International Journal of Basic and Applied Science 11 (3) (2022) 138-141
Published by: IOCSCIENCE

International Journal of Basic and Applied Science

Journal homepage: www.ijobas.pelnus.ac.id

Susceptibility of *Escherichia coli* isolates from mares to ozonated sunflower oil

Gabriel Souza dos Santos[1] , Arthur Azevedo Perpétuo[1], Tiago Santos Fonseca[2], Marcus Vinícius Dias-Souza[3]

[1]Bachelor of Science program in Veterinary Medicine, Pitágoras College, Ipatinga, MG, Brazil.
[2]Bachelor of Science program in Pharmacy, Pitágoras College, Ipatinga, MG, Brazil.
[2]Reference Center for Drug Information, Pitágoras College, Ipatinga, MG, Brazil.

Article Info

Article history:

Received Aug 25, 2022
Revised Nov 20, 2022
Accepted Dec 11, 2022

Keywords:

Antimicrobial;
Escherichia coli;
Ozonation;
Sunflower oil.

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 indicate that it also has antimicrobial potential. The ozonation of oils of vegetable sources has been explored as a way 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. We used clinical isolates of Escherichia coli, obtained from intrauterine lavages of mares with endometritis. Tests were conducted using the minimum inhibitory concentration method. Our data open doors for discussion on the use of sunflower oil, with or without ozone treatment, for therapeutic purposes in veterinary medicine.

This is an open access article under the CC BY-NC license.

Corresponding Author:

Marcus Vinícius Dias-Souza, PhD
Reference Center for Drug Information,
Pitágoras College, Brazil.
401 Jequitiba Street, Horto, Ipatinga, MG.
Email: souzamvd@gmail.com

1. INTRODUCTION

The clinical use of oils from vegetable sources has scientific support regarding different purposes. Sunflower oil, obtained from the seeds, is of wide availability and low cost in several countries [1]. It is an important source of tocopherol and fatty acids, largely explored for health and cooking purposes [2]. In addition to its emollient and wound healing properties, some studies suggest that it has antibacterial potential [3][4][5].

Natural products are poorly used for clinical treatments in veterinary medicine [6]. There is lack of evidence, in many cases, on the efficacy of plants and their extracts (such as oily, aqueous, ethanolic) for the treatment of diseases. Among these is bacterial endometritis in mares, which impairs the animal health and hampers reproduction [7]. Some studies suggest that the ozonated sunflower oil may represent an alternative on the use of antimicrobial drugs in the treatment of this disease [8],[9]. However, experimental evidences in this context remain scarce.

Here we evaluated the antimicrobial efficacy of ozonated sunflower oil, compared to non-ozonated oil, against *Escherichia coli* isolates obtained from uterine lavages of mares.

2. RESEARCH METHOD

2.1. Microorganisms

Ten clinical isolates of *E. coli* were selected from the bacterial collection of the research laboratory of Pitágoras College (Brazil). The isolates had their identity confirmed using classical biochemical tests. The isolates were prepared as to reach 0.5 MacFarland scale turbidity.

2.2. Preparation of oils

The oils with and without ozone treatment were purchased from local compounding pharmacies. For the antimicrobial activity assays, the oils were prepared in sterile aqueous suspensions at 4.1 mg/mL. We used 0.5% v/v Tween 80 to increase their miscibility in water.

2.3. Antimicrobial activity assays

The antimicrobial activity was evaluated by the minimum inhibitory concentration (MIC), following the CLSI method [10]. The concentrations ranged from 1024 to 8 µg/mL. A sterile 0.5% Tween 80 aqueous solution was used as negative control. MIC was conducted in 96 wells microtitration plates.

3. RESULTS AND DISCUSSIONS

Surprisingly, the antimicrobial activity of both the oils was superior to the maximum concentration tested (1014 µg/mL) and could not be determined on our methodological approach. The negative control was also ineffective.

The lack of activity of the oils is an important data, as there is a growing interest on the use of the ozonated sunflower oil for antimicrobial purposes [11][12]. Our data suggests that it might be not as effective as expected, making the antimicrobial susceptibility testing even more relevant. Commercial products with ozonated oils can be freely purchased on pharmaceutical enterprises, in several countries, as they are considered GRAS (generally recognized as safe) products, what open doors for improper use [13][14][15].

One may question why we did not explore higher concentrations of the oil on our studies. Substances of elevated MIC values are not of interest for further research, as they suggest that large amounts of them would be necessary for eventual clinical use [16][17], what can jeopardize patient (human or animal) safety.

4. CONCLUSION

The ozonated oil was not effective against *E. coli* strains, as well as the non-ozonated oil. It is possible that the However, more studies are necessary with a higher number of isolates, and also, *in vivo*, as to overcome the limitations of *in vitro* studies.

REFERENCES

[1] B. S. Adeleke and O. O. Babalola, "Oilseed crop sunflower (*Helianthus annuus*) as a source of food: nutritional and health benefits," *Food Sciences and Nutrition*, vol. 8, no. 9, pp. 4666–4684, 2020.https://onlinelibrary.wiley.com/doi/abs/10.1002/fsn3.1783

[2] R.E. Go, *et al.*, "Effects of palm and sunflower oils on serum cholesterol and fatty liver in rats," *Journal of Medicinal Food*, vol. 18, no. 3, pp. 363–369, 2015.https://www.liebertpub.com/doi/abs/10.1089/jmf.2014.3163

[3] A. Ayerdi-Gotor, *et al.*, "Comparative analysis of fatty acids, tocopherols and phytosterols content in sunflower cultivars (*Helianthus annuus*) from a three-year multi-local study," *Phyton*, vol. 84, no. 1, pp. 14–25, 2015.https://www.hindawi.com/journals/cfg/2008/284508/

[4] M. K. Gupta, "Sunflower oil. Vegetable oils in food technology," in *Vegetable Oils in Food Technology Composition, Properties and Uses*, pp. 128–156, Blackwell Publishing: Oxford, 2002.https://www.researchgate.net/profile/Dimitrios-Boskou-2/publication/6633297_Olive_oil/links/0c96052f8f0432417e000000/Olive-oil.pdf#page=143

[5] M. R. Akkaya, "Fatty acid compositions of sunflowers (*Helianthus annuus* L.) grown in east Mediterranea region," *Rivista Italiana Delle Sostanze Grasse*, vol. XCV, no. 4, pp. 239–247, 2018.https://www.researchgate.net/profile/Murat-Akkaya/publication/328143287_Fatty_acid_compositions_of_sunflowers_Helianthus_annuus_L_grown_in_east_Mediterranea_region/links/5bbb4c9e4585159e8d8c313b/Fatty-acid-compositions-of-sunflowers-Helianthus-annuus-L-grown-in-east-Mediterranea-region.pdf

[6] S. Rastogi, *et al.*, "Veterinary herbal medicines in India," *Pharmacognosy Reviews*, vol. 9, no. 18, pp. 155–163, 2015.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4557239/
[7] E. M. Woodward, *et al.*, "Endometrial inflammatory markers of the early immune response in mares susceptible or resistant to persistent breeding-induced endometritis," *Reproduction*, vol. 145, no. 3, pp. 289–296,2013.https://www.researchgate.net/profile/Mette Christoffersen/publication/236197400_Endometrial_inflammatory_markers_of_the_early_immune_response_in_mares_susceptible_or_resistant_to_persistent_breeding_induced_endometritis_PBIE/links/559b7fe208ae5d8f39381a71/Endometrial-inflammatory-markers-of-the-early-immune-response-in-mares-susceptible-or-resistant-to-persistent-breeding-induced-endometritis-PBIE.pdf
[8] I.S. Tilak, B. Kisan, I. S. Goud et al., "Biochemical and molecular characterization of parents and its crosses for high oleic acid content in sunflower (*Helianthus annuus* L.)," *International Journal of Current MicrobiologyandAppliedSciences*,vol.7,no.4,pp.2000–2020, 2018.https://www.researchgate.net/profile/Mahantesha-Naika/publication/324651141_Biochemical_and_Molecular_Characterization_of_Parents_and_its_Crosses_for_High_Oleic_Acid_Content_in_Sunflower_Helianthus_annuus_L/links/5aed3a54458515f59982f328/Biochemical-and-Molecular-Characterization-of-Parents-and-its-Crosses-for-High-Oleic-Acid-Content-in-Sunflower-Helianthus-annuus-L.pdf
[9] T. F. Abro, P. K. Oad, J. K. Sootaher et al., "Genetic variability and character association between grain yield and oil content traits in sunflower (*Helianthus annuus* L.)," *International Journal of Biology and Biotechnology*, vol. 17, no. 4, pp. 701–706, 2020https://www.ejbio.org/index.php/ejbio/article/view/177.
[10] CLSI/NCCIS, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, CLSI, Wayne, PA, USA, 2013.https://www.academia.edu/download/33848619/High_Prevalence_of_Multidrug-Resistance_MRSA_and_VRSA_of_Different_Infections_from_Al-Jumhuory_Teaching_Hospital_Patients_in_Mosul.pdf
[11] J. J. Salas, *et al.*, "High stearic sunflower oil: latest advances and applications," *Oilseeds and Fats Crops and Lipids*,vol.28,no.35,pp.1–8,2021.https://www.ocl-journal.org/articles/ocl/abs/2021/01/ocl2100225/ocl2100225.html
[12] S. Rauf, *et al.* "Progress in modification of sunflower oil to expand its industrial value," *Journal of the Science ofFoodandAgriculture*,vol.97,no.7,pp.1997–2006, 2017.https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.8214
[13] H. T. Ebeed, *et al.*, "Comparative analysis of seed yield and biochemical attributes in different sunflower genotypes under different levels of irrigation and salinity," *Egyptian Journal of Botany*, vol. 59, no. 2, pp. 339–355, 2019.https://link.springer.com/article/10.1007/s12298-019-00744-7
[14] A. Dimitrijević, *et al.*, "Oleic acid variation and marker-assisted detection of pervenets mutation in high- and low-oleic sunflower cross," *Crop Breeding and Applied Biotechnology*, vol. 17, no. 3, pp. 229–235, 2017.https://www.scielo.br/j/cbab/a/QyxCfV3dwhxbcWFNCd8Z48j/?lang=en&format=html
[15] N. Calero, *et al.*, "Influence of chitosan concentration on the stability, microstructure and rheological properties of O/W emulsions formulated with high-oleic sunflower oil and potato protein," *Food Hydrocolloids*,vol.30,no.1,pp.152–162, 2013.https://www.sciencedirect.com/science/article/pii/S0268005X1200104X
[16] K. V. Nguyen, *et al.*, "Antibiotic use and resistance in emerging economies: a situation analysis for Viet Nam," *BMCPublicHealth*,vol.13,pp.1158–1210, 2013.https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-13-1158
[17] G. Sulis, *et al.*, "Antibiotic prescription practices in primary care in low- and middle-income countries: a systematic review and meta-analysis," *PLoS Medicine*, vol. 17, no. 6, Article ID e1003139, 2020.https://gh.bmj.com/content/5/9/e003393.abstract