Evaluating the Antibacterial Potential and In silico Toxicological Profile of 7-Hydroxycitronellal

João Paulo Ribeiro, Luana Martins Gomes, Leonardo Souza Silva, Maria Eduarda Carvalho, Pedro Henrique Guedes

Department of Microbiology, Instituto Federal do Norte de Minas Gerais, Itabira, Minas Gerais, Brazil.

Citation: João Paulo Ribeiro, Luana Martins Gomes, Leonardo Souza Silva, Maria Eduarda Carvalho, Pedro Henrique Guedes (2025). Evaluating the Antibacterial Potential and In silico Toxicological Profile of 7-Hydroxycitronellal. Psychiatria, 17(12), 60-65. https://doi.org/10.5281/zenodo.19096759

ABSTRACT

The pharmacological selectivity imposed by the micro-organisms enabled the emergence of new perspectives in the research field of natural products, in which the monoterpenic phytoconstituents, especially the 7-hydroxycitronellal (7OH) present antimicrobial activity. Therefore, this study assessed the *in silico* antimicrobial and toxicological activity of the monoterpene 7-OH. The product was solubilized in tween 80 and dimethylsulfoxide (DMSO). Later it was diluted in sterile distilled water up to the concentration of 2048µg/mL. The minimum inhibitory concentration (MIC) of the product was determined by microdilution in doubly concentrated brain heart infusion (BHI) medium. The minimum bactericidal concentration (MBC) was determined by the depletion technique in nutrient agar (NA) with aliquots of 10µL of the MIC, MIC × 2 and MIC × 4. The MIC and the MBC of the 7-OH was 64µg/mL for *S. aureus* (ATCC 6538). However, for *S. epidermides* (LPM 35), *S. aureus* (LPM 45) and *S. aureus* (LPM 55), were 512 and 1024µg/mL respectively. In the *in silico* toxicological analysis the absence of mutagenic, tumorigenic effects and damage to the reproductive system were observed. Therefore, the 7-OH is bactericidal for the strains used in this study and presents a theoretical low oral toxicity.

Keywords: 7-hydroxycitronellal, gram-positive bacteria, *in silico* toxicity.

INTRODUCTION

The resistance inherent to the micro-organisms emerged due to the indiscriminate use of antibiotics resulting in the emergence of highly selective pathogens, capable of producing and/or transferring auto-protective gene molecules[1]. Such procedure reduces the action of the drugs on the bacteria, as well as their eventual destruction. However, the implementation of new pharmacological perspectives was necessary in order to placate such mechanisms and destroy the pathogens[2]. The use of plants for medicinal purposes started at the dawn of humanity and is related to their capacity to possess bioactive compounds capable of generating therapeutic responses[3]. Thus promoting antitumoral, antiviral, antibacterial action, among others[4]. According to Trombetta *et al*. (2005)[5], such pharmacological properties are related to the proportions of the metabolites present in the plant. Among the derivative products of the secondary metabolism is the citronellal, isolated mainly from plants of the genus *Cymbopogon*, *Eucalyptus*, *Melissa*, *Mentha*, *Allium* and *Cinnamomum*, reason why it is mainly found in essential oils of these plants[6]. There by it is broadly used in the cosmetic industry, as well as in the control of biological plagues such as ticks and weeds, however it has an effective action against bacterial and fungal microorganisms of clinical interest, in the latter case the molecule acts in order to promote homeostatic alterations in the plasmatic membrane, in addition to preventing the formation of biofilms[7]. The chiral versatility of the compound propitiates the chemical synthesis for the formation of other constituents, once that the same linear monoterpene is capable of forming two distinct optical isomers [(±)-citronellal][8,9]. In this context, the antimicrobial potential performed by the 7-hydroxycitronellal (7-OH) was assessed, in addition to comparing its toxicological potential with standard drugs, by means of an *in silico* analysis.

MATERIALS AND METHODS

Phytoconstituent

The following substances used in this work were obtained commercially: 7-hydroxycitronellal [7-hydroxy-3,7dimethyloctanal] (purity > 95%), dimethylsulfoxide (DMSO) and tween 80 (0.02%) (all from Sigma-Aldrich, São Paulo, SP, Brazil). The tween 80 and the DMSO were solubilized in a proportion that did not exceed 0.5% in the tests, and subsequently was diluted in sterile distilled water with the 7-OH in order to obtain a doubly concentrated emulsion of 2048µg/mL[10].

Bacterial strains

The tests were carried out with five bacterial strains: *S. epidermides* (LPM 35), *S. aureus* (LPM 45 e 55) (clinical isolates) and two standard strains: *S. aureus* ATCC 6538 and *E. coli* ATCC 8859. All the samples belong to the collection of the Microbiology Research Laboratory (LPM) of the Integrated Faculties of Patos (FIP). All the strains were maintained in NA at 4°C. In the tests were used 24h sown material at 35±2°C.

Inoculum

The suspensions were prepared from the recent bacterial cultures cultivated in NA and incubated at 35±2°C during 24h. After the incubation, approximately 4-5 colonies were transferred (using a sterile loop) to test tubes containing 5.0mL of sterile saline solution (NaCl at 0.85%). The

resulting suspensions were agitated during 15 seconds with the aid of vortex machine (Fanem Ltd., Guarulhos, SP, Brazil). The turbidity of the final inoculum was normalized using a suspension of barium sulphate (tube of 0.5 in the McFarland scale). The final concentration obtained was of 1-5 × 10^8 colony forming units per milliliters (CFU/mL)[11,12].

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The determination of the MIC of the product on the strains used in the biological tests was determined by the broth microdilution method[13-15]. One hundred microliters (100µL) of doubly concentrated BHI liquid medium were transferred to the cavities of a 96-well microdilution plate with a u-shaped bottom (Alamar, Diadema, SP, Brazil). After that, 100µL of the emulsion of the doubly concentrated product was inoculated in the first horizontal line of the dishes' wells. Serial dilutions were carried out at a ratio of two, where an aliquot of 100µL was removed from the most concentrated well to the following one, producing concentrations of 1024-16µg/mL. Finally, 10µL of the bacterial suspensions were added to each well of the plate, in which each column represented a strain. At the same time, the controls were made for the bacterial viability and for the susceptibility to the standard antibiotic chloramphenicol (100IU/mL). The plates were incubated at 35±2°C during 24h. After the appropriate incubation time, the presence (or absence) of growth was visually observed. The formation of cell clusters or "buttons" in the plate wells was considered form. The MIC was defined as the lowest concentration of the product which produced an inhibition of the visible growth of the bacteria. The antimicrobial activity of the product was interpreted (considered active or not) according to the criteria proposed by Morales *et al*. (2008)[16]. strong/good activity (MIC 50-500µg/mL), moderate activity (MIC 6001500µg/mL) and inactive/no antimicrobial effect (MIC> 1500µg/mL). To determine the MBC, aliquots of 10µL of the MIC, MIC × 2 and MIC× 4 of the test product were subcultivated. The chloramphenicol (100IU/mL) was the control of the bacterial growth in the Petri dishes containing NA. After 24h of incubation at 35±2°C a reading was carried out to assess the MBC based on the controls. The MBC was defined as the lowest concentration of the product capable of inhibiting the bacterial growth or allowing a growth inferior to three CFU, thus resulting in a bactericidal activity of 99.9%[13]. The biological activity tests were carried out in duplicate and the results were expressed with the arithmetic average of the MIC and MBC.

In silico analysis (Osiris)

The prediction process of the biological effects executed by the Osiris software (http://www.organicchemistry.org/prog/peo/) is based on a set of precomputerized molecular fragments which result in toxicity alerts, in the case of being found in the currently designed molecular structure. The toxicity predictions of the Osiris result from possible effects of mutagenicity, tumorigenicity, irritability, effects on the reproductive system, cLogP value, druglikeness and drug-score of the molecules[17,18]. Lipinski's rule of five (2004)[19], assesses the similarity of the drugs. It determines if the chemical compound with validated pharmacological or biological activity has the properties which make it a probable active drug by oral administration in human beings. This rule describes the molecular properties, which are important for the pharmacokinetics of a drug in the human body, including its absorption, distribution, metabolism and excretion (ADME). The rule established for the majority of the "drug−like" molecules has cLogP ≤ 5, molecular weight ≤ 500Da, number hydrogen acceptors ≤ 10 (nALH ≤ 10) and the number of hydrogen donors ≤ 5 (nDLH ≤ 5). Molecules, which violate more than one of these parameters, may have problems with its bioavailability[19,20].

RESULTS

The product's MIC value against *S. aureus* (ATCC 6538) was 64µg/mL, as well as the MBC. However, for *S. epidermides* (LPM 35), *S. aureus* (LPM 45) and *S. aureus* (LPM 55), MIC and MBC were 512 and 1024µg/mL respectively. For *E. coli* (ATCC 8859), MIC and MBC were also 512 and 1024µg/mL respectively (Table 1 and 2). The toxicological evaluation of the product, as well as of its pharmacological properties were analyzes *in silico*, enabling the determination of mutagenic, tumorigenic and irritating characteristics and also damage to the reproductive system[21] (Table 3).

DISCUSSION

The bacterial resistance resulting from the prolonged and indiscriminate chemotherapy with antimicrobial drugs, has stimulated the search for new alternatives for the treatment of infections, and the studies directed to the use of plants for medicinal purposes as a viable alternative due to the diversity of biologically active molecules and the low cost of the drugs produced from these natural products. The essential oils (EO) produced by plants of the genus *Cymbopogon*, *Eucalyptus*, *Melissa*, *Mentha*, *Allium* and *Cinnamomum* present phytoconstituents with distinct pharmacological properties[22]. According to Zore et al. (2011)[23] it has been suggested that the monoterpenes such as the 7-OH have the potential of promoting an elevated antimicrobial effect as a consequence of metabolic alterations in the pathogenic cells, as well as the maintenance of the cell wall. Innsan (2011)[24] in researches related to the antibacterial activity of the citronellal against *E. coli* and *S. aureus* proved the bactericide action of this compound. Correlating with the data obtained in this study, it can be inferred that the 7-OH retarded and/or eliminated

Table 1: Values of MIC (µg/mL) of the monoterpene 7-OH against the bacterial strains *Staphylococcus* and *Escherichia*.

Bacterial strains / Treatment	*S. epidermides* LPM 35	*S. aureus* LPM 45	*S. aureus* LPM 55	*S. aureus* ATCC 6538	*E. coli* ATCC 8859
1024µg/mL	+	+	+	+	+
512µg/mL	+	+	+	+	+
256µg/mL	-	-	-	+	-
128µg/mL	-	-	-	+	-
64µg/mL	-	-	-	+	-
Negative control	-	-	-	-	-
Positive control	+	+	+	+	+

(+) inhibition (-) no inhibition

Table 2: Values of MBC (µg/mL) of the ıonoterpene 7- ɔH against the bacterial strains *'taphylococcus* and *Escherichia.*

Bacterial strains / Treatment	*S. epidermides* LPM 35	*S. aureus* LPM 45	*S. aureus* LPM 55	*S. aureus* ATCC 6538	*E. coli* ATCC 8859
1024µg/mL	+	+	+	+	+
512µg/mL	-	-	-	+	-
256µg/mL	-	-	-	+	-
128µg/mL	-	-	-	+	-
64µg/mL	-	-	-	+	-
Negative control	-	-	-	-	-
Positive control	+	+	+	+	+

(+) inhibition; (-) no inhibition

Table 3: Osiris calculations of toxicity risks and drug-score of compounds 7-OH monoterpenes compared to the standard antibiotics drugs.

Compounds	Toxicity risk[a]					Drug score[b]					
	MUT	TUMO	IRRI	REP	CLP	S	D-L	D-S	nALH	nDLH	Da
7-3.13						-2.36	-6.97	0.26	1.00	0.00	154.25
Hydroxycitronellal											
Vancomycin -6.75	:Slighlytoxic; : Highlytoxic;					-9.42	1.82	0.23	33.0	19.0	1449.27
Chloramphenicol -0.42						-2.36	-2.36	0.06	7.00	3.00	323.13

: Nontoxic; [a]MUT: Mutagenic; TUMO: Tumorigenic; IRRI: Irritant; REP: Reproductive effective. [b]CLP: cLogP; S: Solubility; DL: Drug-likeness; DS: Drug-Score; nALH: number of acceptors hydrogen bonding; nDLH: number of hydrogen bond donor groups; Da: Molecular Weight.

the growth of the micro-organisms in this work. As the dilutions of 512µg/mL and 1024µg/mL inhibited and killed 80% of the analyzed strains (Table 1), as a consequence of the antimicrobial action of this molecule[25]. According to Janssen; Scheffer; Svendsen (1986)[26] the MIC suffers variations according to the sensitivity and the species of micro-organisms. Notoriety analyzed among the clinical and standard strains, *S. aureus* (LPM 45), *S. aureus* (LPM 55) and *S. aureus* (ATCC 6538) respectively, seen as the later showed to be more susceptible in the concentrations of the compound (MIC and MBC
64µg/mL). Relating these findings to the researches by Santos et al. (2007)[27] the susceptibility of the gram positive strains in face of the phytoconstituents is measured by the cellular structure, and these were deprived of protective capsule, which facilitates the penetration of the compound. This explains also the vulnerability of the tested strains in the face of the 7-OH. In contrast the MBC of the compound followed the methodology of Hafidh et al. (2011)[28], considering that a phytoconstituent presents bactericide property when the ratio between the MBC/MIC is between 1 and 2, an effect capable of causing the eventual death of the micro-organisms. Therefore, the 7-OH presents bactericide effect, presenting itself as being effective against all the strains present in this study. The pharmacological properties of the 7-OH were based on the principles of Morales et al. (2008)[16] and Ursu, Oprea, (2010)[17], allowing the analysis of the toxicological effects produced by it. However, the determination of the phytoconstituent's pharmacokinetics follows the methodology proposed by Lipinski et al. (2001)[20] and

Abhay et al. (2007)[29], so that the compound has to have at least three of the four proposed requirements; number of hydrogen bond donors (nDLH ≤ 5), number of hydrogen bond acceptors (nALH ≤ 10), molecular weight (DA ≤ 500) and (cLogP ≤ 5). The results obtained demonstrated that the phytoconstituent presents low toxicity levels, presenting values of Drug-likeness (-6.97) and Drug-Score (0.26), characterizing the 7-OH as similar to drugs with a good theoretical oral availability, absence of mutagenic and tumorigenic effect, as well as effects on the reproductive system. However, the vancomycin drug administered during systemic bacterial infections, demonstrated to be within the limitations proposed by Lipinski, (2004)[19]. On the other hand, the chloramphenicol did not obey such rules, and had a strong mutagenic, tumorigenic and irritating effects and effects on the reproductive system (Table 3).

CONCLUSION

In conclusion, the derived compounds of plants be have as an efficient alternative in medicinal therapy, as they are composed of bioactive molecules capable of having pharmacological effects. Based on the results of this study, the 7-OH has a bactericidal activity on the strains of *S. aureus*, as well as *S. epidermides* and *E. coli*. Therefore, the molecule presents itself as a promising antibacterial agent, with a good theoretical oral bioavailability and low toxicity. However, greater investigations of their *in vitro* and *in vivo* pharmacological and toxicological properties are necessary.

REFERENCES

1. Holvoet K, Sampers I, Benedicte C, Dewulf J, Uyttendaele M. Moderate prevalence of antimicrobial resistance in *Escherichia coli* isolates from lettuce, irrigation water, and soil. Applied and Environmental Microbiology 2013; 79(21):6677-6683.
2. Vorn J, Solomon DH. Cultural and economic factors that (mis) shape antibiotic use: the nonpharmacological basis of therapeutics. Annals of Internal Medicine 2000; 133(2):128-135.
3. Ernst M, Lagoudakis HS, Grace OM, Nilsson N, Simonsen HT, Horn JW, Ronsted N. Evolutionary prediction of medicinal properties in the genus *Euphorbia* L. Scientific Reports 2016; 6(30531):1-10. doi: 10.1038/srep30531.
4. Berdy J. Bioactive microbial metabolites. Journal of Antibiotics 2005; 58(1):1-26.
5. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpenes. Antimicrobial agents and chemotherapy 2005; 49(6):2474-2478.
6. Singh S, Fatima Z, Hameed S. Citronellal-induced disruption of membrane homeostasis in *Candida albicans* and attenuation of its virulence attributes. Revista da Sociedade Brasileira de Medicina Tropical 2016; 49(4):465-472.
7. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food and chemical toxicology 2008; 46(2):446-475.
8. Zambon SN, Chamorro ER, Casuscelli SC. Estudio de la Pureza Óptica de Citronelal presente enlos Aceites Esencialesobtenidos de Citronela y de *EucaliptoCitriodora*. Información tecnológica 2015; 26(4):29-36.
9. Lenardao EJ, Ferreira PC, Jacob RG, Perin G, Leite FP. Solvent-free conjugated addition of thiols to citral using KF/alumina: preparation of 3thioorganylcitronellals, potential antimicrobial agents. Tetrahedron Letters 2007; 48(38):6763-6766.
10. Nascimento PFC, Nascimento AC, Rodrigues CS, Antoniolli AR, Santos PO, Junior AMB, Trindade RC. Atividade antimicrobiana dos óleos essenciais: uma abordagem multifatorial dos métodos. Revista Brasileira de Farmacognosia 2007; 17(1):108-113.
11. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Win WCJ. Diagnóstico microbiológico. 6 ed. São Paulo: Médica e Científica Ltda; 2008. p.1565.
12. Ostrosky EA, Mizumoto MK, Lima MEL, Kaneko TM, Nishikawa SO, Freitas BR. Métodos para avaliação da atividade antimicrobiana de determinação de concentração mínima inibitória (CMI) de plantas medicinais. Revista Brasileira de Farmacognosia 2008; 18(2):301-307.
13. Ingroff AE, Chaturvedi V, Fothergill A, Rinaldi MG. Optimal testing conditions for determining MICs and minimum fungicidal concentrations of new and established antifungal agents for uncommon molds: NCCLS collaborative study. Journal of Clinical Microbiology 2002; 40(10):3776-3781.
14. Viljoen A, Vuuren SV, Ernst E, Klepser M, Demirci B, Baser H, Wyk BEV. *Osmitopsis asteriscoides* (Asteraceae) the antimicrobial activity and essential oil composition of a Cape-Dutch remedy. Journal of Ethonopharmacology 2003; 88(2):137-143.
15. Sahin F, Gulluce M, Daferera D, Sokmen A, Sokmen M, Polissiou M, Agar G, Ozer H. Biological activities of the essential oils and methanol extract of *Origanumvulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control 2004; 15(7):549-557.
16. Morales G, Paredes A, Sierra P, Loyola LA. Antimicrobial activity of three Baccharis species used in the traditional medicine of Northern Chile. Molecules 2008; 13(4):790-794.
17. Ursu O, Oprea TI. Model-Free Drug-Likeness from Fragments. Journal Chemical Information and Modeling2010; 50(8):1387-1394.
18. Ursu O, Rayan A, Goldblum A, Oprea TI. Understanding drug-likeness. Wiley Interdisciplinary Reviews: Computational Molecular Science 2011; 1(5):760-781.
19. Lipinski CA. Drug discovery today. Technologies 2004; 1(1):337-341.
20. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to

estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 2001;46(1-3):3-26.
21. Tekto IV. Computing chemistry on the web. Drug Discov Today 2005;10(22):1497-1500.
22. Raimundo WSA, Marcio AO, Sérgio DA, Talita PSF, Manoel MS, Gil RS. Fumigant Antifungal Activity of *Corymbia citriodora* and *Cymbopogon nardus* Essential Oils and Citronellal against Three Fungal Species. The Scientific World Journal 2014; 2014 (492138):1-8. doi:10.1155/2014/492138.
23. Zore GB, Thakre AD, Jadhav S, Karuppayil SM. Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of cell cycle. Phytomedicine 2011; 18(13):1181-1190.
24. Innsan MFMF, Sarilho MH, Samihah MS, Asma OS, Radzi SM, Jalil AKA, Hanina MN. Pharmacodynamic properties of essential oils from *Cymbopogon* species. African Journal of Pharmacy and Pharmacology 2011; 5(24):2676-2679.
25. Millezi AF, Baptista NN, Caixeta DS, Rossoni DF, Cardoso MG, Piccoli RH. Caracterização química e atividade antibacteriana de óleos essenciais de plantas condimentares e medicinais contra *Staphylococcus aureus* e *Escherichia coli*. Revista Brasileira de Plantas Medicinais 2014; 16(1):18-24.
26. Janssen AM, Scheffer JJC, Svendsen A, Baerheim S. Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of the test methods. Planta medica 1987; 53(5):395-398.
27. Santos AL, Santos DO, Freitas CC, Ferreira BLA, Afonso IF, Rodrigues CR, Castro HC. *Staphylococcus aureus*: visitando uma cepa de importância hospitalar. Jornal Brasileiro de Patologia e Medicina Laboratorial 2007; 43(6):413-423.
28. Hafidh RR, Abdulamir AS, Vern LS, Bakar FA, Abas F, Jahanshiri F, Sekawi Z. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. The open microbiology journal 2011; 5(1):96-106.
29. Sangamwar AT, Deshpande UD, Pekmwar SS, Vadvalkar M. Improving decision making for drug candidates: A computational approach for benzthiazoles as antifungal. Indian Journal of Biotechnology 2007; 6(1):389-396.