PRODUCTION AND EVALUATION OF GERANYL ACETATE NANOEMULSIONS FOR ANTIMICROBIAL EFFICACY

João Francisco C. do Nascimento[1], Daniela Remonatto[1], Stéphanie R. Amaral[1], Fernando L. Primo[1], Josemar G. Oliveira Filho[2], Luiz H. C. Mattoso[2], Juliana C. Bassan[3], José V. Oliveira[4], Beatriz B. Teixeira[1] & Ariela V. Paula[1]*

[1] *Department of Engineering of Bioprocesses and Biotechnology, São Paulo State University, Araraquara, Brazil.*
[2] *Nanotechnology National Laboratory for Agriculture, Brazilian Agricultural Research Corporation, São Carlos, Brazil.*
[3] *State Center for Technological Education Paula Souza, Faculty of Technology of Barretos, Barretos, Brazil.*
[4] *Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, Brazil*
* *ariela.veloso@unesp.br*

ABSTRACT

Geranyl acetate is an FDA-approved ester used as a flavoring agent that has antimicrobial properties. However, its limited solubility in water is a challenge for maximizing its antimicrobial effectiveness. Some authors are exploring nanotechnology methods, such as using nanocarrier systems, to enhance solubility of biomolecules. In this context, the aim was to create a polymeric nanoemulsion (NE) with geranyl acetate as the oil-core and evaluate its antimicrobial activity *in vitro*. Dynamic Light Scattering yielded promising results for the developed NE. A monodisperse NE with submicron particle size and a Zeta Potential of -30,2 mV was achieved, indicating stability during analysis. The geranyl acetate nanoemulsion exhibited great antimicrobial activity at low concentrations, particularly against bacteria, suggesting its potential for food and pharmaceutical applications. Overall, the method proved effective in producing a stable and uniform nanocarrier system with significant antimicrobial efficacy.

Keywords: Natural compound. Nanotechnology. Nanocarrier. Geraniol. MIC.

1 INTRODUCTION

Essential oils and their constituents, such as terpenic alcohols and phenols, have been extensively studied for their antimicrobial, larvicidal, insecticidal, and repellent properties. However, the investigation of their derivatives, such as aromatic esters, remains less explored[1].

One of the compounds found in some essential oils is geraniol, a monoterpene alcohol recognized for its bioactive properties, including antioxidant, antitumor, and antimicrobial activities[2]. Despite its significant antimicrobial potential, its application is limited due to high volatility, molecule instability, low solubility, and some toxicity at higher concentrations[3].

To overcome these challenges, some authors have adopted the strategy of esterifying geraniol with carboxylic acids to stabilize the molecule and enhance desirable characteristics such as antimicrobial action. However, the solubility of the compound in aqueous environments remains a challenge for its optimization as an antimicrobial agent.

In turn, to address this challenge, studies propose the application of biocompounds in nanostructured systems. The use of nanocarrier systems, such as nanoemulsions (NEs), enables the creation of a nanoproduct with higher bioavailability, improved water solubility, and controlled release[4,5]. Furthermore, studies in the literature have demonstrated prolonged antimicrobial efficacy following nanoemulsification.

Thus, the aim of the study was to develop a polymeric NE using geranyl acetate ester as the oil-core, as well as to investigate the *in vitro* antimicrobial activity of the compound in its nanostructured form.

2 MATERIAL & METHODS

Geranyl acetate ester was previously synthesized and purified according to Remonatto et al. (2022)[2].

To obtain a reservoir-type nanocarrier system with a lipophilic core (containing geranyl acetate ester as the oil-core), the nanoprecipitation method was applied according to De Paula et al. (2017) modified[6]. The NEs were synthesized at a concentration of 1.25% (w/v) of geranyl acetate.

For physicochemical characterization, the particle size, polydispersity index (PdI), and zeta potential (ζ) were determined using Dynamic Light Scattering (DLS) technique. The analyses were conducted 1, 7, and 27 days after synthesis.

The in-vitro antimicrobial activity assay of geranyl acetate nanoemulsion (NE/GA) followed the microdilution method in Brain Heart Infusion (BHI) broth[7]. The minimum inhibitory concentration (MIC) was investigated against two bacterial strains (*Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 700926). Additionally, two fungal strains, *Rhizopus stolonifer* (CCT 0276) and *Botrytis cinerea* (CCT 1252), were selected, and MIC was determined according to Souza-Moreira (2019)[8].

3 RESULTS & DISCUSSION

The NE/GA demonstrated a particle size preferable for submicron polymeric colloidal particles, with a size below 100nm. The obtained PdI value may indicate some polydispersity in the system, suggesting potential particle aggregation and/or size variation. However, the percentage of particles by number (>97.9%) in the region of the predominant average size indicates a monodisperse system (as shown in Figure 1) with relative variation in particle distribution. The average value of the Zeta Potential suggests potential stability of the nanostructured system.

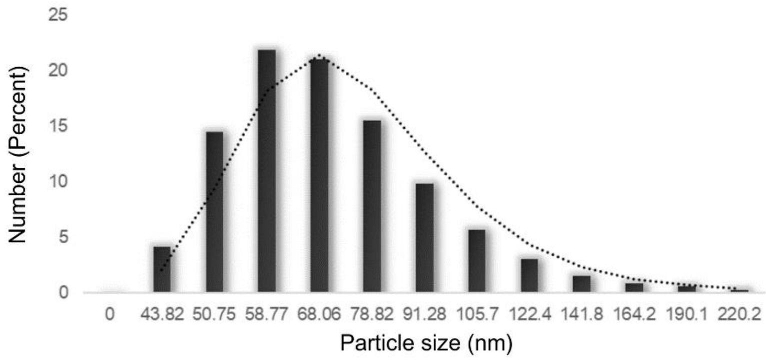

Figure 1 Particle size distribution of the nanoemulsion synthesized with Geranyl acetate

Particle size, polydispersity index, and zeta potential are crucial analyses for characterizing nanoparticles. Zeta potential reflects surface properties, impacting biological performance and safety. Alongside the polydispersity index, these parameters indicate the stability of nanomaterials, essential for their efficacy in biological applications. The results (Table 1) of these analyses showed that the polymeric formulations exhibit the desired physicochemical parameters, with particle size and zeta potential indicating stability during the analysis period.

Table 1 Mean particle size, polydispersity index (PdI), and zeta potential of the synthesized geranyl acetate nanoemulsion (NE/GA)

	Mean particle size (nm)	PdI	Zeta Potential
NE/GA	73,3 ± 20,5	0,7 ± 0,1	-30,2 ± 0,5

The produced NE was then used in the antimicrobial activity assay. As shown in Table 2, the NE/GA inhibited microorganisms at low dosages, especially for bacteria, demonstrating its potential application in food and pharmaceuticals as an antimicrobial agent.

Table 2 Minimum inhibitory concentration (MIC) of nanoemulsions using 1.25% geranyl acetate.

Microorganism	MIC (%)
S. aureus ATCC 6538	0,16
E. coli ATCC 700926	0,31
R. stolonifer CCT 0276	1,25
B. cinerea CCT 1252	0,63

4 CONCLUSION

The method employed for the synthesis of polymeric nanoemulsions of geranyl acetate was satisfactory. A stable and monodisperse nanocarrier system was obtained. The antimicrobial activity assay demonstrated that the ester nanoemulsion as the oil-core has potential application as an antimicrobial agent to be used in food and pharmaceuticals, with notable action against the tested bacteria.

REFERENCES

1 TABARI M. A., YOUSSEFI M. R., ESFANDIARI A., BENELLI G. 2017. Res. Vet. Sci. 114. 36–40.
2 REMONATTO D., FANTATTO R. R., PIETRO R. C. L. R., MONTI R., DE OLIVEIRA J. V., DE PAULA A. V., BASSAN J. C. 2022. Process Biochem. 120. 287–300.
3 WANG P., WU Y. 2021. Food Hydrocoll. 120. 106922.
4 AZIZKHANI M., KIASARI F. J., TOORYAN F., SHAHAVI M. H., PARTOVI R. J. 2021. Food Sci. Technol. 58 (4). 1341–1348.
5 IRACHE J. M., ESPARZA I., GAMAZO C., AGÜEROS M., ESPUELAS S. 2011. Vet. Parasitol. 180 (1–2). 47–71.
6 DE PAULA L. B., PRIMO F. L., PINTO M. R., MORAIS P. C., TEDESCO A. C. 2017. RSC Adv. 7 (15). 9115–9122.
7 PANKEY G. A., SABATH L. D. 2004. Clin. Infect. Dis. 38 (6). 864–870.
8 SOUZA-MOREIRA T. M., SEVERI J. A., RODRIGUES E. R., DE PAULA M. I., FREITAS J. A., VILEGAS W., PIETRO R. C. L. R. 2019. Nat. Prod. Res. 33 (17). 2579–2582.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Finance Code 001; and by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, nº 2020/09592-1). A.V. Paula thanks the National Council of Scientific and Technological Development, Brazil (Conselho Nacional de Desenvolvimento Científco e Tecnológico—CNPq) — proc. no. 304399/2022-1. J. F. C. do Nascimento also acknowledges the support from CAPES proc. no. 88887.900168/2023-00.