Advances in Sample Preparation 7 (2023) 100072

Contents lists available at ScienceDirect

Advances in Sample Preparation

journal homepage: www.elsevier.com/locate/sampre

Antibacterial activity and antibiotic-modifying action of carvacrol against multidrug-resistant bacteria

Ana Raquel Pereira da Silva[a], Maria do Socorro Costa[a], Nara Juliana Santos Araújo[b,f], Thiago Sampaio de Freitas[c], Antônia Thassya Lucas dos Santos[c], Sheila Alves Gonçalves[d], Viviane Bezerra da Silva[e], Jacqueline Cosmo Andrade-Pinheiro[f,*], Catarina Martins Tahim[g], Elaine Cristina Pereira Lucetti[g], Henrique Douglas Melo Coutinho[c,d,*]

[a] Graduate Program in Biotechnology, Universidade Estadual do Ceará - UECE, Fortaleza 60714-903, Brazil
[b] Graduate Program in Health Sciences, Universidade Federal do Cariri - UFCA, Barbalha 63180-000, Brazil
[c] Department of Biological Chemistry, Universidade Regional do Cariri - URCA, Crato 63105-000, Brazil
[d] Laboratory of Microbiology and Molecular Biology - LMBM, Universidade Regional do Cariri - URCA, 63105-000, Brazil
[e] Department of Botany, Universidade Federal de Pernambuco - UFPE, Recife 50670-901, Brazil
[f] Laboratory Applied Microbiology - LAMAP, Universidade Federal do Cariri - UFCA, Brejo Santo CEP 63260-000, Brazil
[g] CECAPE College. Av. Padre Cícero, 3917, Juazeiro do Norte, São José, CE, 63024-015, Brazil

ARTICLE INFO

Keywords:
Antibacterial activity
Monoterpene
Antibiotic
Synergism

ABSTRACT

Bacterial resistance is a serious health problem, which is mostly caused by the inappropriate use of antibiotics. The emergence of strains resistant to these drugs results in difficulties in the treatment of bacterial infections. Therefore, it has been a trend to investigate new alternatives against microbial resistance such as the use of natural products and their major compounds. The monoterpene carvacrol which is mostly extracted from essential oils has been pointed as a promisor. In the search for better results in terms of pharmaceutical applications of associations and complexes, β-cyclodextrin has been promising when complexed with isolated compounds. This study aimed to evaluate the antibacterial activity of the compound carvacrol, pure and complexed with β-cyclodextrin, against the strains of *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*. The antibacterial activity assay was performed using the broth microdilution method, determining the minimum inhibitory concentration (MIC), and from the subinhibitory concentration value (MIC/8) was evaluated the modifier activity of the antibiotic action. The reading of the tests was carried out using sodium resazurin and expressed by geometric mean. Carvacrol showed significant results against standard bacteria: *S. aureus* with a MIC of 81 μg/mL, and *P. aeruginosa* with a MIC of 161 μg/mL. Also, a MIC of 128 μg/mL for the multidrug-resistant strain of *P. aeruginosa*. Regarding the modifier effect on antibiotic action when associated with gentamicin, complexed carvacrol showed synergism against *E. coli*, with a reduction in the MIC from 25 μg/mL to 5 μg/mL. Carvacrol demonstrated relevant clinical antibacterial activity, for both gram-positive and gram-negative bacteria, and a synergistic effect when associated with gentamicin. Therefore, these results showed to be promising in fighting bacterial resistance.

1. Introduction

Bacterial resistance to conventional antibiotic drugs is a matter of worldwide concern. In this context, the search for new antimicrobial agents that can help or enhance the effect of the conventional drugs already used in the treatment of bacterial infections has been the subject of research. Natural products, mostly of vegetal origin, are often investigated in an attempt to discover new pharmacological properties, which can represent a better option for controlling microbial resistance [1–3].

Essential oils are secondary metabolites extracted from different parts of medicinal plants. They have in their composition a set of chemical compounds, mainly terpenes, and these compounds can act individually or synergistically to improve the therapeutic efficacy of other drugs, thus being very important in pharmacological studies [4]. There is a wide interest in the use of the major compounds of essential oils, due to their antimicrobial and antioxidant properties [5–7].

Isolated compounds from natural products can display significant and promising antimicrobial activities, due to their biochemical features, or have them expressed when in combination with other ther-

* Corresponding authors.
E-mail address: hdmcoutinho@gmail.com (H.D.M. Coutinho).

https://doi.org/10.1016/j.sampre.2023.100072
Received 3 April 2023; Received in revised form 22 May 2023; Accepted 22 May 2023

Table 1
Determination of the minimum inhibitory concentration (MIC μg/mL).

Bacteria	S.A ATCC 25,923	P.A ATCC 9027	E.C ATCC 25,922	S.A 10	P.A 24	E.C 06
Carvacrol	81	161	256	256	128	256
Carvacrol /β-CD	≥ 1024	≥ 1024	≥ 1024	≥ 1024	≥ 1024	≥ 1024
β-cyclodextrin	≥ 1024	≥ 1024	≥ 1024	≥ 1024	≥ 1024	≥ 1024

Abbreviations: S.A: *Staphylococcus aureus;* P.A: *Pseudomonas aeruginosa,* E.C: *E. coli,* Carvacrol /β- CD: Carvacrol β- cyclodextrin complexes.

apeutic antibiotics drugs. Also, it must be considered that the molecular diversity of plant-derived compounds is greater than that originated from synthetic standard antibiotics [1,8,9].

Carvacrol is an active pharmaceutical monoterpene, and a primary compound in several essential oils, being one of the most active antioxidants [10,11]. Therefore, the interest in conducting the present study is based on the pharmacological activities already reported in previous studies, in which carvacrol demonstrated antimicrobial [12], antioxidant [13], antinociceptive [14], and anti-inflammatory activities [15], thus presenting interesting bioactivity for use in the pharmaceutical industry.

This study aimed to evaluate the antibacterial activity of carvacrol isolated and in complexes forms, determining the minimum inhibitory concentration (MIC), both isolated or complexed with standard antibiotics.

2. Results and discussion

2.1. Antibacterial activity

The minimum inhibitory concentration (MIC) values showed that most of the substances tested did not display direct antibacterial activity on the strains evaluated. MIC values were greater than or equal to 1.024 μg/mL, for both, standard and multiresistant bacteria. This concentration is considered clinically irrelevant. However, isolated carvacrol showed significant results against the standard bacteria *S. aureus* ATCC 25,923 (MIC of 81 μg/mL), *P. aeruginosa* ATCC 9027 (MIC of 161 μg/mL), and the multiresistant strain *P. aeruginosa* 24 (MIC of 128 μg/mL). Against other strains, *E. coli* ATCC 25,922, *E. coli* 06 and *S. aureus* 10, carvacrol displayed a MIC of 256 μg/mL, as shown in Table 1.

Therefore, these results corroborate the findings described in the literature regarding the antibacterial effect of carvacrol [16–19].It has been reported antibacterial activity of essential oils against clinically relevant pathogenic agents, this being linked to the antibacterial activity of their major compounds.

The lowest MIC presented was observed for the bacteria *S. aureus* ATCC 25,923, corroborating previous findings regarding the antibacterial activity of the essential oils of *Origanum vulgare* and *Lippia sidoides* [20–23]. The compound carvacrol is reported to be more effective against gram-positive bacteria than gram-negative [24,25]. This effect against gram-positive bacteria is due to the differences in the structure of the cell wall and membrane of these types of bacteria, which makes gram-positive species more sensitive to the action of compounds present in the essential oils [26]. On the other hand, gram-negative bacteria display cell membranes with a differentiated nature which restricts the absorption of molecules moving into the cells through porin channels [27].

Regarding carvacrol in complexed form, and pure β-Cyclodextrin, they did not show any detectable antimicrobial effect (MIC ≥ 1.024 μg/mL) for the bacteria strains tested. Similar results were found concerning the non-antimicrobial activity of β-cyclodextrin against the strains of *S. aureus, P. aeruginosa* and *E. coli* [28,29]. However, it has been reported that β-cyclodextrin can increase solubility, and improve adherence to the bacterial cell wall [30,31]. This differentiation can be associated with the fact that the complexation of compounds results in structural changes in the molecules, and also in different physico-chemical properties, which explains the different results involving the complexes [28].

2.2. Modifier effect on antibiotic action

Under the current scenario of increasing antibiotic resistance, it has been a trend in the development of studies combining synthetic drugs with natural products, aiming to understand their combined actions, which can potentialize antibiotic activity or reduce microbial resistance [1].

Carvacrol/β-CD demonstrated antibacterial synergism against *E. coli* 06, with a reduction in MIC from 25 μg/mL to 5 μg/mL, when associated with gentamicin Fig. 1. This effect may be due to the lipophilic nature of terpenes which increases the influx of antibiotics into the cell by changing the permeability of the cell membrane [32,33]. The study of La Storia et al. [34] indeed proved that carvacrol affects the membrane of bacteria. According to Xu et al. [19], the antibacterial activity against *E. coli* 06 is attributed to the ability of this compound to permeabilize and depolarize its cytoplasmic membrane.

The association of carvacrol with gentamicin against *P. aeruginosa* 24 and *S. aureus* 10 was expressed by an antagonistic effect, with a high increase in the MIC Fig. 1. This result was characterized by a decrease in the biological activity of the combined constituents [35]. Freitas et al. [36] evaluated the antimicrobial activity of the compound carvacrol in association with gentamicin against the strains of *S. aureus* ATCC 25,923 and *P. aeruginosa* ATCC 15,442, and the results showed no difference compared to the control, which was evidenced by the absence of synergism or antagonism against the tested strains.

Carvacrol/β-CD complexes, as well as β-CD isolated, did not show any synergic effect when compared to the control, except for *E. coli* 06 Fig. 1. This effect observed in both carvacrol/β-CD and β-CD may be due to the alterations developed in these compounds when they were complexed, being an indication that the complexation process can alter the structure and physical-chemical interactions between compound-antibiotic [28,29].

According to Fig. 2, the results observed for *P. aeruginosa* 24 proved to be antagonistic, that is, this association of carvacrol complex and the isolated β-CD with norfloxacin led to an increase in the MIC from 6.34 μg/mL to 128 μg/mL, 40.37 μg/mL and 64 μg/mL respectively. These results indicated that β-CD did not improve antibiotic activity, similar results were reported in other investigations by Freitas [8] and Oliveira et al. [29].

The association between carvacrol with norfloxacin against *S. aureus* 10 also demonstrated antagonism, evidenced by the increase in the MIC from 161 μg/mL to 322.54 μg/mL. Bahmani et al. [37] evaluated carvacrol in association with norfloxacin against the strain *S. aureus* ATCC 12,600, and the results were not different from the observed for the control, characterized by the absence of synergism against the tested strain. Evaluating the complexed carvacrol and isolated β-CD in association with the antibiotic, both displayed a reduction in the MIC from 161 μg/mL to 128 μg/mL. When comparing only the results of isolated β-CD, it corroborates with the findings reported by Oliveira et al. [29] and Costa et al. [9].

Evaluating the effects against *E. coli* 06, the combination of substances combined with norfloxacin was antagonistic, however, when we compared the isolated carvacrol to the complexed one, we observed a decrease in the MIC from 102 μg/mL to 50.79 μg/mL, respectively

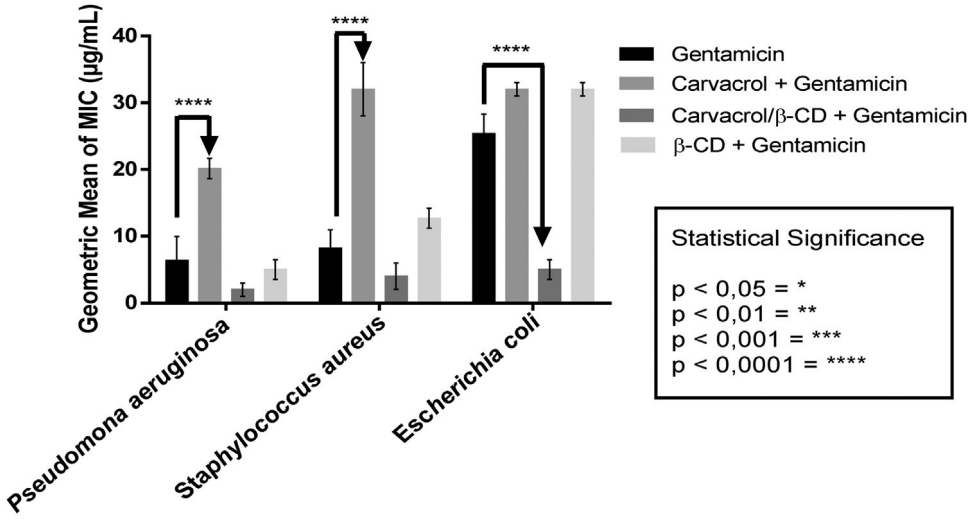

Fig. 1. Modulation effect of carvacrol, complex: carvacrol/β-CD, and β-cyclodextrin: β-CD in association with gentamicin against *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* 10 and *Escherichia coli* 06. Control refers to antibiotic-gentamicin. **** value statistically significant when $p < 0.0001$; not statistically significant when $p > 0.05$.

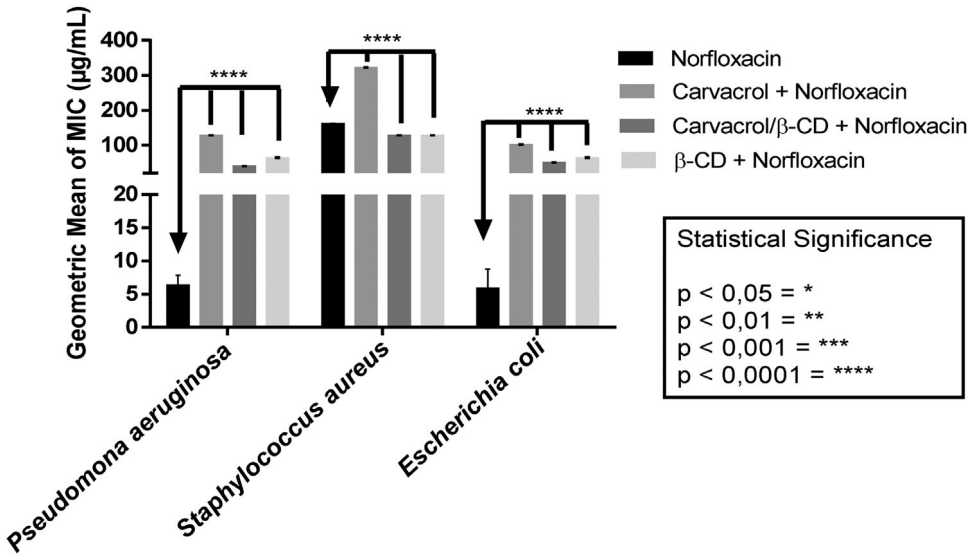

Fig. 2. Modulation effect of carvacrol, complex: carvacrol/β-CD, and β-cyclodextrin: β-CD in association with norfloxacin against *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* 10 and *Escherichia coli* 06. Control refers to the antibiotic-norfloxacin. **** value statistically significant when $p < 0.0001$; not statistically significant when $p > 0.05$.

Fig. 2. This decrease in the complexed substance is related to the action of carvacrol in increasing the permeability of the outer membrane of gram-negative bacteria, also the increase in the interaction capacity of β-cyclodextrin [34,38,39]. When analyzing isolated carvacrol, the results corroborate the study by Lima et al. [18] in which they evaluated carvacrol bacteriostatic action against *E. coli* ESBL strains, where the MIC was found to be 128 µg/mL against the strains C-18, C-20, C-21, C-24, C-25 and 24.

Regarding the antagonistic effect of the substances when associated with the antibiotics, it can be explained by the binding of the compound in the place where the antibiotic would bind, thus having a possible site competition. Also, there is the possibility of chelation of the antibiotic by the compound, which would lead to a decrease in the spectrum of action of the drug [1,29].

Carvacrol in association with imipenem tested against *E. coli* 06 also showed antagonism, with an increase in the MIC from 6.85 μg/mL to 16 μg/mL Fig. 3. While against other bacteria and comparisons, it did not show any significant activity (p>0.05). This is the first study reporting the antimicrobial activity of carvacrol complexed with β-cyclodextrin for improving the antibiotic action, which was based on statistical comparisons of the antagonistic or synergistic activities. However, there are other studies regarding the antimicrobial activity of carvacrol, such as the inhibition of biofilm formation [40] and the evaluation of the antibacterial efficacy by the disk-diffusion method [41].

3. Material and methods

3.1. Antibiotics and chemicals

The antibiotics used in the test were gentamicin, norfloxacin and imipenem (Sigma Co., St. Louis, USA). These antibiotics were dissolved in sterilized distilled water at a concentration of 1.024 μg/mL. Regarding the compounds used in the test, they were weighed 10 mg and subsequently dissolved in 1 mL of dimethylsulfoxide (DMSO) and sterilized distilled water to reach the concentration of 1.024 μg/mL. Test readings were proceeded using sodium resazurin reagent (Sigma - Aldrich, St. Louis, MO), which was used as a colorimetric indicator of bacterial growth by redox.

Carvacrol was provided by the Laboratory of Pharmacology and Molecular Chemistry (LFQM) of the Universidade Regional do Cariri (URCA). β-cyclodextrin was obtained from Sigma-Aldrich® (USA), and the carvacrol/β-cyclodextrin complex was prepared at the Pharmacy Laboratory of the Universidade Federal de Sergipe (UFS).

3.2. Microbiological culture media

During the tests, the following microbiological culture media were used: heart infusion agar (HIA, Difco laboratories Ltda.) prepared according to the manufacturer; and brain heart infusion broth (BHI Acu-

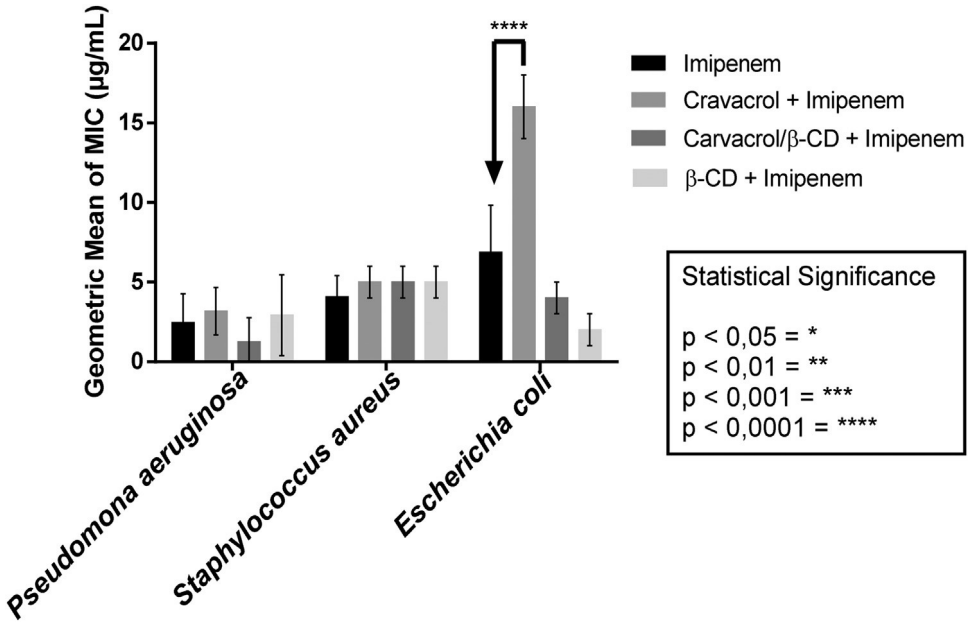

Fig. 3. Modulation effect of carvacrol, complex: carvacrol/β-CD, and β-cyclodextrin: β-CD in association with imipenem against *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* 10 and *Escherichia coli* 06. Control refers to the antibiotic-imipenem. **** value statistically significant when $p < 0.0001$; not statistically significant when $p > 0.05$.

media Manufacturers Inc.), which was prepared at the concentration of 10%. The bacterial strains that were kept under refrigeration at 4 °C before the tests, were previously cultivated in HIA. Subsequently, they were incubated in a bacteriological incubator at the temperature of 37 °C for 24 h before the beginning of the experiments.

3.3. Microbial strains

The microorganisms used in the tests came from the Laboratory of Microbiology and Molecular Biology (LMBM) of the Universidade Regional do Cariri (URCA). The standard strains used were: *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25,923; and the multiresistant strains: *E. coli* 06, *Pseudomonas aeruginosa* 24 and *Staphylococcus aureus* 10.

3.4. Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) test was performed using the CLSI broth microdilution method [42] with modifications. Using a nickel-chromium inoculation loop, the bacterial strains were suspended in test tubes containing 3 mL of sterile saline solution (0.9%). For the standardization of the prepared inoculums, they were compared using the Mcfarland scale (10^5 CFU).

Following, *eppendorfs*® containing 1.350 µL of liquid BHI culture medium and 150 µL of bacterial inoculum were prepared, resulting in a final volume of 1.5 mL, subsequently, 96-well microdilution plates were filled with 100 µL. Serial microdilutions were performed, where 100 µL of each solution was added in the first well was diluted up to the penultimate well 1:1, and the last well of each plate was used as control for microbial growth. And a control was also performed to verify the sterility of the liquid BHI culture medium. Compound concentrations ranged from 512 μg/mL to 4 μg/mL.

The plates were incubated in a microbial incubator for 24 h at a temperature of 37 °C. After this period, MIC readings were performed, where 20 µL of sodium resazurin solution was added to the wells of the plate, the readings were performed one hour later. Based on the oxidation–reduction reaction at room temperature, it was observed the changes in color from blue to pink, as indicative of bacterial growth. MIC was defined as the lowest concentration capable of inhibiting microbial growth. The tests were performed only once in triplicate.

3.5. Evaluation of modifier effect on antibiotic action

To verify the effect of the compounds combined with antibiotics against multidrug-resistant bacteria, it was used the method proposed by Coutinho et al. [43], where the compounds tested were evaluated in subinhibitory concentration (MIC/8). These tests were also performed in triplicate.

Eppendorfs containing carvacrol, carvacrol/β-CD and β-cyclodextrin at concentrations of 16 µg/mL, 32 µg/mL and 128 µg/mL were prepared. And suspensions of 10^5 CFU/mL of the microorganisms were deposited together with liquid BHI culture medium. Control was prepared for the antibiotics (gentamicin, norfloxacin and imipenem) with the same amount of bacterial inoculum corresponding to the 10% volume of the eppendorf and 1350 µL of BHI medium. 96-well microdilution plates were filled with 100 µL of the prepared solutions. Then 100 μL of each antibiotic was added at concentration of 1024 μg/mL and serial microdilutions were performed at 1:1 ratio, ranging from 512 μg/mL in the first well to 0.5 μg/mL in the penultimate well, with the last well of each plate being reserved for the positive control of the test.

3.6. Statistical analyses

The tests were performed in triplicates, and the statistical analyzes were run using the two-way ANOVA test, followed by the post hoc Bonferroni test, where the results were considered significant when $p \langle 0.05$, $p < 0{,}0001$, and not significant when $p \rangle 0.05$. The results were expressed as mean ± standard deviation, using the GraphPad Prism software, version 5.0.

4. Conclusions

Based on the results of this trial, isolated carvacrol showed direct antibacterial activity against both gram-positive and gram-negative strains, however, the complex carvacrol/β-cyclodextrin and isolated β-cyclodextrin did not show any direct antibacterial activity against the strains tested. However, when the complex and β-cyclodextrin were tested in association with gentamicin, they showed a synergistic effect against *E. coli* 06. The modifier effect on the antibiotic action by the substances tested in association with norfloxacin and imipenem showed an antagonistic or no effect against the strains tested. Isolated carvacrol displayed a promising effect on antibacterial activity. Complexed carvacrol and isolated β-cyclodextrin showed promising activities in association

with antibiotics against *E. coli*. These results were promising regarding fighting bacterial resistance, however, further studies are needed to better elucidate the mechanism of action of these compounds tested.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] H.D.M. Coutinho, S.M.O. Brito, N.F. Leite, V.C.S. Vandesmet, M.T.A. Oliveira, G.M.A.B. Martins, A.R.P. Silva, M.S. Costa, Avaliação comparativa da modulação de antibióticos, frente às cepas bacterianas de *Escherichia coli, Staphylococcus aureus*, Rev. Cienc. de la Salud 13 (2015) 345–354.

[2] J.M. Arancibia, Estrategias para el uso de antibióticos en pacientes críticos, Rev. méd. Clín. Las Condes 30 (2019) 151–159.

[3] G.D. Freitas, C.P. de Lima, D.F.S. Coelho, M.O. Moraes, G.L. Lima, W.R. Alves, Use of diferente methods to control the development of *Staphylococcus aureus*: a literature review, Res. Soc. Dev 10 (2021).

[4] E.G. Ferrarini, R.S. Paes, G.M. Baldasso, P.M. de Assis, M.C. Gouvêa, P.de Cicco, N.R.B. Raposo, R. Capasso, E.L.G. Moreira, R.C. Dutra, Broad-spectrum *cannabis* oil ameliorates reserpine-induced fibromyalgia model in mice, Biomed. Pharmacotherapy. 154 (2022) 113552.

[5] J.Q. Gonçalves, R.L. Oliveira, R.B. De Sousa, J.E. De Lima Silva, R.L. De Albuquerque, Estudo químico de óleos essenciais, Mostra Cient. Farmácia. 4 (2018).

[6] P.S.P. Costa Júnior, M.A. Ferreira, A.S.R. Bispo, B.B. Vieira, Antimicrobial activity of essential oils of different spices, Hig. Aliment. 33 (2019) 2697–2701.

[7] N.V. Alves, I.P.M. de Castro, L.F.A. Gellen, J.F. Panontin, Potencial farmacológico dos óleos essenciais: uma atualização, in: Práticas Integrativas e Complementares: Visão Holística e Multidisciplinar, Editora Científica Digital, 2022, pp. 144–160.

[8] T.S. Freitas, Atividade Antibacteriana Do óleo essencial Das Folhas De *Hyptis martiusii* Benth e De Seu Complexo De Inclusão Em β–ciclodextrina, Universidade Regional do Cariri, Crato, 2017.

[9] M.D.S. Costa, J.E. Rocha, F.F. Campina, A.R.P. Silva, R.P. da Cruz, R.L.S. Pereira, L.J. Quintans-Júnior, I.R.A. De Menezes, A.A. Adriano, T.S. De Freitas, A.M.R. Teixeira, H.D.M. Coutinho, Comparative analysis of the antibacterial and drug-modulatory effect of D-limonene alone and complexed with β-cyclodextrin, Eur. J. Pharm. Sci. 128 (2019) 158–161.

[10] M. de Vincenzi, A. Stammati, A. de Vincenzi, M. Silano, Constituents of aromatic plants: carvacrol, Fitoterapia 75 (2004) 801–804.

[11] D.H. de Oliveira, A.M. Farias, M.B. Cleff, M.C.A. Meireles, M.R.A. Rodrigues, Caracterização química do óleo essencial de *Origanum vulgare*: análise da relação timol/carvacrol, XVII Congresso de Iniciação Científica, Pelotas, 2008.

[12] G. Klein, C. Rüben, M. Upmann, Antimicrobial activity of essential oil components against potential food spoilage microorganisms, Curr. Microbiol. 67 (2013) 200–208.

[13] S. Kumar, M.M. Mukherjee, M.F. Varela, Modulation of Bacterial Multidrug Resistance Efflux Pumps of the Major Facilitator Superfamily, Int. J. Bacteriol. (2013) 1–15.

[14] F.H. Cavalcante Melo, E.R.V. Rios, N.F.M. Rocha, M.D.C.D.O. Citõ, M.L. Fernandes, D.P. de Sousa, S.M.M. de Vasconcelos, F.C.F. de Sousa, Antinociceptive activity of carvacrol (5-isopropyl-2-methylphenol) in mice, J. Pharm. Pharmacol. 64 (2012) 1722–1729.

[15] P. Landa, L. Kokoska, M. Pribylova, T. Vanek, P. Marsik, In vitro anti-inflammatory activity of carvacrol: inhibitory effect on COX-2 catalyzed prostaglandin E2 biosynthesis, Pharm. Res. 32 (2009) 75–78.

[16] C.H.S. Santos, R.H. Piccoli, V.M.R. Tebaldi, Atividade antimicrobiana de óleos essenciais e compostos isolados frente aos agentes patogênicos de origem clínica e alimentar, Rev. Instituto Adolfo Lutz. 76 (2017) 1–8.

[17] A. Nostro, A.S. Roccaro, G. Bisignano, A. Marino, M.A. Cannatelli, F.C. Pizzimenti, P.L. Cioni, F. Procopio, A.R. Blanco, Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms, J. Med. Microbiol. 56 (2007) 519–523.

[18] D.S. de Lima, J.C. Lima, R.M.C.B. Calvacanti, B.H.C. dos Santos, I.O. Lima, Study of the antibacterial activity of thymol and carvacrol monoterpenes against strains of *Escherichia coli* producing extended-spectrum β-lactamases, Rev. Pan-Amazônica de Saúde. 8 (2017) 17–21.

[19] J. Xu, F. Zhou, B.P. Ji, R.S. Pei, N. Xu, The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*, Lett. Appl. Microbiol. 47 (2008) 174–179.

[20] M.M. Araujo, P.L. Longo, Teste da ação antibacteriana in vitro de óleo essencial comercial de *Origanum vulgare* (orégano) diante das cepas de *Escherichia coli* e *Staphylococcus aureus*, Arq. Inst. Biol. (Sao Paulo) (2016) 83.

[21] J.C. Santos, C.D. Carvalho-Filho, T.F. Barros, A.G. Guimarães, Atividade antimicrobiana in vitro dos óleos essenciais de orégano, alho, cravo e limão sobre bactérias patogênicas isoladas de vôngole, Semina:Cienc. Agrar. 32 (2011) 1557–1564.

[22] A. Alexopoulos, A.C. Kimbaris, S. Plessas, I. Mantzourani, I. Theodoridou, E. Stavropoulou, M.G. Polissiou, E. Bezirtzoglou, Antibacterial activities of essential oils from eight Greek aromatic plants against clinical isolates of *Staphylococcus aureus*, Anaerobe 17 (2011) 399–402.

[23] P.L.D. Lobo, C.S.R. Fonteles, C.B.M. de Carvalho, D.F. do Nascimento, S.G. da Cruz Fonseca, F.V.F. Jamacaru, M.E.A. de Moraes, Dose-response evaluation of a novel essential oil against *Mutans streptococci* in vivo, Phytomedicine 18 (2011) 551–556.

[24] M.D. Pozzo, J. Viégas, D.F. Santurio, L. Rossatto, I.H. Soares, Atividade antimicrobiana de óleos essenciais de condimentos frente a *Staphylococcus* spp isolados de mastite caprina, Cienc. Rural 41 (2011) 667–672.

[25] A.E. Edris, Pharmaceutical and Therapeutic Potentials of Essential Oils and Their Individual Volatile Constituents: a Review, Phytother. Res. 21 (2007) 308–323.

[26] L.S. Araújo, R.S. Araújo, J.L. Serra, A.R. Nascimento, Composição química e susceptibilidade do óleo essencial de óregano (*Origanum vulgare* L., família lamiaceae) frente à cepas de Escherichia *coli*, *Staphylococcus aureus* e *Salmonella choleraesuis*, Bol. Cent. de Pesqui. de Process. de Aliment. 33 (2015) 73–78.

[27] G.J. Tortora, B.R. Funke, C.L. Case, Microbiologia, 10th ed., Artmed, Porto Alegre, 2012.

[28] T.A. Andrade, T.S. Freitas, F.O. Araújo, P.P. Menezes, G.A.A. Dória, A.S. Rabelo, L.J. Quintans-Júnior, M.R.V. Santos, D.P. Bezerra, M.R. Serafini, I.R.A. Menezes, P.S. Nunes, A.A.S. Araújo, M.S. Costa, F.F. Campina, A.T.L. Santos, A.R.P. Silva, H.D.M. Coutinho, Physico-chemical characterization and antibacterial activity of inclusion complexes of *Hyptis martiusii* Benth essential oil in β-cyclodextrin, Biomed. Pharmacotherapy. 89 (2017) 201–207.

[29] F.S. Oliveira, T.S. de Freitas, R.P. da Cruz, M.S. Costa, R.L.S. Pereira, L.J. Quintans-Júnior, T.A. Andrade, P.P. Menezes, B.M.H. de Sousa, P.S. Nunes, M.R. Serafini, I.R.A. de Menezes, A.A.S. Araújo, H.D.M. Coutinho, Evaluation of the antibacterial and modulatory potential of α-bisabolol, β-cyclodextrin and α-bisabolol/β-cyclodextrin complex, Biomed. Pharmacother. 92 (2017) 1111–1118.

[30] J.A.C.N. Junior, L.N. de Almeida, C.M.L. Silva, M.R. Serafini, análise dos pedidos de patentes recentes envolvendo ciclodextrinas no setor farmacêutico analysis of recent patent applications involving cyclodextrins the pharmaceutical industry, Rev. Interdisc. Pesquisa e Inovação. 1 (2015) 1–7.

[31] K.I.R. Teixeira, Estudo Das Alterações Da Membrana Celular De Microrganismos Por Compostos De Inclusão De Clorexidina: Beta-Ciclodextrina Em Diferentes Proporções Molares Usando Microscopia de Força Atômica e Microscopia Eletrônica De Varredura, Dissertação (Mestrado em Odontologia), Universidade Federal de Minas Gerais, Minas Gerais, 2008.

[32] A.J. Maia, K.R.F. Schwan-Estrada, C.M.D.R. Faria, J.S.B. Oliveira, V.A. Jardinetti, B.N. Batista, Óleo essencial de alecrim no controle de doenças e na indução de resistência em videira, Pesqui. Agropecu. Bras. 49 (2014) 330–339.

[33] A.M.F. Ribeiro, Farmacologia Dos Antibióticos aminoglicosídeos, Dissertação (Mestrado em Ciências Farmacêuticas), Universidade Fernando Pessoa, Porto-Portugal, 2017.

[34] A.La Storia, D. Ercolini, F. Marinello, R. Di Pasqua, F. Villani, G. Mauriello, Atomic force microscopy analysis shows surface structure changes in carvacrol-treated bacterial cells, Res. Microbiol. 162 (2011) 164–172.

[35] N.G. Ntalli, F. Ferrari, I. Giannakou, U. Menkissoglu-Spiroudi, Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece, Pest Manag. Sci. 67 (2011) 341–351.

[36] M.A. Freitas, J.C. Andrade, G.M.M. Guedes, S.R. Tintino, C.E.S. Souza, N.F. Leite, C.N.F.L. Gondim, M.F.B. Morais-Braga, E.F.F. Matias, H.D.M. Coutinho, avaliação in vitro da atividade antimicrobiana do carvacrol através dos métodos de contato direto e gasoso evaluation in vitro of antimicrobial activity of carvacrol by the direct and gaseous contact methods, Bioscience J. 29 (2013) 781–786.

[37] M. Bahmani, M. Taherikalani, M. Khaksarian, S. Soroush, B. Ashrafi, R. Heydari, Phytochemical profiles and antibacterial activities of *Origanum vulgare* and *Hypericum perforatum* and carvacrol and hypericin as a promising anti-*Staphylococcus aureus*, Mini. Rev. Med. Chem. 19 (2019) 923–932.

[38] M. Hyldgaard, T. Mygind, R.L. Meyer, Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components, Front. Microbiol. 3 (2012).

[39] P.V. da Silva, Avaliação Da Atividade Antimicrobiana De Compostos de Inclusão Formados Entre Sulfadiazina De Sódio e Ciclodextrinas, Dissertação (Mestrado em Materiais Para Engenharia), Universidade Federal de Itajubá, Minas Gerais, 2015.

[40] P. Raei, M.Y. Memar, M. Aghamali, M. Asgharzadeh, T. Pourlak, N. Alizadeh, E. Zeinalzadeh, H.S. Kafil, Thymol and carvacrol strongly inhibit biofilm formation and growth of carbapenemase-producing Gram negative bacilli, Cell. Mol. Biol. (Noisy-Le-Grand) 63 (2017) 108–112.

[41] Y. El Atki, I. Aouam, F. El, A. Taroq, A. Gourch, B. Lyoussi, A. Abdellaoui, Antibacterial efficacy of Thymol, Carvacrol, Eugenol and Menthol as alternative agents to control the growth of nosocomial infection-bacteria, J. Pharm. Sci. Res. 11 (2019) 306–309.

[42] CLSI, in: Performance Standards for Antimicrobial Susceptibility Teating; Twenty-Second Informational Supplement. I document. M100-S16CLSI, Clinical and Laboratory Standards Institute, Wayne, PA:NIH, 2015, p. 184.

[43] H.D.M. Coutinho, J.G.M. Costa, O. Lima, V.S. Falcão-Silva, J.P. Siqueira-Júnior, Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L and chlorpromazine, Chemotherapy 54 (2008) 328–330.