

RESEARCH ARTICLE (ORIGINAL) 

Wound healing: *In vitro* effects of the pharmacotherapeutic use of sunflower oil (*Helianthus annuus*)

Tratamento de feridas: Efeitos in vitro de aplicações farmacoterapêuticas do óleo de girassol (Helianthus annuus)

Tratamiento de heridas: Efectos in vitro de las aplicaciones farmacoterapêuticas del aceite de girasol (Helianthus annuus)

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Abstract

Background: Vegetable oils have antimicrobial activity and promote cell proliferation. Sunflower oil is used as an alternative for treating skin wounds, particularly in underdeveloped or developing countries.

Objective: To characterize sunflower oil and evaluate the *in vitro* effects on cell proliferation and antimicrobial activity.

Methodology: The study was carried out using gas chromatography-mass spectrometry (GC-MS) analysis and cell proliferation and antimicrobial activity tests.

Results: The chromatographic analysis identified the main components of sunflower oil, namely: unsaturated fatty acids (82.2%) with linoleic (47.8%), oleic (28.7%), and linolenic (3.9%) acids as the main lipids, followed by saturated (12.70%), palmitic (8.8%) and stearic (3.6%) acids. A difference ($p < 0.001$) in cell proliferation was found between treatments with sunflower oil (100 and 10 µg/ml) and the negative controls. It failed in antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*.

Conclusion: Sunflower oil contains a high concentration of essential fatty acids and promotes cell proliferation but fails to inhibit bacterial activity.

Keywords: sunflower oil; wound healing; cell proliferation; antimicrobial; *in vitro* techniques

Resumo

Enquadramento: Óleos vegetais apresentam ação antimicrobiana e promovem a proliferação celular. O óleo de girassol é usado como alternativa para o tratamento de feridas cutâneas, especialmente nos países subdesenvolvidos ou em desenvolvimento.

Objetivo: Caracterizar o óleo de girassol e avaliar os efeitos *in vitro* na proliferação celular e na atividade antimicrobiana.

Metodologia: Análises por cromatografia a gás acoplada à espectrometria de massas, testes de proliferação celular e atividade antimicrobiana.

Resultados: Na análise cromatográfica do óleo de girassol identificaram-se os compostos majoritários - ácidos gordos insaturados (82,2%) tendo como principais lípidos os ácidos linoleico (47,8%), oleico (28,7%) e linolênico (3,9%), seguidos pelos ácidos saturados (12,70%), palmítico (8,8%) e esteárico (3,6%). Houve diferença ($p < 0,001$) entre os tratamentos com óleo de girassol (100 e 10 µg/ml) e controlos negativos na proliferação celular. Ineficácia na atividade antimicrobiana frente às bactérias *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* e *Klebsiella pneumoniae*.

Conclusão: A composição do óleo de girassol mostrou elevada concentração de ácidos gordos essenciais, promoveu proliferação celular, mas não inibiu atividade bacteriana.

Palavras-chave: óleo de girassol; cicatrização; proliferação de células; antimicrobiano; técnicas *in vitro*

Resumen

Marco contextual: Los aceites vegetales tienen acción antimicrobiana y promueven la proliferación celular. El aceite de girasol se utiliza como alternativa para tratar las heridas cutáneas, especialmente en los países subdesarrollados o en vías de desarrollo.

Objetivo: Caracterizar el aceite de girasol y evaluar los efectos *in vitro* sobre la proliferación celular y la actividad antimicrobiana.

Metodología: Análisis por cromatografía de gases acoplado a espectrometría de masas, pruebas de proliferación celular y actividad antimicrobiana.

Resultados: En el análisis cromatográfico del aceite de girasol, se identificaron los compuestos mayoritarios - ácidos grasos insaturados (82,2%), los principales lípidos son el ácido linoleico (47,8%), oleico (28,7%) y linolénico (3,9%), seguidos del ácido saturado (12,70%), palmítico (8,8%) y esteárico (3,6%). Hubo una diferencia ($p < 0,001$) entre los tratamientos con aceite de girasol (100 y 10 µg/ml) y los controles negativos en la proliferación celular. Actividad antimicrobiana ineficaz contra las bacterias *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* e *Klebsiella pneumoniae*.

Conclusión: La composición del aceite de girasol mostró una alta concentración de ácidos grasos esenciales, promovió la proliferación celular, pero no inhibió la actividad bacteriana.

Palabras clave: aceite de girasol; cicatrización de herida; proliferación celular; antimicrobiano; técnicas *in vitro*



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Introduction

The use of medicinal plants and their derivatives has increased considerably. Approximately one-third (1/3) of all traditional herbal medicines are used for treating wounds. The natural products used in these treatments include vegetable oils rich in essential fatty acids, which possess healing properties (Lania et al., 2019).

Topical treatment of wounds can help tissue repair by stimulating the healing process, as some vegetable oils have antimicrobial, anti-inflammatory, and antioxidant properties. These promote cell proliferation, increase collagen synthesis during the healing process, and stimulate dermal reconstruction and lipid barrier repair (Poljšak et al., 2020). Sunflower oil (*Helianthus annuus L.*) is a vegetable oil widely used for wound care in several healthcare settings.

Easy access, low cost, and simple application to the wound (Das et al., 2017) are some of the arguments favoring the use of vegetable oils in underdeveloped or developing countries. Although healthcare professionals widely use sunflower oil (SFO) as part of care protocols, the scientific evidence supporting its use is scarce. Thus, exploring the potential of herbal medicines is urgent, challenging, and crucial for developing new technologies for wound care (Das et al., 2017).

However, this is a complex issue due to the diversity of factors involving the healing process. Thus, it is vital to understand the action of SFO on fibroblast proliferation and against bacteria commonly found in chronic wounds, as these directly influence the healing process. This study aims to characterize SFO and evaluate the *in vitro* effects on cell proliferation and antimicrobial activity.

Background

Tissue repair is a physiological process essential for maintaining the skin's protective function after its integrity is breached. It is a complex, highly regulated, and crucial process for recovering and maintaining the skin's barrier function (Han & Ceilley, 2017). The process of tissue repair includes the following phases: coagulation, inflammation, proliferation, collagen deposition, epithelialization, wound contraction with granulation tissue formation, remodeling, and/or maturation (Cañedo-Dorantes & Cañedo-Ayala, 2019). Tissue repair begins immediately after the wound is suffered, with the inflammatory phase. During the proliferative phase, keratinocytes, fibroblasts, and endothelial cells migrate to the wound tissue to ensure epithelialization and granulation tissue formation. In the maturation phase, proteolytic enzymes degrade the excessive collagen (Han & Ceilley, 2017).

Acute wounds are injuries that heal within an expected period. Chronic wounds are injuries that undergo the healing process inadequately for different reasons, including underlying metabolic and physiological disorders (such as diabetes, vascular deficits, hypertension, and chronic

kidney disease) and socioeconomic and psychosocial factors (Sen, 2021). Both acute and chronic wounds do not progress seamlessly through the healing phases and persist in the inflammatory phase, remaining incurable despite proper treatment (Han & Ceilley, 2017). The prolonged healing time causes microbial infections to become recurrent. Among the bacterial species commonly found in wounds are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* (Dydak et al., 2021; Garcia et al., 2021).

Pharmaceutical products based on essential fatty acids from vegetable oils are widely available in Brazil. Their therapeutic effect is mainly due to the oleic, linoleic, and linolenic acids, which are essential for maintaining epidermal integrity and the skin's water barrier (Lania et al., 2019; Poljšak et al., 2020). Linoleic acid, in particular, has shown promising results when applied to wounds due to its action in regulating biochemical events. It stimulates specific cell proliferation factors and consequently improves the formation of granulation and epithelial tissues. However, free fatty acids can also harm the skin's lipid barrier as they can enhance the skin's permeability to their other components (Poljšak et al., 2020).

Being primarily composed of linoleic and linolenic acids, SFO has served as an alternative for wound treatment. It impacts cell proliferation through the increase in collagen synthesis (Lania et al., 2019), preserves the stratum corneum, and prevents transepidermal water loss (TEWL, Poljšak et al., 2020) in addition to its antimicrobial activity (Lin et al., 2017). These aspects possibly explain the idea that it reduces wound healing time.

It is important to mention that the quality of sunflower oil mainly depends on the oil extraction method, processing and storage conditions. (Castro et al., 2021; Rabonato et al., 2017). Thus, the composition of SFOs on the market can vary according to the producer.

Oil extraction is primarily done using artisanal and industrialized methods. Artisanal production is done on a small scale, and the oil is obtained through the continuous cold pressing of the seeds, followed by filtration or decanting to separate residues. This process does not require solvents, refining, or high temperatures (Rabonato et al., 2017). The industrialized method uses pressed whole grains, which undergo an extraction process using solvents (Alves et al., 2019). Thus, there are significant differences in the composition of the SFOs available on the market, possibly due to the different extraction, division, or storage processes. These processes aim to meet specific food industry standards, so there is no concern for the quality characteristics and composition of the product expected for medicinal use.

Research Question

What are the *in vitro* effects of SFO on cell proliferation and antimicrobial activity for wound treatment?

Methodology

This pre-clinical *in vitro* study included sample preparation phases, SFO characterization, and *in vitro* cell proliferation and antimicrobial sensitivity tests.

Cold-pressed SFO was purchased on a Brazilian market. Serial dilutions were prepared from a pure product sample to carry out the cell proliferation and antimicrobial activity experiments.

The volatile components of pure SFO were identified using gas chromatography-mass spectrometry analysis in the HP7820A equipment (with the Agilent EZChrom Elite Compact data acquisition software program), equipped with a flame ionization detector. The SUPELCOWAX 30m x 0.25mm x 0.25 μ m (Supelco) column was used. This analysis was performed at the Analytical Center of the Chemistry Department/Institute of Exact Sciences of the Federal University of Minas Gerais.

The chromatographic conditions were: temperature gradient 120°C (2 min), 2.5°C/min up to 240°C; injector (1/10 split) at 240°C and detector at 260°C. Hydrogen was used as the carrier gas (4.0ml/min) with an injection volume of 2 μ l. Peaks were identified by establishing the comparison with the FAME C14-C22 methylated fatty acid standards (Supelco cat. no 18917) and a mixture of methyl esters containing methyl dodecanoate, methyl stearate, and methyl linoleate. The results show the percentage (%) of the components' area in relation to the sample's total area.

A Mouse Embryonic Fibroblast (MEF) strain grown in Dulbecco's Modified Eagle's Medium (DMEM - Gibco, California, USA), supplemented with 10% Fetal Bovine Serum (FBS) and 1mg/ml of 10% penicillin/streptomycin (Gibco, California, USA) was used. For the proliferation test, 0.85 x 10⁵ cells were grown in a 96-well plate in DMEM solution at 37°C, 5% CO₂ for 24 hours. The cells were then treated with 100 μ g/ml and 10 μ g/ml of SFO, with 20% FBS, in the positive control and 50% dimethyl sulfoxide (DMSO) in the negative control. After 48 hours of incubation, cell viability was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay of colorimetric analysis. Cultures were incubated with 10 μ l of MTT for three hours. After incubation, 100 μ l of DMSO was added to all wells until the precipitate's complete solubility was achieved. The absorbance was measured using a spectrophotometer at 570nm. Two independent rounds of experiments were performed, with eight technical repetitions for each treatment on each plate (n = 32) and the controls. The test result was given using optical density (OD) measures, and the percentage of viable cells in relation to the negative control was calculated. They were presented as mean + SEM (standard error of the mean) and subjected to one-way statistical analysis of variance (ANOVA), followed by a Tukey's test. Values of p < 0.001 were considered significantly different in relation to the negative control. The analyses were performed using the GraphPad Prism 9.0 software.

The antimicrobial activity of SFO was tested against five strains of bacteria frequently present in chronic wound infections (Dydak et al., 202; Garcia et al., 2021).

The standard strains *Escherichia coli* (ATCC 25723) and *Pseudomonas aeruginosa* (ATCC 25853) are gram-negative, and the *Enterococcus faecalis* (ATCC 19433), and *Staphylococcus aureus* (ATCC 29213) are gram-positive. These strains were provided by the Laboratory of Ecology and Physiology of Microorganisms of the Institute of Biological Sciences of the Federal University of Minas Gerais. The Microbiology Laboratory of the Hospital das Clínicas, also from the Federal University of Minas Gerais, provided the *Klebsiella pneumoniae* (wild-type/multi-sensitive).

The pathogens were activated in BHI agar (Acumedia®) plates and incubated for 24h at 37 \pm 2°C. Bacterial suspensions were prepared to match the McFarland 0.5 standard (1x10⁸UFC/ml). The disk diffusion and broth dilution tests were applied to assess antimicrobial activity. The agar disk diffusion test was performed according to the Clinical and Laboratory Standards Institute ([CLSI], 2020). Discs with the antibiotics tetracycline (Laborclin®; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*) or amikacin (*Klebsiella pneumoniae*) were used in the positive control. The negative control was carried out with 0.85% saline solution (SS). The standardized samples were spread on the Mueller Hinton Agar surface, where 6mm sterile filter paper discs were placed and impregnated with 10 μ L of each sample. The plates were incubated at 37°C for 24-48h. The diameter of the zone of inhibition (millimeters) formed around the discs was measured using a digital pachymeter. The results were presented in the form of the mean, followed by the standard deviation.

In the broth dilution test, 100 μ L of BHI + Tween80 1% broth was put in 96-well plates, then 100 μ L of the pure SFO, 2% bacterial inoculum, was added and left incubating at 37°C for 24h. The colorimetric analysis method, described by Araújo and Longo (2016), was used to assess the zone of inhibition of bacterial growth. After 24h of incubation, 20 μ L of 0.01% sodium resazurin (R) solution was added to each well and incubated at 37°C for one hour. Blue color signals bacterial inactivity, while red reveals bacterial activity. At the end of the incubation period, a portion of each well was striated with BHI agar to confirm the bactericidal activity. The plates were then incubated under microbial growth conditions according to the CLSI (2020) guidelines. BHI + Tween80 broth + bacterial inoculum was used for growth control. BHI + Tween80 broth served as the negative control, and BHI + Tween80 broth + bacterial inoculum + tetracycline (Laborclin®) 30 μ g/ml as the positive control for growth inhibition. Both tests were performed in triple samples.

Results

The characterization of SFO is presented in the results of the chromatographic analysis (GC/MS; Table 1). The SFO samples contain primarily unsaturated fatty acids (82.2%), with linoleic (47.8%), oleic (28.7), and linolenic (3.9%) acids as the main lipids, followed by saturated (12.70%), palmitic (8.8%) and stearic (3.6%) acids.

Table 1*SFO components identified in the chromatographic analysis*

COMPONENTS	SFO
Nomenclature	area %
C16:0 - Palmitic	8.8
C16:1 - Palmitoleic	0.1
C16:1 ω 7	0.4
C18:0 - Stearic	3.6
C18:1 - Oleic	26.5
C18:1 ω 7	1.1
C18:1 ω 5	1.1
C18:2- Linoleic	47.8
C18:2 ω 4	1.3
C18:3- γ - Linolenic	3.6
C18:3- α - Linolenic	0.3
C20:0- Arachidic	0.3
Other	5.1
saturated	12.7
monounsaturated	29.2
polyunsaturated	53.0

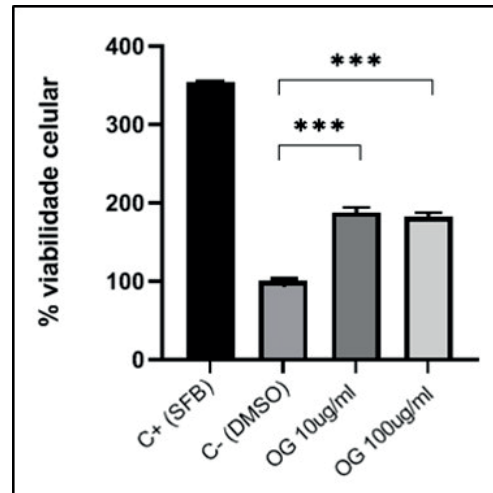
Note. SFO = sunflower oil.

The in vitro test of cell proliferation showed an increase in the proliferation of fibroblasts treated with SFO in both treatments compared to the negative control (DMSO), considered 100%. The in vitro test of cell proliferation showed an increase in the proliferation of fibroblasts treated with sunflower oil in both treatments compared to the negative control (DMSO), considered 100%. The 10 μ g/ml SFO sample seems more effective than the 100 μ g/ml sample.

However, this difference is not statistically significant ($p > 0.001$). The results are described as the mean of the observed OD \pm SEM, followed by the percentage of viable cells in the treatment wells, with the 10 μ g/ml SFO sample = 0.836 ± 0.045 ($87.86 \pm 4.71\%$) and the 100 μ g/ml SFO sample = 0.767 ± 0.027 ($72.36 \pm 2.55\%$), in relation to the negative control with OD = 0.445 ± 0.013 . Positive control shows an OD = 1.576 ± 0.155 (Figure 1; $p < 0.001$).

Figure 1

Cell proliferation of mouse embryonic fibroblasts treated with SFO (10µg/ml and 100 µg/ml)



Note. SFB = FBS = Fetal bovine serum; DMSO = dimethyl sulfoxide, C+ = positive control; C- = negative control; OG = SFO = sunflower oil. *** highly significant values $p < 0.001$ (ANOVA, Tukey).

The experiments on antimicrobial activity, performed using the agar disk diffusion technique (Table 2), reveal that SFO treatment fails to inhibit the tested bacteria's

growth. Similar results are observed in the negative control, whereas the antibiotic used in the positive control inhibited bacterial growth, as expected.

Table 2

SFO antibacterial evaluation against the bacteria S. aureus, E. coli, E. faecalis, P. aeruginosa, and K. pneumoniae

TREATMENTS	MEAN OF THE ZONE OF INHIBITION (mm)				
	Tested pathogens				
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
C+	26.925 (0.355)	23.745 (0.295)	25.21 (0.268)	26.535 (0.555)	22.57 (0.156)
C-	n/a	n/a	n/a	n/a	n/a
SFO	n/a	n/a	n/a	n/a	n/a

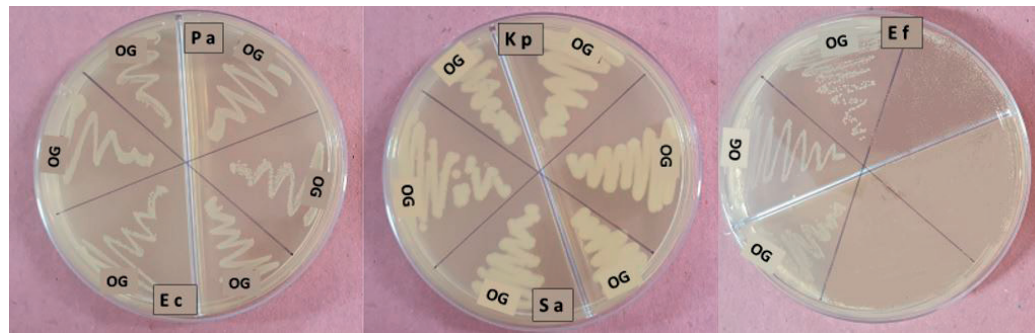
Note: C+ = Positive control (tetracycline for *E. coli*, *S. aureus*, *P. aeruginosa*, *E. faecalis* and amikacin for *K. pneumoniae*); C- = Negative control (0.85% saline); SFO = sunflower oil; n/a = not apparent, SD = Standard deviation.

The antimicrobial sensitivity test applying the broth dilution technique confirms the result obtained using the disk diffusion technique. The wells containing SFO samples became red, indicating no inhibition of growth of the

bacteria *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Figure 2 confirms the result showing the growth of all bacterial colonies tested on the BHI plates.

Figure 2

Growth of the bacterial colonies tested on BHI agar plates



Note. Pa = *P. aeruginosa*; Ec = *E. coli*; Kp = *K. pneumoniae*; Sa = *S. aureus*; Ef = *E. faecalis*; OG = SFO = sunflower oil

Discussion

In general, medicinal plants occupy a prominent position among wound treatment options as they have low costs, are easily found, and are less susceptible to adverse events (Das et al., 2017; Lordani et al., 2018). The pharmaceutical market offers several wound treatment-related products, yet less than 3% are listed in Western pharmacopeias as healing agents. Of these, at least a third is obtained from plants (Lordani et al., 2018). SFO is described as a wound healing promoting agent and is widely used in clinical practice in Brazilian health services (Lania et al., 2019). However, its effects are not yet confirmed due to the lack of robust evidence-based studies.

The sunflower oil used in food has a high content of mono-unsaturated and polyunsaturated fatty acids, especially linoleic and oleic acids (Castro et al., 2021; Rabonato et al., 2017). The present study also observed the same high percentage of components, with the SFO's chromatographic analysis identifying the presence of unsaturated fatty acids (82.20%), primarily linoleic (47.8%), oleic (28.70), and linolenic (3.9%) acids, followed by saturated (12.70%), palmitic (8.8%), and stearic (3.6%) acids. These results align with those of other studies, such as the research conducted on edible oils by Chowdhury et al. (2007) in Bangladesh, which analyzed samples of sunflower ($n = 5$), soybean ($n = 3$), palm ($n = 3$), mustard ($n = 5$), and coconut ($n = 6$) oils. Chowdhury et al.'s (2007) chromatography analysis shows that SFO contains the highest percentage of long-chain mono- and polyunsaturated fatty acids (91.49 ± 1.91 %) compared to other oils.

Alves et al. (2019) analyzed the fatty acid components of 21 commercial vegetable oils purchased on the Brazilian market, including SFO. They compared them with a commercial reference product for wound care in Brazil (Dersani®). The fatty acids found more abundantly in the vegetable oils are: caprylic ($10.45\% \pm 0.07\%$), capric ($5.8\% \pm 0.75\%$), lauric ($45.63\% \pm 0.93\%$), myristic ($16.33\% \pm 2.23\%$), linoleic (omega 6; $57.09\% \pm 8.47\%$) and oleic (omega 9; $52.94\% \pm 12.54\%$) acids. The study concluded that the vegetable oils' profile is similar to the

reference product, which is composed of the following acids: capric ($18.8\% \pm 0.8\%$), caprylic ($17.4\% \pm 0.4\%$), palmitic ($3.8\% \pm 0.0\%$), stearic ($2.7\% \pm 0.0\%$), linoleic (omega 6; $28.1\% \pm 0.5\%$) and oleic (omega 9; $27.5\% \pm 0.5\%$; Alves et al., 2019). Nevertheless, when specifically analyzing linoleic acid (omega 6), the percentage of the total composition in pure SFO (64.1 ± 7.2) differs from the reference product. Likewise, linoleic acid (omega 6; 47.8%) also stands out in the results of this study's results. Lin et al. (2017) relate the components of vegetable oils to their wound healing properties. The fatty acids responsible in SFO for its therapeutic effect are oleic, linoleic, and linolenic. Thus, this study chose to perform a cell proliferation test. The results demonstrate that $10\mu\text{g/ml}$ (87.86%) and $100\mu\text{g/ml}$ of SFO (72.36%) caused increased proliferation of fibroblasts when compared to the negative control (DMSO). In particular, the $10\mu\text{g/ml}$ SFO sample revealed a higher percentage of viable cells. Thus, the benefit of SFO for wound treatment at the tested concentrations is confirmed. However, it is worth noting that the SFO's composition used in clinical practice is not standardized, which can compromise the outcome of *in situ* wound treatments.

Lania et al. (2019) conducted a study with 30 mice with 6mm skin wounds on their backs and treated topically with SFO (essential fatty acids), mineral oil, or SS. They observed that SFO induces the local production of cytokines such as IGF-1, leptin, IL-6, and IFN- γ . However, it shows slower wound healing results, as it took 7.2 days to reach 25% of the total healed area and about 10 days to reach 50%. There was no difference in collagen density between the animals treated with mineral oil and SFO. Lania et al. (2019) argue that further studies are needed to evaluate the treatment safety and possible adverse effects, bearing in mind that SFO is widely used to decrease the healing time of ulcers.

The analysis of the positive results of fibroblast proliferation compared to the negative control and the wound healing results of the animal model obtained by Lania et al. (2019) encourages reflection. However, as nurses must guide their practice based on ethics and scientific evidence and bearing in mind the results obtained, this

study questions the scientific evidence for clinical practice professionals' use of SFO in chronic wound treatment in health services.

The antimicrobial activity tests performed by this study did not demonstrate the inhibition of bacteria growth under the effect of SFO, contrary to other studies in the literature. Liu et al. (2020) found that 0.2 mg/ml of SFO is the minimum inhibitory concentration (MIC) against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, 3.2 mg/ml inhibited the growth of *Saccharomyces cerevisiae*, and 6.4 mg/ml prevents the growth of *Escherichia coli* and *Candida albicans*. Their data also prove the antibacterial and antifungal activity of SFO. Similarly, in the study conducted in India by Tabassum & Vidyasagar (2014), in which dried sunflower seeds were semi-pulverized, and about 250g of the powder was extracted using solvents, SFO showed high antimicrobial activity against *Escherichia coli* bacteria and *Trichophyton rubrum* and *Candida albicans* fungi with MIC values ranging from 0.62 to 40 mg/ml.

Silva et al. (2021) also evaluate the antimicrobial activity of SFO. Their disc diffusion test showed zones of inhibition of 9mm for *Staphylococcus aureus*, 11mm for *Enterococcus faecalis*, and 12mm for *Klebsiella pneumoniae*, demonstrating the antimicrobial activity of SFO. Thus, Silva et al. (2021) describe SFO as potentially promising for infection prevention and skin restoration. However, these results are not unanimous in the literature. The results of Silva et al. (2021) may be influenced by the chemical composition of the samples they analyzed. Some of their components were used in different proportions than the oils presented in other research. This fact reinforces the need for standardizing the chemical composition of the SFOs used to treat wounds in clinical practice.

The results of Silva et al. (2021) may be influenced by the product's composition or the type of sunflower from which the oil was extracted. SFO can be produced from partially dehulled seeds using different production processes (Liu et al., 2020). The oil's quality and stability are the main factors in the production, acceptance, and marketing phases. The processes are influenced by some components, such as free fatty acids, tocopherols, phospholipids, trace metals, and waxes, which have pro- or antioxidant properties (Rhazi et al., 2022). Some vegetable oils can undergo chemical changes if stored for a long time, an aspect confirmed by Castro et al. (2021), whose study assessed the quality of commercial cold-pressed SFO. Their chemical analyses of SFO were done using the variables: iodine, peroxide, acidity indexes, and percentage of free fatty acids. Castro et al. (2021) also demonstrate that the extraction process, industrial processing, and storage conditions influence the product's quality.

The present study has limitations as it is based on pre-clinical *in vitro* research. When treating chronic wounds with SFO, nurses must use clinical reasoning in their decision-making. They must consider the many complex factors involving the wound healing process and that clinical studies with SFO are still limited. Thus, nurses must seek studies that provide information and clarify new treatment possibilities.

The search for knowledge must involve the implementation of clinical trials comparing the effectiveness of SFO with other antimicrobial agents used in clinical practice, besides further analyzing the effect of SFO on reducing the bacterial load and the healing process. The results demonstrate the need for further studies investigating the action of the SFOs available on the market, including those considered reference healthcare products for wound treatment.

Conclusion

SFO contains a high concentration of unsaturated fatty acids, impacts cell proliferation, when compared to negative controls, and demonstrates no antimicrobial activity. Depending on the formulation used, these results may be influenced by the product's chemical composition and effect. Bacterial colonization poses an ongoing challenge in treating and healing skin wounds. Translating the results to support the use of SFO for wound care in clinical practice must be done cautiously. Therefore, further studies are recommended to support the use of SFO in human wound treatment.

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Writing – review & editing: Leite, V. V., Borges, E. L., Ruas, C. M., Januário, L. H.

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