



HAL
open science

Selection of nerolidol among a series of terpenic and phenolic compounds for its potent activity against *Lactobacillus fermentum* ATCC 9338

Elissa Ephrem, Amal Najjar, Catherine Charcosset, H el ene Greige-Gerges

► **To cite this version:**

Elissa Ephrem, Amal Najjar, Catherine Charcosset, H el ene Greige-Gerges. Selection of nerolidol among a series of terpenic and phenolic compounds for its potent activity against *Lactobacillus fermentum* ATCC 9338. *Process Biochemistry*, Elsevier, 2019, 80, pp.146-156. 10.1016/j.procbio.2019.02.015 . hal-03033836

HAL Id: hal-03033836

<https://hal.archives-ouvertes.fr/hal-03033836>

Submitted on 8 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin ee au d ep ot et  a la diffusion de documents scientifiques de niveau recherche, publi es ou non,  emanant des  tablissements d'enseignement et de recherche franais ou  trangers, des laboratoires publics ou priv es.

1 **Selection of nerolidol among a series of terpenic and phenolic compounds for**
2 **its potent activity against *Lactobacillus fermentum* ATCC 9338**

3 **Elissa Ephrem^{a,b}, Amal Najjar^a, Catherine Charcosset^b, H el ene Greige-Gerges^{a,*}**

4 ^a Bioactive Molecules Research Laboratory, Faculty of Sciences, Lebanese University, B.P.
5 90656 Jdaidet El-Matn, Lebanon.

6 ^b Laboratoire d'Automatique et de G enie des Proc ed es, Universit e Claude Bernard Lyon I,
7 France, 43 Boulevard du 11 Novembre 1918, B atiment CPE 69 622, Villeurbanne Cedex,
8 France.

9 *Corresponding author: Faculty of Sciences, Bioactive Molecules Research Laboratory,
10 Lebanese University, B.P. 90656 Jdaidet El-Matn, Lebanon.

11 Tel.: +961 3 341011; Fax: +961 1 689647

12 E-mail addresses: greigegeorges@yahoo.com, hgreige@ul.edu.lb (H el ene Greige-Gerges).

13

14

15

16

17

18

19

20

21

22 **Abstract**

23 Essential oil components are widely used for their antibacterial activity against spoilage
24 microorganisms in food. *Lactobacillus fermentum* is a Gram-positive lactic acid bacteria
25 responsible for the deterioration of various food products, including beverages and dairy
26 products. In this study, 17 terpenic and 11 phenolic compounds were screened against the food
27 spoilage microorganism *Lactobacillus fermentum* ATCC 9338. The antibacterial activity of the
28 tested compounds was dependent on hydrophobicity and particular chemical features. Nerolidol
29 solubilized in dimethylsulfoxide exhibited the highest antibacterial activity and showed low
30 minimal inhibitory (MIC: 25 μ M) and minimal bactericidal (MBC: 50 μ M) concentrations.
31 Moreover, no viable cells were detected within 16 h of incubation at 50 μ M. The important
32 antibacterial activity of nerolidol against *L. fermentum* is probably related to the high
33 hydrophobicity, the aliphatic chain length, and the presence of the hydroxyl group.
34 Hydroxypropyl- β -cyclodextrin/nerolidol inclusion complex showed MIC and MBC values of
35 100 and 200 μ M, respectively. The total bacterial kill was observed after 12 h of incubation. The
36 results obtained with the inclusion complex are probably due to the time required to allow
37 nerolidol to be released from the inclusion complex.

38 **Keywords:** Cyclodextrins; *Lactobacillus fermentum*; nerolidol; phenolic compounds; terpenes.

39 **List of abbreviations:** CFU: colony forming unit; DMSO: dimethylsulfoxide; HP- β -CD:
40 hydroxypropyl- β -cyclodextrin; HPLC: high performance liquid chromatography; LAB: lactic
41 acid bacteria; MBC: minimal bactericidal concentration; MIC: minimal inhibitory concentration;
42 Ner: nerolidol.

44 1. Introduction

45 *Lactobacillus fermentum* is an ubiquitous Gram-positive, rod-shaped, anaerobic, thermo-
46 acidophilic lactic acid bacteria (LAB), which can originate from plants, animals, meat, dairies,
47 fruits, and cereals. *L. fermentum* is an obligate heterofermentative bacteria which ferments
48 various types of sugars (lactose, fructose, maltose, sucrose) under anaerobic conditions. In
49 addition to lactic acid, *L. fermentum* produces acetic acid, ethanol, carbon dioxide, and minor by-
50 products, such as diacetyl, hydrogen peroxide, and different alcohols (e.g. 3-methylbutanol),
51 esters (e.g. ethyl acetate), and carbonyls (e.g. nonanal) compounds. *L. fermentum* is not harmful
52 to humans and its presence is necessary for the fermentation of different food products, including
53 sourdough, cocoa, and certain beverages. Certain *L. fermentum* strains are probiotic agents and
54 express outstanding health-promoting characteristics when consumed [1]. Nonstarter lactic acid
55 bacteria, including *L. fermentum*, cause defects in certain food products, such as slits or cracks in
56 hard cheeses, lack of flavors, or bloated packaging in dairy products [2–4]. *L. fermentum* also
57 spoils various types of beverages and can grow in fruit juice, leading to the production of
58 undesirable compounds [5–9]. It is one of the LABs responsible for the desired malolactic
59 fermentation in wine, resulting in the conversion of malic acid into lactic acid, acetate, succinate,
60 and carbon dioxide [10]. However, the presence of LABs could lead to wine spoilage as the
61 control of bacterial growth and malolactic fermentation is difficult to achieve, thus altering the
62 wine organoleptic properties [10–12]. The growth of heterofermentative LABs (such as *L.*
63 *fermentum*) in wine causes an increased acidity, cloudiness, and mousy odor [13]. On the other
64 hand, *L. fermentum* can grow in beer, as it is resistant to hop-compounds [14], leading to beer
65 spoilage [14,15] and aroma alteration [14].

66 Different strategies have been adopted to overcome the microbial spoilage of food, including the
67 addition of chemical additives and physical treatments. Physical treatments include various
68 preservation techniques, such as thermal, ultraviolet light, ultrasound, pulsed electric field, and
69 high hydrostatic pressure technologies [16]. However, the application of these treatments is
70 limited due to changes in the organoleptic properties of the food product [17–19] and sometimes
71 due to high cost [20]. On the other hand, chemical preservatives used against food microbial
72 spoilage, including benzoates, sorbates, propionates, nitrates, and nitrites, can cause allergic
73 responses and could be converted to potential carcinogens [21]. The high demand of fresh and
74 “safe” food, free of synthetic additives and contaminants, have increased the interest of using
75 natural products for food preservation. Natural antimicrobials may derive from plants, animals,
76 and microorganisms. Plant essential oils are largely exploited due to their wide spectrum of
77 antimicrobial activity against spoilage bacteria and food-borne pathogens [22]. Plant
78 antimicrobials include different chemical classes, among which are saponins, tannins, flavonoids,
79 terpenes, simple phenols, and phenolic acids [23,24].

80 The majority of natural antimicrobials are hydrophobic and poorly stable, which limit their use in
81 aqueous media. Solvents, such as dimethylsulfoxide (DMSO) [25], dimethylformamide [26], and
82 ethanol [27, 28], are used to dissolve hydrophobic compounds in aqueous solutions. However,
83 the use of organic solvents in food products is not desirable. Various encapsulation systems
84 (nanoemulsions, liposomes, nanoparticles, solid lipid nanoparticles, cyclodextrins, etc.) were
85 introduced to the food industry as a novel strategy to overcome the poor water solubility of food
86 antimicrobials and to enhance their stability in food matrices [29,30]. Cyclodextrins are natural
87 oligosaccharides widely used in food products for their safety and their ability to deliver
88 hydrophobic compounds (e.g. antioxidants, antimicrobials, etc.) [31]. Moreover, different

89 derivatives of cyclodextrin have been synthesized to enhance the aqueous solubility of native
90 cyclodextrins [32].

91 The objective of this study is to select a natural potent antibacterial agent against *L. fermentum*.

92 The selected antibacterial is then complexed with hydroxypropyl- β -cyclodextrin (HP- β -CD) and
93 the obtained inclusion complex is tested against the chosen bacterium in culture medium.

94 Therefore, a wide range of terpenic and phenolic phytochemicals was screened against the
95 bacterium, under its optimal growth conditions. The tested compounds belong to different

96 subclasses and possess variable structures. Seventeen terpenes were tested, among which eight
97 monoterpene hydrocarbons (camphene, *p*-cymene, limonene, α -phellandrene, α -pinene, β -

98 pinene, α -terpinene, γ -terpinene), a sesquiterpene hydrocarbon (β -caryophyllene), four
99 monoterpene alcohols (borneol, geraniol, linalool, menthol), a sesquiterpene alcohol (nerolidol),

100 two monoterpene esters (bornyl acetate, linalyl acetate), and a monoterpene ketone (camphor)
101 (Fig. 1). In addition, eleven phenols were tested, among which four phenylpropenes (*trans*-

102 anethole, eugenol, isoeugenol, estragole), a phenylpropene ester (eugenyl acetate), two
103 hydroxycinnamic acids (*p*-coumaric acid, *trans*-ferulic acid), a phenol ether (anisole), a

104 methylphenol (*o*-cresol), a flavonoid (quercetin), and a stilbenoid (resveratrol) (Fig. 2).
105 Nerolidol (Ner) showed the most potent antibacterial activity against *L. fermentum* among the 28

106 tested compounds. The potency of nerolidol solubilized in DMSO and hydroxypropyl- β -
107 cyclodextrin/nerolidol (HP- β -CD/Ner) complex was assessed against *L. fermentum* by

108 determining the minimal inhibitory concentration (MIC) and the minimal bactericidal
109 concentration (MBC) values under the optimal conditions for *L. fermentum* growth. The time

110 required for both forms of nerolidol to cause a total bacterial kill was determined by time-kill
111 analysis.

112 2. Materials and methods

113 2.1. Materials

114 β -Caryophyllene, geraniol, isoeugenol (98% mixture *cis* and *trans*), nerolidol (98%, mixture of
115 *cis* (40%) and *trans* (60%) isomers), (-)- β -pinene, ethanol, and DMSO were purchased from
116 Sigma-Aldrich (Missouri, United States). *trans*-Anethole (99%) and linalyl acetate were
117 purchased from Sigma-Aldrich (Madrid, Spain). Anisole, camphene, *o*-cresol, eugenol, eugenyl
118 acetate, (-)-menthol, and α -phellandrene were purchased from Sigma-Aldrich (Schnelldorf,
119 Germany). (-)-Borneol, (-)-bornyl acetate, *p*-cymene, (R)-(t)-limonene, linalool, (+)- α -pinene, α -
120 terpinene, and γ -terpinene were purchased from Sigma-Aldrich (Buchs, Switzerland). (\pm)-
121 Camphor and *trans*-ferulic acid were purchased from Sigma-Aldrich (Hong Kong, China).
122 Quercetin was purchased from Sigma-Aldrich (Haryana, India). *p*-Coumaric acid was purchased
123 from Sigma-Aldrich (Irvine, United kingdom). Hydroxypropyl- β -cyclodextrin was purchased
124 from Wacker-Chemie (Lyon, France). De Man, Rogosa, and Sharpe (MRS) broth and MRS agar
125 were purchased from Laboratorios Conda (Madrid, Spain).

126 2.2. Bacterial strain and culture

127 *L. fermentum* ATCC 9338 was purchased from American Type Culture Collection (*Manassas*,
128 Virginia, USA). *L. fermentum* cultures were routinely maintained at 4 °C on MRS agar. Before
129 each antimicrobial assay, fresh cultures were prepared in sterile MRS broth and incubated at 37
130 °C for 22 h under anaerobic conditions. A bacterial suspension was prepared by diluting the
131 bacterial culture in MRS broth to a final concentration of 25×10^5 colony forming unit (CFU)/ml.

132 2.3. Screening of natural terpenic and phenolic compounds for antibacterial activity

133 2.3.1. Screening rounds

134 Twenty-eight natural terpenic and phenolic compounds were screened for their antibacterial
135 activity against *L. fermentum* at 3500, 500, 250, and 100 μM . The tested molecules were
136 dissolved in DMSO, except β -caryophyllene and camphene which were dissolved in ethanol, and
137 the obtained solutions were homogenized by hand agitation prior to each test. Antibacterial agent
138 solutions (25 μl) were mixed with MRS broth (4.8 ml) in glass culture tubes (20 x 100 mm). The
139 tubes were then inoculated with 200 μl of a diluted *L. fermentum* suspension (25×10^5 CFU/ml)
140 to yield a bacterial concentration of 10^5 CFU/ml at baseline. Bacterial cultures (5 ml) exempt of
141 any agent, or containing 25 μl of DMSO or ethanol, served as controls. All cultures were
142 incubated at 37 °C for 22 h under anaerobic conditions. Screening rounds were conducted with
143 bioactive compounds at 3500, 500, and 250 μM , successively. After each screening round,
144 molecules demonstrating an anti-proliferative activity against *L. fermentum* at a given
145 concentration were identified. Whereas, compounds demonstrating a bactericidal effect against
146 *L. fermentum* were selected for another screening round at a lower concentration. The
147 compounds exhibiting a total bactericidal activity or a total anti-proliferative activity against *L.*
148 *fermentum* at 250 μM were screened at 100 μM . Each test was performed in triplicate and under
149 sterile conditions.

150 2.3.2. Determination of the anti-proliferative activity of molecules

151 The anti-proliferative activity of the molecules against *L. fermentum* was assessed by UV-visible
152 spectroscopy at 660 nm using Uviline 9100-9400 spectrophotometer (GmbH, Germany). The
153 optical density of each tube was measured and compared to the control. The percentage of
154 bacterial proliferation inhibition was calculated as follows:

155
$$\text{Bacterial proliferation inhibition (\%)} = 100 \times \left[1 - \frac{OD_{660m}}{OD_{660c}} \right],$$

156 where OD_{660m} and OD_{660c} are the optical densities of the tubes containing the molecule and the
157 control tube, respectively. Each test was done in triplicate.

158 2.3.3. Determination of the bactericidal activity of the natural molecules

159 Cultures showing a total inhibition of bacterial proliferation were analyzed in duplicate by
160 spreading an aliquot of 100 μ l on MRS agar. The bacterial concentration in the control was
161 determined by enumeration. The bactericidal capacity was then evaluated according to the
162 decrease in the initial bacterial concentration and calculated as follows:

163
$$\text{Bacterial kill (\%)} = 100 \times \left[1 - \frac{[bac]_m}{[bac]_c} \right],$$

164 where $[bac]_m$ and $[bac]_c$ are the bacterial concentrations in the tube containing the molecule and
165 the control tube, respectively.

166 2.4. Preparation of HP- β -CD/Ner inclusion complex

167 HP- β -CD/Ner inclusion complex was prepared by freeze-drying, as previously described by Azzi
168 et al. [33]. Briefly, an aqueous solution of HP- β -CD (25 mM) containing an excess of nerolidol
169 was kept under magnetic stirring at 300 rpm for 24 h at room temperature. The suspension was
170 then filtered (0.45 μ m, cellulose acetate membrane) to remove the excess of nerolidol. The
171 filtrate was frozen at -80 $^{\circ}$ C, and lyophilized. HP- β -CD/Ner complex in powder form was stored
172 at 4 $^{\circ}$ C until usage. The amount of nerolidol encapsulated in the cyclodextrin cavity was
173 determined by HPLC as described previously by Azzi et al. [33], and the result was expressed as
174 mass of nerolidol (μ g) per mg of powder (μ g_{Ner}/mg_{powder}).

175 2.5. Study of the antibacterial activity of nerolidol and HP- β -CD/Ner complex

176 2.5.1. Determination of MIC and MBC values

177 Bacterial cultures were prepared as previously described in section 2.3.1, in presence of nerolidol
178 dissolved in DMSO, and added at a final concentration ranging from 0.1 to 3500 μ M. The MIC
179 was determined as the minimal concentration at which no bacterial growth was observed in MRS
180 broth, whereas the MBC was determined as the minimal concentration at which no bacterial
181 growth was observed on agar. Each experiment was performed in triplicate and under sterile
182 conditions. On the other hand, HP- β -CD was investigated for its capacity to replace DMSO for
183 nerolidol solubilization in aqueous solution. The inclusion complex was tested at a final
184 concentrations of nerolidol ranging between 50 and 4000 μ M. Bacterial cultures (5 ml) exempt
185 of any agent, or containing DMSO (25 μ l) or blank HP- β -CD added in similar amounts to that of
186 the inclusion complex, served as controls.

187 2.5.2. Time-kill analysis

188 Time-kill assay was performed in triplicate on nerolidol and HP- β -CD/Ner complex. Cultures
189 were prepared as described in section 2.3.1. Nerolidol solubilized in DMSO and the complex
190 were added to cultures at their respective MBC values. Starting from an initial bacterial
191 concentration of 10^5 CFU/ml (5 log CFU/ml), bacterial growth was followed during 22 h in
192 cultures maintained at 37 °C under anaerobic conditions. The viable plate count was determined
193 at different time intervals using the spread plate method [34]. Therefore, 100 μ l of samples with
194 appropriate dilutions was spread on MRS agar. The plates were incubated under *L. fermentum*
195 optimal growth conditions for 22 h. The colonies were then counted and the bacterial
196 concentration was determined.

197 2.6. Statistical Analysis

198 Statistical analysis was performed using the Student T test. *P* values equal or less than 0.05 were
199 considered statistically significant.

200 3. Results

201 3.1. Antibacterial activity of terpenic and phenolic compounds

202 Terpenic compounds exhibited a significantly higher antibacterial activity against *L. fermentum*
203 compared to phenolic compounds, with α -terpinene being the only exception. In fact at 3500 μ M,
204 16 among the 17 tested terpenes were bactericidal, whereas α -terpinene strongly inhibited the
205 proliferation of *L. fermentum* (89.1%) (Table 1). Eugenol and its ester derivative eugenyl acetate
206 were the only phenolic compounds exhibiting a strong bactericidal activity against *L. fermentum*
207 at 3500 μ M. Indeed, eugenol and eugenyl acetate exhibited a bactericidal activity of 100% and
208 95%, respectively, within 22 h of incubation at 37 °C under anaerobic conditions (Table 2).
209 Among the phenolic compounds, *trans*-anethole, estragole, and isoeugenol showed a significant
210 anti-proliferative activity against *L. fermentum* at the highest concentration (3500 μ M), where
211 bacterial growth inhibition was higher than 88% (Table 2). At this concentration, *p*-coumaric
212 acid, *trans*-ferulic acid, and anisole showed a weak bacterial growth inhibition of 21.9, 17.3, and
213 16.1%, respectively (Table 2). Whereas, quercetin, *o*-cresol, and resveratrol possessed no
214 antibacterial activity against *L. fermentum* (Table 2). In fact, quercetin and resveratrol were
215 insoluble in MRS at this concentration, which was marked by their strong precipitation.

216 The stronger antibacterial property of terpenes compared to phenolic compounds was highlighted
217 by the strong bactericidal and anti-proliferative effect of different studied terpenes at 500 μ M.
218 Indeed, at this concentration, the two phenolic compounds, eugenol and eugenyl acetate, showed
219 no antibacterial activity against *L. fermentum* (Table 2). On the other hand, β -pinene, bornyl

220 acetate, linalyl acetate, and nerolidol exhibited a total bactericidal activity at 500 μ M (Table 1).
221 Camphene, *p*-cymene, limonene, α -phellandrene, α -pinene, and γ -tepinene showed a total
222 inhibition of *L. fermentum* proliferation but were not bactericidal (Table 1). Furthermore, β -
223 caryophyllene, geraniol, and menthol showed a bacterial inhibition percentage higher than 75%,
224 compared to control, whereas borneol demonstrated a weak anti-proliferative activity (28.5%)
225 (Table 1). However, linalool and camphor, which exhibited a bactericidal activity against *L.*
226 *fermentum* at 3500 μ M, showed no antibacterial activity at 500 μ M (Table 1).

227 When β -pinene, bornyl acetate, linalyl acetate, and nerolidol were tested at 250 μ M, nerolidol
228 was the only compound exhibiting a bactericidal activity (100%) (Table 1). Whereas, β -pinene,
229 bornyl acetate, and linalyl acetate showed a strong anti-proliferative activity against *L.*
230 *fermentum* at 250 μ M, as no bacterial growth was observed in MRS broth within 22 h of
231 incubation (Table 1). Nerolidol maintained a strong bactericidal activity (100%) against *L.*
232 *fermentum* at 100 μ M, whereas, β -pinene strongly inhibited bacterial proliferation (~91%), and
233 bornyl acetate and linalyl acetate showed no antibacterial activity (Table 1). Thus, among the 28
234 tested compounds, nerolidol was retained for further studies as it was the most effective studied
235 compound against *L. fermentum*.

236 3.2. Antibacterial activity of nerolidol

237 Nerolidol was the only molecule that exhibits a bactericidal activity against *L. fermentum* at a
238 low concentration (100 μ M) (Tables 1 and 2). Therefore, the study was taken further to
239 determine the MIC and MBC values of nerolidol. Nerolidol exhibited a strong antibacterial
240 activity against *L. fermentum* with low MIC (25 μ M; 5.56 mg/l) and MBC (50 μ M; 11.12 mg/l)
241 values. Moreover, *L. fermentum* survival was approximately 1% at a nerolidol concentration of

242 35 μM . However, the adopted MBC was 50 μM , as no bacterial growth was observed at this
243 concentration.

244 The antibacterial activity profile (bactericidal or bacteriostatic) of nerolidol was evaluated using
245 the MBC to MIC ratio. The *in vitro* antimicrobial activity of nerolidol can be described as
246 bactericidal as the MBC to MIC ratio ($\text{MBC}/\text{MIC}= 2$) is lower than 4 [35].

247 3.3. Antibacterial activity of HP- β -CD/Ner complex

248 The amount of nerolidol in the freeze-dried inclusion complex, determined by HPLC, was 40
249 $\mu\text{g}_{\text{Ner}}/\text{mg}_{\text{powder}}$. The antibacterial activity of HP- β -CD/Ner complex was investigated for the first
250 time against *L. fermentum* in culture medium under the optimal conditions for bacterial growth.
251 HP- β -CD without nerolidol had no effect on the bacterial growth (data not shown). The MIC and
252 MBC values of HP- β -CD/Ner against *L. fermentum* were 100 and 200 μM , respectively. At 50
253 μM , a 4 log increment was observed in the bacterial concentration within 22 h of incubation
254 (data not shown).

255 3.4. Nerolidol and HP- β -CD/Ner complex time-kill analysis

256 A time-kill analysis was conducted to determine the time required to achieve a total bacterial kill
257 in presence of nerolidol solubilized in DMSO at 50 μM and HP- β -CD/Nero complex at 200 μM
258 (MBC). Free nerolidol exhibited a bactericidal activity against *L. fermentum* within the first few
259 hours of incubation (Fig. 3). Indeed, a 1.44 log reduction of *L. fermentum* concentration was
260 observed within 4 h of incubation. The bacterial concentration continued to decrease over time,
261 as a 2.82 log reduction was obtained after 10 h. At 16 h of incubation, no viable cells were
262 observed.

263 In presence of HP- β -CD/Ner complex (200 μ M), the bacterial concentration decreased by 1.22
264 log and 2.44 log after 4 and 10 h, respectively, and a total bacterial death was obtained within 12
265 h (Fig. 3).

266 **4. Discussion**

267 4.1. Antibacterial efficiency of nerolidol

268 Plants produce a wide range of antimicrobial agents highly desired by consumers due to their
269 natural origin. However, many of these antimicrobials are only effective at high concentrations
270 (for example at millimolar range), thus exhibiting a weak activity compared to common
271 antibiotics [36]. In this study, nerolidol exhibited the highest antibacterial activity among the 28
272 tested terpenic and phenolic compounds. The outstanding antibacterial potency of nerolidol
273 against *L. fermentum* was marked by the low MIC (25 μ M; 5.56 mg/l) and MBC (50 μ M; 11.12
274 mg/l) values, as well as the rapid bactericidal activity (Fig. 3). This compound acts by disrupting
275 and by damaging the bacterial cell membrane, and by interfering with genes responsible for the
276 microbe pathogenicity [37]. Besides, Brehm-Stecher and Johnson [27] demonstrated the
277 permeabilizing effect of nerolidol on *L. fermentum* membrane. The permeabilization of the
278 bacterial membrane leads to the leakage of the cytoplasmic molecules, thus causing cell lysis
279 [38]. Also, the disruption of the bacterial membrane would allow the permeation of exogenous
280 molecules into the bacterial cytoplasm [27]. Moreover, Brehm-Stecher and Johnson [27]
281 suggested that the permeabilizing activity of nerolidol may be due to its structural resemblance to
282 the lipids of the bacterial membrane. This was previously highlighted by Cornwell and Barry
283 [39] which attributed the enhancement of skin penetration by nerolidol to its long hydrocarbon
284 tail which promotes the interaction of the molecule with the interior of the cell bilayer.

285 Nerolidol is a sesquiterpene alcohol widely used in the food industry as a flavoring agent. It
286 demonstrated a potent antimicrobial activity against some fungi, in addition to different Gram-
287 positive and Gram-negative bacterial strains, such as *Staphylococcus aureus* (MIC: 3.9 mg/l; 50
288 mg/l) [26,40,41], *Streptococcus mutans*, *Propionibacterium acnes* (MIC: 25 mg/l) [26],
289 *Salmonella enterica* (MIC: 15.6 mg/l) [41], *Trichophyton mentagrophytes* (MIC: 12.5 mg/l) [26],
290 and *Aspergillus niger* (15.6 mg/l) [41]. On the other hand, nerolidol was able to enhance the
291 susceptibility of *S. aureus* and *Escherichia coli* to antibiotics, including ciprofloxacin,
292 erythromycin, gentamicin, vancomycin [42], and amoxicilline/clavulanic acid [43]. In this study,
293 nerolidol antibacterial potency against *L. fermentum* (MIC: 25 μ M; 5.56 mg/l) was weaker than
294 that of gentamicin [44] and chloramphenicol [45], and close to that of novobiocin [46] and N-
295 alkyl dimethylbenzyl ammonium chloride [47] (Table 3). On the other hand, *L. fermentum* strains
296 were less sensitive to well-known antibiotics including vancomycin [45,48], teicoplanin [45],
297 streptomycin [44,45,48], erythromycin, tobramycin, clindamycin, kanamycin, polymixin B
298 [44,46,48], metronidazole, and nitrofurantoin [49] (Table 3).

299 4.2. Antibacterial assay of HP- β -CD/Ner complex

300 Cyclodextrin inclusion complexes have been widely studied for their capacity to enhance the
301 stability and solubility of antimicrobials [28,50,51].

302 Some studies reported similar antibacterial activities of free and complexed antimicrobials (e.g.
303 clarithromycin, chlorogenic acid, peptide CM4) [28,50,52], while others proved modifications of
304 the potency of the antimicrobial agent following complexation with cyclodextrins. Compared to
305 the free form, the inclusion complex of *Hyptis martiusii* essential oil [53] and coriander essential
306 oil [51] showed a lower antimicrobial activity, whereas that of carvacrol showed a higher
307 antimicrobial activity [54].

308 The incorporation of nerolidol into HP- β -CD inclusion complex increased the MIC and MBC
309 values by 4 fold. Azzi et al. [33] studied the release of nerolidol from the inclusion complex in
310 water and at room temperature by dialysis. Around 45% of nerolidol were released from HP- β -
311 CD/Ner complex within 8 h, followed by a slow release rate over 7 days [33]. In fact, when HP-
312 β -CD/Ner complex is added at 50 μ M, the actual concentration of nerolidol interacting with *L.*
313 *fermentum* in MRS is significantly lower than 50 μ M during the whole experiment, thus
314 explaining the ability of the inoculated bacteria to proliferate (data not shown). However, at 200
315 μ M the concentration of nerolidol in the culture medium should be approximately 100 μ M after 8
316 to 10 h of incubation, which could explain the rapid drop in the bacterial concentration (Fig. 3).
317 The slow release and the photo-protection of nerolidol provided by the encapsulation systems
318 [33] would prevent and limit the proliferation of spoilage bacteria in food products.

319 4.3. Relationship between the antibacterial activity of terpenic and phenolic compounds and their 320 structural and physicochemical parameters

321 Various parameters may modulate the activity of antibacterial agents, including the drug
322 hydrophobicity, the presence of functional groups, the bacterial cell envelope characteristics, and
323 the incubation conditions (for instance, aeration).

324 4.3.1. The influence of hydrophobicity on the antibacterial activity of molecules

325 It has been reported that molecules with high hydrophobicity exhibit a high affinity to the
326 lipophilic structures in the target microorganisms, such as the bacterial membrane [55].
327 Therefore, they may disrupt the membrane integrity, thus affecting membrane permeability and
328 enzymes activity [55]. However, many exceptions could be revealed from this work, which
329 suggests that the hydrophobicity is not always the key parameter governing the antibacterial

330 activity; and some structural features could be taken into consideration when analyzing the
331 structure-antibacterial activity relationship.

332 Nerolidol, which has shown the strongest antibacterial activity against *L. fermentum*, has a LogP
333 value (5.33-5.36) considerably higher than that of the other tested molecules, except β -
334 caryophyllene (6.87) (Table 1). The latter, showed a modest antibacterial activity against *L.*
335 *fermentum* as it lost its bactericidal potential at 500 μ M (Table 1). In fact, β -caryophyllene have
336 been found to exhibit a weak antibacterial activity against different Gram-positive and Gram-
337 negative bacteria [56].

338 On the other hand, the antibacterial potency of the compounds exhibiting an antibacterial activity
339 at a concentration lower than 500 μ M was influenced by hydrophobicity (Table 1). Indeed, β -
340 pinene showed a higher antibacterial activity than the less hydrophobic molecules, bornyl acetate
341 and linalyl acetate (Table 1). The acetylation of linalool and borneol increased their
342 hydrophobicity and their antibacterial activity (Table 1). Indeed, bornyl acetate and linalyl
343 acetate retained their total anti-proliferative activity at 250 μ M, whereas borneol and linalool lost
344 partially and totally the activity at 500 μ M, respectively (Table 1). Similarly, Knobloch et al.
345 [57] reported a higher inhibition of H⁺-translocation by linalyl acetate, compared to linalool.
346 Also, Dorman and Deans [58] observed a higher antibacterial activity of bornyl acetate compared
347 to borneol against a wide range of bacterial strains, among which *Lactobacillus plantarum*.
348 Moreover, phenolic compounds with LogP values close to or lower than 2, such as anisole (2.11)
349 and *o*-cresol (1.95-1.98), showed no or a weak antibacterial activity against *L. fermentum* (Table
350 2).

351 Acyclic monoterpenoids showed an increased antibacterial activity with the increment of their
352 LogP value. Indeed, linalyl acetate showed a higher antibacterial activity than geraniol, which

353 was more potent than linalool (Table 1). This was also observed for bicyclic monoterpenes, as
354 borneol and camphor had the lowest LogP values, and showed the weakest antibacterial activities
355 (Table 1). However, despite β -pinene not being the most hydrophobic compound in the bicyclic
356 monoterpenes chemical class, it exhibited the strongest antibacterial activity (Table 1).

357 For monocyclic monoterpenes, limonene, α -phelladrene, and γ -terpinene, having higher LogP
358 values than menthol, showed a stronger antibacterial activity (Table 1). α -Terpinene was found
359 to be an exception as it showed the weakest antibacterial potency, despite a high LogP value
360 (Table 1).

361 4.3.2. Structure-activity analysis of terpenic compounds

362 Different studies have demonstrated the role of terpenoid functional groups in the antimicrobial
363 activity. Carvacrol showed a better antimicrobial activity compared to its derivatives, carvacrol
364 methyl ether and *p*-cymene, which lack the hydroxyl group [58]. On the other hand, Kotan et al.
365 [59] reported a better antibacterial activity for alcohol derivatives of oxygenated monoterpenes,
366 when compared to ketone and acetate derivatives.

367 The weak antibacterial activity of β -caryophyllene, despite its high hydrophobicity, may be due
368 to the absence of a hydrophilic functional group in the chemical structure of the molecule. The
369 combination of a lipophilic character of the skeleton and the presence of a hydrophilic functional
370 group was found to be important for the antimicrobial activity of essential oils components [60].

371 The structural features of nerolidol are in line with the previous findings. Indeed, the
372 antibacterial activity of aliphatic terpene alcohols was demonstrated to be dependent on the
373 hydrophobic chain length starting from the carbon connected to the hydroxyl group [38,61]. In
374 fact, farnesol (C₁₂) exhibited a stronger antibacterial activity than nerolidol (C₁₀) followed by
375 geraniol (C₈), whereas linalool (C₆) showed no antibacterial activity against *Staphylococcus*

376 *aureus* [61]. Similarly, Togashi et al. [38] reported a very weak antibacterial activity of geraniol
377 and linalool against *S. aureus*. Additionally, farnesol (C₁₂), nerolidol (C₁₀), and plaunotol (C₁₁),
378 showed a strong antibacterial activity against *S. aureus*, in that order [38,62]. Also, no or weak
379 antibacterial activity against *S. aureus* was reported for alcohols with chains containing more
380 than 12 carbon atoms like farnesylacetol (C₁₄) [61], geranylgeraniol, and phytol (C₁₆) [38].
381 Therefore, to exhibit a potent antibacterial effect, the authors suggested that the chain, starting
382 from the hydroxyl group, should contain from 10 to 12 carbon atoms [38], or less than 12 carbon
383 atoms but as close to 12 as possible [61]. Although the previous studies were conducted on *S.*
384 *aureus*, our study supports the pattern of the antibacterial activity of terpene alcohols against *L.*
385 *fermentum*. Indeed, nerolidol (C₁₀; sesquiterpene) exhibited a stronger antibacterial activity than
386 geraniol (C₈; monoterpene), the latter being more potent than linalool (C₆; monoterpene) (Table
387 1).

388 4.3.3. Structure-activity analysis of phenolic compounds

389 The importance of the propenyl side chain was noted among the tested phenolic compounds, as
390 eugenol, *trans*-anethole, isoeugenol, and estragole, showed a significantly higher antibacterial
391 activity against *L. fermentum* compared to anisole (Table 2). The propenyl side chain is absent in
392 anisole compared to eugenol, *trans*-anethole, isoeugenol, and estragole (Fig. 2), which could be
393 the reason for the weaker antibacterial activity of anisole.

394 On the other hand, eugenol showed the highest antibacterial activity among the tested phenolic
395 compounds against the Gram-positive bacterium *L. fermentum*. However, Gharib et al. [63]
396 reported a higher antibacterial activity for anethole and estragole compared to eugenol, against
397 the Gram-negative bacterium *Escherichi coli*. Indeed, the hydrophobicity of the compound
398 seemed to play a role in the potency of the antibacterial activity against *E. coli*, as anethole and

399 estragole have a higher hydrophobicity than eugenol [63] (Table 2). Whereas, the hydrophobicity
400 of phenylpropenes did not seem to influence their antibacterial activity against the Gram-positive
401 bacterium, *L. fermentum*. Therefore, our results strongly suggest the presence of other factors
402 that modulate the antibacterial activity of phenylpropenes against *L. fermentum*. The higher
403 antibacterial activity of eugenol compared to its ester, eugenyl acetate (Table 2), highlights the
404 importance of the hydroxyl group in the phenolic structure (Fig. 2). Additionally, the absence of
405 the hydroxyl group in *trans*-anethole and estragole structure (Fig. 2) could explain the lower
406 antibacterial activity against *L. fermentum*, compared to eugenol (Table 2). Indeed, the hydroxyl
407 group of eugenol and isoeugenol has been found to reinforce the lipid membrane-fluidizing
408 effect, compared to anethole and estragole [63]. Also, the bacterial membrane characteristics
409 play a crucial role in the differential bacterial susceptibility to a given antibacterial agent [64–
410 66]. In fact, eugenol has previously shown lower MIC values against the Gram-positive bacteria
411 *Staphylococcus aureus* (MIC= 2.5 mg/l) and *Bacillus subtilis* (MIC= 1.25 mg/l), compared to *E.*
412 *coli* (MIC= 5 mg/l) [67].

413 Both ferulic acid and *p*-coumaric acid exhibited a weak anti-proliferative activity against *L.*
414 *fermentum* in MRS broth (pH ~6.2) (Table 2). The antibacterial activity of hydroxycinnamic
415 acids depends on pH, which controls the concentration of their undissociated form. The
416 undissociated form can easily penetrate the cytoplasmic membrane of the bacterium [68]. The
417 pKa values of ferulic acid and *p*-coumaric acid are between 4 and 5, thus a greater proportion of
418 their dissociated forms is found at the pH of the culture medium (~6.2). In fact, the antibacterial
419 activity of ferulic acid and *p*-coumaric acid was found to increase in culture media at pH values
420 below 6 [69]. Moreover, *L. fermentum* is able to metabolize ferulic acid and coumaric acid into

421 less potent metabolites, such as phloretic acid, p-vinyl phenol, or dihydroferulic acid [69]. This
422 also may explain the weak antibacterial activity of both acids against *L. fermentum*.

423 **5. Conclusion**

424 In this study, 28 terpenic and phenolic compounds were screened against *L. fermentum*, an
425 ubiquitously present bacterium which could cause spoilage of different food products. Among
426 the tested compounds, nerolidol exhibited the strongest antibacterial activity marked by the low
427 MIC and MBC values. Moreover, a total bacterial kill was obtained within 16 h in presence of
428 nerolidol (50 μ M). The antibacterial activity of nerolidol was dependent on different factors,
429 including the hydrophobicity of the compound, as well as the position of the hydroxyl group.
430 Compared to nerolidol solubilized in DMSO, HP- β -CD/Ner inclusion complex exhibited 4 fold
431 higher MIC and MBC values and a more rapid bactericidal activity. Indeed, HP- β -CD/Ner
432 inclusion complex was proven effective against *L. fermentum* in culture medium. The high
433 demand for the replacement of synthetic food additives by natural molecules encourages the
434 application of nerolidol in food. Moreover, due to the physico-chemical limitations of the
435 application of natural bioactive components in food, the use of encapsulation systems has
436 recently become a widely investigated novel approach for the delivery of bioactive compounds in
437 food products. Therefore, further studies could be realized to investigate the antibacterial activity
438 of free and encapsulated nerolidol in different food products including fruit juices, alcoholic
439 beverages, and milk products. This evaluation would lead to a better understanding of the
440 antibacterial potency of natural molecules such as nerolidol, under their free and complexed
441 form, in various types of food matrices and under various conditions.

442 **Acknowledgments**

443 The authors thank the Research Funding Program at the Lebanese for supporting this project.

444 **Conflict of Interest**

445 The authors declare no conflict of interest.

446

447

448

449

450 **References**

451 [1] C. Lacerda, L. Thorsen, R. Freitas, L. Jespersen, Strain-specific probiotics properties of
452 Lactobacillus fermentum , Lactobacillus plantarum and Lactobacillus brevis isolates from
453 Brazilian food products, Food Microbiol. 36 (2013) 22–29. doi:10.1016/j.fm.2013.03.010.

454 [2] L.H. Ledenbach, R. T. Marshall, Microbiological spoilage of dairy products, in: W.H.
455 Sperber, M.P. Doyle (Eds.), Compendium of the Microbiological Spoilage of Foods and
456 Beverages, Springer, New York, USA, 2009, pp. 41–67. doi:10.1007/978-1-4419-0826-
457 1_2.

458 [3] K.R. Nath, B.J. Kostak, Etiology of white spot defect in swiss cheese made from pasteurized
459 milk, J. Food Prot. 49 (1986) 718–723.

460 [4] J.A. Kurmann, Studies of the defect excessive development of eyes in Gruyere cheese, Dtsch.
461 Molkereiztg. 84 (1963) 1364–1366.

462 [5] B.J. Juven, Identification of chemical constituents of tomato juice which affect the heat
463 resistance of Lactobacillus fermenturn, J. of Appl. Bacteriol. 54 (1983) 335–338.
464 <https://doi.org/10.1111/j.1365-2672.1983.tb02625.x>

- 465 [6] M. Parish, D. Higgins, Isolation and identification of lactic acid bacteria from samples of
466 citrus Molasses and unpasteurized orange juice, *J. Food Sci.* 53 (1988) 645–646.
467 <https://doi.org/10.1111/j.1365-2621.1988.tb07775.x>
- 468 [7] A.E.H. Shearer, A.S. Mazzotta, R. Chuyate, D.E. Gombas, Heat resistance of juice spoilage
469 microorganisms, *J. Food Prot.* 65 (2002) 1271–1275.
- 470 [8] B. Ray, *Fundamental food microbiology*, Third ed., CRC Press, Boca Raton, Florida, 2005.
- 471 [9] R. Robinson, C. Batt, *Encyclopedia of Food Microbiology*, second ed., Elsevier Science,
472 2014.
- 473 [10] A. Matthews, A. Grimaldi, M. Walker, E. Bartowsky, P. Grbin, V. Jiranek, Lactic acid
474 bacteria as a potential source of enzymes for use in vinification, *Appl. Environ. Microbiol.*
475 70 (2004) 5715–5731. doi:10.1128/AEM.70.10.5715–5731.2004.
- 476 [11] C.G. Edwards, K.M. Haag, M.D. Collins, R.A. Hutson, Y.C. Huang, *Lactobacillus kunkeei*
477 sp . nov. : a spoilage organism associated with grape juice fermentations, *J. Appl.*
478 *Microbiol.* 84 (1998) 698–702.
- 479 [12] E.J. Bartowsky, Bacterial spoilage of wine and approaches to minimize it, *Lett. Appl.*
480 *Microbiol.* 48 (2009) 149–156. doi:10.1111/j.1472-765X.2008.02505.x.
- 481 [13] O. Erkmen, T.F. Bozoglu, *Food Microbiology: Principles Into Practice*, 2 Volume Set, John
482 Wiley & Sons, 2016.
- 483 [14] N.A. Bokulich, C.W. Bamforth, N.A. Bokulich, W. Bamforth, The microbiology of malting
484 and brewing, *Microbio.l Mol. Biol. Rev.* 77 (2013) 157–172. doi:10.1128/MMBR.00060-
485 12.

- 486 [15] L. Dolezil, B.H. Kirsop, Variations amongst beers and lactic acid bacteria relating to beer
487 spoilage, *J. Inst. Brew.* 86 (1980) 122–124. <https://doi.org/10.1002/j.2050->
488 0416.1980.tb03969.x
- 489 [16] K.R. Aneja, R. Dhiman, N.K. Aggarwal, A. Aneja, Emerging preservation techniques for
490 controlling spoilage and pathogenic microorganisms in fruit juices, *Int. J. Microbiol.* (2014).
491 doi:10.1155/2014/758942.
- 492 [17] A. Mayer, E. Harel, Phenoloxidase and their significance in fruit and vegetables, in: P.F.
493 Fox (Ed.), *Food Enzymology*, Elsevier applied science, London, UK, 1991, pp. 373–398.
- 494 [18] W. Messens, J. Van Camp, A. Huyghebaert, The use of high pressure to modify the
495 functionality of food proteins, *Trends Food Sci. Technol.* 8 (1997) 107–112.
496 [https://doi.org/10.1016/S0924-2244\(97\)01015-7](https://doi.org/10.1016/S0924-2244(97)01015-7)
- 497 [19] A.I. V Ross, M.W. Griffiths, G.S. Mittal, H.C. Deeth, Combining nonthermal technologies
498 to control foodborne microorganisms, *Int. J. Food Microbiol.* 89 (2003) 125–138.
499 doi:10.1016/S0168-1605(03)00161-2.
- 500 [20] J. Raso, R. Paga, S. Condon, F.J. Sala, Influence of temperature and pressure on the lethality
501 of ultrasound, *Appl. Environ. Microbiol.* 64 (1998) 465–471.
- 502 [21] S.P. Anand, N. Sati, Artificial preservatives and their harmful effects: looking toward nature
503 for safer alternatives, *Int. J. Pharm. Sci. Res.* 4 (2013) 2496–2501.
504 doi:10.13040/IJPSR.0975-8232.4(7).24960-01
- 505 [22] J. Gutierrez, G. Rodriguez, C. Barry-Ryan, P. Bourke, Efficacy of plant essential oils
506 against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables:
507 antimicrobial and sensory screening, *J. Food Prot.* 71 (2008) 1846–1854.

- 508 [23] M.M. Cowan, Plant products as antimicrobial agents, *Clin. Microbiol. Rev.* 12 (1999) 564–
509 582. doi:10.1016/j.tibtech.2009.09.002.
- 510 [24] M. Gutiérrez-Iarraínzar, J. Rúa, I. Caro, C. De Castro, D. De Arriaga, M.R. García-armesto,
511 P. del Valle, Evaluation of antimicrobial and antioxidant activities of natural phenolic
512 compounds against foodborne pathogens and spoilage bacteria, *Food Control.* 26 (2012)
513 555–563. doi:10.1016/j.foodcont.2012.02.025.
- 514 [25] S.J. Lee, J.I. Han, G.S. Lee, M.J. Park, I.G. Choi, K.J. Na, E.B. Jeung, Antifungal effect of
515 eugenol and nerolidol against *Microsporium gypseum* in a guinea pig model, *Biol. Pharm.*
516 *Bull.* 30 (2007) 184–188. <https://doi.org/10.1248/bpb.30.184>
- 517 [26] I. Kubo, H. Muroi, M. Himejima, Antimicrobial activity of green tea flavor components and
518 their combination effects, *J. Agric. Food Chem.* 40 (1992) 245–248.
519 doi:10.1021/jf00014a015.
- 520 [27] B.F. Brehm-Stecher, E.A. Johnson, Sensitization of *Staphylococcus aureus* and *Escherichia*
521 *coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone,
522 *Antimicrob. Agents Chemother.* 47 (2003) 3357–3360. doi:10.1128/AAC.47.10.3357-
523 3360.2003
- 524 [28] M. Zhao, H. Wang, B. Yang, H. Tao, Identification of cyclodextrin inclusion complex of
525 chlorogenic acid and its antimicrobial activity, *Food Chem.* 120 (2010) 1138–1142.
526 doi:10.1016/j.foodchem.2009.11.044.
- 527 [29] A. Blanco-Padilla, K.M. Soto, M. Hernández Iturriaga, S. Mendoza, Food antimicrobials
528 nanocarriers, *Sci. World J.* 2014 (2014) 1–11. doi:10.1155/2014/837215.
- 529 [30] J. Weiss, S. Gaysinsky, M. Davidson, J. McClements, Nanostructured Encapsulation
530 Systems: Food Antimicrobials, in: E. by, G. Barbosa-Cánovas, A. Mortimer, D. Lineback,

531 W. Spiess, K. Buckle, P. Colonna (Eds.), *Global Issues in Food Science and Technology*,
532 Academic Press, San Diego, 2009, pp. 425–479. doi:10.1016/B978-0-12-374124-0.00024-
533 7.

534 [31] G. Astray, C. Gonzalez-Barreiro, J.C. Mejuto, R. Rial-Otero, J. Simal-Gándara, A review on
535 the use of cyclodextrins in foods, *Food Hydrocoll.* 23 (2009) 1631–1640.
536 doi:10.1016/j.foodhyd.2009.01.001.

537 [32] E.M.M. Del Valle, Cyclodextrins and their uses: a review, *Process Biochem.* 39 (2004)
538 1033–1046. doi:10.1016/S0032-9592(03)00258-9.

539 [33] J. Azzi, L. Auezova, P.E. Danjou, S. Fourmentin, H. Greige-Gerges, First evaluation of
540 drug-in-cyclodextrin-in-liposomes as an encapsulating system for nerolidol, *Food Chem.*
541 255 (2018) 399–404. doi:10.1016/j.foodchem.2018.02.055.

542 [34] E.R. Sanders, *Aseptic Laboratory Techniques : Plating Methods 2. Streak Plate Procedure :*
543 *Isolation of bacterial colonies using the quadrant method*, *J. Vis. Exp.* 63 (2012) 1–18.
544 doi:10.3791/3064.

545 [35] E.R.S. Nkanwen, D. Gatsing, D. Ngamga, S.P.C. Fodouop, P. Tane, Antibacterial agents
546 from the leaves of *Crinum purpurascens* herb (Amaryllidaceae), *Afr. Health Sci.* 9 (2009)
547 264–269.

548 [36] K. Lewis, F.M. Ausubel, Prospects for plant-derived antibacterials, *Nat Biotech.* 24 (2006)
549 1504–1507. doi:10.1038/nbt1206-1504

550 [37] W.K. Chan, L.T.H. Tan, K.G. Chan, L.H. Lee, B.H. Goh, Nerolidol: A sesquiterpene
551 alcohol with multi-faceted pharmacological and biological activities, *Molecules.* 21 (2016)
552 529. doi:10.3390/molecules21050529.

- 553 [38] N. Togashi, H. Hamashima, A. Shiraishi, Y. Inoue, A. Takano, Antibacterial activities
554 against *Staphylococcus aureus* of terpene alcohols with aliphatic carbon chains, *J. Essent.*
555 *Oil Res.* 22 (2010) 263–269. doi:10.1080/10412905.2010.9700321.
- 556 [39] P.A. Cornwell, B.W. Barry, Sesquiterpene components of volatile oils as skin penetration
557 enhancers for the hydrophilic permeant 5-fluorouracil, *J. Pharm. Pharmacol.* 46 (1994)
558 261–269.
- 559 [40] T. Hada, A. Shiraishi, S. Furuse, Y. Inoue, Y. Hamashima, H. Matsumoto, K. Masuda, K.
560 Shiojima, J. Shimada, Inhibitory effects of terpenes on the growth of *Staphylococcus*
561 *aureus*, *Nat. Med.* 57 (2003) 64–67.
- 562
- 563 [41] R. Tao, C.Z. Wang, Z.W. Kong, Antibacterial/antifungal activity and synergistic
564 interactions between polyprenols and other lipids isolated from *Ginkgo Biloba* L. leaves,
565 *Molecules.* 18 (2013) 2166–2182. doi:10.3390/molecules18022166.
- 566 [42] aM. Simoes, S. Rocha, M. Coimbra, M. Vieira, Enhancement of *Escherichia coli* and
567 *Staphylococcus aureus* antibiotic susceptibility using sesquiterpenoids, *Med. Chem.* 4
568 (2008) 616–623. doi:10.2174/157340608786242016.
- 569 [43] O. Gonçalves, R. Pereira, F. Gonçalves, S. Mendo, M.A. Coimbra, S.M. Rocha, Evaluation
570 of the mutagenicity of sesquiterpenic compounds and their influence on the susceptibility
571 towards antibiotics of two clinically relevant bacterial strains, *Mutat. Res.* 723 (2011) 18–
572 25. doi:10.1016/j.mrgentox.2011.03.010.
- 573 [44] M. Egervärn, M. Danielsen, S. Roos, H. Lindmark, S. Lindgren, Antibiotic susceptibility
574 profiles of *Lactobacillus reuteri* and *Lactobacillus fermentum*, *J. Food Prot.* 70 (2007) 412–
575 418.

- 576 [45] I. Klare, C. Konstabel, G. Werner, G. Huys, V. Vankerckhoven, G. Kahlmeter, B.
577 Hildebrandt, S. Müller-Bertling, W. Witte, H. Goossens, Antimicrobial susceptibilities of
578 *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for
579 probiotic or nutritional use, *J. Antimicrob. Chemother.* 59 (2007) 900–912.
580 doi:10.1093/jac/dkm035.
- 581 [46] K.Y. Gfeller, M. Roth, L. Meile, M. Teuber, Sequence and genetic organization of the 19.3-
582 kb erythromycin- and dalbapristin-resistance plasmid pLME300 from *Lactobacillus*
583 *fermentum* ROT1, *Plasmid.* 50 (2003) 190–201. doi:10.1016/j.plasmid.2003.08.001.
- 584 [47] P. De Oliva-Neto, F. Yokoya, Susceptibility of *Saccharomyces cerevisiae* and lactic acid
585 bacteria from the alcohol industry to several antimicrobial compounds, *Braz. J. Microbiol.*
586 32 (2001) 10–14. doi:10.1590/S1517-83822001000100003.
- 587 [48] A.B. Florez, M.S. Ammor, B. Mayo, A.H.A.M. van Hoek, H.J.M. Aarts, G. Huys,
588 Antimicrobial susceptibility profiles of 32 type strains of *Lactobacillus*, *Bifidobacterium*,
589 *Lactococcus* and *Streptococcus* spp., *Int. J. Antimicrob. Agents.* 31 (2008) 484–504.
590 doi:10.1016/0002-9610(92)90118-B.
- 591 [49] O. Neto, P. De, F.A. de Lima, K.C. da Silva, D.F. da Silva, A.F.A. Carvalho, C. dos Santos,
592 Chemical inhibition of the contaminant *Lactobacillus fermentum* from distilleries
593 producing fuel bioethanol, *Braz. Arch. Biol. Technol.* 57 (2014) 441–447.
594 doi:10.1590/S1516-8913201401214.
- 595 [50] J.F. Li, J.X. Zhang, Z.G. Wang, Y.J. Yao, X. Han, Y.L. Zhao, J.P. Liu, S.Q. Zhang,
596 Identification of a cyclodextrin inclusion complex of antimicrobial peptide CM4 and its
597 antimicrobial activity, *Food Chem.* 221 (2017) 296–301.
598 doi:10.1016/j.foodchem.2016.10.040.

- 599 [51] C. Dima, M. Cotarlet, tiberius Balaes, G. Bahrim, P. Alexe, S. Dima, Encapsulation of
600 Coriander essential oil in beta-cyclodextrin: Antioxidant and antimicrobial properties
601 evaluation, Rom. Biotechnol. Lett. 19 (2014) 9128–9140.
- 602 [52] I.I. Salem, N. Düzgünes, Efficacies of cyclodextrin-complexed and liposome-encapsulated
603 clarithromycin against Mycobacterium avium complex infection in human macrophages,
604 Int. J. Pharm. 250 (2003) 403–414. [https://doi.org/10.1016/S0378-5173\(02\)00552-5](https://doi.org/10.1016/S0378-5173(02)00552-5)
- 605 [53] T.A. Andrade, T.S. Freitas, F.O. Araújo, P.P. Menezes, G.A.A. Dória, A.S. Rabelo, L.J.
606 Quintans-Júnior, M.R.V. Santos, D.P. Bezerra, M.R. Serafini, I.R.A. Menezes, P.S. Nunes,
607 A.A.S. Araújo, M.S. Costa, F.F. Campina, A.T.L. Santos, A.R.P. Silva, H.D.M. Coutinho,
608 Physico-chemical characterization and antibacterial activity of inclusion complexes of
609 Hyptis martiusii Benth essential oil in β -cyclodextrin, Biomed. Pharmacother. 89 (2017)
610 201–207. doi:10.1016/j.biopha.2017.01.158.
- 611 [54] E.H. Santos, J.A. Kamimura, L.E. Hill, C.L. Gomes, Characterization of carvacrol beta-
612 cyclodextrin inclusion complexes as delivery systems for antibacterial and antioxidant
613 applications, LWT - Food Sci. Technol. 60 (2015) 583–592. doi:10.1016/j.lwt.2014.08.046.
- 614 [55] J. Sikkema, J.A. de Bont, B. Poolman, Mechanisms of membrane toxicity of hydrocarbons,
615 Microbiol. Rev. 59 (1995) 201–222. doi:0146-0749/95/\$04.00+0.
- 616 [56] M.C. Selestino Neta, C. Vittorazzi, A.C. Guimarães, J.D.L. Martins, M. Fronza, D.C.
617 Endringer, R. Scherer, Effects of β -caryophyllene and *Murraya paniculata* essential oil in
618 the murine hepatoma cells and in the bacteria and fungi 24-h time–kill curve studies,
619 Pharmaceut. Biol. 55 (2017) 190–197. doi:10.1080/13880209.2016.1254251.

- 620 [57] K. Knobloch, A. Pauli, B. Iberl, H. Weigand, N. Weis, Antibacterial and antifungal
621 properties of essential oil components, *J. Essent. Oil Res.* 1 (1989) 119–128.
622 doi:10.1080/10412905.1989.9697767.
- 623 [58] H. Dorman, S. Deans, Antimicrobial agents from plants: antibacterial activity of plant
624 volatile oils, *J. Appl. Microbiol.* 88 (2000) 308–316.
- 625 [59] R. Kotan, S. Kordali, A. Cakir, Screening of Antibacterial activities of twenty-one
626 oxygenated monoterpenes, *Z. Naturforsch. C.* 62 (2007) 507–513.
- 627 [60] D. Kalemba, A. Kunicka, Antibacterial and antifungal properties of essential oils, *Curr.*
628 *Med. Chem.* 10 (2003) 813–829.
- 629 [61] I. Kubo, H. Muroi, M. Himejima, A. Kubo, Antibacterial activity of long-chain alcohols:
630 The role of hydrophobic alkyl groups, *Bioorg. Med.Chem. Lett.* 3 (1993) 1305–1308.
631 [https://doi.org/10.1016/S0960-894X\(00\)80336-4](https://doi.org/10.1016/S0960-894X(00)80336-4)
- 632 [62] Y. Inoue, A. Shiraishi, T. Hada, K. Hirose, H. Hamashima, J. Shimada, The antibacterial
633 effects of terpene alcohols on *Staphylococcus aureus* and their mode of action, *FEMS*
634 *Microbiol. Lett.* 237 (2004) 325–331. doi:10.1016/j.femsle.2004.06.049.
- 635 [63] R. Gharib, A. Najjar, L. Auezova, C. Charcosset, Interaction of selected phenylpropenes
636 with dipalmitoylphosphatidylcholine membrane and their relevance to antibacterial activity,
637 *J. Membr. Biol.* 250 (2017) 259–271. doi:10.1007/s00232-017-9957-y.
- 638 [64] S. Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao, D. Dash, Characterization of
639 enhanced antibacterial effects of novel silver nanoparticles, *Nanotechnol.* 18 (2007)
640 225103. doi:10.1088/0957-4484/18/22/225103.

- 641 [65] D.P. Tamboli, D.S. Lee, Mechanistic antimicrobial approach of extracellularly synthesized
642 silver nanoparticles against gram positive and gram negative bacteria, *J. Hazard. Mater.* 260
643 (2013) 878–884. doi:10.1016/j.jhazmat.2013.06.003.
- 644 [66] F. Nazzaro, F. Fratianni, L. De Martino, R. Coppola, V. De Feo, Effect of essential oils on
645 pathogenic bacteria, *Pharmaceut.* 6 (2013) 1451–1474. doi:10.3390/ph6121451.
- 646 [67] H. Liang, Q. Yuan, F. Vriesekoop, F. Lv, Effects of cyclodextrins on the antimicrobial
647 activity of plant-derived essential oil compounds, *Food Chem.* 135 (2012) 1020–1027.
648 doi:10.1016/j.foodchem.2012.05.054.
- 649 [68] J.B. Russell, Another explanation for the toxicity of fermentation acids at low pH: anion
650 accumulation versus uncoupling, *J. Appl. Bacteriol.* 73 (1992) 363–370.
651 doi:10.1111/j.1365-2672.1992.tb04990.x.
- 652
- 653 [69] A.F. Sánchez-Maldonado, A. Schieber, M.G. Gänzle, Structure-function relationships of the
654 antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria, *J. Appl.*
655 *Microbiol.* 111 (2011) 1176–1184. doi:10.1111/j.1365-2672.2011.05141.x.
- 656 [70] S. Griffin, S.G. Wyllie, J. Markham, Determination of octanol–water partition coefficient
657 for terpenoids using reversed-phase high-performance liquid chromatography, *J.*
658 *Chromatogr. A.* 864 (1999) 221–228. doi:10.1016/S0021-9673(99)01009-2.
- 659 [71] S. Ohtsubo, T. Fujita, A. Matsushita, E. Kumamoto, Inhibition of the compound action
660 potentials of frog sciatic nerves by aroma oil compounds having various chemical
661 structures, *Pharmacol. Res. Perspect.* 3 (2015) e00127. doi:10.1002/prp2.127.
- 662 [72] O. Sensch, W. Vierling, W. Brandt, M. Reiter, Effects of inhibition of calcium and
663 potassium currents in guinea-pig cardiac contraction: comparison of β -caryophyllene oxide,

664 eugenol, and nifedipine, *Brit. J. Pharmacol.* 131 (2000) 1089–1096.
665 doi:10.1038/sj.bjp.0703673.

666 [73] C. Hansch, A. Leo, D. Hoekman, *Exploring QSAR: Hydrophobic, electronic, and steric*
667 *constants*, American Chemical Society, Washington DC, 1995.

668 [74] A.F. El-Kattan, C.S. Asbill, N. Kim, B.B. Michniak, The effects of terpene enhancers on the
669 percutaneous permeation of drugs with different lipophilicities, *Int. J. Pharms.* 215 (2001)
670 229–240. doi:10.1016/S0378-5173(00)00699-2.

671 [75] J. Li, E.M. Perdue, S.G. Pavlostathis, R. Araujo, Physicochemical properties of selected
672 monoterpenes, *Environ. Int.* 24 (1998) 353–358. doi:10.1016/S0160-4120(98)00013-0.

673 [76] C. Schmid, R. Steinbrecher, H. Ziegler, Partition coefficients of plant cuticles for
674 monoterpenes, *Trees.* 6 (1992) 32–36. doi:10.1007/BF00224496.

675 [77] H.S. Camargos, R.A. Moreira, S.A. Mendanha, K.S. Fernandes, M.L. Dorta, A. Alonso,
676 Terpenes increase the lipid dynamics in the *Leishmania* plasma membrane at concentrations
677 similar to their IC50 values, *PLOS ONE.* 9 (2014) e104429.
678 doi:10.1371/journal.pone.0104429.

679 [78] A. Ben Arfa, S. Combes, L. Preziosi-Belloy, N. Gontard, P. Chalier, Antimicrobial activity
680 of carvacrol related to its chemical structure, *Lett. Appl. Microbiol.* 43 (2006) 149–154.
681 doi:10.1111/j.1472-765X.2006.01938.x.

682 [79] A.A. Taherpour, H. Maroofi, O. Bajelani, K. Larijani, Chemical composition of the essential
683 oil of *Valeriana alliariifolia* Adams of Iran, *Nat. Prod. Res.* 24 (2010) 973–978.
684 doi:10.1080/14786410902900010.

685 [80] J.R. Clare, Automatic dishwashing compositions comprising diacyl peroxide bleach and
686 blooming perfume, EP1360269B1, 2005.

- 687 [81] R. Lun, D. Varhanickova, W.-Y. Shiu, D. Mackay, Aqueous solubilities and octanol–water
688 partition coefficients of cymenes and chlorocymenes, *J. Chem. Eng. Data.* 42 (1997) 951–
689 953. doi:10.1021/je970069v.
- 690 [82] M. Kfoury, D. Landy, L. Auezova, H. Greige-Gerges, S. Fourmentin, Effect of cyclodextrin
691 complexation on phenylpropanoids’ solubility and antioxidant activity, *Beilstein J. Org.*
692 *Chem.* 10 (2014) 2322–2331. doi:10.3762/bjoc.10.241.
- 693 [83] N.C. Dias, M.I. Nawas, C.F. Poole, Evaluation of a reversed-phase column (Supelcosil LC-
694 ABZ) under isocratic and gradient elution conditions for estimating octanol–water partition
695 coefficients, *Analyst.* 128 (2003) 427–433. doi:10.1039/B300574G.
- 696 [84] J.A. Rothwell, A.J. Day, M.R.A. Morgan, Experimental determination of octanol–water
697 partition coefficients of quercetin and related flavonoids, *J. Agric. Food Chem.* 53 (2005)
698 4355–4360. doi:10.1021/jf0483669.
- 699 [85] M.E. Herbig, D.H. Evers, Correlation of hydrotropic solubilization by urea with logD of
700 drug molecules and utilization of this effect for topical formulations, *Eur. J. Pharm.*
701 *Biopharm.* 85 (2013) 158–160. doi:10.1016/j.ejpb.2013.06.022.
- 702 [86] S. Ritter, W.H. Hauthal, G. Maurer, Partition coefficients of some environmentally
703 important organic compounds between 1-octanol and water from reversed-phase high-
704 performance liquid chromatography, *J. Chem. Eng. Data.* 39 (1994) 414–417.
705 doi:10.1021/je00015a003.
- 706 [87] G.N. Reiner, D.O. Labuckas, D.A. García, Lipophilicity of some GABAergic phenols and
707 related compounds determined by HPLC and partition coefficients in different systems, *J.*
708 *Pharm. Biomed. Anal.* 49 (2009) 686–691. doi:10.1016/j.jpba.2008.12.040.

709 [88] O. Wesołowska, M. Kuzdzał, J. Strancar, K. Michalak, Interaction of the chemopreventive
710 agent resveratrol and its metabolite, piceatannol, with model membranes, *Biochim.*
711 *Biophys. Acta.* 1788 (2009) 1851–1860. doi:10.1016/j.bbamem.2009.06.005.

712

713

714

715

716

717

718

719 **Figure legends**

720 **Fig.1.** Chemical structure of terpenic compounds.

721 **Fig. 2.** Chemical structure of phenolic compounds.

722 **Fig. 3.** Nerolidol (50 μ M) and HP- β -CD/Ner complex (200 μ M Ner) time-kill analysis against *L.*
723 *fermentum* at 37 °C under anaerobic conditions.

724

725

726

727

728

729
730
731
732
733
734
735
736
737

738 **Table 1: Bactericidal and anti-proliferative activity of terpenes against *L. fermentum*.**

	Borneol	Bornyl acetate	Camphene	Camphor	β -Caryophyllene	<i>p</i> -Cymene	Geraniol	Limonene	Linalool	Linalyl acetate	Menthol	Nerolidol	α -Phellandrene	α -Pinene	β -Pinene	α -Terpinene	γ -Terpinene
LogP	3.01 [70]	3.86 [71]	4.22 [70]	2.74 [70]	6.87 [72]	4.1 [73]	3.56 [70]	4.58 [74] 4.23 [77] 4.57 [75] 4.38 [70] 4.83 [79]	2.97 [75]	3.93 [70]	3.4 [70] 3.38 [78]	5.36 [74]	4.83 [75] 4.49 [76] 4.48 [70]	4.16 [70] 4.42 [76]		4.25 [70]	4.5 [75] 4.36 [70]
3500 μM Bacterial kill (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	100
Inhibition (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	89.1 \pm 0.5	100
500 μM Bacterial kill (%)	0	100	0	0	0	0	0	0	0	100	0	100	0	0	100	ND	0

Inhibition (%)	28.5 ± 1.8	100	100	0	89 ± 2.4	100	80.9 ± 1.4	100	0	100	75.7 ± 4.4	100	100	100	100	ND	100
250 μM Bacterial kill (%)	ND	0	ND	ND	ND	ND	ND	ND	ND	0	ND	100	ND	ND	0	ND	ND
Inhibition (%)	ND	100	ND	ND	ND	ND	ND	ND	ND	100	ND	100	ND	ND	100	ND	ND
100 μM Bacterial kill (%)	ND	0	ND	ND	ND	ND	ND	ND	ND	0	ND	100	ND	ND	0	ND	ND
Inhibition (%)	ND	0	ND	ND	ND	ND	ND	ND	ND	0	ND	100	ND	ND	91.4 ± 0.8	ND	ND

739 ND: Not determined

740

741

742

743

744 **Table 2: Bactericidal and anti-proliferative activity of phenolic compounds against *L.***
 745 ***fermentum*.**

	<i>trans</i> -Anethole	Anisole	<i>p</i> -Coumaric acid	<i>o</i> -Cresol	Estragole	Eugenol	Eugenyl acetate	<i>trans</i> -Ferulic acid	Isoeugenol	Quercetin	Resveratrol
LogP	3.31[80]			1.95[73]	3.13[80]	2.40[83] 2.45[72]		1.51[73]			3.10 [85]
	3.0961[82]	2.11[81]	1.43[82]	1.975[86]	2.818[82]	2.29[87] 2.73[78] 2.99[70]	2.9[72]	1.249[82]	3.04[70]	1.82 [84]	3.06 [88]
3500 μM											
Bacterial kill (%)	0	0	0	0	0	100	95 ± 0.47	0	0	0	0
Inhibition (%)	91.4 ± 1.80	16.1 ± 0.92	21.9 ± 0.94	0	84.4 ± 0.13	100	100	17.3 ± 0.14	89.3 ± 0.09	0	0
500 μM											
Bacterial kill (%)	ND	ND	ND	ND	ND	0	0	ND	ND	ND	ND
Inhibition (%)	ND	ND	ND	ND	ND	0	0	ND	ND	ND	ND

746 ND: Not determined

747
748

749

750

751

752

753

754

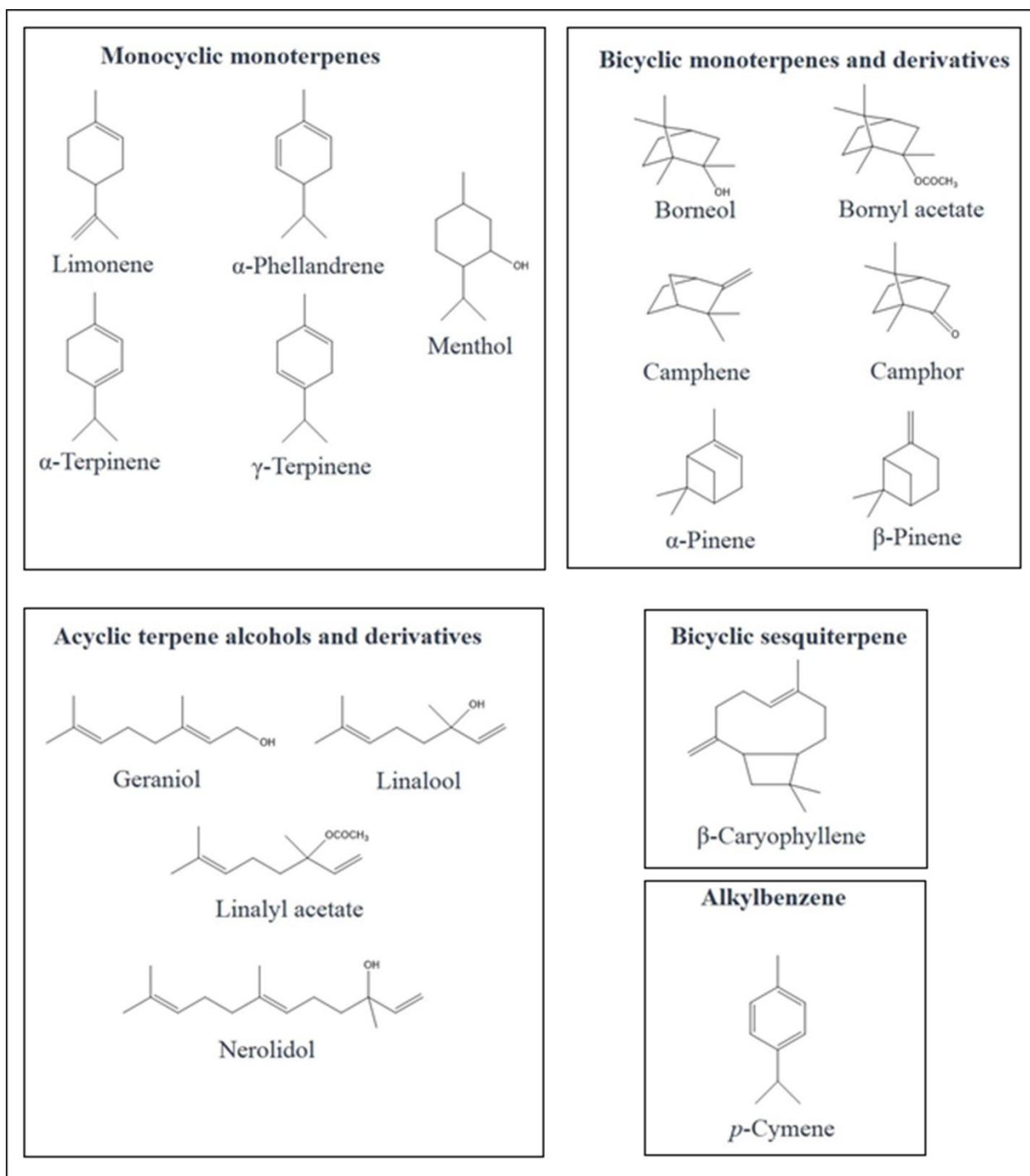
755

756 **Table 3: MIC values of common antibiotics against *L. fermentum*.**

Antibiotic	MIC (mg/l)	MIC (μM)	Reference
Clindamycin	>64	>150.60	[46]
Chloramphenicol	4 ^a	12.38	[45]
Erythromycin	>128 - 256	>174.40 - 348.81	[46]
Gentamicin	4 - 8	8.38 - 16.75	[44]
Kanamycin	64	132.10	[48]
Metronidazole	>40	>233.7	[49]
N-alkyl dimethylbenzyl ammonium chloride	8	25.11	[47]
Nitrofurantoin	15	62.98	[49]
Novobiocin	>16	>26.12	[46]
Polymixin B	64	49.17	[48]
Streptomycin	16 ^a	27.51	[45]
	16	27.51	[48]
	8 - 128	13.76 - 220.09	[44]
	>128	>220.09	[46]
Teicoplanin	>256 ^a	>134.19-163.65	[45]
Tobramycin	>128	>273.79	[46]
Vancomycin	96	66.24	[48]
	>256 ^a	>176.64	[45]

757 ^aValues of MIC₉₀

758

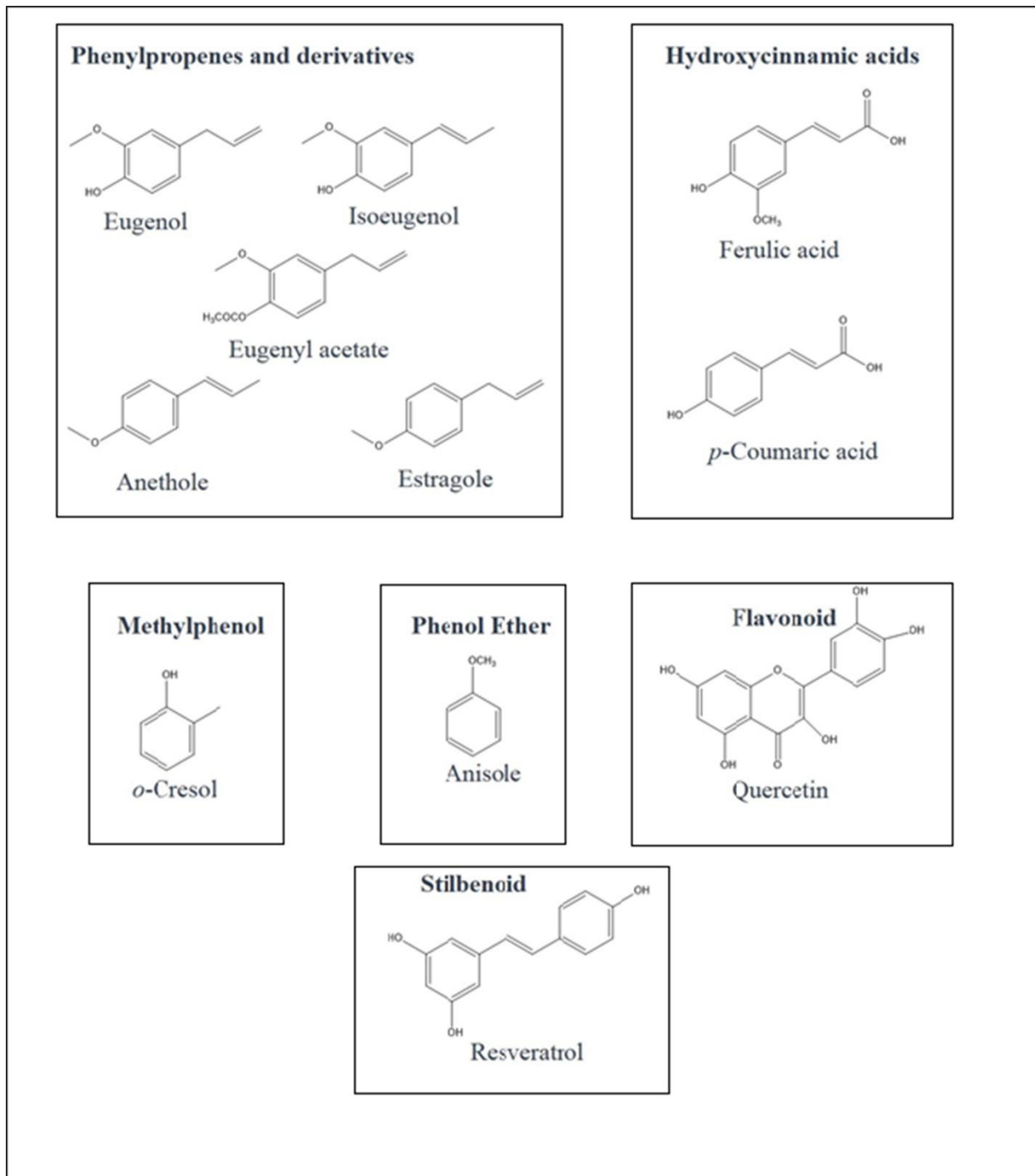


759

760 **Fig. 1**

761

762

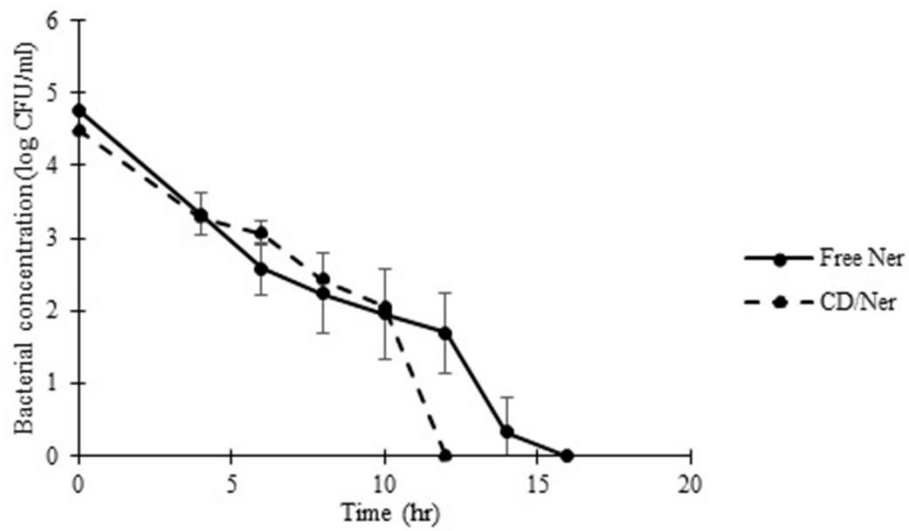


763

764 **Fig. 2**

765

766



767

768 **Fig. 3**

769

770

771

772

773

774

775

776

777

778

779