

1 **Antibacterial activity of *Cinnamomum verum* and *Thymus vulgaris* essential oils on**
2 **multidrug-resistant zoonotic bacteria isolated from dogs in southern Benin**

3

4 Ayaovi Bruno Yaovi¹, Arpita Das², Rama N. Behera², Paulin Azokpota³, Souaïbou
5 Farougou¹, Lamine Baba-Moussa⁴, Franck Michels⁵, Marie-Laure Fauconnier⁵, Kiran
6 Ambatipudi², Philippe Sessou¹

7

8 ¹Research Unit on Communicable Diseases, Polytechnic School of Abomey-Calavi,
9 University of Abomey-Calavi, Abomey-Calavi, Benin

10 ²Department of Biosciences and Bioengineering, Indian Institute of Technology Roorkee,
11 Roorkee, Uttarakhand 247667, India

12 ³Laboratory of Food Science and Technology, University of Abomey-Calavi, 03 B.P. 2819
13 Jericho-Cotonou, Benin

14 ⁴Laboratory of Biochemistry and Molecular Typing in Microbiology, Department of
15 Biochemistry and Cell Biology, Faculty of Science and Technology, University of Abomey-
16 Calavi, Abomey-Calavi 05 BP 1604, Benin

17 ⁵Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, Liege University,
18 Passage des Déportés 2, Gembloux 5030, Belgium

19 **Corresponding author:** sessouphilippe@yahoo.fr, philippe.sessou@uac.bj

20 **Key words:** Essential oils, *Thymus vulgaris*, *Cinnamomum verum*, *Escherichia coli*,
21 *Staphylococcus aureus*, Benin

22 **Abstract**

23 Antibiotic resistance is a major public health problem. The search for new therapeutic
24 alternatives is becoming urgent. Essential oils are a promising alternative. This study aimed to
25 evaluate the antibacterial activities of essential oils from selected plants on multidrug-resistant
26 zoonotic strains isolated from dogs. Essential oils from dried *Thymus vulgaris* leaves,
27 *Cinnamomum verum* bark, and *Cuminum cyminum* seeds were extracted and tested on five
28 multidrug-resistant *Escherichia coli* and four *S. aureus* isolated from dogs in southern Benin.
29 The study showed that *Thymus vulgaris* essential oil was bacteriostatic, with a Minimum
30 Inhibitory Concentration (MIC) equal to 2.5 µl/ml and a Minimum Bactericidal Concentration
31 (MBC) of 17 µl/ml for *E. coli* strains and 11.25 µl/ml for *S. aureus* strains. Regarding

32 *Cinnamomum verum* essential oil, its bacteriostatic power was characterized by an MIC of
33 1.25 µl/ml for the isolates tested and an average MBC of 11.50 µl/ml for *E. coli* and 12.19
34 µl/ml for *S. aureus*. On the other hand, *Cuminum cyminum* essential oil was ineffective on the
35 strains investigated. Additionally, *T. vulgaris* essential oil contained predominantly thymol
36 (36.57%), p-cymene (30.51%), and carvacrol (7.62%), while *C. verum* essential oil contained
37 cinnamaldehyde (88.76%). This study reveals the antibacterial activity of *T. vulgaris* dry leaf
38 and *C. verum* bark essential oils on multi-resistant *E. coli* and *S. aureus* isolated from dogs.
39 These two essential oils may be alternative candidates for combating antibiotic-resistant *E.*
40 *coli* and *S. aureus* infections.

41

42 **Data Summary**

43 The authors confirm all supporting data, code, and protocols have been provided within the
44 article.

45 **Introduction**

46 Antibiotic resistance is a major threat to human and animal health. Indeed, the misuse of
47 antibiotics in humans and animals has been identified as the main cause of the development of
48 antibiotic-resistant pathogens (Mugwaneza et al., 2024). This misuse includes the use of
49 antibiotics (administration, dispensing, or prescribing) for reasons other than treatment, non-
50 completion of prescribed treatments, and/or incorrect doses of antibiotics (under- or over-
51 dosing). The study conducted by O'Neil (O'Neill, 2016) showed that resistance to anti-
52 infectives could be responsible for more than 10 million deaths a year, making it the leading
53 cause by 2050, with an economic cost of US\$100 billion if precautions are not taken.

54 Furthermore, close contact between pets and humans (petting, licking, or physical injury), as
55 well as the domestic environment, facilitates the transmission of antibiotic-resistant bacteria
56 and genes (Caneschi et al., 2023). Studies have reported antibiotic resistance in strains of
57 *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*,
58 *Streptococcus pyogenes*, *coagulase-negative Staphylococcus* and *Staphylococcus*
59 *pseudintermedius* isolated from dogs (Yaovi et al., 2022).

60 In Benin, multi-resistant strains of *E. coli* and *S. aureus* have been isolated from both free-
61 ranging and caged dogs, posing a health threat to all those who come into contact with dogs
62 harboring these microorganisms.

63 Nowadays, faced with the increase in drug-resistant bacteria and the limited availability of
64 new, effective antibacterial agents, researchers are prompted to look for new strategies to
65 replace or assist synthetic antibiotics (Usai and Di Sotto, 2023). Essential oils are known to
66 possess a broad spectrum of bioactivities, including antimicrobial, anti-inflammatory,
67 antioxidant, antiviral, and antiproliferative (Sharifi-Rad et al., 2021; Micucci et al., 2020;
68 Sessou et al., 2018). The antimicrobial properties of essential oils have been known since
69 antiquity and represent the most exploited to date. They can act as both bacteriostatic and
70 bactericidal agents, being capable of inhibiting bacterial growth, thus blocking bacteria's
71 ability to reproduce, and killing bacterial cells (Usai and Di Sotto, 2023).

72 Given the consequences of infections caused by antimicrobial-resistant agents, there is an
73 urgent need to look for alternatives to combat multi-resistant bacteria infections, which are
74 emerging in dogs in our country. The present study aimed to evaluate the antibacterial activity
75 of *Thymus vulgaris*, *Cinnamomum verum*, and *Cuminum cyminum* essential oils on multi-
76 resistant *Escherichia coli* and *Staphylococcus aureus* isolated from dogs in southern Benin.

77 **Materials and methods**

78 This study was approved by the Ethical Committee of Research Unit on Communicable
79 Diseases (URMAT in French) of the Polytechnic School of Abomey-Calavi of the University
80 of Abomey-Calavi (N°004/EPAC/LARBA/URMAT/CE/R).

81 **Plant samples used**

82 *Cinnamomum verum* bark (YH 1014/HNB), *Cuminum cyminum* seeds (YH 1015/HNB), and
83 dry leaves of *Thymus vulgaris* (YH 1016/HNB) purchased at the market and identified at the
84 National Herbarium of Benin were used in this study.

85 **Essential oils extraction**

86 Essential oils were extracted from dried *Thymus vulgaris* leaves, *Cinnamomum verum* bark,
87 and *Cuminum cyminum* seeds purchased at the market, at the “Laboratoire d’Etude et de
88 Recherche en Chimie Appliquée” (LERCA). The hydrodistillation method described by
89 Kpatinvoh et al. (Kpatinvoh et al., 2017) was used. 100 g of each plant material was
90 introduced into 1 liter of distilled water and the mixture was distilled for 3h in a Clevenger-
91 type hydrodistillation apparatus. The essential oil obtained was separated from the aqueous
92 phase, then dried with sodium sulfate (Na₂SO₄), and kept cool at 4°C in a refrigerator for later
93 use (Sharifi-Rad et al., 2021).

94 **Determination of inhibition diameters of essential oils**

95 The antibacterial activities of essential oils from dry *Thymus vulgaris* leaves, *Cinnamomum*
 96 *verum* bark, and *Cuminum cyminum* seeds were evaluated on five multi-resistant strains of
 97 *Escherichia coli* (Ec01, Ec02, Ec03, Ec04 and Ec05) and four multi-resistant *S. aureus* strains
 98 (Sa01, Sa02, Sa03 and Sa04) isolated from dogs in Southern Benin (Table 1). The disk
 99 diffusion method described by Sessou et al. (Sessou et al., 2018) and slightly modified was
 100 used. A bacterial suspension was prepared by placing one colony in 5 ml of Mueller Hinton
 101 broth (Oxoid, Basingstoke, UK) and adjusted to 10⁸ CFU/ml. The inoculum thus prepared
 102 was inoculated onto Muller-Hinton Agar (Oxoid, Basingstoke, UK). Sterile Whatman paper
 103 discs (6 mm in diameter) coated with 10 µl of the essential oil were placed on the previously
 104 seeded agar. A negative control was prepared in the same way as the experimental test, and
 105 sterile distilled water was added in place of the essential oil. The experimental plates were
 106 incubated at 37°C for 24 h. After incubation, the diameters of the zone of inhibition around
 107 the discs were measured. Essential oils with a diameter greater than 14 mm at 10 µl were
 108 selected for determination of MIC, MBC, and antibiotic potency (Sessou et al., 2018).

109 Table 1 : Antibiotic susceptibility profile of multi-resistant *E. coli* and *S. aureus* strains
 110 studied

Bactéries	<i>E. coli</i>					<i>S. aureus</i>			
	Ec01	Ec02	Ec03	Ec04	Ec05	Sa01	Sa02	Sa03	Sa04
Antibiotics									
Penicillin G	R	R	R	R	R	R	R	R	R
Amoxicillin-clavulanic acid	S	S	S	S	I	S	S	S	S
Tetracycline	R	R	R	R	R	R	R	R	R
Gentamicin	S	S	S	S	S	S	S	S	S
Streptomycin	R	R	R	R	R	R	R	R	R
Erythromycin	-	-	-	-	-	R	S	R	S
Chloramphenicol	S	S	S	S	S	R	S	S	S
Ceftazidime	S	S	S	S	S	I	S	S	I
Cefotaxime	S	S	S	S	S	S	S	S	S
Cotrimoxazole	R	R	R	R	R	R	R	R	S

R : resistant to the antibiotic; S : susceptible to the antibiotic ; I : intermediate resistance

112 **Determination of the Minimum Inhibitory Concentration (MIC) and Minimum**
113 **Bactericidal Concentration (MBC) of effective essential oils**

114 The minimum inhibitory concentration and minimum bactericidal concentration of the
115 essential oils effective on the bacteria tested were determined following the method described
116 by Sessou et al. (Sessou et al., 2018).

117 Concerning MIC, a stock solution of each essential oil was prepared from 2 ml of Mueller
118 Hinton broth (MHB), 40 µl of the essential oil, and one drop of Tween 80. Thus, 100 µl of
119 MHB was dispensed into each well of a 96-well microplate. 100 µl of the oil stock solution
120 was added to the first well, and successive dilutions of reason 2, well by well, were made up
121 to the 11th well in each row. All wells except those in the eleventh row were inoculated with
122 100 µl of bacterial suspension at 10⁶ Colony Forming Units (CFU)/ml, equal density on the
123 Mc Farland scale. The microplate was then covered with parafilm and incubated at 37°C for
124 18 to 24 hours. The eleventh row represents the negative control, while the twelfth represents
125 the positive control. The MIC is the lowest concentration for which no visible growth was
126 noted. The MIC was considered to be the concentration of the well in which no visible
127 bacterial growth was observed.

128 The MBC was determined successively to the MIC. Briefly, the contents of each well, ranging
129 from the MIC value to the highest concentrations, were streaked onto the surface of Muller-
130 Hinton Agar poured into Petri dishes. The wells of the positive and negative control rows
131 were also streaked on the same agar to ensure the absence of bacterial growth in the wells of
132 the negative control row and the presence of bacterial strains in the wells of the positive row.
133 Inoculated agar plates were incubated at 37°C for 24 hours. On reading, CMB was the lowest
134 extract concentration for which there were 0.01% surviving bacteria for the wells (Sessou et
135 al., 2018).

136 **Determining the antibiotic potency of essential oils**

137 The antibiotic potency of the strain is determined by calculating the MBC/MIC ratio. When
138 this ratio is less than or equal to 4, the essential oil is said to be bactericidal, and when the
139 ratio is greater than 4, the essential oil is said to be bacteriostatic (Mamadou et al., 2014).

140 **Analysis of the constituents of essential oils effective against multi-resistant bacteria**

141 GC/MS

142 *Thymus vulgaris* leaves and *Cinnamomum verum* bark essential oils were analyzed on a
143 Hewlett-Packard gas chromatograph model 7890, coupled to a Hewlett-Packard MS model
144 5875, equipped with a DB5 MS column (30 m x 0.25mm; 0.25 μ m), programmed from 50°C
145 (5 minutes) to 300°C at 5°C/minutes, 5 minutes hold. Helium as carrier gas (1.0 ml/minutes);
146 split mode injection (1:30); injector and detector temperatures: 250 and 280°C respectively.
147 Mass spectrometer operated in electron impact mode at 70 eV; electron multiplier: 2500 eV;
148 ion source temperature: 180°C; mass spectra data were acquired in scan mode in the m/z 33-
149 450 range.

150 GC/FID

151 *Thymus vulgaris* leaves and *Cinnamomum verum* bark essential oils were analyzed on a
152 Hewlett-Packard gas chromatograph model 6890, equipped with a DB5 MS column (30 m x
153 0.25 mm; 0.25 μ m), programmed from 50°C (5 minutes) to 300°C at 5°C/minutes, 5 minutes
154 hold. Hydrogen was used as carrier gas (1.0 ml/minutes); split mode injection (1:60); injector
155 and detector temperatures, 280 and 300°C respectively. The essential oil was diluted in
156 hexane: 1/30. Compounds determined by GC in the various essential oils were identified by
157 comparing their retention indices with those of reference compounds in the literature and
158 confirmed by GC-MS by comparing their mass spectra with those of reference substances
159 (Rösch et al., 1999; Adams, 1989; Swigar and Silverstein, 1981).

160 **Statistical analysis**

161 Data from the analyses were entered into an Excel spreadsheet and analyzed using R 4.3.1
162 software. Inhibition diameters were expressed as mean \pm standard deviation. A one-factor
163 analysis of variance (ANOVA) and an unpaired Student's t-test were performed to compare
164 the means of inhibition diameters. For a p-value<0.05, the difference between the means
165 compared was statistically significant, and for a p-value>0.05, it was statistically non-
166 significant.

167 **Results**

168 **Antibacterial activity of the essential oils tested**

169 Analysis of Table 2 shows that *E. coli* and *S. aureus* strains are sensitive to *T. vulgaris* and *C.*
170 *verum* essential oils, but are resistant to *C. cynimum* essential oil. The average diameter of the
171 zone of inhibition of *T. vulgaris* was 31 mm and 36.5 mm respectively for *E. coli* and *S.*

172 *aureus* strains, while that of *C. verum* was 33.8 mm and 35.25 mm respectively for the same
 173 strains.

174

175

176

177 Table 2: Average diameters of the inhibition zones of the essential oils of the plants studied

Strains	Code	<i>T. vulgaris</i>	<i>C. verum</i>	<i>C. cyminum</i>
		Mean	Mean	Mean
<i>E. coli</i>	Ec01	32	35	0
	Ec02	34	32	0
	Ec03	30	35	0
	Ec04	29	34	0
	Ec05	30	33	0
	Mean± SD	31±2.00^{ba}	33.8±1.30^{ca}	0.00^{aa}
<i>S. aureus</i>	Sa01	27	35	10
	Sa02	44	35	13
	Sa03	32	37	8
	Sa04	43	34	13
	Mean± SD	36.5±8.35^{ba}	35.25±1.26^{ba}	11±2.44^{ab}

SD: Standard deviation

Means followed by different letters are statistically different at the 5% threshold.

The first letters indicate comparisons of averages within the same bacterium, and the second comparisons of means of the diameters of each oil between the two bacterial species studied.

178

179 MIC and MBC of essential oils effective on multi-resistant strains

180 The minimum inhibitory concentration of *T. vulgaris* essential oil was the same for *E. coli* and
 181 *S. aureus* strains (2.5±0.00 µl/ml), while the average minimum bactericidal concentration was
 182 17 µl/ml for *E. coli* strains and 11.25 µl/ml for *S. aureus* strains. For *C. verum* essential oil, a
 183 MIC equal to 1.25 µl/ml was obtained for *E. coli* and *S. aureus* strains, while a mean MBC
 184 equal to 11.50 µl/ml was recorded for *E. coli* strains and 12.19 µl/ml for *S. aureus* strains
 185 (Table 3).

186 Table 3: MIC and MBC of *T. vulgaris* and *C. verum* essential oils

Strains	Code	<i>T. vulgaris</i>		<i>C. verum</i>	
		MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)

		Mean±SD	Mean±SD	Mean±SD	Mean±SD
<i>E. coli</i>	Ec01	2.5±00	20±00	1.25±00	10±00
	Ec02	2.5±00	5±00	1.25±00	2.5±00
	Ec03	2.5±00	20±00	1.25±00	15±00
	Ec04	2.5±00	20±00	1.25±00	15±00
	Ec05	2.5±00	20±00	1.25±00	15±00
	Mean±SD	2.5±00 ^a	17±6.71 ^b	1.25±00 ^a	11.50±5.48 ^a
<i>S. aureus</i>	Sa01	2.5±00	20±00	1.25±00	15±00
	Sa02	2.5±00	15±00	1.25±00	15±00
	Sa03	2.5±00	5±00	1.25±00	15±00
	Sa04	2.5±00	5±00	1.25±00	3.75±00
	Mean±SD	2.5±00 ^a	11.25±7.5 ^a	1.25±00 ^a	12.19±5.62 ^a

SD: Standard deviation

Means followed by different letters are statistically different at the 5% threshold.

187

188 **Antibiotic potency of *T. vulgaris* and *C. verum* essential oils effective on multi-resistant**
189 **strains**

190 Analysis of Table 4 shows that *T. vulgaris* and *C. verum* essential oils were bacteriostatic
191 against the multi-resistant *E. coli* and *S. aureus* strains tested.

192 Table 4: Antibiotic power of *T. vulgaris* and *C. verum* essential oils

Strains	<i>T. vulgaris</i>		<i>C. verum</i>	
	MBC/CMI	Antibiotic potency	MBC/CMI	Antibiotic potency
<i>E. coli</i>	6.80	Bacteriostatic	9.20	Bacteriostatic
<i>S. aureus</i>	4.50	Bacteriostatic	9.75	Bacteriostatic

193

194 **Chemical composition of *T. vulgaris* and *C. verum* essential oils**

195 Analysis of the essential oils revealed thymol (36.57%), p-cymene (30.51%), and carvacrol
196 (7.62%) as the majority compounds in *T. vulgaris* essential oil (Table 5). In *C. verum* essential
197 oil, cinnamaldehyde was found to be the major component with a percentage of 88.76%
198 (Table 6).

199

200

201

202 Table 5: Chemical composition of *T. vulgaris* essential oil

N°	Components	RT	RI	Percentage (%)
1	Camphene	3.5094	1074	0.72
2	D-Limonene	5.6248	1194	0.44
3	Eucalyptol	5.7768	1202	1.42
4	gamma-Terpinene	6.6085	1240	0.43
5	p-Cymene	7.2039	1267	30.51
6	1-Octen-3-ol	11.5015	1444	1.42
7	(+)-2-Bornanone	12.8575	1498	0.48
8	Linalool	13.9004	1540	2.45
9	2-isopropyl-5-methyl-anisole	14.9475	1582	3.08
10	Terpinen-4-ol	15.1333	1589	1.10
11	2-Isopropyl-1-methoxy-4-methylbenzene	15.205	1592	1.07
12	Estragole	16.7715	1656	0.82
13	endo-Borneol	17.4639	1685	2.18
14	γ -Cadinene	18.8446	1739	0.62
15	Anethole	20.6982	1810	1.65
16	p-Cymen-8-ol	21.3231	1833	0.51
17	Caryophyllene oxide	24.7769	1955	1.68
18	2-Propanone, 1-(4-methoxyphenyl)-	29.9196	2129	0.39
19	3-Allyl-6-methoxyphenol	30.2869	2142	2.52
20	Thymol	31.0891	2169	36.57
21	3-Methyl-4-isopropylphenol	31.5029	2183	1.15
22	Carvacrol	31.8027	2193	7.62
23	Eugenol acetate	32.9976	2234	1.16

RT : Retention Time ; RI : Retention Index

203

204

205

206

207 Table 6: Chemical composition of *C. verum* essential oil

N°	Components	RT	RI	Percentage (%)
1	Anethole	20.6984	1810	1.79
2	2-Propenal, 3-phenyl-	22.4084	1872	2.47
3	Cinnamaldehyde, trans	26.7362	2022	88.76
4	Cinnamyl acetate	29.8438	2127	3.20
5	Cinnamaldehyde, o-methoxy	37.914	2403	3.78

RT : Retention Time ; RI : Retention Index

208

209 Discussion

210 This study assessed the antibacterial activity of *T. vulgaris*, *C. cynimum*, and *C. verum* oils on
211 multidrug-resistant *E. coli* and *S. aureus* isolated from dogs in Southern Benin and determined
212 the chemical composition of the effective essential oils.

213 The results of this study revealed the bacteriostatic power of *T. vulgaris* dry leaf and *C. verum*
214 bark essential oils on multi-resistant *E. coli* and *S. aureus* strains.

215 The antimicrobial activity of *T. vulgaris* essential oil depends on the percentage composition
216 of its main components (Kowalczyk et al., 2020). In this study, thymol presented the highest
217 percentage in the *T. vulgaris* essential oil tested (36.57%). This percentage may explain the
218 bacteriostatic power of this oil. Indeed, thymol, chemically known as 2-isopropyl-5-
219 methylphenol, is an edible monoterpene phenol found in abundance in certain plants such as
220 *Thymus vulgaris* (Nagoor Meeran et al., 2017; Amiri, 2012). Studies investigating the
221 mechanism of thymol's antibacterial activity have indicated that its ability to integrate into the
222 lipid layer of the cell membrane increases surface curvature. The hydrophilic part of the
223 thymol interacts with the polar part of the membrane, while the hydrophobic benzene ring and
224 aliphatic side chains sink into the inner part of the biological membrane (Kowalczyk et al.,
225 2020). This interaction leads to major changes in membrane structure, with destabilization of
226 the lipid layer, reduced elasticity, and increased fluidity. The change leads to increased

227 permeability to potassium and hydrogen ions and also affects the activity of internal
228 membrane proteins such as enzymes and receptors. After incorporation into the cell
229 membrane, thymol interacts with the proteins embedded in it via a variety of non-specific
230 mechanisms, leading to changes in the conformation and activity of internal and membrane
231 proteins. As a result, cell membrane tension and destabilization can be induced by the
232 presence of thymol (Kowalczyk et al., 2020).

233 The bacteriostatic power of *T. vulgaris* essential oil may also be linked to the presence of p-
234 cymene in the oil tested. P-cymene, known as p-cymol or p-isopropyltoluene, is an alkyl-
235 substituted aromatic compound naturally present in the essential oils of various aromatic
236 plants, including *Artemisia*, *Protium*, *Origanum* and *Thymus*. It is related to the terpene
237 family, in particular the monocyclic monoterpenes (Balahbib et al., 2021). Studies on the
238 biological activities of this molecule have shown that it has synergistic antibacterial activity
239 with other molecules such as carvacrol (Ersanli et al., 2023; Rattanachaikunsopon and
240 Phumkhachorn, 2010).

241 Carvacrol is a natural monoterpene phenol that is particularly abundant in the essential oils of
242 *Origanum vulgare*, *Thymus vulgaris*, *Lepidium flavum*, *Citrus aurantium* bergamia and other
243 plants (Marinelli et al., 2019; Sharifi-Rad et al., 2018). Carvacrol interacts with the cell
244 membrane via hydrogen bonding, making membranes and mitochondria more permeable and
245 disintegrating the outer cell membrane (Imran et al., 2022). Its antibacterial activity on *E. coli*
246 (Khan et al., 2020) and methicillin-resistant *S. aureus* (ATCC-33591) strains has been
247 demonstrated (Selvaraj et al., 2020). The bacteriostatic power of *Thymus vulgaris* essential oil
248 can also be attributed to the synergistic activity of thymol, p-cymene, and carvacrol (Kachur
249 and Suntres, 2020; Micucci et al., 2020; Andrade-Ochoa et al., 2015).

250 *Cinnamomum verum* essential oil's bacteriostatic power against *E. coli* and *S. aureus* strains
251 can be linked to its high cinnamaldehyde content. Cinnamaldehyde is a chemical compound
252 that occurs naturally as a trans-stereoisomer, namely (2 E)-3-phenylprop-2-enal or trans-
253 cinnamaldehyde, which is particularly abundant in the essential oils of plants in the
254 *Cinnamomum* genus (Usai and Di Sotto, 2023). Trans-cinnamaldehyde has been shown to
255 possess substantial antimicrobial activity, as well as a range of medicinal properties (Ahmed
256 et al., 2020; Zhu et al., 2017; Vazirian et al., 2015). The antibacterial activities of this
257 molecule extend to a range of Gram-positive and negative bacteria, such as *E. coli*, *Bacillus*
258 *subtilis*, *Staphylococcus* spp, *Listeria* spp, *Salmonella* spp, *Lactobacillus sakei*,

259 *Campylobacter jejuni*, *Vibrio* spp, *Pseudomonas* spp, *Porphyromonas gingivalis*,
260 *Streptococcus pyogenes*, *Cronobacter sakazakii*, etc. (Doyle and Stephens, 2019).

261 In this study, the essential oil of *C. cynimum* seeds proved ineffective on the multidrug-
262 resistant *E. coli* and *S. aureus* strains investigated. However, in the study by Sharifi et al. , *C.*
263 *cynimum* seed oil showed bacteriostatic and bactericidal activities on multidrug-resistant
264 *Staphylococcus aureus* isolated from patients and from the milk of cattle suffering from
265 mastitis (Sharifi et al., 2021). This difference in results is linked to the different sources of
266 bacteria studied, the resistance mechanisms developed by these strains, and the chemical
267 composition of the oils tested.

268 **Conclusion**

269 This study demonstrated the antibacterial activity of *Thymus vulgaris* dry leaf and
270 *Cinnamomum verum* bark essential oils on multi-resistant *E. coli* and *S. aureus* strains. Both
271 oils are bacteriostatic on the zoonotic agents studied. The study also revealed the components
272 responsible for antibacterial activity. Thymol, p-cymene, and carvacrol were the main
273 components of *T. vulgaris* essential oil, while cinnamaldehyde was the main component of *C.*
274 *verum* essential oil. The two oils investigated in this study may be alternative candidates for
275 combating infections with resistant bacterial agents, such as *E. coli* and *S. aureus*.

276 **Funding information**

277 This work received the funding from Ministry of Science and Technology (DST), the
278 Ministry of External Affairs (MEA) of the Government of India (Gol), and the Federation of
279 Indian Chambers of Commerce and Industry (FICCI) through the C.V. Raman International
280 Fellowship for African Researchers to Mr Yaovi Bruno.

281 **Acknowledgements**

282 The authors thank the Ministry of Science and Technology (DST), the Ministry of External
283 Affairs (MEA) of the Government of India (Gol), and the Federation of Indian Chambers of
284 Commerce and Industry (FICCI) who through the C.V. Raman International Fellowship for
285 African Researchers, funded the work on antibacterial activity of *Thymus vulgaris* and
286 *Cinnamomum verum* essential oils on the strains.

287 **Conflicts of Interest**

288 The authors declare that there are no conflicts of interest.

289 **Author contributions**

290 A.B.Y: Conceptualization, statistical analysis, writing original draft, review, and editing; A.D
291 and R.N.B: Methodology; F.M and M.L.F: essential oil analysis; A.Z, L.B, S.F, K.A, P.S:
292 Supervision, review, and editing.

293 **Ethical Approval**

294 The study was approved by the Ethical Committee of Research Unit on Communicable
295 Diseases (URMAT in French) of the Polytechnic School of Abomy-Calavi of the University
296 of Abomey-Calavi (N°004/EPAC/LARBA/URMAT/CE/R).

297 **References**

- 298 Ahmed, H.M., Ramadhani, A.M., Erwa, I.Y., Ishag, O.A.O., Saeed, M.B., 2020.
299 Phytochemical Screening, Chemical Composition and Antimicrobial Activity of
300 Cinnamon verum Bark. *International Research Journal of Pure and Applied Chemistry*
301 36–43. <https://doi.org/10.9734/irjpac/2020/v21i1130222>
- 302 Amiri, H., 2012. Essential Oils Composition and Antioxidant Properties of Three Thymus
303 Species. *Evid Based Complement Alternat Med* 2012, 728065.
304 <https://doi.org/10.1155/2012/728065>
- 305 Andrade-Ochoa, S., Nevárez-Moorillón, G.V., Sánchez-Torres, L.E., Villanueva-García, M.,
306 Sánchez-Ramírez, B.E., Rodríguez-Valdez, L.M., Rivera-Chavira, B.E., 2015.
307 Quantitative structure-activity relationship of molecules constituent of different
308 essential oils with antimycobacterial activity against *Mycobacterium tuberculosis* and
309 *Mycobacterium bovis*. *BMC Complement Altern Med* 15, 332.
310 <https://doi.org/10.1186/s12906-015-0858-2>
- 311 Balahbib, A., El Omari, N., Hachlafi, N.E.L., Lakhdar, F., El Menyiy, N., Salhi, N., Mrabti,
312 H.N., Bakrim, S., Zengin, G., Bouyahya, A., 2021. Health beneficial and
313 pharmacological properties of p-cymene. *Food and Chemical Toxicology* 153,
314 112259. <https://doi.org/10.1016/j.fct.2021.112259>
- 315 Caneschi, A., Bardhi, A., Barbarossa, A., Zaghini, A., 2023. The Use of Antibiotics and
316 Antimicrobial Resistance in Veterinary Medicine, a Complex Phenomenon: A
317 Narrative Review. *Antibiotics (Basel)* 12, 487.
318 <https://doi.org/10.3390/antibiotics12030487>
- 319 Doyle, A.A., Stephens, J.C., 2019. A review of cinnamaldehyde and its derivatives as
320 antibacterial agents. *Fitoterapia* 139, 104405.
321 <https://doi.org/10.1016/j.fitote.2019.104405>
- 322 Ersanli, C., Tzora, A., Skoufos, I., Fotou, K., Maloupa, E., Grigoriadou, K., Voidarou, C.
323 (Chrysa), Zeugolis, D.I., 2023. The Assessment of Antimicrobial and Anti-Biofilm
324 Activity of Essential Oils against *Staphylococcus aureus* Strains. *Antibiotics (Basel)*
325 12, 384. <https://doi.org/10.3390/antibiotics12020384>
- 326 Imran, M., Aslam, M., Alsagaby, S.A., Saeed, F., Ahmad, I., Afzaal, M., Arshad, M.U.,
327 Abdelgawad, M.A., El-Ghorab, A.H., Khames, A., Shariati, M.A., Ahmad, A.,
328 Hussain, M., Imran, A., Islam, S., 2022. Therapeutic application of carvacrol: A
329 comprehensive review. *Food Science & Nutrition* 10, 3544–3561.
330 <https://doi.org/10.1002/fsn3.2994>

331 Kachur, K., Suntres, Z., 2020. The antibacterial properties of phenolic isomers, carvacrol and
332 thymol. *Critical Reviews in Food Science and Nutrition* 60, 3042–3053.
333 <https://doi.org/10.1080/10408398.2019.1675585>

334 Khan, I., Bahuguna, A., Shukla, S., Aziz, F., Chauhan, A.K., Ansari, M.B., Bajpai, V.K.,
335 Huh, Y.S., Kang, S.C., 2020. Antimicrobial potential of the food-grade additive
336 carvacrol against uropathogenic *E. coli* based on membrane depolarization, reactive
337 oxygen species generation, and molecular docking analysis. *Microb Pathog* 142,
338 104046. <https://doi.org/10.1016/j.micpath.2020.104046>

339 Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A., Fecka, I., 2020. Thymol and
340 Thyme Essential Oil—New Insights into Selected Therapeutic Applications.
341 *Molecules* 25, 4125. <https://doi.org/10.3390/molecules25184125>

342 Kpatinvoh, B., Adjou, E.S., Dahouenon-Ahoussi, E., Konfo, T.R.C., Atrevi, B., Soumanou,
343 M.M., Sohounhloue, D.C.K., 2017. Efficacité des huiles essentielles de trois plantes
344 aromatiques contre la mycoflore d’altération du niébé (*Vigna unguiculata* L., Walp)
345 collecté dans les magasins de vente du Sud-Bénin. *Journal of Applied Biosciences*
346 109, 10680–10687. <https://doi.org/10.4314/jab.v109i1.12>

347 Mamadou, R., Moussa, I., Philippe, S., Yehouenou, B., Agbangnan D., C.P., Illagouma, A.,
348 Abdoulaye, A., Sohounhloue, D., Ikhiri, K., Soc, J., Ouest, 2014. Etude
349 phytochimique, activités antiradicalaire, antibactérienne et antifongique d’extraits de
350 *Sebastiania chamaelea* (L.) Müll.Arg. *Journal de la Société Ouest Africaine de Chimie*
351 037, 10–17.

352 Marinelli, L., Fornasari, E., Eusepi, P., Ciulla, M., Genovese, S., Epifano, F., Fiorito, S.,
353 Turkez, H., Örtücü, S., Mingoia, M., Simoni, S., Pugnaloní, A., Di Stefano, A.,
354 Cacciatore, I., 2019. Carvacrol prodrugs as novel antimicrobial agents. *Eur J Med*
355 *Chem* 178, 515–529. <https://doi.org/10.1016/j.ejmech.2019.05.093>

356 Micucci, M., Protti, M., Aldini, R., Frosini, M., Corazza, I., Marzetti, C., Mattioli, L.B.,
357 Tocci, G., Chiarini, A., Mercolini, L., Budriesi, R., 2020. *Thymus vulgaris* L.
358 Essential Oil Solid Formulation: Chemical Profile and Spasmolytic and Antimicrobial
359 Effects. *Biomolecules* 10, 860. <https://doi.org/10.3390/biom10060860>

360 Mugwaneza, D., Rwagasore, E., El-Khatib, Z., Dukuziyaturemye, P., Omolo, J., Nsekuye, O.,
361 Rwunganira, S., Manzi, M., 2024. Factors Associated with Inappropriate Use of
362 Antibiotics Among Animal Health Professionals in Selected Districts of Rwanda,
363 2021. *J Epidemiol Glob Health* 14, 265–273. [https://doi.org/10.1007/s44197-024-](https://doi.org/10.1007/s44197-024-00192-x)
364 [00192-x](https://doi.org/10.1007/s44197-024-00192-x)

365 Nagoor Meeran, M.F., Javed, H., Al Taei, H., Azimullah, S., Ojha, S.K., 2017.
366 Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its
367 Therapeutic Potential and Pharmaceutical Development. *Front Pharmacol* 8, 380.
368 <https://doi.org/10.3389/fphar.2017.00380>

369 O’Neill, J., 2016. Tackling drug-resistant infections globally: final report and
370 recommendations (Report). Government of the United Kingdom.

371 Rattanachaikunsopon, P., Phumkhachorn, P., 2010. Assessment of factors influencing
372 antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food.
373 *Journal of bioscience and bioengineering* 110, 614–619.
374 <https://doi.org/10.1016/j.jbiosc.2010.06.010>

375 Selvaraj, A., Valliammai, A., Muthuramalingam, P., Priya, A., Suba, M., Ramesh, M.,
376 Karutha Pandian, S., 2020. Carvacrol Targets SarA and CrtM of Methicillin-Resistant
377 *Staphylococcus aureus* to Mitigate Biofilm Formation and Staphyloxanthin Synthesis:
378 An In Vitro and In Vivo Approach. *ACS Omega* 5, 31100–31114.
379 <https://doi.org/10.1021/acsomega.0c04252>

380 Sessou, P., Yaovi, B.A., Yovo, M., Gamedjo, J., Dossa, F., Aguidissou, O.N., Boko, K.C.,
381 Alitonou, G., Farougou, S., Sohounhloue, D., 2018. Phytochemistry and antibacterial
382 activity of plants extracts compared with two commercial antibiotics against E coli
383 responsible for avian colibacillosis in Benin. *ijpm* 10, 168.
384 <https://doi.org/10.5138/09750185.2259>

385 Sharifi, A., Mohammadzadeh, A., Salehi, T.Z., Mahmoodi, P., Nourian, A., 2021. Cuminum
386 cyminum L. Essential Oil: A Promising Antibacterial and Antivirulence Agent
387 Against Multidrug-Resistant Staphylococcus aureus. *Front Microbiol* 12, 667833.
388 <https://doi.org/10.3389/fmicb.2021.667833>

389 Sharifi-Rad, J., Dey, A., Koirala, N., Shaheen, S., El Omari, N., Salehi, B., Goloshvili, T.,
390 Cirone Silva, N.C., Bouyahya, A., Vitalini, S., Varoni, E.M., Martorell, M.,
391 Abdolshahi, A., Docea, A.O., Iriti, M., Calina, D., Les, F., López, V., Caruntu, C.,
392 2021. Cinnamomum Species: Bridging Phytochemistry Knowledge, Pharmacological
393 Properties and Toxicological Safety for Health Benefits. *Front Pharmacol* 12, 600139.
394 <https://doi.org/10.3389/fphar.2021.600139>

395 Sharifi-Rad, M., Varoni, E.M., Iriti, M., Martorell, M., Setzer, W.N., del Mar Contreras, M.,
396 Salehi, B., Soltani-Nejad, A., Rajabi, S., Tajbakhsh, M., Sharifi-Rad, J., 2018.
397 Carvacrol and human health: A comprehensive review. *Phytotherapy Research* 32,
398 1675–1687. <https://doi.org/10.1002/ptr.6103>

399 Usai, F., Di Sotto, A., 2023. trans-Cinnamaldehyde as a Novel Candidate to Overcome
400 Bacterial Resistance: An Overview of In Vitro Studies. *Antibiotics* 12, 254.
401 <https://doi.org/10.3390/antibiotics12020254>

402 Vazirian, M., Alehabib, S., Jamalifar, H., Fazeli, R., Toosi, A., Khanavi, M., 2015.
403 Antimicrobial effect of cinnamon (*Cinnamomum verum* J. Presl) bark essential oil in
404 cream-filled cakes and pastries. *Research Journal of Pharmacognosy* 2, 11–16.

405 Yaovi, A.B., Sessou, P., Tonouhewa, A.B.N., Hounmanou, G.Y.M., Thomson, D., Pelle, R.,
406 Farougou, S., Mitra, A., 2022. Prevalence of antibiotic-resistant bacteria amongst dogs
407 in Africa: A meta-analysis review. *Onderstepoort j. vet. res.* 89.
408 <https://doi.org/10.4102/ojvr.v89i1.1970>

409 Zhu, R., Liu, H., Liu, C., Wang, L., Ma, R., Chen, B., Li, L., Niu, J., Fu, M., Zhang, D., Gao,
410 S., 2017. Cinnamaldehyde in diabetes: A review of pharmacology, pharmacokinetics
411 and safety. *Pharmacological Research* 122, 78–89.
412 <https://doi.org/10.1016/j.phrs.2017.05.019>

413