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Research article

Infuse herbal oils: a comparative study of wheat germ and tomato seed oils

Firat Baris Barlas*[1,2]

[1] *Istanbul University-Cerrahpasa, Institute of Nanotechnology and Biotechnology, 34500, Istanbul, Türkiye*
[2] *Health Biotechnology Joint Research and Applications Center of Exxellence, 34220, Istanbul, Türkiye*

Abstract

The use of traditional herbal medicine products has recently been revived, with special emphasis on the use of herbal oils in non-invasive wound treatment. Extraction conditions are very important in seed oils and especially suitable temperatures at which the extraction process is carried out. is vital for the preservation of primary and secondary metabolites. In this study, wheat germ (WG) was collected from the Central Anatolia Region and tomato seed (TS) was collected from the Aegean Region. Oils were obtained from these two seeds by cold pressing and their infused forms (WGO-TSO) were prepared. To investigate the *in vitro* activities of these study groups, cytotoxicity, wound healing, and antioxidant capacity tests were performed on HaCaT (Human keratinocyte) and Vero (Monkey kidney fibroblast-like) cell lines. According to the results of the intracellular cytotoxicity analysis, a common dose of 25 µg/mL was determined to be non-toxic for all groups, and this dose was then used as the working dose. Antioxidant capacity studies, in cells under oxidative stress with hydrogen peroxide, yielded positive results for all working groups and the WGO/TSO group showed statistically superior outcomes. In addition, similar results were obtained in wound healing experiments. As a result, using oils in the form of infusion may be more effective in combating oxidative stress and promoting wound healing than using oil alone.

Keywords: *Antioxidant capacity; cell culture; infuse oils; wound healing*

1. Introduction

Mankind has used plant-derived ingredients in the treatment of diseases since ancient times. Due to their natural antibacterial, antifungal, and wound-healing properties, they played an important role in the survival of people in these times (Shankar and Liao, 2004; Allaw et al., 2021). After the discovery of modern antibiotics and the subsequent side effects and resistance problem, there is a return to herbal ingredients (Munuswamy et al., 2013). In addition, the fact that herbal materials are cheap, have fewer side effects, and have a high variety of active substances attracts the attention of researchers (Budala et al., 2023).

Traditional medicine relies on experience and natural ingredients, especially herbal ingredients. Seed oils and their derivatives, such as creams, soaps, and liposomes, have been extensively used in the treatment of wound healing and dermatological diseases to prevent infection and promote rapid tissue repair without scarring (Ahmad et al., 2013; Ehterami et al., 2019). While plant extracts or oils have demonstrated therapeutic activity on their own, combining them with other biologically active substances can produce even more effective products through a synergistic or additive effect (Khan et al., 2022; Razzaq et al., 2022). As a result, the flavonoid and phenolic compound content of herbal sources can be enhanced and diversified by mixing different sources together.

The tomato (*Solanum lycopersicum*) is an edible berry and one of the most widely cultivated crops that are used for human nutrition around the world. The tomato, a species with origins in western South America, Mexico, and Central America, was first encountered by the Spanish following their contact with the Aztecs. During the 16th century, the plant was brought to Europe

* Corresponding author.
E-mail address: fbbarlas@iuc.edu.tr (F. B. Barlas).
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by the Spanish, marking its introduction to the continent. They contain important compounds like lycopene (LYC) (Machmudah et al., 2012; Osma et al., 2012). Lycopene belongs to the family of carotenoids, which have been shown to have health benefits such as anti-inflammatory, antioxidant, anti-microbial, neuroprotective, and cardiovascular disease prevention (Hung et al., 2008; Shakir et al., 2018; Przybylska, 2020; Binsuwaidan et al., 2022; Przybylska and Tokarczyk, 2022). Also, there are so many studies about lycopene and tomato processing industries produce a large amount of waste, including tomato pomace, which is typically used as animal feed or disposed of as solid waste and the seeds of tomatoes also contain oil that is high in lycopene and beta-carotene, which are important nutrients (Gumus et al., 2020).

Triticum aestivum L., commonly known as wheat, has been a significant part of human evolution and continues to serve as a staple food source for humanity. The utilization of wheat extends beyond human consumption and encompasses the production of oil and feed. Wheat germ oil (WGO), a specialty product derived from wheat, is rich in vitamin E and tocopherols, particularly Alpha-tocopherol (α-TOH). The α-TOH, which is one of the most common tocols in WGO, has been the subject of many studies today. Numerous medical properties have been discovered in the light of these studies, including anti-diabetic, anti-inflammatory, anti-microbial and anti-nephrotoxic activities (Lee et al., 2004; Ajith et al., 2007; Harrabi et al., 2021; Liu et al., 2021; Guven et al., 2022). Removal of wheat germ during flour production from wheat is a common process, while WGO to be produced from it has high medicinal properties. This product is known for its high concentration of vitamin E and tocopherol, making it a valuable ingredient in a variety of applications (Catzeddu et al., 2023).

In this research text, cold-pressed oils were obtained from wheat germ and tomato seeds, and infused versions of these oils were prepared. Then, cytotoxicity, antioxidant capacity, and wound healing experiments were performed with these formulations, and the results were compared with the control groups. The objective of this study was to identify the most biologically active formulation.

2. Materials and methods

2.1. Material

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), H_2O_2 (Hydrogen Peroxide Solution 30 % (w/w) in H_2O), DMSO (Dimethyl sulfoxide, cell culture grade), DMEM (Dulbecco's Modified Eagle Medium), PBS (Phosphate Buffered Saline, pH:7,4), FBS (Fetal Bovine Serum), P/S (Penicillin/Streptomycin) purchased from Sigma Aldrich. Ultra-pure water (Millipore), Microplate Readers (Thermo Fisher Scientific), inverted microscope (Leica).

2.2. Sampling

Wheat germ oil (WGO), tomato seed oil (TSO) and their mix oils obtained by cold-press extraction (CPE) were produced in different regions in Türkiye and were kindly donated from EGE-LS (Türkiye). Tomato seeds (TS) were obtained from İzmir (Aegean Region), and wheat germ (WG) was purchased from Konya city (Central Anatolia Region). Three different working groups were formed, WGO, TSO and WGO/TSO (v/v).

2.3. Cell culture studies

To investigate the biological activities of the samples, two cell lines were utilized: HaCaT (Human keratinocyte from ATCC) and Vero (Monkey kidney fibroblast cell from ATCC). These cell lines were maintained in a humidified incubator set at 37°C with 5.0% CO_2, using DMEM as the growth medium. The DMEM was supplemented with 10% FBS, 100 UI/mL Penicillin/Streptomycin, and 2.0 mM L-Glutamine to ensure optimal cell growth. The cells were sub-cultured twice a week by trypsinization when they reached 70% confluence.

2.3.1. Cell viability

In this study, by following the method used by Barlas et al. (2019), 8000 HaCaT and Vero cells were grown in a 96-well plate (37°C, 5% CO_2, and 95% humidity) in a controlled environment for 48 hours. At the end of this period, the normal medium was removed from the cells and added to the medium containing the working groups. Subsequently, a solution of MTT was added to each well and incubated for 4 hours. The living cells convert MTT into formazan crystals, which are insoluble in the MTT solution. The number of living cells was quantified by measuring the absorbance of the solution at 570 nm and 630 nm reference wavelengths. The control group in the assay consisted of cells treated only with DMEM, which were considered to be 100% viable. The relative cell viability of the sample-treated cells was then calculated as a percentage of the absorbance of the control group. The results were reported as the mean of four replicates.

2.3.2. Scratch assay for wound healing

The *in vitro* wound healing assay was used to assess the cell migration ability, or the capacity of cells to move towards and fill in a damaged area. Two monolayer cell line, HaCaT and Vero cells were cultivated in 24-well plates and waited to grow into a confluent. Then, a wound was created by gently scratching the cells using a pipette tip. The cells at the edge of the wound were expected to move towards the opening and close it until new cell-cell contacts were established. After the wound was formed, various samples were added to the wells, and the scratch area was documented at different time intervals (1^{st}, 4^{th}, and 8^{th} hours) using an inverted microscope equipped with a CCD camera. Migration in the cavity of the cell monolayer was photographed and then using a computer program (ImageJ) the results were calculated as percent migration compared to the control group. The results were reported as the mean of three replicates (Liang et al., 2007; Roy et al., 2023).

2.3.3. Antioxidant activity

Here is a description of the cell-based hydrogen peroxide (H_2O_2) assay without numbered steps or individual items described by Guler et al., (2014). H_2O_2 stimulated antioxidant capacity test is a frequently used method. According to this experiment, the cells are first left to pre-incubate with the samples for two hours, then certain doses of H_2O_2 are added to see how much this stress is prevented by viability tests. For this purpose, 8000 HaCaT and Vero cells were seeded in 96-well plates and incubated for 48 hours under normal cell culture conditions. Then, the media were drawn from them and incubi-

ted for two hours with the study media containing the samples. After this pre-treatment, 1.25 mM H_2O_2 was added to the cells and incubated for 24 hours. At the end of the period, cell viability was measured with the MTT method. The results are calculated and compared with the control groups to determine the antioxidant activity of the sample.

2.4. Statistical analysis

All statistical analyses were performed using one-way analysis of variance (ANOVA) and Tukey multiple comparison test with the help of GraphPad 5 Prism software. The results were presented as the mean and standard deviation. The significance of the comparison between groups was determined by a *p* value of less than 0.05.

3. Results and discussion

The cell viability analyse was performed with MTT method on HaCaT and Vero cell lines to evaluate cytotoxicity and dose dependent effect of samples. Cells were treated with samples at different doses and then allowed to incubate for 24 hours. Subsequently, cell viability was measured by the MTT method and non-cytotoxic doses were determined and used in subsequent studies. The MTT assay was performed to evaluate the cell viability of the samples in two different cell lines (HaCaT and Vero). The results, depicted in Fig. 1, showed that the cell viability for WGO, WGO-TSO, and TSO at a dose of 25 µg/mL was 100.025 ± 1.214, 100.025 ± 1.671, and 101.921 ± 2.671, respectively, in the HaCaT cell line compared to the control group. In the Vero cell line, the cell viability was 105.32 ± 5.029, 105.32 ± 4.083, and 114.364 ± 1.957, respectively. Based on these results, the dose of 25 µg/mL was selected as the study dose as it did not demonstrate high proliferative effects and was not toxic for all groups.

Cell-based antioxidant activity was assayed on the cells those are exposed to hydrogen peroxide destruction. The cells were pre-treated with seed oils 2 h before and then, treated with 100 mM hydrogen peroxide and allowed to incubate for 24 h. Afterwards, viability was measured, and comparisons were made between the groups (Fig. 2). The results showed that the viability of the HaCaT cell line decreased to 48.314 ± 3.00 when exposed to hydrogen peroxide (H_2O_2) alone. However, the viability of WGO, WGO-TSO, and TSO remained at the levels of 61.240 ± 4.565, 71.832 ± 3.691, and 60.279 ± 2.914, respectively. In the Vero cell line, the viability decreased to 50.738 ± 4.096 with H_2O_2 alone, whereas the viability of WGO, WGO-TSO, and TSO remained at the levels of 61.384 ± 3.227, 69.201 ± 3.217, and 60.940 ± 4.258, respectively. Antioxidant activity assays have shown that the mixture of seed and oils is more effective against to the oxidative stress compared to their use alone (Fig. 2). α-TOH present in wheat germ oils constitute the most important part of the antioxidant capacities. Also, the antioxidant properties of the tomato seed oil are well known because of LYC (Barnes, 1982; Eller et al., 2010; Kumar et al. 2023). Furthermore, cold pressed oils may contain higher level of lipophilic phytochemicals including important natural components and biologically active compounds. Also, these oils are free from chemical contamination (Yuenyong et al., 2021; Sumara et al., 2023).

The scratch assay is a commonly used method in the field of tissue regeneration for evaluating the migration of cells across a wounded area. This assay is particularly important for studying the roles of keratinocytes and fibroblast cells in the process of tissue repair. In this study, a gap was opened with a 100 µM pipette and photographs were taken at three different time points (0, 4, and 8 hours). As can be seen in Fig. 3, the migrations of the cells were found by comparison with the control groups with the help of the image j program. Considering the measurements made after 8 hours, the cell migration rate for the HaCaT cell

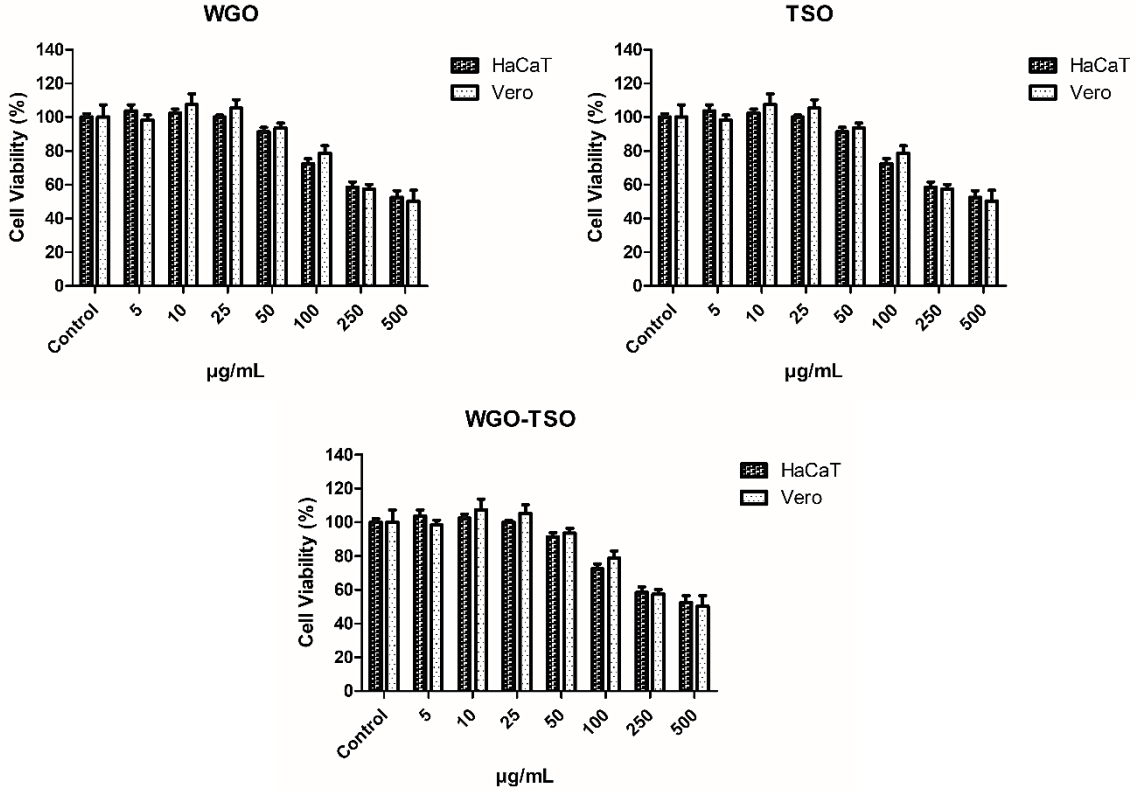

Fig. 1. *In vitro* cytotoxicity effect of seed oils and infuse form on HaCaT and Vero cell lines.

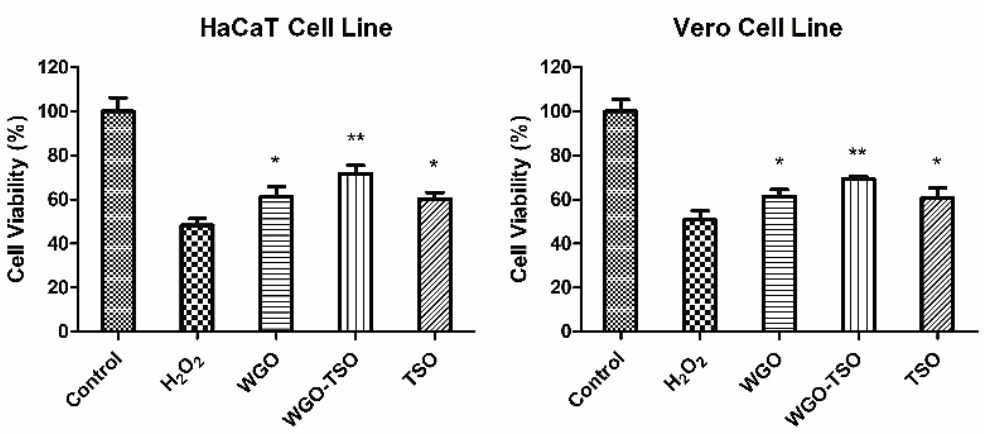

Fig. 2. Effect of seed oils and their infuse form on hydrogen peroxide-induced oxidative stress.

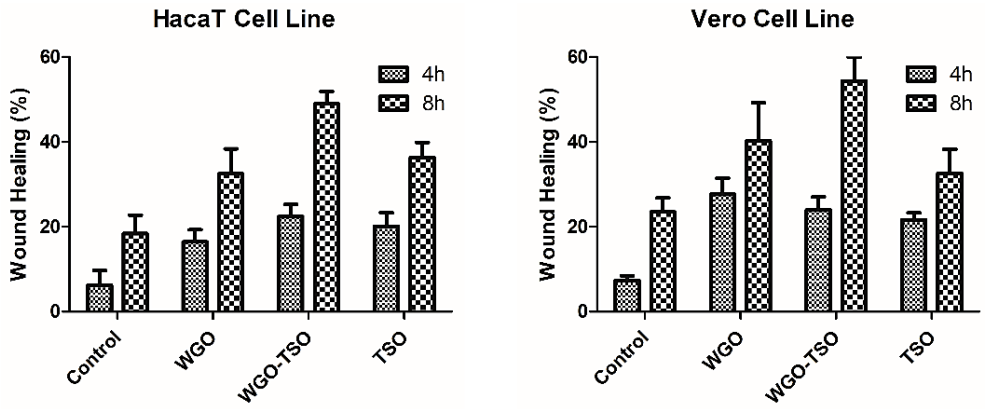

Fig 3. The wound healing effect of seed oils and infuse form on HaCaT and Vero cell lines.

line was 18.3741 ± 4.371 in the control group. On the other hand, it is 32.4700 ± 5.914, 48.9915 ± 2.902 and 36.2790 ± 3.574 for WGO, WGO-TSO and TSO, respectively. for the Vero cell line at the same time, the control group had a migration rate of 23,4850 ± 3,278, while it was 40.2280 ± 8.996, 54.3470 ± 5.614 and 32.6180 ± 5.672 for WGO, WGO-TSO and TSO, respectively. In light of the scratch assay results, it is observed that the use of seed oils in an infused form gives more successful results than using them alone. This is evidenced by the increased migration rates observed in the WGO, WGO-TSO, and TSO treatment groups compared to the control group. These findings show that seed oils and their combination have a beneficial effect on wound healing and have great potential to be a potential therapeutic option in the field of tissue regeneration. In previous studies, wheat germ oil and tomato germ oil have been extensively studied for potential health benefits such as lowering blood cholesterol levels, improving physical strength, promoting healing, exhibiting antioxidant wound activity, and anti-aging therapeutic effects have been reported (Małecka, 2002; Ghafoor et al., 2017; Zahid et al., 2019). Some studies have reported that combining oils is more effective than using them individually, similar to our results (El-Marasy et al., 2012). Additionally, for these oils used, a combination of intracellular activities of key components such as Alpha-tocopherol (α-TOH) and lycopene (LYC) may be more effective. Furthermore, the synergistic effects of minor compounds in oils from both plants may contribute to improved wound healing and antioxidant properties of the combined use of seed oils (Zaid and Al Ramahi, 2019; Bengi et al., 2022).

4. Conclusion

In this study, infused oils (WGO-TSO) (v/v) were prepared from a mixture of cold-pressed wheat germ oil (WGO) and tomato seed oil (TSO) in equal volumes. Then, *in vitro* cytotoxicity analyses of these samples were examined in two different cell lines (HaCaT and Vero). As a result of this study, 25µg/mL was chosen as the working dose. After that, *in vitro* antioxidant capacity test was evaluated with hydrogen peroxide stimulation, and wound healing studies were evaluated with scratch assay. In light of the results, it was determined that infused oils showed better therapeutic effects than the use of oils alone. As a result, they exhibit a synergistic effect in combinations of active and minor compounds found in WGO and TSO. This suggests that the use of infused forms of seed oils is more promising for medical and cosmetic applications.

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Conflict of interest: The author declares that he has no conflict of interests.

Informed consent: The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

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