well diffusion method and the serial broth macrodilution method.

104 The well diffusion method was carried out in accordance with CLSI recommendations [13].  
105 Sample were dissolved in 10% DMSO then diluted to 2 final concentrations of 2000 ppm and  
106 1000 ppm for the essential oils and of 1000 ppm and 500 ppm for antibiotics. These tested  
107 concentrations are different due to the fact that antibiotics are pure reference molecules and  
108 specific; therefore, the activity has already been proven. Briefly, 1 mL bacterial culture (10<sup>5</sup>  
109 cells/mL) were inoculated on a solidified Mueller Hinton agar with 5% glucose in a Petri dish;  
110 then circular wells (3 wells per dish sealed at the bottom with the same medium) of 6 mm  
111 were filed with a 50 µL of diluted samples. Wells filed with DMSO were used as negative  
112 control. The Petri dishes were then incubated at 37°C for 24 h. The growth inhibition zone  
113 diameter (IZ, mm) was measured to the nearest mm. Each experiment was performed in  
114 triplicate and the results presented in terms of the concentration that produced the highest  
115 inhibition diameter.

116 The serial broth macrodilution method was carried out in accordance with CLSI  
117 recommendations [13] in order to evaluate the antimicrobial activity of essential oils and  
118 antibiotics on selected lactic acid bacteria. A stock solution was first prepared by diluting the  
119 respective essential oils (150 000 ppm) and antibiotics (100 000 ppm) in 10% DMSO.  
120 Simultaneously, 10<sup>5</sup> cells/mL of bacteria inoculum was prepared in Mueller Hinton broth from  
121 an overnight milk broth culture. Subsequently, 40 µL of the stock solution was added to 3960  
122 µL of bacteria inoculum to reach 1500 ppm and 1000 ppm as first test concentration for  
123 essential oils and antibiotics respectively. Then, from these concentrations, we proceeded to  
124 twofold dilution using bacteria inoculum to obtain concentrations ranging from 1500 ppm to

125 0.18 ppm for the essential oils and from 1000 ppm to 0.12 ppm for the antibiotics followed by  
 126 incubation at 37°C for 24 h (after mixing with vortex). Minimal inhibitory concentration (MIC)  
 127 and minimal bactericidal concentration (MBC) were defined as in [13]. The presence of  
 128 viable bacterial after incubation was assessed through their capacity of acidifying the  
 129 environment. This was done by adding two drops of bromocresol purple as a colored  
 130 indicator: color change to purple indicated the metabolic activity of viable cells.

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## 132 **2.5 Evaluation of the effect of different combinations of essential oils and** 133 **antibiotics on growth kinetics, assessed indirectly through growth medium** 134 **titrable acidity**

135 For this purpose, two strains (the most sensitive one and the most resistant one), two broad  
 136 spectrum antibiotics and two essential oils were selected. The selection of the strains and  
 137 antibiotics were done base on the means of inhibition diameter while for the essential oils, it  
 138 was done using the MIC and MBC values.

139 To evaluate the effect of different combination made of essential oils and antibiotics on  
 140 bacterial strains, a calibration curve was first developed to correlate the microbial growth to  
 141 the titrable acidity as a function of time. Then, we evaluated the vitality of bacteria exposed  
 142 to the combinations using a  $3^{(k-1)}$  fractional design experimental plan.

143 The calibration curve was realized according to the [14] protocol. Briefly, 25  $\mu$ L of a bacterial  
 144 suspension was introduced in 250 mL of milk broth to obtain a final concentration of  
 145  $10^5$  cells/mL. After 0, 1, 2, 4, 6, 8 and 24 hours, 1 mL of the solution corresponding to each  
 146 time was used for titration. The operation was performed in duplicate. Three drops of  
 147 phenolphthalein were added and a volume of NaOH (0.1 mol) until the pink titration solution  
 148 turned. The volume of NaOH was used to calculate the mass concentration of lactic acid  
 149 using formula as follows:

$$150 \quad C_m = \frac{(C_b * V_b * M_a)}{V_a} \quad \text{Equation 1}$$

151 With  $C_m$  the mass concentration of lactic acid in g/L,  $C_b$  the NaOH concentration in mol/L,  
 152  $V_b$  the volume of NaOH in L and  $M_a$  the mass molar molecular in g/mol.

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154 The microbial enumeration was performed according to [15]. 1ml of the previous batches  
 155 was sampled and introduced into 9 mL of physiological water follow by serial dilution. The  
 156 dilutions was sowed in Petri dishes and incubated at 37°C for 24 hours. The number of  
 157 colonies count allowed to calculate the cellular concentration using the formula as follows:

$$158 \quad C = (n * Fd) / V \quad \text{Equation 2}$$

159 With  $C$  the cellular concentration in UFC/mL,  $n$  the number of colonies,  $Fd$  the dilution factor  
 160 and  $V$  the sow volume in mL.

161

162 The evaluation of the combine effect of antibiotics and essential oils on the vitality of bacteria  
 163 was assessed using a  $3^{(k-1)}$  fractional design experimental plan [16] with two variables at  
 164 three levels (Table 1).

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166 **Table 1. Coding of independent variables**

Levels	-1	0	1
Essential oils	MIC/2	MIC	3/2 MIC
Antibiotics	MIC/2	MIC	3/2 MIC

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168 For this realization, a milk broth was prepared in vials and antimicrobials (essential oils and  
169 antibiotics) were introduced at different concentrations according to a fractional design  
170 experimental plan so as to obtain a final volume of 100 mL; then 10  $\mu$ L of a bacterial pre-  
171 culture were introduced to obtain in the broth a concentration of  $10^5$  UFC/mL of the various  
172 lactic acid bacteria. Each run was repeated 10 times, and the incubation was performed for  
173 24 hours at 37°C. A series of samples during the incubation were titrated to determine the  
174 mass concentration of lactic acid produced in the presence of the different antimicrobial  
175 concentrations according to the above equation 1.

### 177 3. RESULTS AND DISCUSSION

#### 178 3.1 Chemical composition of essential oils

179 Gas chromatography (GC) and solid-phase micro extraction (SPME) analyses of the  
180 essential oils allowed the identification of several components (Table 2). *Origanum*  
181 *compactum* showed the presence of thirteen components amounting to 82.62% of the total  
182 chemical composition; the oil was characterized by three major monoterpenes compounds:  
183 carvacrol (53.2%), thymol (15.3%) and p-cymene (14.1%). *Thymus vulgaris* presented seven  
184 components amounting to 88.64% of the total chemical composition; the oil was  
185 characterized by two major monoterpenes compounds: thymol (56.19%) and m-cymene  
186 (32.45%). *Eucalyptus globulus* presented six components amounting to 95.89% of the total  
187 chemical composition; the oil was characterized by the monoterpenes eucalyptol (95.89%)  
188 as major compound. *Cymbopogon winterianus* presented eight components amounting to  
189 89.66% of the total chemical composition; the oil was characterized by three major  
190 monoterpenes and one sesquiterpenes compounds: citronellal (38.34%), trans-geraniol  
191 (21.05%), beta-citronellol (18.58%) and elementol (11.69%). *Rosmarinus officinalis*  
192 presented eleven components amounting to 79.14% of the total chemical composition; the  
193 oil was characterized by two major monoterpenes compounds: eucalyptol (63.83%) and  
194 camphor (15.31%).

196 Analysis of the same EO obtained from leaves collected in Boulemane region [17] or leaves  
197 and stems in Cerrado region of Brazil [18] showed chemical composition dominated by  
198 eucalyptol (42.24-28.5%), camphor (10.81-27.7%) and  $\alpha$ -pinene (16.31-21.3%) respectively.  
199 Thymol (56.191%) and m-cymene (32.455%) were obtained as major compounds for  
200 *Thymus vulgaris*. Analysis of the same EO obtained from flowering tops from France [19] or  
201 aerial plant from Romania [20] showed chemical composition dominated by thymol (36.58-  
202 45.5%), p-cymene (16.51-8.41%) and  $\Delta$ -terpene (13.70-30.90%) respectively.

203 Eucalyptol (95.830%) is the major compound for *Eucalyptus globulus*. Analysis of the same  
204 EO obtained from leaves in Brazil [21] or leaves in Haramaya University, Ethiopia [22]  
205 showed chemical composition dominated by eucalyptol (83.89%), limonene (8.16%) and  $\alpha$ -  
206 pinene (4%); eucalyptol (55.29%), spathulenol (7.44%) and  $\alpha$ -terpineol (5.46%) respectively.  
207 m-cymene (14.097%), thymol (15.288%) and carvacrol (53.242%) were obtained as major  
208 compounds for *Origanum compactum*. Analysis of the same EO obtained from 200g of  
209 powder from plant in Morocco [23] or leaves in Belgium [24] showed chemical composition  
210 dominated by carvacrol (58.1%), p-cymene (11.4%), thymol (9%) and  $\square$ -terpinene (7.1%) ;  
211 carvacrol (30.53%), thymol (27.5%) and  $\Delta$ -terpinene (18.20%) respectively. Citronellal  
212 (38.348%),  $\beta$ -citronellol (18.581%), trans-geraniol (21.053%) and 11.689% elemental were  
213 obtained as major compounds for *Cymbopogon winterianus*. Analyses of the same EO  
214 obtained from leaves and stems in holzminden, Germany [25] showed chemical composition  
215 dominated by citronellal (27%), trans-geraniol (22.78%) and citronellol (10.9%).

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219 **Table 2. Chemical composition of the five essential oils expressed as percentage of total compounds revealed by the GC-MASS**  
 220 **spectrum**

Great family	Compounds	Retention index (IR/SPB5)	<i>Origanum compactum</i>	<i>Thymus vulgaris</i>	<i>Rosmarinus officinalis</i>	<i>Eucalyptus globulus</i>	<i>Cymbopogon winterianus</i>
<b>Monoterpenes</b>	$\alpha$ -pinene	939	/	0.3	<b>5.9</b>	0.3	/
	Camphene	954	/	0.2	2.4	/	/
	$\beta$ -pinene	979	/	/	4.7	0.2	/
	(+)-4-carene	1001	1.3	/	/	/	/
	$\beta$ -carene	1011	0.8	/	/	/	/
	1,4-cineole	1014	/	/	/	0.2	/
	$\alpha$ -terpinene	1017	<b>9.4</b>	/	/	/	/
	p-cymene	1024	<b>14.1</b>	<b>32.4</b>	/	/	/
	Eucalyptol	1031	/	/	<b>63.8</b>	<b>95.9</b>	/
	$\alpha$ -thuyone	1102	0.5	/	/	/	/
	1,3,8-p-menthatrien	1110	/	/	4.1	/	/
	$\beta$ -thujene	1114	0.6	/	/	/	/
	camphor, (1R,4R)-(+)-	1146	/	/	<b>15.3</b>	/	/
	(R)-(+)-citronellal	1153	/	/	/	/	<b>38.3</b>
	borneol acetate	1169	/	/	0.3	/	/
	p-cymen-8-ol	1182	0.1	/	/	/	/
	$\alpha$ -terpineol	1188	/	/	0.2	/	/
	(R)-(+)- $\beta$ -citronellol	1225	/	/	/	/	<b>18.6</b>
	trans-geraniol	1252	/	/	/	/	<b>21.1</b>
	Thymol	1290	<b>15.3</b>	<b>56.2</b>	/	/	/
	Carvacrol	1299	<b>53.2</b>	/	/	/	/
	thymol acetate	1352	/	3.5	/	/	/
	isobornyl format	1239	0.500	/	/	/	/
Eugenol	1359	/	/	/	/	1.8	
<b>Terpenes</b>	D-limonene*	/	/	/	/	1.5	1.9
	$\alpha$ -phellandrene	1002	/	/	/	0.1	/
	Limonene	1029	/	/	0.5	/	/
	$\beta$ -linalool	1096	1.3	3.7	/	/	/
	Borneol	1169	/	1.5	2.05	/	/

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<b>Sesquiterpenes</b>	(E)- $\beta$ -caryophyllene	1424	2.5	/	0.4	/	/
	$\beta$ -gurjunene	1433	/	/	/	/	0.6
	Elemol	1549	/	/	/	/	<b>11.7</b>
	caryophyllene oxide	1583	0.1	/	/	/	/
	Ledol	1602	/	/	/	/	<b>5.9</b>
	<b>Total compounds identified (%)</b>	<b>/</b>	<b>99.7</b>	<b>97.8</b>	<b>99.65</b>	<b>98.2</b>	<b>99.9</b>

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221 \* Compound identify from the mass spectrum

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231 **3.2 Antimicrobial activities**

232 The antimicrobial activities of these essential oils and antibiotics on the lactic acid bacteria  
 233 were assessed by well diffusion method through the inhibition zone (IZ) diameter  
 234 measurement and by macrodilution method determining the MIC and MBC values [13]. The  
 235 IZ diameters expressed in mm are presented (Table 3). All the selected bacteria were not  
 236 sensitive to the five essential oils at concentrations of 2000 ppm and 1000 ppm. However,  
 237 for the antibiotics, the highest IZ diameters (2.63-4.07 mm) were observed with the  
 238 ciprofloxacin at 1000 ppm for all the bacteria, the two more sensitive strains being  
 239 *Lactobacillus casei* (4.07 mm) and *Lactobacillus rhamnosus* C24 (4.13 mm); the less  
 240 sensitive strain was *Lactobacillus rhamnosus* C1112 (2.63 mm). Globally, the inhibition zone  
 241 diameters were not proportional to the concentration of antibiotics. *Lactobacillus casei* was  
 242 sensitive to the antibiotics, *Lactobacillus plantarum* was not sensitive to streptomycin. The  
 243 two *Lactobacillus rhamnosus* strains were the less sensitive to amoxicillin and ampicillin.

244

245 **Table 3. Sensitivity of bacteria to the essential oils and antibiotics expressed as**  
 246 **inhibition zone diameter  $\pm$  sd (mm)**

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Sample concentrations (ppm)		LC	LP	LRH1	LRH2
<b>1000 ppm</b>	Streptomycin	2.20 $\pm$ 0.35	00 $\pm$ 00	1.47 $\pm$ 0.50	1.33 $\pm$ 0.15
	Amoxicillin	2.73 $\pm$ 0.31	3.27 $\pm$ 0.23	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	Ampicillin	3.60 $\pm$ 0.53	3.53 $\pm$ 0.50	0.00 $\pm$ 0.00	1.60 $\pm$ 0.17
	Ciprofloxacin	4.07 $\pm$ 0.12	3.47 $\pm$ 0.46	4.13 $\pm$ 0.81	2.63 $\pm$ 0.06
<b>500 ppm</b>	Streptomycin	2.73 $\pm$ 0,31	0.00 $\pm$ 0.00	1.10 $\pm$ 0.14	1.33 $\pm$ 0.06
	Amoxicillin	3.13 $\pm$ 0.12	2.93 $\pm$ 0.50	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	Ampicillin	2.87 $\pm$ 0.23	3.27 $\pm$ 0.23	0.00 $\pm$ 0.00	1.27 $\pm$ 0.12
	Ciprofloxacin	4.07 $\pm$ 0.12	3.00 $\pm$ 0.20	3.40 $\pm$ 0.92	2.57 $\pm$ 0.21
<b>2000 ppm</b>	<i>Origanum compactum</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Eucalyptus globulus</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Rosmarinus officinalis</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Cymbopogon winterianus</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Thymus vulgaris</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
<b>1000 ppm</b>	<i>Origanum compactum</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Eucalyptus globulus</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Rosmarinus officinalis</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Cymbopogon winterianus</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	<i>Thymus vulgaris</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

248 LC (*Lactobacillus casei*), LP (*Lactobacillus plantarum*), LRH2 (*Lactobacillus rhamnosus*  
 249 C24), LRH1 (*Lactobacillus rhamnosus* C1112).

250

251 The antibacterial activities of the essential oils and antibiotics were also evaluated using  
 252 macrodilution method. The corresponding antibacterial activities (MIC and MBC) are  
 253 presented (Table 4). The classification of the activity of essential oils was done based on



254 [26] in proposal. According to these authors, the antimicrobial activity can be high  
 255 (MIC<100ppm), moderate (100<MIC<625ppm) or low (MIC>625ppm) depending on the MIC  
 256 values. On the basis of this classification, most of the essential oils whose antimicrobial  
 257 activity was evaluated showed low activity on all the bacteria studied.

258 Bacterial strains were more sensitive to ATB compared to essential oils, *Lactobacillus*  
 259 *plantarum* is the most sensitive strain (low value of MIC and MBC). The essential oils at the  
 260 maximum concentration tested were less active on most bacterial strains.

261

262 **Table 4. Antibacterial activities (MIC and MBC) of the essential oils and antibiotics**

Antimicrobials		MIC (µg/ml)				MBC (µg/ml)			
		LC	LP	LRH1	LRH2	LC	LP	LRH1	LRH2
Antibiotics	<b>Streptomycin</b>	31.25	3.90	31.25	31.25	62.50	15.62	31.25	31.25
	<b>Amoxicillin</b>	3.90	3.90	>1000	>1000	62.50	31.25	1000	>1000
	<b>Ampicillin</b>	3.90	3.90	>1000	500	125	31.25	>1500	>1000
	<b>Ciprofloxacin</b>	1000	500	62.50	1000	250	500	62.50	>1000
Essential oils	<b><i>Origanum compactum</i></b>	1500	1500	375	1500	>1500	>1500	>1500	>1500
	<b><i>Eucalyptus globulus</i></b>	>1500	>1500	>1500	>1500	>1500	>1500	>1500	>1500
	<b><i>Rosmarinus officinalis</i></b>	>1500	>1500	>1500	>1500	>1500	>1500	>1500	>1500
	<b><i>Cymbopogon winterianus</i></b>	750	>1500	>1500	>1500	1500	1500	>1500	>1500
	<b><i>Thymus vulgaris</i></b>	>1500	>1500	>1500	>1500	>1500	>1500	>1500	>1500

263 LC (*Lactobacillus casei*), LP (*Lactobacillus plantarum*), LRH2 (*Lactobacillus rhamnosus*  
 264 C24), LRH1 (*Lactobacillus rhamnosus* C1112)

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266 The sensitivity and inhibition parameters evaluated in this work revealed that the different  
 267 essential oils had no antibacterial activity in general on our target probiotic germs for  
 268 concentration lower or equal to 1500 ppm. Particularly, only the essential oil of *Origanum*  
 269 *Compactum* and *Cymbopogon winterianus* had low activities on the tested strain. *C.*  
 270 *winterianus* could be bactericidal on *Lactobacillus casei* and *Lactobacillus plantarum* at 1500  
 271 ppm and inhibit *Lactobacillus casei* at 750 ppm. On the other hand, *O. Compactum* was not  
 272 bactericidal but could inhibit the growth of all the selected strains at a concentration of 1500  
 273 ppm and 375 ppm for *Lactobacillus rhamnosus* C1112. This antibacterial activity could be  
 274 attributed to the presence in these oils of secondary metabolites with antimicrobial  
 275 properties. Indeed, studies have reported that secondary metabolites such as tannins,  
 276 phenols, flavonoids, saponins, phenolic compounds have antibacterial activity [27].

277 The antibacterial activity varies from one oil to another, it could be explained by the  
 278 difference in composition and concentration of secondary metabolite [28] present in each  
 279 essential oil. In addition, [29] reports that several essential oils exhibit antimicrobial activity  
 280 against many bacteria and fungi at high concentrations. However, the bactericidal activity  
 281 detected in *Origanum compactum* in this study can be related to the presence of carvacrol,  
 282 which is the major compound in this oil and is generally known for its antimicrobial properties  
 283 [30]. In addition, bacterial strains were more sensitive to antibiotics than essential oils. This  
 284 could be due to the fact that antibiotics are pure active compounds while essential oils are  
 285 mixtures of substances that contain in addition to active compounds; other substances such

286 as polysaccharides, polypeptides that could bind to active compounds mask or decrease  
287 their activity [31, 32].

288

### 289 **3.3 Effect of different combinations of essential oils and antibiotics on the** 290 **growth kinetics of the probiotic strains used**

291 The determination of the inhibition zone diameters, MIC and MBC allowed the selection of  
292 two antibiotics: Ampicillin (AMP) and Ciprofloxacin (CPF), two essential oils: *Cymbopogon*  
293 *winterianus* (CW) and *Origanum compactum* (OC) and two bacteria: *Lactobacillus casei* (LC)  
294 and *Lactobacillus rhamnosus* C 24 (LRH2) based on the different averages obtained. For  
295 this evaluation, a calibration line was constructed in order to predict microbial concentration  
296 from the acidity data (Figure 1). From this calibration line, it could be observed that the  
297 increase of medium acidity could be correlated to the level of lactic acid bacteria growth.

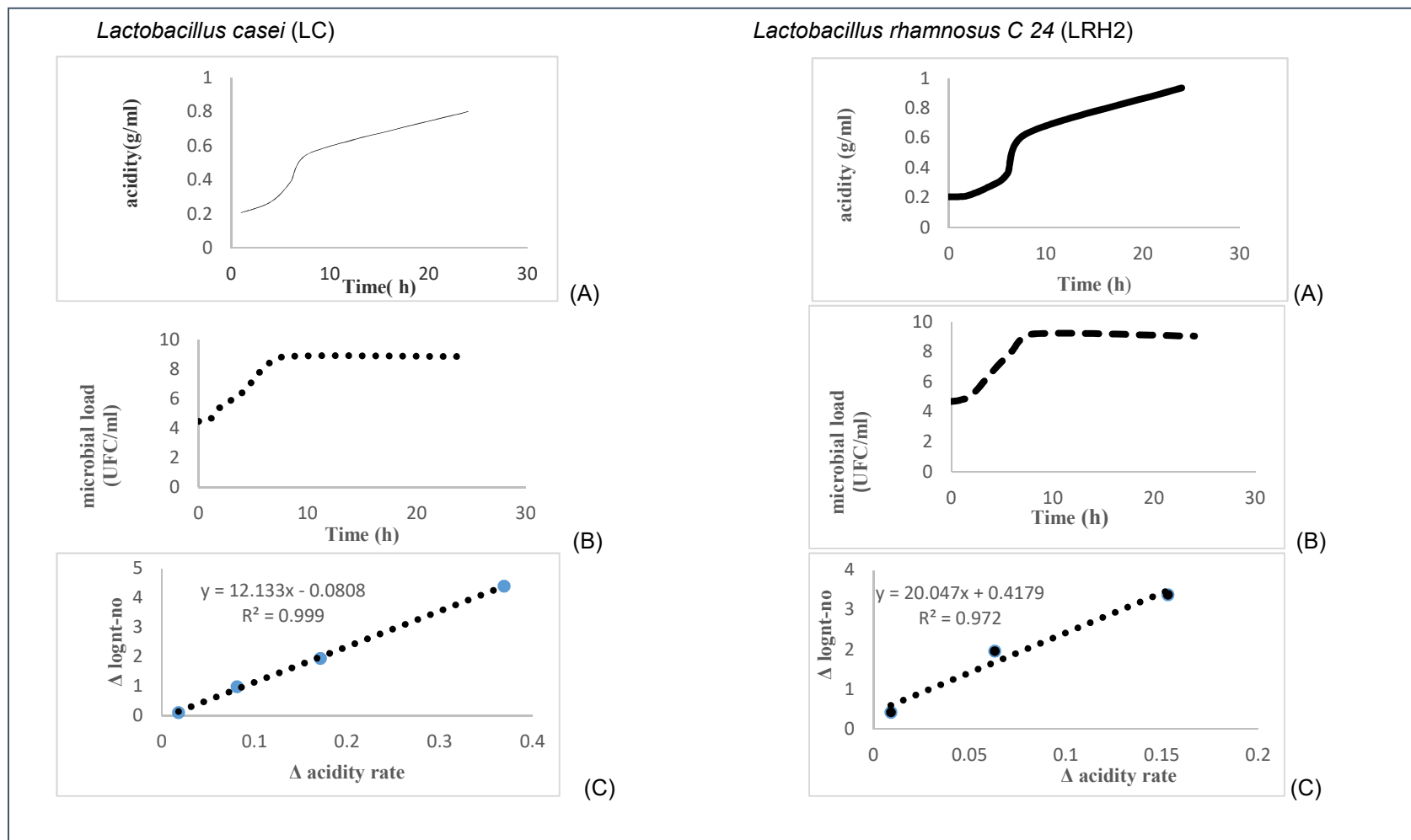
298 From these figures we observe that both strains have a correlation coefficient more than 0.9  
299 reflecting a satisfactory correlation between the titrable acidity and the microbial growth.

300 According to the fractional design experimental plan, we evaluated the effect of the  
301 combinations of essential oils and antibiotics after 24 hours of incubation by the expression  
302 of acidity increments (Table 5 and Table 6).

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**Fig. 1. Kinetics evolution of titratable acidity (A), bacterial load (B) and bacterial load calibration curve (C) for *Lactobacillus casei* and *Lactobacillus rhamnosus* C 24**

308 **Table 5. Acidity variation after 24 hours of the culture of *Lactobacillus casei* in the presence of combinations of essential oils and**  
 309 **antibiotics**

<i>Lactobacillus casei</i>												
TEST	AMP (ppm)	CW (ppm)	acidity	CPF (ppm)	CW (ppm)	acidity	AMP (ppm)	OC (ppm)	acidity	CPF (ppm)	OC (ppm)	acidity
1	1.95	375	0.189	500	375	0.000	1.95	750	0.630	500	750	0.180
2	3.90	375	0.135	1000	375	0.000	3.90	750	0.630	1000	750	0.180
3	5.85	375	0.045	1500	375	0.000	5.85	750	0.270	1500	750	0.360
4	1.95	750	0.360	500	750	0.030	1.95	1500	0.540	500	1500	0.000
5	3.90	750	0.063	1000	750	0.036	3.90	1500	0.540	1000	1500	0.090
6	3.90	750	0.063	1000	750	0.036	3.90	1500	0.540	1000	1500	0.090
7	5.85	750	0.252	1500	750	0.090	5.85	1500	0.540	1500	1500	0.180
8	1.95	1125	0.333	500	1125	0.098	1.95	2250	0.630	500	2250	0.190
9	3.90	1125	0.243	1000	1125	0.036	3.90	2250	0.630	1000	2250	0.135
10	5.85	1125	0.000	1500	1125	0.126	5.85	2250	0.540	1500	2250	0.110

310 CW/AMP= *Cymbopogon winterianus* and Ampicillin, CW/CPF= *Cymbopogon winterianus* and Ciprofloxacin, OC/AMP= *Origanum*  
 311 *compactum* and CW/AMP= *Cymbopogon winterianus* and Ampicilline, CW/CPF= *Cymbopogon winterianus* and Ciprofloxacin, OC/AMP=  
 312 *Origanum compactum* and Ampicillin, OC/CPF= *Origanum compactum* and Ciprofloxacin.

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317 **Table 6. Acidity variation after 24 hours of the culture of *Lactobacillus rhamnosus* C 24 in the presence of combinations of**  
 318 **essential oils and antibiotics**

<i>Lactobacillus rhamnosus</i> C24												
TEST	AMP (ppm)	CW (ppm)	acidity	CPF (ppm)	CW (ppm)	acidity	AMP (ppm)	OC (ppm)	acidity	CPF (ppm)	OC (ppm)	acidity
1	250	750	0.009	500	750	0.000	250	750	0.495	500	750	0.000
2	500	750	0.036	1000	750	0.000	500	750	0.360	1000	750	0.000
3	750	750	0.000	1500	750	0.180	750	750	0.450	1500	750	0.090
4	250	1500	0.000	500	1500	0.000	250	1500	0.270	500	1500	0.000
5	500	1500	0.000	1000	1500	0.000	500	1500	0.315	1000	1500	0.090
6	500	1500	0.000	1000	1500	0.000	500	1500	0.360	1000	1500	0.090
7	750	1500	0.000	1500	1500	0.090	750	1500	0.090	1500	1500	0.180
8	250	2250	0.000	500	2250	0.000	250	2250	0.450	500	2250	0.540
9	500	2250	0.000	1000	2250	0.000	500	2250	0.360	1000	2250	0.000
10	750	2250	0.000	1500	2250	0.000	750	2250	0.000	1500	2250	0.360

319 CW/AMP= *Cymbopogon winterianus* and Ampicillin, CW/CPF= *Cymbopogon winterianus* and Ciprofloxacin, OC/AMP= *Origanum*  
 320 *compactum* and Ampicillin, OC/CPF= *Origanum compactum* and Ciprofloxacin.

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324 The acidity after 24 hours varies according to the different combinations of essential oils and  
325 antibiotics. The evaluation of the effect of combinations on bacteria has shown that acid  
326 accumulation synonymous of growth, varies according to the different concentrations of  
327 antimicrobials and the different combinations of essential oils and antibiotics. In addition, it is  
328 generally observed that when the values below the MICs of essential oils are combined with  
329 the MIC values of antibiotics, acid is produced indicating microorganism growth. According  
330 to [33], it could be described as an antagonistic phenomenon of oil and antibiotics.  
331 Antibiotics generally act very specifically on certain structures of the bacterial cell; and this  
332 high specificity of action explains why they are active at very low concentrations [34]. On the  
333 other hand, some researchers have shown that the potency of essential oils varies according  
334 to their major constituents and that the mode of action is mainly related to the chemical  
335 profile of the constituents of each essential oil [35]. The presence of oils could therefore limit  
336 access to the site of action of antibiotics, thus reducing their effects. In situations where  
337 essential oils and antibiotics are combined with values lower than their MICs, no acid  
338 production is observed, example of OC/CPF combination on *Lactobacillus rhamnosus* C24.  
339 Ampicillin is a broad-spectrum bêta-lactam bacteria that acts on Gram+ bacteria and some  
340 Gram- bacteria it inhibits the enzymes of transpeptidation involved in the bridging of the  
341 polysaccharide chains of the peptidoglycan of the wall [36]. Ciprofloxacin is an ATB  
342 belonging to the fluoroquinolone family .it works by killing the bacteria responsible for  
343 infection by inhibiting bacteria DNA gyrase and therefore interferes with DNA replication  
344 transcription and other activities involving DNA (inhibition of nucleic acids) [34].  
345 Essential oils act on both Gram+ bacteria as well as Gram- bacteria nevertheless, Gram-  
346 bacteria seem less sensitive to their action and this is directly linked to the structure of their  
347 cell wall [37]. Several chemical components of EOs make it possible to modulate the  
348 intestinal flora and thus reduce the number of certain bacteria [38]. Carvacrol and thymol are  
349 able to form hydrogen bonds with the actives sites of microbial enzymes and thus may  
350 contribute to the antimicrobial effects of essential oils [24,39]. This antibacterial efficacy of  
351 essential oils rich in carvacrol and thymol is explained by the position of the hydroxyl group  
352 on the phenolic structure of the molecules and hydroxylamine groups of the bacteria causing  
353 an ultracellular leak from the cells. All these alterations and changes lead to cell death  
354 [40,41].  
355 It can be observed that the combination of CPF/CW and CPF/OC are not favorable to the  
356 growth of *L. casei* as the increase in acidity is very low independently oh the combination  
357 levels. This strain was more affected by the combination of AMP/OC while combining  
358 AMP/CW had irregular activity on the strain growth. Regarding *L. rhamnosus*, it can be  
359 observed that the combination of AMP/CW, CPF/CW and CPF/OC did not favor the strain  
360 growth as very low or no acid increase was observed. On the other hand, the combination of  
361 AMP/OC generally did not affect *L. rhamnosus* growth and acid production. Taking into  
362 consideration the *L. casei* and *L. rhamnosus* demonstrated to has almost the same  
363 acidification capacity (Figure 1) it can be observed that *L. rhamnosus* is more affected by the  
364 combination of AMP/OC than *L. casei*.

#### 366 4. CONCLUSION

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368 This work has showed that, no matter the composition of essential oils, they have in most of  
369 the cases, very low activities on presumptive probiotics when used alone. Antibiotics tested  
370 were generally active on all the bacteria tested. Combinations between essential oils at  
371 values below the MIC with antibiotics greater than or equal to the MIC reduced the effect of  
372 antibiotics on probiotics. However, combinations of essential oils and antibiotics at  
373 concentrations greater than or equal to their MICs lead in most cases to inhibition of the  
374 growth of the probiotics studied. Finally, our results suggest that the effect of essential oils  
375 and medicinal plant solvent extracts used alone or in combination to antibiotics on the gut

376 microbiota have to be evaluated for validation as well as their toxicity activities before using  
377 them for human therapy.

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**ABBREVIATIONS**

506

MIC: Minimum inhibitory concentration

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MBC: Minimum bactericidal concentration

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UFC: Units Forming colony

509

Cm: Mass concentration of lactic acid (g/L)

510

Cb: NaOH concentration (mol/L)

511

Vb: Volume of NaOH (L)

512

Ma: Mass molecular (g/mol)

513

C: Cellular concentration (UFC/mL)

514

n: number of colonies

515

Fd: Dilution factor

516

V: Sow volume (mL)

517

GC: Gas Chromatography

518

SPME: Solid-phase micro extraction

519

EO: Essential oil

520

ATB: Antibiotic

521

IZ: Inhibition zone

522

OC: *Origanum compactum*

523

CW: *Cymbopogon winterianus*

524

AMP: Ampicilin

525

AMOX: Amoxicillin

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STREP: Streptomycin

527

CPF: Ciprofloxacin

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LC: *Lactobacillus casei* LBLDL

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LP: *Lactobacillus plantarum* ATCC 14197

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LRH1: *Lactobacillus rhamnosus* C1112

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LRH2: *Lactobacillus rhamnosus* C24

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COMPETING INTERESTS DISCLAIMER:

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Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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