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Evaluation of the antioxidant, antibacterial, and antibiofilm activity of the sesquiterpene nerolidol

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Abstract

The aim of this study was to evaluate the antioxidant, antibacterial, and antibiofilm activities of nerolidol. The antioxidant activity of nerolidol was determined using the total antioxidant activity method. Antibacterial activity was performed using the microdilution method to determine the minimum inhibitory concentration (MIC) against seven standard strains of the ATCC and four bacterial clinical isolates with a resistance profile, following the Clinical and Laboratory Standards Institute (CLSI). The antibiofilm activity of nerolidol was performed using the crystal violet method. The results of the antioxidant test revealed a total antioxidant activity of 93.94%. Nerolidol inhibited the growth of *Staphylococcus aureus* (MIC = 1 mg/mL), *Streptococcus mutans* (MIC = 4 mg/mL), *Pseudomonas*

aeruginosa (MIC = 0.5 mg/mL), and *Klebsiella pneumoniae* (MIC = 0.5 mg/mL). For clinical isolates, nerolidol showed an inhibitory potential against multidrug-resistant *P. aeruginosa*, *K. pneumoniae* carbapenemase (MIC = 0.5 mg/mL), methicillin-susceptible *S. aureus* (MIC = 2 mg/mL), and methicillin-resistant *S. aureus* (MIC = 2 mg/mL). Nerolidol showed similar antibacterial activity against ATCC strains and hospital clinical isolates with resistance profile, suggesting that even though these strains are resistant to antibiotics, they are still sensitive to nerolidol. Nerolidol exerted a dose-dependent effect on the inhibition of biofilm formation, even at subinhibitory concentrations. Nerolidol inhibited bacterial biofilms of ATCC strains at a rate ranging from 51 to 98%, at concentrations ranging from 0.5 to 4 mg/mL. For clinical bacterial isolates, biofilm inhibition ranged from 6 to 60%. Therefore, the present study showed the antioxidant, antibacterial, and antibiofilm properties of nerolidol.

Availability of data and materials (data transparency)

All relevant data are available in the manuscript.

References

Adamczak A, Ożarowski M, Karpiński TM (2020) Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J Clin Med 9(1):109. https://doi.org/10.3390/jcm9010109

Ahmed A, Khan AK, Anwar A, Ali SA, Shah MR (2016) Biofilm inhibitory effect of chlorhexidine conjugated gold nanoparticles against *Klebsiella pneumoniae*. Microb Pathog 98:50–56. https://doi.org/10.1016/j.micpath.2016.06.016

Alam MN, Bristi NJ, Rafiquzzaman M (2013) Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J 21(2):143–152. https://doi.org/10.1016/j.jsps.2012.05.002

Arruda DC, D'Alexandri AMK, Uliana SRB (2005)

Antileishmanial Activity of the Terpene Nerolidol. Antimicrob Agents Chemother 49:1679–1687. https://doi.org/10.1128/AAC.49.5.1679-1687.2005

Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42–51. https://doi.org/10.1038/nrmicro3380

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200. https://doi.org/10.1038/1811199a0

Borges KA, Furian TQ, Souza SN, Menezes R, Tondo EC, Salle CT, Moraes HLS, Nascimento VP (2018) Biofilm formation capacity of Salmonella serotypes at different temperature conditions. Pesq Veterinario Bras 38:71–76. https://doi.org/10.1590/1678-5150-pvb-4928

Borut P, Raja D (2012) Free radicals and extrinsic skin aging. Dermatol Res Pract 2012:1–4. https://doi.org/10.1155/2012/135206

Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH (2016) Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules 21:1–40. https://doi.org/10.3390/molecules21050529

Clinical and Laboratory Standards Institute (2019)

Performance standards for antimicrobial susceptibility testing, 29th edn. CLSI document M100. Clinical and Laboratory Standards Institute, Wayne

Clutterbuck AL, Woods EJ, Knottenbelt DC, Clegg PD, Cochrane CA, Percival SL (2007) Biofilms and their relevance to veterinary medicine. Vet Microbiol 121(1–2):1–17. https://doi.org/10.1016/j.vetmic.2006.12.029

Di T, Chen GJ, Sun Y, Ou SY, Zeng XX, Ye H (2017) Antioxidant and immunostimulating activities in vitro of sulfated polysaccharides isolated from *Gracilaria rubra*. J Funct Foods 28:64–75. https://doi.org/10.1016/j.jff.2016.11.005

Diniz EFC, Moura LR, Carvalho RB, Nunes LCC (2016) Prospecção tecnológica: estudos sobre complexo de inclusão do nerolidol com a β-ciclodextrina. Cad Prospecção 9(3):307–315. https://doi.org/10.9771/cp.v9i3.16169

Dong G, Li J, Chen L, Bi W, Zhang X, Liu H, Zhi X, Zhou T, Cao J (2019) Effects of sub-minimum inhibitory concentrations of ciprofloxacin on biofilm formation and virulence factors of *Escherichia coli*. Bras J Infectar Dis 23(1):15–21. https://doi.org/10.1016/j.bjid.2019.01.004

El-Kattan AF, Asbill CS, Kim N, Michniak BB (2001) The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. Int J

Pharm 215:229–240. https://doi.org/10.1016/S0378-5173(00)00699-2

Ephrem E, Najjar A, Charcosset C, Greige-Gerges H (2019) Selection of nerolidol among a series of terpenic and phenolic compounds for its potent activity against *Lactobacillus fermentum* ATCC 9338. Process Biochem 80:146–156. https://doi.org/10.1016/j.procbio.2019.02.015

Escobar P, Leal SM, Herrera LV, Martinez JR, Stashenko E (2010) Chemical composition and antiprotozoal activities of *Colombian Lippia* spp. essential oils and their major components. Mem Inst Oswaldo Cruz 105:184–190. https://doi.org/10.1590/S0074-02762010000200013

Fang JY, Leu YL, Hwang TL, Cheng HC, Hung CF (2003) Development of sesquiterpenes from *Alpinia oxyphylla* as novel skin permeation enhancers. Eur J Pharm Sci 19:253–262. https://doi.org/10.1016/S0928-0987(03)00118-0

Fontes JT, Nogueira BVS, Farias EO, Martins MR (2020) Medidas de prevenção à infecção hospitalar em unidades de terapia intensiva. Enferm Bras 19:67–74. https://doi.org/10.33233/eb.v19i1.2658

Gonçalves O, Pereira R, Gonçalves F, Mendo S, Coimbra MA, Rocha SM (2011) Evaluation of the mutagenicity of sesquiterpenic compounds and their influence on the

susceptibility towards antibiotics of two clinically relevant bacterial strains. Mutat Res Genet Toxicol Environ 723:18–25. https://doi.org/10.1016/j.mrgentox.2011.03.010

Guimarães AG, Oliveira GF, Melo MS, Cavalcanti SC, Antoniolli AR, Bonjardim LR, Silva FA, Santos JPA, Rocha RF, Moreira JCF, Araújo AA, Gelainn DP, Quintans Júnior LJ (2010) Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. Basic Clin Pharmacol Toxicol 107:949–957. https://doi.org/10.1111/j.1742-7843.2010.00609.x

Gutteridge JMC, Halliwell B (2010) Antioxidants: molecules, medicines, and myths. Biochem Biophys Res Commun 393:561–564. https://doi.org/10.1016/j.bbrc.2010.02.071

Hada T, Shiraishi A, Furuse S, Inoue Y, Hamashima H, Matsumoto Y, Masuda K, Shimada J (2003) Inhibitory effects of terpenes on the growth of *Staphylococcus aureus*. Nat Med 57(2):64–67

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim YK, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH (2007) Antimicrobial effects of silver nanoparticles. Nanomed Nanotech Biol Med 3(1):95–101. https://doi.org/10.1016/j.nano.2006.12.001

Kiryu M, Hamanaka M, Yoshitomi K, Mochizuki S,

Akimitsu K, Gomi K (2018) Rice terpene synthase 18 (OsTPS18) encodes a sesquiterpene synthase that produces an antibacterial (E)-nerolidol against a bacterial pathogen of rice. J Gen Plant Pathol 84(3):221–229. https://doi.org/10.1007/s10327-018-0774-7

Kyriacou SV, Brownlow WJ, Xu XN (2004) Using nanoparticle optics assay for direct observation of the function of antimicrobial agents in single live bacterial cells. Biochemistry 43(1):140–147. https://doi.org/10.1021/bi0351110

Laureti L, Matic I, Gutierrez A (2013) Bacterial responses and genome instability induced by subinhibitory concentrations of antibiotics. Antibiotics 2(1):100–114. https://doi.org/10.3390/antibiotics2010100

Lee K, Lee JH, Kim SI, Cho MH, Lee J (2014) Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against *Staphylococcus aureus*. Appl Microbiol Biotechnol 98:9447–9457. https://doi.org/10.1007/s00253-014-5903-4

Lima JLDC, Alves LR, Paz JNPD, Rabelo MA, Maciel MAV, Morais MMC (2017) Analysis of biofilm production by clinical isolates of *Pseudomonas aeruginosa* from patients with ventilator-associated pneumonia. Rev Bras Ter Intensiva 29(3):310–316. https://doi.org/10.5935/0103-507x.20170039

Liu Y, Shi L, Su L, van der Mei HC, Jutte PC, Ren Y, Busscher HJ (2019) Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev 48(2):428–446. https://doi.org/10.1039/c7cs00807d

Martínez JL (2017) Effect of antibiotics on bacterial populations: a multi-hierachical selection process. F1000Research 6:1–10. https://doi.org/10.12688/f1000research.9685.1

Miranda CASF, Graças Cardoso M, Batista LR, Rodrigues LMA, Silva Figueiredo AC (2016) Óleos essenciais de folhas de diversas espécies: propriedades antioxidantes e antibacterianas no crescimento espécies patogênicas. Rev Ciênc Agron 47:213–220. https://doi.org/10.5935/1806-6690.20160025

Monteiro RFS, Santos VRR, Ferreira AACT, Abreu JRG (2020) O uso indiscriminado de antimicrobianos para o desenvolvimento de micro-organismos resistentes. REAS/EJCH 53:1–10. https://doi.org/10.25248/reas.e3597.2020

M'sou S, Alifriqui M, Romane A (2017) Estudo fitoquímico e efeitos biológicos do óleo essencial de *Fraxinus dimorpha* Coss & Durieu. Nat Prod Res 31:2797–2800. https://doi.org/10.1080/14786419.2017.1294173

Nguikwie SK, Nyegue MA, Belinga FNF, Ngane RAN, Romestand B, Kouzayha A, Menut C (2013) The chemical composition and antibacterial activities of the essential oils from three Aframomum species from Cameroon, and their potential as sources of (E)-(R)-nerolidol. Nat Prod Commun 8(6):829–834. https://doi.org/10.1177/1934578X1300800638

Nogueira Neto JD, Sousa DPD, Freitas RM (2013) Avaliação do potencial antioxidante in vitro do nerolidol. Rev De Cienc Farm Basica e Apl 34(1):125–130

Pacifico S, Abrosca B, Golino A, Mastellone C, Piccolella S, Fiorentino A, Monaco P (2008) Antioxidant evaluation of polyhydroxylated nerolidols from redroot pigweed (*Amaranthus retrofexus)* leaves. Food Sci Technol 41(9):1665–1671. https://doi.org/10.1016/j.lwt.2007.10.006

Patil S, Chen H, Zhang X, Lian M, Ren PG, Wen F (2019) Antimicrobial resistance and resistance determinant insights into multi-drug resistant gram-negative bacteria isolates from paediatric patients in China. Infect Drug Resist 12:3625. https://doi.org/10.2147/IDR.S223736

Pinto-Zevallos DM, Martins CB, Pellegrino AC, Zarbin PH (2013) Compostos orgânicos voláteis na defesa induzida das plantas contra insetos herbívoros. Quím Nova

36:1395–1405. https://doi.org/10.1590/S0100-40422013000900021

Prasanthi D, Lakshmi PK (2012) Terpenes: Effect of lipophilicity in enhancing transdermal delivery of alfuzosin hydrochloride. J Adv Pharm Technol Res 3:216–223. https://doi.org/10.4103/2231-4040.104712

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: specific application to the determination of vitamin E. Anal Biochem 269(2):337–341. https://doi.org/10.1006/abio.1999.4019

Qi L, Li H, Zhang C, Liang B, Li J, Wang L, Du X, Liu X, Qiu S, Song H (2016) Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in *Acinetobacter baumannii*. Front Microbiol 7:483. https://doi.org/10.3389/fmicb.2016.00483

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rubini D, Banu SF, Nisha P, Murugan R, Thamotharan S, Percino MJ, Nithyanand P (2018) Essential oils from unexplored aromatic plants quench biofilm formation and

virulence of Methicillin resistant *Staphylococcus aureus*. Microb Pathog 122:162–173. https://doi.org/10.1016/j.micpath.2018.06.028

Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CDG, Pérez-Jiménez J, Saura-Calixto FD (2007) Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. Embrapa Agroindústria Tropical-Comunicado Técnico (INFOTECA-E)

Serafini MR, Santos RC, Guimarães AG, Santos JPA, Santos ADC, Alves IA, Gelain DP, Nogueira PCL, Quintans Júnior LJ, Bonjardim LR, Araújo AAS (2011) Morinda citrifolia linn leaf extract possesses antioxidant activities and reduces nociceptive behavior and leukocyte migration. J Med Food 14:1159–1166. https://doi.org/10.1089/jmf.2010.0254

Silva NCC, Fernandes A Jr (2010) Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins Incl Trop Dis 16(3):402–413. https://doi.org/10.1590/S1678-91992010000300006

Stefanello S, Kozera C, Ruppelt BM, Fumagalli D, Camargo MP, Sponchiado D (2018) Levantamento do uso de plantas medicinais na Universidade Federal do Paraná, Palotina–PR. Brasil Ext Foco 1(15):15–27. https://doi.org/10.5380/ef.v1i15.52776

Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahovic M (2000) A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 40(20):175–179. https://doi.org/10.1016/S0167-7012(00)00122-6

Szerwieski LLD, Cortez DAG, Bennemann RM, Silva ES, Cortez LER (2017) Uso de plantas medicinais por idosos da atenção primária. Rev Eletr Enferm 19:1–10. https://doi.org/10.5216/ree.v19.42009

Tao J, Rajan K, Ownley B, Gwinn K, D'Souza D, Moustaid-Moussa N, Tschaplinskif TJ, Labbé N (2019) Natural variability and antioxidant properties of commercially cultivated switchgrass extractives. Ind Crops Prod 138(5):111474. https://doi.org/10.1016/j.indcrop.2019.111474

Tinkel J, Hassanain H, Khouri SJ (2012) Cardiovascular Antioxidant Therapy: a review of supplements, pharmacotherapies, and mechanisms. Cardiol Rev 20(2):77–83. https://doi.org/10.1097/CRD.0b013e31823dbbad

Togashi N, Hamashima H, Shiraishi A, Inoue Y, Takano A (2010) Antibacterial activities against *Staphylococcus aureus* of terpene alcohols with aliphatic carbon chains. J Essent Oil Res 22:263–269. https://doi.org/10.1080/10412905.2010.9700321

Wang X, Zhang Y, Liu Z, Zhao M, Liu P (2017) Purification, characterization, and antioxidant activity of polysaccharides isolated from Cortex Periplocae. Molecules 31:1–15. https://doi.org/10.3390/molecules22111866

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Contributions

DFM—designed the project, executed the laboratorial methodology, analyzed the data, and wrote the article. TAR—assisted in the laboratory experiments and data analysis. DMB—assisted in the laboratory experiments, data analysis, and writing the manuscript. MMS—assisted in the

tabulation, statistical analysis of the data. MSS—assisted in setting up and carrying out laboratory experiments. BMN—participated in collecting samples and carrying out experiments. IMFC—supervised the laboratory experiments and contributed to the critic evaluation of the manuscript. RDM—assisted in the data analysis and writing the manuscript. MVS—supported research planning and writing the manuscript. All the authors have read the manuscript and approved its submission.

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Ethics declarations

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

This article does not involve studies with humans and animals.

Additional information

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