www.nclinmed.com
Novelty in Clinical Medicine. 2022; 1(4): 192-196
https://doi.org/10.22034/NCM.2022.350997.1051

Research Article Open Access

Antibacterial activity of modified Carvacrol against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*

Mona Ghods Elahi [1], **Malak Hekmati** [1*], **Davoud Esmaeili** [2*], **Parisa Ziarati** [3], **Mohamad Yousefi** [4]

[1] Department of Organic Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[2] Department of Microbiology and Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[3] Department of Medicinal Chemistry, Pharmacy Faculty, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[4] Department of Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

[*] **Corresponding author:** Davoud Esmaeili, Department of Microbiology and Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. **Email:** esm114@gmail.com

Received: 9 July 2022 **Revised:** 7 August 2022 **Accepted:** 10 August 2022 **e-Published:** 18 August 2022

Abstract

Background: Carvacrol, a prominent phenolic compound found in Satureja and thyme, possesses a wide range of beneficial properties, including antibacterial, antifungal, anti-inflammatory, and anti-cancer activities.

Objectives: This study aimed to determine the antibacterial effects of peptide-modified carvacrol on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

Methods: Using disk diffusion and MIC methods, the antibacterial activity of peptide-modified carvacrol against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* was evaluated.

Results: The modified carvacrol demonstrated growth inhibition diameters of 25mm and 20mm against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* using the disk diffusion method. The minimum inhibitory concentration (MIC) against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* was 1 g/ml and 0.51 g/ml, respectively.

Conclusion: Peptide-modified carvacrol displays antibacterial effects.

Keywords: Antibacterial, Modified carvacrol, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*.

Introduction

Carvacrol, with the chemical formula $C_{10}H_{14}O$, is a chemical compound with a molar mass of 150.217 g/mol. Carvacrol is the main compound found in plants such as marjoram, thyme, and wild bergamot, and the beneficial properties of these plants are due to the presence of this compound. Carvacrol has numerous therapeutic properties.[1] Carvacrol is a phenolic monoterpene found largely in oregano essential oil, one of the key constituents of EO, with an antibacterial action against various bacteria.[1,2] This substance is present in the essential oils of thyme oil, coconut oil, and wild coconut. Thyme essential oil contains 5 to 75% carvacrol. Thyme is a member of the mint family, which is considered an evergreen perennial aromatic medicinal plant.

Carvacrol and thymol are two important phenolic compounds found in the thyme plant.[3,4] These compounds are known for their strong antioxidant and anti-inflammatory properties.[5]

This substance has excellent antimicrobial properties, making it an appropriate choice for use in cosmetics, food preservatives, and some other medicines. One interesting fact is that pure carvacrol has only around half the ability to destroy pathogens as pure oregano oil, according to laboratory tests.[6,7]

Although antibiotics are the main way to control infection, one of the most worrying features of Pseudomonas aeruginosa and Staphylococcus epidermidis is their low antibiotic sensitivity. The prevalence of resistance to these bacteria is increasing, and it is

considered one of the major public health problems worldwide, as it raises some other side effects, complications, mortality, and increasing limitations of therapeutic use, as well as significant economic costs.[8,9]

Natural products in traditional medicine play an important role in the treatment of infectious diseases.[10] Given the importance of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* in burns, pneumonia, urinary tract infections, and meningitis, as well as their high resistance to drugs and disinfectants and the potential threat to patients with immune system defects, it is critical to discover new compounds and find complementary treatments.

The use of medicinal plants for the treatment of diseases has been common since ancient times. With the prosperity of urban life and the increase in population, the consumption of medicinal plants has gradually decreased, and synthetic drugs have replaced them.[1-3]

As medicinal plants are widely distributed in Iran, the study of these plants and their antimicrobial properties provides some scientific evidence for greater use of them. Additionally, this can reduce the use of chemical drugs and their side effects.[1]

Thymol and carvacrol are the main compounds of essential oils in the mint family. These two compounds are chemically very similar, with only the position of the hydroxyl group differing between them. Thymol and carvacrol are highly effective antimicrobial components in essential oils. Their antimicrobial effect is due to their permeabilization of the cell membrane, which can bind to cations on the surface of the membrane and disrupt vital activities.[1,5,7,9]

Carvacrol is an isomer of thymol. This substance is an edible plant extract that is insoluble in water but soluble in alcohol and ether. This substance is present in the structure of vegetable edible oils such as oregano oil, which are used as food seasonings.[11] Carvacrol has a wide range of antimicrobial effects. The substance in question inhibits ATPase activity and increases the non-specific permeability of the bacterial cell membrane, not only inhibiting the microbial population but also rendering it susceptible and vulnerable to other antibacterial agents.[1,12]

Objectives

In this study, the effects of modified carvacrol on two important pathogens, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, were investigated.

Methods

Investigating the antibacterial effect of modified carvacrol by disk diffusion method

The antibacterial effect of modified carvacrol was investigated using the disk diffusion method. On sterile swabs, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were cultured in Mueller-Hinton agar medium at half McFarland density. Sterile blank discs with a diameter of 6 mm and a concentration of 0.5 µg/ml were then impregnated and dried using sterile forceps. The discs were placed in Mueller-Hinton agar medium containing bacteria and incubated for 24 hours at 37 ^{oc}.[13]

Determination of MIC of modified carvacrol essential oil by broth microdilution method

To determine the MIC, 2 mL of Mueller-Hinton broth was added to 10 tubes. Concentrations of 32, 16, 8, 4, 2, 1, 0.5, and 0.25 micrograms of modified carvacrol were added to each tube, and the final concentration of bacteria in each tube was 1 x 106 CFU/mL. The ninth and tenth tubes were positive and negative controls, respectively. The tubes were incubated for 24 hours at 37°C, and the first concentration that inhibited bacterial growth was considered the MIC.[13]

Results

Disk diffusion test with modified carvacrol

Carvacrol's antibiotic property was confirmed using blank discs impregnated with 20 µg of modified Carvacrol, and the growth inhibition zone for each bacterial species was recorded [Figure 1] [Figure 2].

MIC determination of modified carvacrol against pathogens

The MIC for *Staphylococcus epidermidis* was 0.5 µg/mL and for *Pseudomonas aeruginosa* was 1 µg/mL. The growth inhibition zone for *Staphylococcus epidermidis* was reported to be 25 mm, and for *Pseudomonas aeruginosa*, it was 20 mm [Figure 3].

Figure 1. The diffusion disc containing modified carvacol demonstrates inhibitory properties against *Staphylococcus epidermidis* (25 mm)

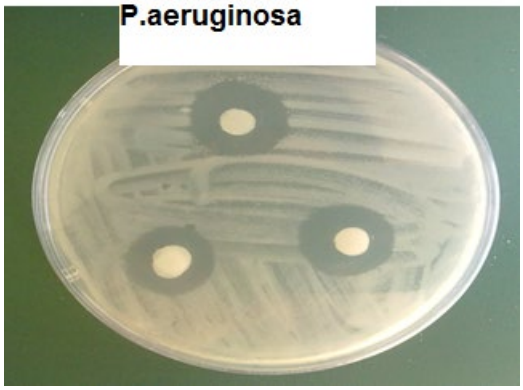

Figure 2. The diffusion disc with modified carvacol shows that it has inhibitory properties against *Pseudomonas aeruginosa* (20 mm)

Figure 3. MIC results of modified carvacol against *Staphylococcus epidermidis* (0.5 µg/ml) and *Pseudomonas aeruginosa* (1 µg/ml)

Discussion

Carvacrol is one of the most effective compounds of *Satureja khuzestanica*. Carvacrol has antimicrobial and anti-aging properties. In this research, a modified form of carvacrol that is fused with a peptide was used for this purpose.[1]

The investigation revealed that the modified carvacrol possesses antibacterial properties against both gram-positive and gram-negative bacteria. *Staphylococcus epidermidis*, a gram-positive coccus and a natural inhabitant of the skin, plays a significant role in various infections such as wounds, surgical sites, the urinary tract, and meningitis. Additionally, it contributes to the occurrence of infections in prosthetic devices. Hence, it is crucial to diminish or eradicate this microorganism from the skin surface before and after medical procedures. On the other hand, *Pseudomonas aeruginosa*, a gram-negative bacillus, exhibits high resistance to antibiotics and is a prevalent cause of wound and burn infections. This bacterium can cause infections in the skin, meninges, lungs, urinary tract, and ears. Consequently, it is imperative to explore complementary medicinal approaches to prevent, treat, and mitigate antibiotic resistance against this bacterium.[1,4]

Carvacrol is a monoterpenoid phenol used as a flavoring agent and intermediate in the production of drugs, fungicides, insecticides, and germicides. Supercritical fluid extraction (SFE) of plant essential oils and isopropylation of o-cresol with propylene over activated alumina at 633 K and 50 bars are the two main methods of carvacrol production. The antimicrobial effect of carvacrol and thymol is due to their capacity to permeate the cell membrane and bind to the cations on the surface of the pathogen's membrane, disrupting their critical functions. Moreover, it exhibits antimicrobial properties through its interaction with the membrane of microorganisms, resulting in alterations in the permeability of certain compounds like potassium and hydrogen. Within the culture medium, the inclusion of thyme and Satureja essential oils, along with carvacrol and thymol substances, restricts bacterial proliferation by 6.5, 7, and 6 mm, respectively.[1-3]

Nakamura et al., investigated thyme, its derivatives, and the combination of carvacrol and the anti-tuberculosis

drug rifampicin. It was found that the combination of carvacrol and rifampicin has a synergistic effect against tuberculosis. Therefore, it can be concluded that carvacrol is a substance with anti-tuberculosis activity that has a synergistic effect when combined with rifampicin.[14] In research conducted by Ultee et al., carvacrol has been shown to have both bactericidal and bacteriostatic properties.[15] Amiri et al., investigated the antibacterial effect of Satureja khusestanica essential oil on respiratory tract infectious bacteria. The results demonstrated that the essential oil had a significant antibacterial effect on these bacteria.[16] In another study, the antimicrobial activities of Satureja khuzestanica against *Candida albicans*, E. coli, Staphylococcus epidermidis, and Bacillus cereus were investigated using two methods: disk diffusion and microbroth dilution. The highest activity was observed against *Candida albicans*, and the lowest was observed against *Staphylococcus epidermidis* and E. coli, with a MIC of 0.1-0.19 μg/ml.[17] In the research of Moradi et al., the antibacterial properties of *Satureja khuzestanica* were compared with antibiotic discs, and it was shown that the zone of growth inhibition in *Pseudomonas aeruginosa* varied from 23 mm to 26 mm.[7] In the research of Esmaeili et al., the inhibitory effect of *Satureja khuzestanica* against Pseudomonas aeruginosa was investigated. The MIC concentration of *Satureja khuzestanica* essential oil showed gene inhibition activity.[6]

In this context, researchers found that carvacrol, cinnamaldehyde, and thymol had the most potent antibacterial action against *Staphylococcus aureus* and *Escherichia coli*, which is accomplished via modifying membrane permeability. In the present study, it was also found that the modified carvacrol has an inhibitory effect against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*.

Conclusions

The findings demonstrated that new compounds with enhanced antibacterial properties can be synthesized by modifying carvacrol. Peptide-modified carvacrol exhibited antibacterial activity against both gram-positive and gram-negative bacteria.

Acknowledgment

The paper is the result of a thesis submitted in partial fulfillment of the requirements for the degree of Ph.D. The authors thank the Department of Microbiology and Applied Virology Research Center of Baqiyatallah University of Medical Sciences, Tehran, Iran for their cooperation and support.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

MIC: *minimum* inhibitory concentration;

DDM: Disk diffusion method;

Supercritical fluid extraction: SFE.

Authors' contributions

MGE drafted the project proposal. MGE and DE performed the experiments and data analysis. MGE and MH interpreted the data analysis in the manuscript. PZ and MY contributed to data collection and tests. MH contributed to statistical analysis. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

None.

Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

References

1. Elahi M, Hekmati M, Esmaeili D, Ziarati P, Yousefi M. Evaluation

and efficacy modified carvacrol and anti-cancer peptide against cell line gastric AGS. Int J Pept Res Ther. 2022;28(4):1-3. doi:10.1007/s10989-022-10426-w
2. Davoodi M, Nejad-Ebrahimi S, Rustaiyan A, Esmaeili D. The chemical composition and antibacterial activity of a methanolic extract of Satureja khuzistanica. Braz J Pharm Sci. 2022;58, e192033 doi:10.31579/2640-1053/004/
3. Mohammadi-Ziveh Z, Mirhosseini SA, Hosseini HM. Satureja khuzestanica mediated synthesis of silver nanoparticles and its evaluation of antineoplastic activity to combat colorectal cancer cell line. Iran J Pharm Res. 2020;19(4):169.
4. Meskini M, Ghorbanalizadegan M, Esmaeili D, Importance of Herbal Ointment for Pseudomonas aeruginosa infection in burn patients. Int J Med Invest. 2015; 4(2); 257-261
5. Baban Zadeh B, Esmaeili D, Moradi F, Mashayekh F. The Study of Satureja khuzestanica Essence Inhibitory Effects against Housekeeping Gene DNA Gyrase A and T3ss and T2ss of Pseudomonas aeruginosa with RT-PCR Technique. J Pure Appl Microbiol. 2015; 9(1); 491-495.
6. Bahador A, Saghi H, Ataee R, Kachoei K, Esmaeili D. The Study of Inhibition Effects Satureja khuzestaniea Essence against Gene Expression bap Acinetobacter baumannii with Real-time PCR Technique. Iran J Med Microbiol. 2015;9 (1)
7. Moradi F, Imani Fooladi AA, Bahador A, Satarzadeh TM. The Study of Resistant Genes of aac(6/)-I, aac(3)-III and Integron Class 1 of MDR Pseudomonas aeruginosa in Tehran Hospital. Sch J Appl Med Sci. 2015; 3(4B):1698-1702
8. Iman Islamieh D, Esmaeili D, Goudarzi H, Moradi F. Applicability of Genotypic and Phenotypic Methods to the Assessment of the SKEO Effect on MexXY-OprM and MexEF-OprN Efflux Pumps in MDR and Clinical Isolates of Pseudomonas aeruginosa. J Pure Appl Microbiol. 2015; 9(2); 377-384.
9. Jalalvandi N, Bahador A, Zahedi B, Saghi H, Esmaeili D. The study of inhibitory effects of satureja khuzestanica essence against mexa and mexr efflux genes of pseudomonas aeruginosa by RT-PCR. Int J Biotechnol. 2015; 4(1):1-8. doi:10.18488/journal.57/2015.4.1/57.1.1.8
10. Jalalvand N, Esmaeili D, Raiszadeh M, Naeimi S. Evaluation of physicochemical activity of anticancer fusion proteins; enterocin A-R type pyocin-lactocin-ligand against gastric cancer cell line by real-time RT PCR technique. Int J Pept Res Ther.2021;27(2):1167-75 doi:10.1007/s10989-021-10158-3
11. Abbasi A, Bahador A, Esmaeili D, Mahbubi A, Amiri M, Amiri M. The Study of Inhibitory Effects of Satureja khuzestanica against MDR Isolates of Pseudomonas aeruginosa. Int J Curr Microbiol Appl Sci. 2014; 2(3):614-8.
12. Amanlou M, Dadkhah F, Salehnia A, Farsam H. An anti-nociceptive effects of hydroalcoholic extract of Satureja khuzistanica Jamzad extract. J Pharm Pharmaceut Sci. 2005; 8 (1): 102-6.
13. Fathizadeh H, Saffari M, Esmaeili D, Moniri R, Salimian M. Evaluation of antibacterial activity of enterocin A-colicin E1 fusion peptide. Iran J Basic Med Sci. 2020; 23:1471-1479.
14. Nakamura de Vasconcelos SS, Caleffi-Ferracioli KR, Hegeto LA, Baldin VP, Nakamura CV, Stefanello TF, et al. Carvacrol activity & morphological changes in Mycobacterium tuberculosis. Futur Microbiol. 2018;13(08):877-88 doi:10.2217/fmb-2017-0232 PMid:29877104
15. Ultee A, Bennik HJ, Moezelaar R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Appl Environ Microbiol. 2002;68:1561-8. doi:10.1128/AEM.68.4.1561-1568.2002 PMid:11916669 PMCid:PMC123826
16. Amiri M, Esmaeili D, Sahlehnia An Ariana M, Alam F, Beiranvand H. Study of antibacterial effects of Satureja essence against some common nosocomial pathogenic bacteria. Int J Curr Microbiol. 2013;2(7): 249-254.
17. Ghodrati L, Alizadeh A. Essential oil constituents and antimicrobial activities of Iranian Satureja khuzistanica Jamzad. Int J Biosci. 2015; 6(3); 249-257. doi:10.12692/ijb/6.3.249-257

Cite this article as:
Ghods Elahi M, Hekmati M, Esmaeili D, Ziarati P, Yousefi M. Antibacterial activity of modified Carvacrol against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. Novel Clin Med. 2022; 1(4):192-196. doi: 10.22034/NCM.2022.350997.1051