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Research Article

Susceptibility of bacterial species isolated from mares to ozonated sunflower oil

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Abstract: Sunflower oil is known for its therapeutic properties and culinary use. It is an important alimentary source of tocopherol and unsaturated fatty acids, and is used especially for wound healing. Studies on its antimicrobial potential, however, are lacking. The ozonation of oils of vegetable sources has been explored to enhance their therapeutic properties; however, studies that provide evidence of such benefits are still lacking. In the field of veterinary medicine, such data are even more scarce. In this study, the antimicrobial activity of ozonated sunflower oil was compared to that of non-ozonated oil, in an in vitro system, against strains of *Staphylococcus aureus* and *Escherichia coli*, isolated from intrauterine lavages of mares with endometritis. Tests were conducted using the minimum inhibitory concentration method. The ozonated oil was effective against *S. aureus*, whereas it was not against *E. coli* isolates. Our data open doors for discussion on the use of sunflower oil, with or without ozone treatment, for therapeutic purposes in veterinary medicine.

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1. INTRODUCTION

The world is facing a growing scenario of bacterial, fungal, and viral resistance to the currently available antimicrobial drugs, and the pharmaceutical industries do not show interest in developing new synthetic or semi-synthetic active molecules (Lai *et al*., 2022; Weis *et al*., 2022). This situation is even more complex in the field of Veterinary Medicine, as there are few studies that provide solid evidence in this context, but high levels of resistance are expected (Schwars *et al*., 2017).

In this context, exploring natural products as antimicrobials is more important than it sounds, as they have the potential to open doors for more effective and less expensive treatments, especially regarding infectious diseases (Dias-Souza *et al*., 2017). Studies with natural products usually do not require immediate technology transfer or very advanced technologies (although they are widely necessary in further steps of the research). The complex structure of

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phytomolecules, the availability of the plants in the environment, and the possibility of cultivation in controlled conditions make research in this field even more compelling (Dias-Souza *et al*., 2018).

Sunflower (*Helianthus annus* L.) oil, obtained from the seeds (one of the main oil crops in the world), is widely used for culinary and food technology purposes, which include cooking by frying, food packaging, and preservation (Kassab *et al*., 2019; Filho & Egea, 2021). The byproducts of the oil, often associated to negative environmental impacts and considered unuseful, are now relevant in the current global trend of implementation of greener industrial processes (Rai *et al*., 2016; Rauf *et al*., 2017). Concerning its biological properties, sunflower oil is mostly known by its wound healing and anti-inflammatory potentials, which are associated to its unsaturated fatty acids (Poljšak *et al*., 2020). Its antimicrobial activity, however, remains poorly described.

Here we investigated the antimicrobial potential of ozonated and conventional (non-ozonated) sunflower oil. There is a growing interest in the use of ozonated oils for the treatment of infectious and/or inflammatory diseases, and some commercial formulations are already available in the pharmaceutical market for human and veterinary use. Ozone is largely known for its biological properties such as wound healing and antimicrobial activity (Di Mauro *et al*., 2019); however, the efficacy and safety of ozone-treated oils remain in discussion. Ozone reacts in different ways with the molecules present at the oils, and products such as peroxides can cause DNA and cell membrane damage in determined conditions (Krkl *et al*., 2016).

In this study we show that conventional and ozonated sunflower oil are effective against *Staphylococcus aureus* and *Escherichia coli* strains isolated from mares with endometritis, a common condition that prevents mares of having a successful pregnancy. These bacterial species are among the most prevalent in the altered intrauterine microbiota of mares with the disease (Ávila *et al*., 2022). Our data open doors for more studies exploring sunflower oil to treat endometritis in mares.

2. MATERIAL and METHODS

2.1. Microorganisms

Ten clinical isolates of *S. aureus* and of *E. coli* from mares with endometritis were selected from the bacterial collection of the research laboratory from Pitágoras College (MG, Brazil). The isolates were cultivated in sterile BHI broth (35±2 °C, 18 h) prior to the experiments.

2.2. Preparation of the Oils

The oils with and without ozone treatment were purchased from local compounding pharmacies. For the antimicrobial activity assays, the oils were prepared in 0.9% sterile saline solution with 0.5% Tween 80 to increase their miscibility in water. A stock solution (4.1 mg/mL) was prepared on the same day of the experiments.

2.3. Antimicrobial Assays

Minimal inhibitory concentration (MIC) of the oils was determined in triplicate following CLSI standards and a protocol standardized by our group using 96-wells polystyrene plates (Dias-Souza *et al*., 2017; CLSI, 2018), with slight modifications. The stock solution of the oils was diluted in sterile 0.9% saline to reach final concentrations in the 96-wells plates ranging from 1024 to 8 µg/mL (100 µL). The overnight-grown bacterial inoculum was diluted in sterile Mueller-Hinton broth (Himedia) to reach the final concentration of $5x10^4$ CFU/mL (100 µL). The plates were incubated overnight at 35±2 °C. Next, we performed resazurine staining (0.1 g/L, 20 µL). The lowest concentration in which no color change from blue to pink was observed in the plates was considered the MIC. We used a 0.9% sterile saline solution with 0.5% Tween 80 as a negative control.

3. RESULTS and DISCUSSION

The ozonated sunflower oil was active against *S. aureus*, whereas the conventional oil was not active to both species even at the highest concentration tested. The ozonated oil was also not active against *E. coli* strains as well (Table 1). Negative control was also not active, as expected.

Table 1. Susceptibility of the isolates to ozonated and conventional sunflower oil

Bacterial species	MIC (μg/mL)	
	Ozonated sunflower oil	Conventional sunflower oil
E. coli	NA	NA
S. aureus	512	NA

Data is referent to all strains. NA: not active.

One may question why we did not explore higher concentrations of the oil in this study. MIC tests are conducted from values ranging from 1024 to 8 μg/mL as to simulate safe *in vivo* concentrations for treating infectious diseases (CLSI, 2018). Substances of elevated MIC values are not of interest for eventual clinical use, as large (and potentially toxic) amounts of them would be necessary (Karasawa & Steyger, 2011).

E. coli is a Gram-negative bacterial species naturally present at the intestinal tract of mammals. It can also behave a pathogen associated to several diseases such as urinary tract infections, intestinal infections, and, in mares and other animals, endometritis (Ávila *et al*., 2012). *S. aureus* is a Gram-positive bacterial species that is commensal to humans and animals, being associated to several skin and internal soft tissues infectious diseases. Recent studies suggest that both *E. coli* and *S. aureus* are among the most frequent bacterial species isolated from endometritis in mares. In the present study, the conventional oil was not effective against any of the species. However, the ozonated oil was effective against *S. aureus*, a possible effect from the peroxides generated by the ozonation process (Krkl *et al*., 2016; Di Mauro *et al*., 2019). Interestingly, the ozonated oil was also not effective against *E. coli.*

Studies that adequately investigated the antimicrobial potential of sunflower oil remain scarce. Although sunflower oil is not clearly associated to any cytotoxic events, previous studies indicate that concentrations in mg/mL scale would present considerable antimicrobial activity. A commercial formulation of ozonated sunflower oil was active against ATCC strains of *S. aureus* and *E. coli* at 9.5 and 4.75 mg/mL, respectively (Sechi *et al*., 2001). Curiously, a study described that both ozonated and conventional sunflower oils were active against ATCC strains of *S. aureus* and *E. coli*, with MIC values of 4.5 and 9.5 mg/mL, respectively (Diaz *et al*., 2006). Our data is partially in agreement with that of these studies, considering the effectiveness of the oil on *S. aureus* isolates.

Interestingly, an investigation suggested that fungal and yeast species associated to dermatophytosis can be more susceptible to sunflower oil than *E. coli* (Tabassum and Vidyasagar, 2014). A recent *in vivo* study described that a commercial ozonated sunflower oil reduced the bacterial counts of mares with endometritis, including *E. coli*, using 50 mL of the formulation *in uterus* (Ávila *et al*., 2022). Full eradication was not achieved, and high concentrations were necessary to reduce bacterial counts.

4. CONCLUSION

The ozonated sunflower oil was active against *S. aureus* isolated from mares with endometritis, but was not effective against *E. coli*, suggesting that treatments with this product may not be effective. The treatment of infectious diseases is mostly empiric in clinical routines, and the use of substances of known effectiveness is of paramount relevance to prevent bacterial resistance.

More studies are necessary to confirm the safety of the oil in higher concentrations, in which they might be effective.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Gabriel Souza dos Santos: Conducted the experiments, drafted the manuscript. **Arthur Azevedo Perpétuo**: Conducted the experiments, drafted the manuscript. **Marcus Vinícius Dias-Souza**: Designed the study, supervised experiments, wrote the final version of the manuscript.

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REFERENCES

Ávila, A., Diniz, N.C., Serpa, R.T., Chaves, M., Viu, M., & de Oliveira, R.A. (2022). Effectiveness of Ozone Therapy in The Treatment of Endometritis in Mares. *Journal of Equine Veterinary Science*, *112*, 103900. https://doi.org/10.1016/j.jevs.2022.103900

Clinical and Laboratory Standards Institute - CLSI. 2018. Performance standards for antimicrobial susceptibility testing; 27th informational supplement. M100-S28. Clinical and Laboratory Standards Institute, Wayne, PA.

Di Mauro, R., Cantarella, G., Bernardini, R., Di Rosa, M., Barbagallo, I., Distefano, A., Longhitano, L., Vicario, N., Nicolosi, D., Lazzarino, G., Tibullo, D., Gulino, M. E., Spampinato, M., Avola, R., & Li Volti, G. (2019). The Biochemical and Pharmacological Properties of Ozone: The Smell of Protection in Acute and Chronic Diseases. *International Journal of Molecular Sciences*, *20*(3), 634. https://doi.org/10.3390/ijms20030634

Dias-Souza, M.V., Dos Santos, R.M., de Siqueira, E.P., & Ferreira-Marçal, P.H. (2017). Antibiofilm activity of cashew juice pulp against Staphylococcus aureus, high performance liquid chromatography/diode array detection and gas chromatography-mass spectrometry analyses, and interference on antimicrobial drugs. *Journal of Food and Drug Analysis*, *25*(3), 589–596. https://doi.org/10.1016/j.jfda.2016.07.009

Dias-Souza, M.V., Dias, C.G., & Ferreira-Marçal, P.H. (2018). Interactions of natural products and antimicrobial drugs: Investigations of a dark matter in chemistry. *Biointerface Research in Applied Chemistry*, *8*(3). 3259–3264

Filho, J. G.O., & Egea, M. B. (2021). Sunflower seed byproduct and its fractions for food application: An attempt to improve the sustainability of the oil process. *Journal of Food Science*, *86*(5), 1497–1510. https://doi.org/10.1111/1750-3841.15719

Karasawa, T., & Steyger, P.S. (2011). Intracellular mechanisms of aminoglycoside-induced cytotoxicity. *Integrative Biology: Quantitative Biosciences from Nano to Macro*, *3*(9), 879–886. https://doi.org/10.1039/c1ib00034a

Kassab, Z., El Achaby, M., Tamraoui, Y., Sehaqui, H., Bouhfid, R., & Qaiss, A. (2019). Sunflower oil cake-derived cellulose nanocrystals: Extraction, physico-chemical

characteristics and potential application. *International Journal of Biological Macromolecules*, *136*, 241–252. https://doi.org/10.1016/j.ijbiomac.2019.06.049

Krkl, C., Yiğit, M.V., Özercan, İ.H., Aygen, E., Gültürk, B., & Artaş, G. (2016). The Effect of Ozonated Olive Oil on Neovascularizatıon in an Experimental Skin Flap Model. *Advances in Skin & Wound Care*, *29*(7), 322-327. https://doi.org/10.1097/01.ASW.0000484172.04260.46

Lai, X., Han, M.L., Ding, Y., Chow, S.H., Le Brun, A.P., Wu, C.M., Bergen, P.J., Jiang, J.H., Hsu, H.Y., Muir, B.W., White, J., Song, J., Li, J., & Shen, H.H. (2022). A polytherapy based approach to combat antimicrobial resistance using cubosomes. *Nature Communications*, *13*(1), 343. https://doi.org/10.1038/s41467-022-28012-5

Poljšak, N., Kreft, S., & Kočevar Glavač, N. (2020). Vegetable butters and oils in skin wound healing: Scientific evidence for new opportunities in dermatology. *Phytotherapy Research: PTR*, *34*(2), 254–269. https://doi.org/10.1002/ptr.6524

Rai, A., Mohanty, B., & Bhargava, R. (2016). Supercritical extraction of sunflower oil: A central composite design for extraction variables. *Food Chemistry*, *192*, 647–659. https://doi.org/10.1016/j.foodchem.2015.07.070

Rauf, S., Jamil, N., Tariq, S.A., Khan, M., Kausar, M., & Kaya, Y. (2017). Progress in modification of sunflower oil to expand its industrial value. *Journal of the Science of Food and Agriculture*, *97*(7), 1997–2006. https://doi.org/10.1002/jsfa.8214

Schwarz, S., Loeffler, A., & Kadlec, K. (2017). Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. *Veterinary Dermatology*, *28*(1), 82–e19. https://doi.org/10.1111/vde.12362

Sechi, L.A., Lezcano, I., Nunez, N., Espim, M., Duprè, I., Pinna, A., Molicotti, P., Fadda, G., & Zanetti, S. (2001). Antibacterial activity of ozonized sunflower oil (Oleozon). *Journal of Applied Microbiology*, *90*(2), 279–284. https://doi.org/10.1046/j.1365-2672.2001.01235.x

Tabassum N., & Vidyasagar, G. M. (2014). *In-vitro* antimicrobial activity of edible oils against human pathogens causing skin infections. *International Journal of Pharmaceutical Sciences and Research*, 5(10), 4493-4498. https://doi.org/10.13040/IJPSR.0975-8232.5(10).4493-98

Weis, C., Cuénod, A., Rieck, B., Dubuis, O., Graf, S., Lang, C., Oberle, M., Brackmann, M., Søgaard, K.K., Osthoff, M., Borgwardt, K., & Egli, A. (2022). Direct antimicrobial resistance prediction from clinical MALDI-TOF mass spectra using machine learning. *Nature Medicine*, *28*(1), 164–174. https://doi.org/10.1038/s41591-021-01619-9