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3 **Susceptibility Of Extended Spectrum Beta**

4 **Lactamase Producing *Klebsiella Pneumoniae***

5 **Clinical Isolates To Antibiotics And Essential**

6 **Oils**

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11 **ABSTRACT**

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Aims: To determine the susceptibility of extended-spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* clinical isolates to antibiotics and essential oils - *O. compactum*, *O. marjoram* and *Thymus serpyllum*, to define the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and synergistic effect between essential oils and antibiotics.

Study design: This research included 30 isolates of *Klebsiella pneumoniae* obtained from clinical material provided from the University Clinical Center Tuzla.

Place and Duration of Study: Department of Biology, Faculty of science, Univerity of Tuzla, between September 2019 and September 2020.

Methodology: The phenotypic disk diffusion method was used to determine sensitivity profile of isolates to antibiotics and essential oils and a confirmatory CLSI test with clavulanic acid for ESBL conformation. The MIC and MBC for essential oils were determined by microdilution test, and checkerboard method was used for determination of interactions between antibiotics and tested oils.

Results: *O. compactum* and *O. majorana* essential oils showed the same antimicrobial activity with 80.0% effect on ESBL *K. pneumoniae* isolates, *Thymus serpyllum* EO showed antimicrobial activity of 60.0%. The lowest MIC value had the *O. compactum* essential oil (MIC 6 mg/ml-10.5 mg/ml), followed by the *T. serpyllum* (MIC 17.2 mg/ml-43 mg/ml), while the *O. majorana* essential oil showed MIC values in range from 11 mg/ml to 39 mg/ml.

Conclusion: The results of the study showed the exceptional sensitivity of ESBL *K. pneumoniae* clinical isolates to the essential oils from *Origanum* and *Thymus* genera, which highly suggests their potential application in the struggle against these pathogens in the future.

14

15 **Keywords:** *Klebsiella pneumoniae*, essential oils, susceptibility

16 **1. INTRODUCTION**

17

18 In this era of antibiotic resistance, *Klebsiella pneumoniae* represents one of the most
19 concerning pathogens involved in antibiotic resistance and as such, together with other
20 highly important multiple drug resistant (MDR) pathogens, it has been classified as an
21 ESKAPE organisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella*
22 *pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*)
23 [1]. Treatment of these organisms is a deep scientific concern. Recently, a significant
24 increase in the incidents of ESBL-related infections has been observed throughout the globe
25 [2].

26 Bacterial resistance has its own characteristics and will continue to worsen if not addressed
27 properly. A growing number of studies are based on the research of the effect of
28 unconventional antimicrobial agents which are widely present in nature and can be used to
29 save the mankind concerning bacterial resistance [3]. Nowadays, the field of essential oils
30 has been widely researched and it is one of the leading trends related to the use of "natural
31 antibiotics". Plant essential oils have been used for hundreds of years as natural medicines
32 to combat a multitude of pathogens, including bacteria, fungi, and viruses [4].

33 Essential oils, also known as volatile oils, are products of the secondary metabolism of
34 aromatic plants. Essential oils are lipophilic and complex chemical compounds with high
35 terpenic and phenolic contents [5]. Several essential oils confer antimicrobial activity by
36 damaging the cell wall and membrane, leading to cell lysis, leakage of cell contents, and
37 inhibition of proton motive force [6]. It is reported that they affect bacterial biofilms
38 specifically by interfering with quorum sensing, inhibiting the peptidoglycan synthesis or
39 reducing cell adherence [7]. In addition, there is no evidence of the development of bacterial
40 resistance to them.

41 The aim of this study was to investigate antimicrobial activity of three essential oils,
42 *Origanum compactum*, *Origanum majorana* and *Thymus serpyllum*, against *K. pneumoniae*
43 clinical isolates that produce extended-spectrum beta-lactamases.

44

45 **2. MATERIAL AND METHODS**

46

47 **2.1 Bacterial isolates and essential oils**

48 The study included thirty ESBL isolates of *K. pneumoniae*, isolated from different human
49 specimens (urine, sputum, gastric lavage, aspirate, throat swab) in the period from 2018. to
50 2019., at the University Clinical Centre in Tuzla. Isolation and identification of *K. pneumoniae*
51 were performed by standard microbiological methods [8].

52 Further analyses and testing were conducted in the laboratory for microbiology, at the
53 Faculty of Natural Sciences and Mathematics in Tuzla.

54 For this study, three different essential oils were tested: *O. compactum*, *O. majorana* and *T.*
55 *serpyllum* produced by Pranarom (B-7822 Ghislenghien, Belgique), indicating that the major
56 components (>90%) of the essential oils *O. compactum* and *T. serpyllum* are carvacrol and
57 thymol, while the major component of the essential oil of *O. majorana* is terpinen-4-ol. The
58 reference strain of *K. pneumoniae* ATCC (American Type Culture Collection) 2342 was used
59 as a control strain.

60

61 2.2 Methods

62 Antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method [9].
63 The following commercially available antibiotic discs were used: amoxicillin (30µg), cefalexin
64 (30 µg), gentamicin (10 µg), amikacin (30 µg), imipenem (10 µg), piperacillin (75µg),
65 ampicillin (10 µg), meropenem (10 µg), ciprofloxacin (10 µg), ceftazidim (30 µg), cefotaksim
66 (30 µg), ceftriaxone (30 µg), cefepime (30 µg) and aztreonam (30 µg) (Mast Group LTD,
67 UK). The results were interpreted according to the European Committee on Antimicrobial
68 Susceptibility Testing (EUCAST) guidelines [10].

69 *K. pneumoniae* isolates resistant to cephalosporins of the third and fourth generations were
70 further analyzed using the phenotypic methods to confirm the production of extended-
71 spectrum beta-lactamases. The confirmation of ESBL production was performed by
72 combined disc method [11].

73 The antibacterial effect of the essential oils of *O. compactum*, *O. majorana* and *T. serpyllum*
74 was tested for ESBL *K. pneumoniae* isolates using the diffusion method according to Clinical
75 laboratory standards institute (CLSI) guidelines [12]. Bacterial suspension inoculated on
76 Mueller-Hinton agar was tested with 10, 20 and 50µL of each essential oil. After incubation
77 period (35°C/24h), interpretation of the results was performed by measuring the diameters of
78 the inhibition zones in millimetres [13].

79 MIC was determined by the microdilution method using microtiter plates with 96 wells in
80 accordance with instructions provided by the CLSI [12]. For this test, a double dilution series
81 of essential oil or antibiotic dissolved in Dimethyl Sulfoxide (DMSO), were applied on plates,
82 in volume of 10 µL, and 90 µL of bacterial strain inoculated in Mueller-Hinton broth (MHB)
83 were added. The final volume in each well was 100 µL, the final density of the bacterial cells
84 was 10⁶ Colony-forming units (CFU)/ml. Concentrations of the tested essential oil ranged
85 from 86 mg/ml to 6 mg/ml and initial antibiotic concentrations was 10 mg/ml. After incubation
86 the bacterial growth was detected by adding 20 µL of 0.5% triphenyl tetrazolium chloride
87 (TTC). The MIC is defined as the lowest concentration of the examined essential oils at
88 which there is no visible bacterial growth. The minimum bactericidal concentration (MBC)
89 matched the lowest concentration of the tested antimicrobial agent producing negative
90 subcultures after the incubation period at appropriate temperature during 24-hour period.

91 2.3 FIC analysis

92 The synergistic effect between two antimicrobial substances was determined by the
93 *checkerboard* method [14]. based on the previously determined MIC values. The obtained
94 MIC values were used for the determination of the FIC (Fractional inhibitory concentration)
95 index and for interpretation of the type of interaction between the antimicrobial agents
96 according to the following formula:

97
$$\text{FIC of antimicrobial agent A} = \text{MIC of agent A in combination} / \text{MIC of agent A alone}$$

98
$$\text{FIC of antimicrobial agent B} = \text{MIC of agent B in combination} / \text{MIC of agent B alone}$$

99
$$(\text{FIC index}) = \text{FIC of agent A} + \text{FIC of agent B.}$$

100
101
102

103 **3. RESULTS AND DISCUSSION**

104

105 A total of 30 *K. pneumoniae* clinical isolates were tested for antibiotic susceptibility. The test
 106 results are summarized and shown in Table 1.

107

108 **Table 1. Cumulative results of *K. pneumoniae* clinical isolates susceptibility to**

109 **antibiotics**

110

Class of antibiotics	Antibiotic	Sensitive		Intermediate		Resistant	
		n	%	n	%	n	%
Aminoglycosides	AK	12	40,0	0	0,0	18	60,0
	GM	4	13,3	0	0,0	26	86,0
Carbapenems	MEM	3	10,0	0	0,0	27	90,0
	IMP	20	66,6	0	0,0	10	33,3
Beta lactams	AX	0	0,0	0	0,0	30	100
	PIP	0	0,0	0	0,0	30	100
	AMP	0	0,0	0	0,0	30	100
Cephalosporins	I CEF	12	40	0	0,0	18	60,0
	III CTX	2	6,0	0	0,0	28	93,3
	IV FEP	5	16,6	0	0,0	25	83,3
	III CAZ	2	6,0	0	0,0	28	93,3
	III CRO	11	36,6	0	0,0	19	63,3

Fluoroquinolon	CIP	4	13,3	2	6,0	24	80,0
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es

Macrolides	ATM	8	26,6	0	0,0	22	73,3
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111 *n – total number of isolates

112 *AK- amikacin, GM - gentamicin, AX - amoxicillin, MEM - meropenem, IMP - imipenem, CIP
 113 – ciprofloxacin, PIP - piperacillin, AMP - ampicillin, CEF - cefalexin, CTX - cefotaxim, FEP -
 114 cefepime, CAZ - ceftazidime, ATM - aztreonam, CRO - ceftriaxon.

115

116 Antibiotic susceptibility of *K. pneumoniae* clinical isolates showed 100% (30/30) resistance to
 117 beta lactam antibiotics amoxicillin, piperacillin and ampicillin. A high degree of resistance
 118 was recorded towards first-generation cephalosporins - 93.3% isolates were resistant to
 119 cefotaxime (28/30). Resistance was particularly expressed for third and fourth generation of
 120 cephalosporins, is 93.3% isolates were resistant to ceftazidime (28/30), 83.3% to cefepime
 121 (25/30), and 93.3% to cefotaxime (28/30). For aminoglycoside antibiotics amikacin and
 122 gentamicin, resistance of 60% (18/30) and 86% (26/30), respectively, was observed.
 123 Resistance to fluoroquinolones (ciprofloxacin) manifested 80% of isolates (24/30), and to
 124 macrolides (azithromycin) 73% (22/30). Sensitivity to carbapenems was diverse, 90.0% of
 125 isolates were resistant to meropenem (27/30), while among all tested antibiotics, the highest
 126 sensitivity isolates expressed to imipenem- 66.6% were sensitive to this antibiotic (20/30).
 127 The production of extended-spectrum beta-lactamases enzymes with combined disk method
 128 was confirmed in 30/30 tested *K. pneumoniae* clinical isolates.

129 Since imipenem showed the best antimicrobial effect on ESBL *K. pneumoniae* isolates, it
 130 was selected as a reference antibiotic for further tests in the microdilution method, Table 2.

131

132 **Table 2. Imipenem MIC and MBC values for 10 beta lactamase positive *K. pneumoniae***

133 **isolates**

Isolate	Imipenem (10 µg)	
	MIC (mg/ml)	MBC (mg/ml)
31	0,0075	0,909
33	0,0826	0,909
39	0,0075	0,909

41	0,0075	0,909
50	0,0000056	0,00068
52	0,0075	0,909
53	0,0000056	0,00068
54	0,0000056	0,00068
56	0,0075	0,909
59	0,0826	10
ATCC 2342	0,0000056	0,00068

134

135

136 The antibacterial activity of *O. compactum*, *O. marjorana* and *T. serpyllum* essential oils was
 137 tested on all 30 ESBL positive *K. pneumoniae* isolates, and the cumulative sensitivity results
 138 were shown in Table 3.

139

140 **Table 3. Cumulative results sensitivity of ESBL isolates of *K. pneumoniae* to essential**

141 **oils**

Essential oil	Volume 10 µl		Volume 20 µl		Volume 50 µl							
	Sensitive		Resistant		Sensitive		Resistant					
	n	%	n	%	n	%	n	%				
<i>Origanum compactum</i>	0	0	30	100	23	76,6	7	23,3	24	80,0	6	20,0

Origanum

0 0 30 100 20 66,6 10 33,3 24 80,0 6 20,0

majorana

Thymus

0 0 30 100 17 56,6 13 43,3 18 60,0 12 40,0

serpyllum

142

143

144 Essential oils of the *Origanum* genera had the greatest antimicrobial effect at 50 µL of
145 volume. 80.0% (24/30) of beta-lactamase-producing isolates of *K. pneumoniae* showed
146 sensitivity to *O. compactum* and *O. majorana* oils. *T. serpyllum* essential oil also showed a
147 high antimicrobial activity of 60.0% with effective results on 18/30 beta-lactamase producing
148 isolates.

149 For further analysis, ten randomly selected ESBL *K. pneumoniae* isolates were tested to
150 determine the minimum inhibitory and minimum bactericidal concentrations of the essential
151 oils, Table 4.

152

153 **Table 4. MIC and MBC of essential oils tested on 10 ESBL positive *K. pneumoniae***

154 **isolates.**

155

Isolates	<i>O. compactum</i>		<i>O. majorana</i>		<i>T. serpyllum</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
31	6.4	8,4	28,6	86	13	26
33	9,3	14	43	86	26	78
39	8,4	10,5	28,6	86	39	78
41	8,4	10,5	21,5	43	15,6	39
50	6	9,3	17,2	43	13	26
52	6.4	8,4	28,6	86	26	78

53	7	10,5	17,2	43	19,5	39
54	6,4	9,3	21,5	43	26	78
56	10,5	16,8	43	86	13	26
59	9,3	14	43	86	26	78
<hr/>						
ATCC						
2342	6	9,3	17,2	43	11,1	15,6

156 *Concentrations MIC and MBC are expressed in mg/ml

157

158 The minimum inhibitory concentration of *O. compactum* essential oil tested on 10 ESBL *K. pneumoniae* clinical isolates of shows a range of values from 6 mg/ml to 10.5 mg/ml, for *O. marjorana* essential oil 17.2 mg/ml to 43 mg/ml, and for *T. serpyllum* essential oil, MIC values vary from 11.1 mg/ml to 39 mg/ml. For the reference strain *K. pneumoniae* ATCC 2342, the following results were obtained: *O. compactum* had the lowest MIC value of 6 mg/ml and MBK 9.3 mg/ml, for *O. marjorana* MIC value was 17.2 mg/ml, while for *T. serpyllum* the MIC value was 11.1 mg/ml.

165 The checkerboard method was performed on 2 ESBL *K. pneumoniae* isolates and ATCC 2342 reference strain. Due to the previous detection of imipenem as the antibiotic with the highest antibacterial activity on beta lactamase positive isolate, this strong conventional antimicrobial agent was selected for checkerboard method analysis, in combination with three essential oils *O. compactum*, *O. marjorana* and *T. serpyllum*. The obtained results are presented in Table 5.

167

168 **Table 5. Synergistic effect of essential oils and imipenem tested on two ESBL *K.***

169

170 ***pneumoniae* isolates and a reference strain**

171

Isolates and reference strain	<i>O. compactum</i>		<i>O. majorana</i>		<i>T. serpyllum</i>	
	Imipenem	Effect	Imipenem	Effect	Imipenem	Effect
ATCC	FIC	Effect	FIC	Effect	FIC	Effect
2342	1,61	Indifferent	2,89	Indifferent	1,11	Indifferent

	FIC	Effect	FIC	Effect	FIC	Effect
31	1,54	Indifferent	1,26	Indifferent	2,18	Indifferent
	FIC	Effect	FIC	Effect	FIC	Effect
33	1,03	Indifferent	1,41	Indifferent	1,23	Indifferent

174

175 The use of essential oils in treating various human diseases, especially infectious ones
 176 caused by multiple-resistant bacterial strains, can be an interesting alternative to synthetic
 177 medicine that can have side effects. Essential oils in combination with antibiotics can prevent
 178 the creation of the strains resistant to antibiotics. Due to the therapeutic problems associated
 179 with particularly resistant strains, essential oils can be useful in fighting infections caused by
 180 nosocomial pathogens.

181 The antibacterial characteristics of the essential oil from *Origanum* genera and their effect
 182 against pathogenic bacteria have been described before in many studies. Ibišević et al.
 183 (2019), tested antibacterial activity of the *O. compactum* essential oil on different Gram-
 184 positive and Gram-negative bacterial strains. This research included 75 clinical bacterial
 185 strains of *S. aureus*, *E. faecalis*, *E. coli*. and *K. pneumoniae*, and gained results confirmed its
 186 strong and significant antibacterial activity [15].
 187 *O. compactum*, the essential oil with the highest antimicrobial activity against ESBL *K.*
 188 *pneumoniae* isolates, had the lowest MIC values (6 mg/ml to 10.5 mg/ml), then *T. serpyllum*
 189 (with MIC values from 11.1 mg/ml to 39 mg/ml), while the essential oil of *O. majorana* ranked
 190 the lowest in terms of antimicrobial activity (MIC 17.2 mg/ml to 43 mg/ml). Mohamed et al.
 191 (2018) also reported the positive effect of the *Origanum* and *Thymus* of the essential,
 192 especially *T. serpyllum* on *K. pneumoniae*, with MIC values of 9.4 mg/ml [16]. Orhan et al.
 193 (2011) examined the inhibitory effect of different types of essential oils, including the
 194 essential oils of oregano (*O. compactum* and *O. majorana*) on 10 beta-lactamase positive
 195 isolates of *K. pneumoniae* [17]. All the essential oils and their components used in that
 196 study showed incredible bacterial growth inhibition with MIC values ranging from 32 to 64
 197 mg/ml, which is in accordance with our results.

198 In the study by Bedenić et al. (2015), which covers *in vitro* antibiotic susceptibility testing of
 199 *K. pneumoniae* clinical isolates that produce different types of beta-lactamase enzymes, it is
 200 noted that imipenem remains the medicine of choice for beta-lactamase positive isolates of
 201 *K. pneumoniae* because a relatively low rate of resistance to this antibiotic has been
 202 reported [18]. In our study, imipenem also showed the most effective activity and its
 203 significantly lower minimum inhibitory concentrations were determined in relation to the
 204 minimum inhibitory concentrations of all the tested essential oils. However, one of the major
 205 problems today is that many bacteria have become resistant to almost all of the available
 206 antibiotics. The strains of *K. pneumoniae* resistant to imipenem have already been described
 207 worldwide. The results of our study also show the rate of resistance to this antibiotic, which
 208 was 33.3% (10/30).

209 Examining the combinatory effects of essential oils and imipenem, in this research synergy
 210 was not established. On the contrary, the effect was indifferent in combinations with all three

211 essential oils. In a study conducted by Rosato et al. it was reported that oregano oil in
212 combination with gentamicin exhibited synergism against *B. cereus*, *B. subtilis* and one
213 strain of *S. aureus*. In contrast, the combination with gentamicin against *E. coli*,
214 *Acinetobacter baumannii* and another strain of *S. aureus* was less effective and more likely
215 to be additive than synergistic [19]. A study investigating thyme oil by Van Vuuren et al.
216 reported a synergistic effect in combination with ciprofloxacin against *S. aureus* and *K.*
217 *pneumoniae*. However, with a FIC between 0.5 and 1.0, this combination would not be
218 classed as synergistic by other researchers [5].
219 The number of studies in which alternative plant agents are combined with conventional
220 antibiotics in control of Gram-negative bacteria is still limited. Combined therapy is a
221 relatively new concept. However, most studies involved with this matter have detected the
222 presence of synergy *in vitro* but have not completely examined basic mechanisms of this
223 common activity. Considering the fact that most components of essential oils have general
224 harmful effects on cell membranes of bacteria and that most antibiotics have specific target
225 molecules involved in synthetic processes in the cell, it is probable that synergy in most
226 cases is a consequence of a multi-target effect.

227 It is known that gram-negative bacteria are more resistant to antibiotics than the gram-
228 positive bacteria. The use of terpenes as a therapeutic alternative combined with antibiotics
229 could amplify their competence of income to the cell. Moreover, antibiotics could permit an
230 effective transport of the latter until reaching its bacterial cell target owing to the lipidic nature
231 of the terpene. This type of synergism raises the question if the presence of beta-lactamases
232 should be discouraging by a potential drastic effect of essential oils [20].

233 The use of essential oils in treating various human diseases caused by multiple-resistant
234 bacterial strains can be an interesting alternative to synthetic medicine. Studies to date have
235 indicated that these unconventional antimicrobial agents can be an interesting choice and
236 alternative for reducing the use of antibiotics [21]. The results of our study are in accordance
237 with this statement, indicating high susceptibility of *K. pneumoniae* beta-lactamase
238 producing isolates to essential oils, namely 80% (24/30) to *O. compactum* and *O. majorana*
239 and 60% (18/30) to *T. serpyllum*.

240

241 **4. CONCLUSION**

242

243 In this study, the susceptibility of 30 ESBL *K. pneumoniae* clinical isolates to three types of
244 essential oils (*Thymus serpyllum*, *Origanum majorana* and *Origanum compactum*) was
245 tested. The antibacterial activity of these unconventional antimicrobials varied among the
246 isolates. The essential oils of *O.compactum* and *O.majorana* showed the greatest
247 antimicrobial activity against the majority of isolates, with the susceptibility of 80% (24/30) of
248 the tested isolates. The essential oil of *Thymus serpyllum* also showed a high antimicrobial
249 activity of 60.0% affecting 18/30 ESBL positive isolates. Studies to date have indicated that
250 unconventional antimicrobial agents can be an interesting choice and alternative for reducing
251 the use of antibiotics. Since new antibiotics have not been developed in the fight against
252 resistant enterobacteria, future research should be directed towards the use of
253 unconventional antimicrobials such as essential oils, which can minimize resistance to
254 antibiotics.

255

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265 **COMPETING INTERESTS**

266

267 None to declare

268

269 **AUTHORS' CONTRIBUTIONS**

270

271 All authors read and approved the final manuscript.

272 **COMPETING INTERESTS DISCLAIMER:**

273

274 Authors have declared that no competing interests exist. The products used for this research
275 are commonly and predominantly use products in our area of research and country. There is
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286 **REFERENCES**

287

288 [1] Boucher HW, Talbot GH, Bradley JS. Bad bugs, no drugs: no ESKAPE! An update from
289 the Infectious Diseases Society of America. *Clinical Infectious Diseases* 2009; 48:1–12.

290 [2] Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended
291 spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological
292 Sciences* 2015; 22 (1): 90-101.

293 [3] Yap PS, Yiap BC, Ping HC, Lim SH. Essential oils, a new horizon in combating bacterial
294 antibiotic resistance. *Open Microbiol Journal* 2014; 8:6-14.

295 [4] Kavanaugh NL, Ribbeck K. Selected antimicrobial essential oils eradicate *Pseudomonas*
296 spp. and *Staphylococcus aureus* biofilms. *Appl Environ Microbiol* 2012; 78(11):4057-4061.

- 297 [5] Van Vuuren SF, Suliman S, Viljoen AM. The antimicrobial activity of four commercial
298 essential oils in combination with conventional antimicrobials. *Lett Appl Microbiol* 2009; 48:
299 440-446.
- 300 [6] Burt S. Essential oils: their antibacterial properties and potential applications in foods-a
301 review. *Int J Food Microbiol* 2004; 94(3): 223-253.
- 302 [7] Nazzaro F, Fratianni F, D'Acierno A, Coppola R, Ayala-Zavala FJ, Da Cruz AG, De Feo
303 V. [Essential Oils and Microbial Communication](#). In: *Essential Oils - Oils of Nature* Ed Hany
304 El-Shemy 2020; 165-185.
- 305 [8] Murray PR. *Manual of Clinical Microbiology*. 8th ed. Washington, D. C: American Society
306 for Microbiology Press, 2003.
- 307 [9] Clinical and Laboratory Standards Institute. M100- Performance Standards for
308 Antimicrobial Susceptibility Testing. 30th ed. CLSI, January 2020.
- 309 https://clsi.org/media/3481/m100ed30_sample.pdf
- 310 https://clsi.org/media/3481/m100ed30_sample.pdf
- 311 [10] The European Committee on Antimicrobial Susceptibility Testing. Guidelines for
312 detection of resistance mechanisms and specific resistances of clinical and/or
313 epidemiological importance. Version 2.0: EUCAST, 2017.
- 314 https://euca.st/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCA
315 [ST_detection_of_resistance_mechanisms_170711.pdf](https://euca.st/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCA)
- 316 [11] Drieu L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum
317 β -lactamase production in *Enterobacteriaceae*: review and bench guide. *Clinical*
318 *Microbiology and infections* 2008; 1:90-103.
- 319 [12] Clinical laboratory standards institute. M07 Methods for dilution, antimicrobial
320 susceptibility tests for bacteria that grow aerobically, approved standard, 11th ed. CLSI,
321 2018.
- 322 https://clsi.org/media/1928/m07ed11_sample.pdf
- 323 [13] Pirvu L, Hlevca C, Nicu I, Bubueanu C. Comparative Studies on analytical, antioxidant
324 and antimicrobial activities of a series of vegetal extracts prepared from eight plant species
325 growing in Romania. *JPC- Journal of Planar Chromatography - Modern TLC* 2014; 27: 346–
326 356.
- 327 [14] Fadli M, Saad A, Sayadi S, Chevalier J, Mezrioui NE, Pagès JM, Hassani L.
328 Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against
329 nosocomial infection - bacteria and their synergistic potential with antibiotics. *Phytomedicine*
330 2012; 19(5):464-71.
- 331 [15] Ibisevic M, Husejnagic D, Kazanovic R, Arsić I. Antibacterial activity of *Origanum*
332 *compactum* essential oil tested on vaginal and cervical clinical bacterial strains. *Acta*
333 *Facultatis Medicae Naissensis* 2019; 36(3):219-228.
- 334 [16] Mohamed SH, Mohamed MSM, Khalil MS, Azmy M, Mabrouk MI. Combination of
335 essential oil and ciprofloxacin to inhibit/eradicate biofilms in multidrug-resistant *Klebsiella*
336 *pneumoniae*. *J Appl Microbiol* 2018 Jul;125(1):84-95.

- 337 [17] Orhan IE, Ozcelik B, Kan Y, Kartal M. Inhibitory effects of various essential oils and
338 individual components against extended-spectrum beta-lactamase (ESBL) produced by
339 *Klebsiella pneumoniae* and their chemical compositions. J Food Sci 2011 Oct; 76(8): M538-
340 46.
- 341 [18] Bedenić B, Zujčić-Atalić V, Jajić I, Djuras-Cuculić B, Godić-Torkar K, Vraneš J, Zarfel
342 G, Grisold A. [Clonal spread of *Klebsiella pneumoniae* producing KPC-2 beta-lactamase in
343 Croatian University Hospital](#). Journal of Chemotherapy 2015, 27(4): 241-245.
- 344 [19] Rosato A, Piarulli M, Corbo F, Muraglia M, Carone A, Vitali ME, Vitali C. In vitro
345 synergistic antibacterial action of certain combinations of gentamicin and essential oils. Curr
346 Med Chem 2010; 17(28): 3289-95.
- 347 [20] Fournomiti M, Kimbaris A, Mantzourani I, Plessas S, Theodoridou I, Papaemmanouil V,
348 Kapsiotis I, Panopoulou M, Stavropoulou E, Bezirtzoglou EE, Alexopoulos A. Antimicrobial
349 activity of essential oils of cultivated oregano (*Origanum vulgare*), sage (*Salvia officinalis*),
350 and thyme (*Thymus vulgaris*) against clinical isolates of *Escherichia coli*, *Klebsiella oxytoca*,
351 and *Klebsiella pneumoniae*. Microb Ecol Health Dis 2015; 26:23289.
- 352 [21] Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present
353 Status and Future Perspectives. Medicines (Basel) 2017; 4 (3):58.