Proceedings of Science and Mathematics
Faculty of Science, Universiti Teknologi Malaysia
https://science.utm.my/procscimath
Volume 19 (2023) 78-87

# The Effect of *Leptospermum Scoparium* Essential Oil Combined with Hyperthermia During *In Vitro* Dermal Wound Healing

**Goh Yu Yan[a], Wan Fatin Amira Binti Wan Mohd Zawawi[a*] and Khairunadwa Binti Jemon[a]**
[a]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
*Corresponding author: wanfatinamira@utm.my

**Abstract**
Wound healing is a complex process that involves multiple stages including hemostasis, inflammation, proliferation and remodeling. *Leptospermum scoparium* essential oil is able to reduce bacterial burden in infected open wounds due to its antimicrobial properties. On the other hand, hyperthermia improves resistance to infection and regeneration while lowering inflammatory reactions and increasing wound secretion, which aids in both normal and impaired wound healing. However, there is a limited understanding of the combined effect of *Leptospermum scoparium* essential oil and hyperthermia on wound healing. Therefore, this study aimed to evaluate the *in vitro* cytotoxicity and wound healing activity of *Leptospermum scoparium* essential oil combined with hyperthermia combined treatment on human primary fibroblast cells. To perform cytotoxicity study, cells were seeded in 96-well plates and treated with different concentrations of essential oil, hyperthermia and combined treatment of selected concentration of essential oil and hyperthermia and analysed MTT Assay. The results of the MTT assay demonstrated that 0.125 µL/ml *Leptospermum scoparium* essential oil, 2 minutes hyperthermia, and the combined treatment, exhibited no significant cytotoxicity on human skin fibroblast cells. Next, scratch assay was used to assess wound healing activity by scratch assay where a scratch was created across the cell monolayer using a sterile pipette tip. Photographs were taken for every 6 hour interval for 24 hours and analyzed using ImageJ software. In the scratch wound assay, the combined treatment of 0.125 µL/ml *Leptospermum scoparium* essential oil and 2 minutes of hyperthermia demonstrated a significant enhancement in cell migration activity compared to the untreated control. This suggests that the increased migration rate observed in the combined treatment could be attributed to the high monoterpene content of *Leptospermum scoparium* essential oils, along with a reduction in oxidative stress in the wound area caused by hyperthermia. In conclusion, this study highlights the potential of combining Leptospermum scoparium essential oil with hyperthermia for wound healing. The results provide insights into their synergistic effects on promoting cell migration and suggest their potential as alternative treatments for clinical wound injuries.

**Keywords:** *Leptospermum scoparium*; hyperthermia; human primary fibroblast; MTT assay; scratch assay

## Introduction

The process of wound healing is a complex sequence of events that involves continuous communication between cells, soluble molecules, and interactions with the extracellular matrix (Mazutti da Silva et al., 2018). The normal healing of wounds involves three consecutive phases that overlap with each other: hemostasis/inflammatory phase, proliferative phase, and remodeling phase (Wang et al., 2018). Through the healing process, capillary sprouts with angiogenic properties infiltrate the wound clot, which is rich in fibrin and fibronectin. These sprouts quickly establish a network of micro vessels within the granulation tissue. By delving into the molecular mechanisms governing wound angiogenesis, there is potential for developing novel therapies to address chronic wounds (Tonnesen et al., 2000).

Nowadays, there are various types of wound treatment available for a shorter time frame of wound healing. Wound healing often involves the use of *Leptospermum scoparium* essential oil (E.O), which is a blend of various aromatic or aliphatic compounds, including terpenes (Sharmeen et al., 2021). These compounds are naturally produced by the plant as secondary metabolites and have been found to be beneficial in promoting the healing of wounds (Sharmeen et al., 2021). Strong data

supporting the pro-healing abilities of naturally occurring bioactive chemicals have high possibilities in

the application of wound healing (Vivcharenko, & Przekora, 2021). In our research project, we are utilizing manuka essential oil, derived from the *Leptospermum scoparium* plant, which has been traditionally used as a medicinal resource by indigenous communities in New Zealand and Australia due to its antimicrobial properties (Mathew et al., 2020). Manuka belongs to the Myrtaceae plant family and is often classified as a type of "tea tree." The volatile essential oil extracted from the leaves, bark, and seeds of the *Leptospermum scoparium* plants is known as manuka essential oil (Mathew et al., 2020).

Apart from that, study has also shown that wound healing was positively correlated with high periwound temperature detected by infrared thermography (Lin et al., 2018). Therefore*, Leptospermum scoparium* essential oil treatment was combined along with hyperthermia to examine the wound healing activity. Hyperthermia refers to the deliberate or spontaneous elevation of body temperature or a targeted area beyond a certain threshold (Chicheł et al., 2007). This increase in temperature can be achieved through natural means or artificially induced processes (Chicheł et al., 2007). Hyperthermia can be generated through various energy-based methods, such as microwaves, radio waves, ultrasound, hot water baths, resistive wire implants, ferromagnetic seeds, nanoparticles, and infrared radiators (Chicheł et al., 2007). In our research project, we employed Water-filtered infrared-A (wIRA) as the method to induce hyperthermia conditions. wIRA is a specific form of heat radiation that utilizes infrared radiation. It possesses the characteristic of deep tissue penetration while minimizing thermal stress on the surface of the skin (Winkel et al., 2014). Through both thermal and non-thermal mechanisms, wIRA has been observed to promote the healing of both acute and chronic wounds (Winkel et al., 2014).

Hence, owing to the biological properties reported in the introduction, an attempt to study the effect of *Leptospermum scoparium* essential oil combined with hyperthermia during wound healing was conducted to validate the effectiveness of *Leptospermum scoparium* essential oil combined with hyperthermia in wound healing treatment.

**Materials and methods**

*Leptospermum scoparium* essential oil used in this study was purchased from dōTERRA International, United States. Human primary fibroblast cells were purchased from Universiti Kebangsaan Malaysia and revived from a -80°C freezer into a T25 flask supplemented with complete media of of Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12). The frozen cells were thawed in a 37°C water bath before being used. After thawing, the cell suspension was transferred to a 15 ml falcon tube and 10 ml of complete medium F12 was added drop by drop. For 5 minutes, the cell suspension was centrifuged at 2350 x g. The supernatant was discarded and the cell pellet was flicked gently at the base of the tube. Another 10 ml of complete media F12 was added into the tube to allow resuspension of the cell pellet. Lastly, the entire cell suspension was transferred to a labeled T25 flask and observed every day using an inverted microscope.

Once the confluency has reached 80%, cells were transferred from T25 flask to T75 flask for further growth and maintenance. The waste media of the flask was discarded and washed with 2 ml of PBS. After discarding the PBS, 2 ml of trypsin was added to the flask and incubated for 3 to 5 minutes to allow detachment of adherent cell cultures and monolayers. An inverted microscope was used to observe the detachment of the cells. To stop the activity of trypsin, 8 ml of complete medium F12 was added to the flask, and the mixture was transferred to a falcon tube and centrifuged at 2350 x g for 5 minutes. The cells pellet was resuspended in 10 ml of complete medium F12 and transferred to a labelled T75 flask. The cells were incubated overnight and observed every day.

In order to optimize the *Leptospermum scoparium* essential oil that exhibited no cytotoxicity effect on human primary fibroblast cells, different concentrations of essential oil ranging from 0.0625 µl/ml to 1.0 µl/ml were prepared using serial dilution. Once the cells had undergone at least four passages, the cells were transferred to a 96-well plate for cell seeding. The cell count was determined using a hemocytometer. The cells were stained with trypan blue and inspected under an inverted microscope to accomplish the cell count. Live cells appeared transparent and white, while dead cells, which had undergone apoptosis, appeared blue. The number of viable cells was determined using the formula:

$$\text{Number of Viable cells} = \frac{Total\ viable\ cells}{Number\ of\ quadrant}\ x10000\ x\ dilution\ factor$$

The treatment groups were divided into 4 groups of untreated control, essential oil group, hyperthermia group and combined treatment group. The MTT (3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide) assay was used to determine the presence of viable cells in the well. The cells were first seeded in 96-well plates at a density of $2x10^4$ cells/well, and then incubated overnight at 37°C. Hyperthermia was applied to the cells for 2 minutes (Shellman et.al., 2004). The cells that served as negative control were treated with 0.5% of DMSO. Then, each well of the microtiter plate was treated with 10 ul of 5 mg/ml MTT solution, continued with incubation at 37°C for 3-4 hours. The generated formazan crystals were dissolved by adding 100 ul of 100 percent DMSO. Finally, the absorbance (OD) was measured with a spectrometer at 560 nm. The absorbance of the untreated cells was considered as 100%. The cell viability was counted using the following formula:

$$\% \text{ Viable cells} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\ x100\%$$

The *in vitro* scratch wound experiment was carried out in accordance with the procedure provided by Suarez-Arnedo et al (2020). Cells were seeded into 6-well plates to a final cell density of $5x10^5$ cells/well, then incubated overnight. The incubation process was done at 37°C enriched with 5% $CO_2$ in order to allow the cells to adhere. Each well was treated with serum-free medium the next day and incubated for 24 hours. When the confluent monolayer was obtained, the cell culture monolayer was then scratched by using a sterile 200 µl micropipette tip, which simulates the wound formation. This was followed by replacing the cultured medium with a medium containing 0.125µl/ml (concentrations were selected from the previous existing result). *Leptospermum scoparium* essential oil and under 2 minutes of hyperthermia. The plates were incubated at 37°C enriched with 5% $CO_2$ and photographs were taken at 4x magnification at 0, 6, 12, 18 and 24 hours. The following formula was used to compute the percentage of gap closure:

$$\% \text{ Gap Closure} = \frac{Gap\ 0h - Gap\ xh}{Gap\ 0h}\ x100\%$$

The experiments were performed in triplicate and repeated in two independent studies to ensure reproducibility. The data were presented as the mean ± standard deviation. To compare significant differences of means between groups at different timepoints, ANOVA was employed. Image J (https://imagej.nih.gov/ij/) and GraphPad Prism version 8.0 software (GraphPad Software, CA, USA) were used for all statistical analyses.

**Results and discussion**

Before investigating the cytotoxic effect of combined treatment, the optimization of *Leptospermum scoparium* essential oil concentrations against human primary fibroblast cells were carried out using MTT assay in order to choose the best concentration. The cell viability of treated cells with 0.0625 µL/ml, 0.125µL/ml, 0.25µL/ml, 0.5µL/ml, and 1.0 µL/ml was compared to untreated control. The cell viability of untreated control was assumed as 100%.

The MTT assay results indicated that the *Leptospermum scoparium* essential oil exhibited varying degrees of cytotoxicity at different concentrations. Notably, at 0.125 µL/ml concentration, the essential oil demonstrated no significant cytotoxicity towards human primary fibroblast cells. Cell viability remained comparable to the control group, suggesting a non-toxic effect of this particular concentration. Apart from that, MTT assay also demonstrated that the cells viability was decreased to 81%, 65%, 55% and 47% when treated with 0.0625µL/ml, 0.25µL/ml, 0.5µL/ml, and 1.0µL/ml *Leptospermum scoparium* essential oil respectively.

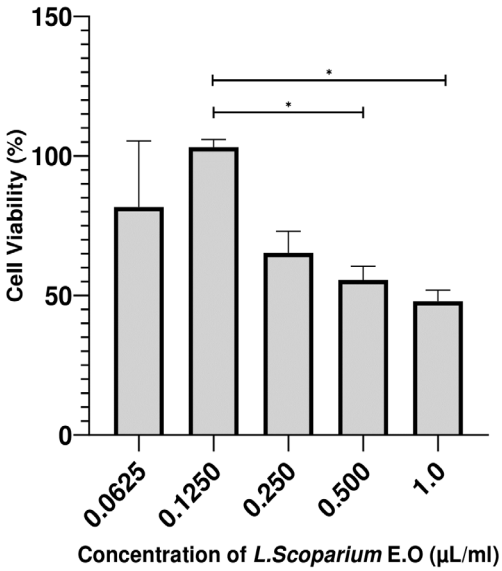


**Figure 1** The percentage of cell viability against concentrations of Leptospermum scoparium essential oil. *P< 0.05 and **P<0.01 using one-way ANOVA in GraphPad Prism

According to Mathew et.al. (2020), the chemical composition of *Leptospermum scoparium* essential oil are reported to be leptospermone (0.8–19.4%), calamenene (2.5–18.5%), δ-cadinene (0.9–6.9%), cadina-1,4-diene (0.1–5.9%), flavesone (0.7–5.8%), cadina-3,5-diene (3.0–10.0%), α-copaene (4.3–6.5%) and α-selinene (1.3–5.0%). Skin epidermoid cancer cells treated with essential oil had resulted in several apoptotic changes, including nuclear condensation, loss of membrane integrity, and DNA fragmentation (Pavithra et.al., 2017). The induction of apoptosis by essential oil was found to be associated with specific cellular events, including the loss of mitochondrial membrane potential, release of cytochrome c, activation of caspases (cleaved forms of caspase-3, caspase-8, and caspase-9), and cleavage of PARP (Pavithra et.al., 2017).

Furthermore, exposure to 1.5 % (v/v) *Leptospermum scoparium* essential oil for 4 hours resulted in notable morphological changes and cell lysis in methicillin-resistant *Staphylococcus aureus*. Moreover, treatment with a higher concentration (3% v/v) of mānuka oil led to complete disruption of the bacterial cells (Mathew et.al., 2020). This antibacterial activity is thought to be due to the presence of β-triketones in the oil (Mathew et.al., 2020). Hence, this explained that at a higher concentration of *Leptospermum scoparium* essential oil, the bioactive compounds have the ability to interact with and disrupt the integrity of the cell membrane, hence exhibit cytotoxicity effect to the fibroblast cells. These compounds could potentially penetrate the cell membrane, causing damage and leading to cell lysis.

However, the absence of cytotoxicity observed at the concentration of 0.125 µL/ml is promising for the potential therapeutic use of *Leptospermum scoparium* essential oil in cell migration and proliferation. This concentration range was considered safe in humans. Therefore, 0.125 µL/ml of *Leptospermum scoparium* essential oil was chosen to determine in the cytotoxicity assay and wound healing of combined treatment.

The cytotoxicity of *Leptospermum scoparium* essential oil combined with hyperthermia was assessed using the MTT assay. In this study, human primary fibroblast cells were treated using 0.125 µL/*ml Leptospermum scoparium* essential oil and 2 minutes of hyperthermia. The viability of the cells was calculated as a percentage, and the results were compared to control groups (100%).

According to Figure 2, the MTT assay showed that cells exposed to the selected 0.125 µL/ml essential oil exhibited no significant cytotoxicity effect. Interestingly, the hyperthermia treatment alone did not affect cell viability, suggesting that it does not have a significant cytotoxic effect on cells under the conditions tested (2 minutes). In addition, when combined with essential oil, cells also showed no significant toxic effect, thus indicating that 0.125 µL/ml essential oil and 2 minutes of hyperthermia is safe to apply on human skin and may be potentially beneficial for further research or application in wound healing or related fields.

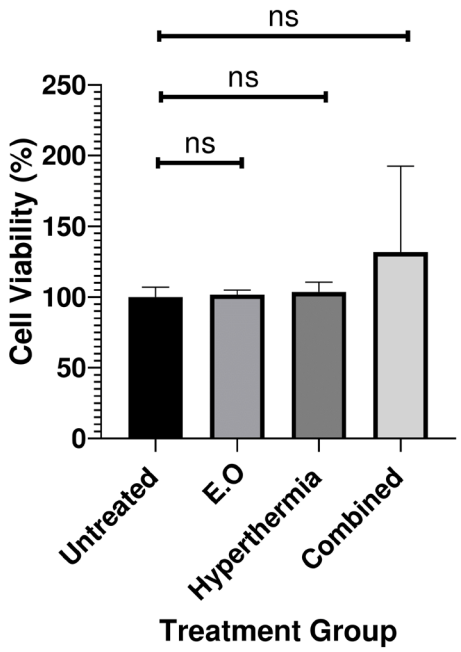


**Figure 2** The percentage of cell viability against essential oil, hyperthermia and combined treatment. Data were expressed as mean ± SEM, from 3 independent experiments.

Hyperthermia treatment involves raising the temperature of the targeted area to create thermal stress, typically with an average temperature of approximately 40 °C (Lee et.al., 2018). According to Hoffmann (2009), heat functions as a stimulus for the human body, elevating the temperature of tissues and accelerating various metabolic processes. Hence, this explained, cells treated with hyperthermia exhibited no significant cytotoxic effect. Hoffmann (2009) also reported that even when wounds were directly exposed to water-filtered ultrared A radiation, no pain or discomfort was experienced, indicating that hyperthermia treatment is safe to be used on humans.

Apart from that, a study by Orchard et al. (2019) concluded that the combination of essential oil and carrier oils such as Aloe vera and *S. chinensis* had resulted in a decrease in overall cytotoxicity. The use of low temperature or fever-like mild hyperthermia, which entails applying a temperature range of 39°C-40°C, has been shown to provide benefits such as reduced toxicity in cells (Kai et.al., 2009).

In conclusion, the *in vitro* cytotoxicity assay relieved that the combined treatment of *Leptospermum scoparium* essential oil and hyperthermia showed no significant cytotoxic effect on human skin fibroblast cells, indicating that both treatments are safe to be used on humans. For the next objective, 0.125µL/ml of *Leptospermum scoparium* essential oil and 2 minutes of hyperthermia were selected for the wound healing activity in scratch assay.

A scratch experiment was used to evaluate the wound healing activity of *Leptospermum scoparium* essential oil in combination with heat. In this study, cells were grown to confluence and a wound-like gap was created to imitate cell migration during wound healing (Buranasukhon et.al., 2017).

Different treatments were applied, including the essential oil alone, hyperthermia alone, and the combination of essential oil and hyperthermia. The closure of the wound area was monitored and quantified in a time interval of 0, 6, 12, 18, and 24 hours using an inverted microscope. After that, the wound healing activity of respective treatment groups were analyzed using ImageJ software.

It was observed that there was significant gap closure when cells are treated with combined treatment as compared to untreated control. The quantitative data presented in Figure 3 demonstrated that combined treatment of *Leptospermum scoparium* essential oil combined with hyperthermia significantly increased the migration of fibroblast cells into the scratched area. Although the result showed that essential oil with hyperthermia and without hyperthermia has no significant difference, but all the treated cells exhibited faster recovery within 24 hours of incubation, with all gaps being closed more rapidly compared to untreated cells.

These data support the hypothesis that combination treatment stimulates the migration of fibroblast cells into the scratched area, hence aiding in the wound healing process.

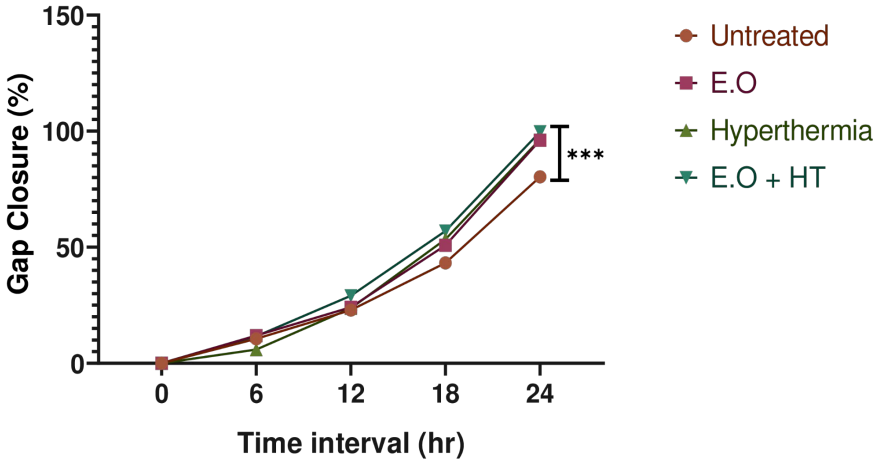


**Figure 3** The effect of untreated control, essential oil, hyperthermia and combined treatment on fibroblast cells migration. Data were expressed as mean ± SEM, from 2 independent experiments. (***P<0.001 using two way ANOVA in GraphPad Prism)

The significant difference in the closure of gaps of combined treatment can be attributed to the phytochemical constituents present in *Leptospermum scoparium* essential oil, particularly their high content of monoterpenes such as alpha-pinene. This observation aligns with a previous study by Lin et al. (2015) that reported a significant stimulation effect on fibroblast migration through the presence of monoterpene compounds in the scratch wound assay. Apart from that, excision, incision and burn wound model treated with *Leptospermum scoparium* were reported to have a significant wound healing performance (Rathinamoorthy & Sasikala, 2019).

On the other hand, Hoffmann (2009) concluded that ultrared water-filtered localized hyperthermia induced by radiation improved wound healing in chronic ulcers by boosting regional blood flow, oxygen partial pressure, and metabolism. The combination of tea tree and rosemary essential oils has shown promising results in promoting various stages of wound healing by a reduced oxidative stress in the wound area (Labib et.al., 2019). Based on these findings, it is proposed that the increased migration rate of combined treatment treated human fibroblast cells can be attributed to the high monoterpene content of *Leptospermum scoparium* essential oils and a reduced oxidative stress in wound by hyperthermia.

However, there is no significant difference between the essential oil group, hyperthermia group and combined group. Firstly, it is possible that the individual treatments (essential oils and hyperthermia) already have strong effects on wound healing, making it difficult to observe additional benefits when combined. Additionally, the sample size of the study might not be large enough to detect subtle differences between the groups (Kim et.al., 2007). The specific protocols used for essential oil application and hyperthermia treatment, such as concentration, duration, or timing, could also influence the outcomes. Further studies, including *in vivo* experiments and elucidation of the underlying mechanisms, are warranted to validate these findings and explore the clinical implications of this combined treatment approach on wound healing.

## Conclusion

In summary, the research study demonstrated the promising possibilities of utilizing a combination of *Leptospermum scoparium* essential oil and hyperthermia as effective agents for wound healing. The optimization process revealed that a concentration of 0.125 µL/ml essential oil demonstrated no significant cytotoxicity towards human primary fibroblast cells. Next, the subsequent cytotoxicity assay demonstrated that the different treatment groups, including 0.125 µL/ml *Leptospermum scoparium* essential oil, 2 minutes hyperthermia, and the combined treatment, were non-toxic to the cells. This indicates that the combined treatment is safe for use on humans. The scratch wound assay revealed a significant enhancement in cell migration activity in fibroblast cells when treated with a combination of 0.125 µL/ml *Leptospermum scoparium* essential oil and 2 minutes of hyperthermia as compared to untreated control. These findings suggest that the increased migration rate observed in the combined

treatment may be attributed to the high monoterpene content of *Leptospermum scoparium* essential oils, along with a reduction in oxidative stress in the wound area caused by hyperthermia. Future research can explore the effect of combining *Leptospermum scoparium* essential oil with hyperthermia on wound healing molecules such as adhesion molecules and angiogenesis molecules. Further research is also needed to assess the combined treatment's healing activity at the tissue level, including tissue remodelling, re-epithelization, and collagen deposition.

**Acknowledgement**

The authors are grateful to the Laboratory Management of Bioscience Department, Faculty of Science, University Teknologi Malaysia for providing assistance during the research progress.

**References**

Boncler, M., Różalski, M., Krajewska, U., Podsędek, A., & Watala, C. (2014). Comparison of PrestoBlue and MTT assays of cellular viability in the assessment of anti-proliferative effects of plant extracts on human endothelial cells. *Journal of Pharmacological and Toxicological Methods*, 69(1), 9–16. doi:10.1016/j.vascn.2013.09.003

Boniakowski, A. E., Kimball, A. S., Joshi, A., Schaller, M., Davis, F. M., denDekker, A., … Gallagher, K. A. (2018). Murine macrophage chemokine receptor CCR2 plays a crucial role in macrophage recruitment and regulated inflammation in wound healing. European *Journal of Immunology*, 48(9), 1445–1455. https://doi.org/10.1002/eji.201747400

Buranasukhon, W., Athikomkulchai, S., Tadtong, S., & Chittasupho, C. (2017). Wound healing activity of Pluchea indica leaf extract in oral mucosal cell line and oral spray formulation containing nanoparticles of the extract. *Pharmaceutical Biology*, 55(1), 1767–1774. doi:10.1080/13880209.2017.1326511

Carmeliet, P. (2000). Mechanisms of angiogenesis and arteriogenesis. *Nature Medicine*, 6(4), 389–395. doi:10.1038/74651

Chicheł, A., Skowronek, J., Kubaszewska, M., & Kanikowski, M. (2007). Hyperthermia – description of a method and a review of clinical applications. *Reports of Practical Oncology & Radiotherapy,* 12(5), 267–275. doi:10.1016/s1507-1367(10)60065-x

Cossarizza, A., Chang, H. D., Radbruch, A., Acs, A., Adam, D., Adam-Klages, S., Agace, W. W., Aghaeepour, N., Akdis, M., Allez, M., Almeida, L. N., Alvisi, G., Anderson, G., Andrä, I., Annunziato, F., Anselmo, A., Bacher, P., Baldari, C. T., Bari, S., Barnaba, V., … Zychlinsky, A. (2019). Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). *European journal of immunology,* 49(10), 1457–1973. https://doi.org/10.1002/eji.201970107

Demidova-Rice, T. N., Hamblin, M. R., & Herman, I. M. (2012, July). Acute and impaired wound healing: Pathophysiology and current methods for drug delivery, part 1: Normal and chronic wounds: Biology, causes, and approaches to care. *Advances in Skin and Wound Care*. https://doi.org/10.1097/01.ASW.0000416006.55218.d0

Dosoky, N. S., & Setzer, W. N. (2018). Chemical composition and biological activities of essential oils of curcuma species. *Nutrients. MDPI AG.* https://doi.org/10.3390/nu10091196

Fratini, F., Mancini, S., Turchi, B., Friscia, E., Pistelli, L., Giusti, G., & Cerri, D. (2017). A novel interpretation of the Fractional Inhibitory Concentration Index: The case Origanum vulgare L. and Leptospermum scoparium J. R. et G. Forst essential oils against Staphylococcus aureus strains. *Microbiological Research*, 195, 11–17. doi:10.1016/j.micres.2016.11.005

Gonzalez, A. C., Costa, T. F., Andrade, Z. A., & Medrado, A. R. (2016). Wound healing - A literature review. *Anais brasileiros de dermatologia*, 91(5), 614–620. https://doi.org/10.1590/abd1806-4841.20164741

Greaves, N. S., Ashcroft, K. J., Baguneid, M., & Bayat, A. (2013). Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound healing. *Journal of Dermatological Science,* 72(3), 206–217. doi:10.1016/j.jdermsci.2013.07.00

Guo, S., & Dipietro, L. A. (2010). Factors affecting wound healing. *Journal of dental research*, 89(3), 219–229. https://doi.org/10.1177/0022034509359125

Hoffmann, G. (1994). Improvement of Wound Healing in Chronic Ulcers by Hyperbaric Oxygenation and by Waterfiltered Ultrared a Induced Localized Hyperthermia. *Oxygen Transport to Tissue XV*, 181–188. doi:10.1007/978-1-4615-2468-7_24

Hoffmann, G. (2009). Water-filtered infrared-A (wIRA) in acute and chronic wounds. *GMS Krankenhaushygiene Interdisziplinär*, 4(2), Doc12. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2831245&tool=pmcentrez&rendertype=abstract

Hoffmann, G., Harte, M., & Mercer, J. B. (2016). Heat for wounds – Water-filtered infrared-a

(wIRA) for wound healing – A review. GMS German Medical Science. *Association of the Scientific Medical Societies in Germany*. https://doi.org/10.3205/000235
Kai, H., Ann Suico, M., Morino, S., Kondo, T., Oba, M., Noguchi, M., ... & Araki, E. (2009). A novel combination of mild electrical stimulation and hyperthermia: general concepts and applications. *International Journal of Hyperthermia*, 25(8), 655-660.
Kim, W.-S., Park, B.-S., Sung, J.-H., Yang, J.-M., Park, S.-B., Kwak, S.-J., & Park, J.-S. (2007). Wound healing effect of adipose-derived stem cells: A critical role of secretory factors on human dermal fibroblasts. *Journal of Dermatological Science*, 48(1), 15–24. doi:10.1016/j.jdermsci.2007.05.01
Kumar, P., Nagarajan, A., & Uchil, P. D. (2018). Analysis of Cell Viability by the MTT Assay. *Cold Spring Harbor Protocols*, 2018(6), pdb.prot095505. doi:10.1101/pdb.prot095505
Labib, R. M., Ayoub, I. M., Michel, H. E., Mehanny, M., Kamil, V., Hany, M., Magdy, M., Moataz, A., Maged, B., & Mohamed, A. (2019). Appraisal on the wound healing potential of Melaleuca alternifolia and Rosmarinus officinalis L. essential oil-loaded chitosan topical preparations. *PloS one*, 14(9), e0219561. https://doi.org/10.1371/journal.pone.0219561
Lee, S., Son, B., Park, G., Kim, H., Kang, H., Jeon, J., … Youn, B. (2018). Immunogenic Effect of Hyperthermia on Enhancing Radiotherapeutic Efficacy. *International Journal of Molecular Sciences*, 19(9), 2795. doi:10.3390/ijms19092795
Lin, Y. H., Chen, Y. C., Cheng, K. S., Yu, P. J., Wang, J. L., & Ko, N. Y. (2021). Higher periwound temperature associated with wound healing of pressure ulcers detected by infrared thermography. *Journal of Clinical Medicine*, 10(13). https://doi.org/10.3390/jcm10132883
Martin, B.M. (2010) Tissue culture techniques: An introduction. Basel: Birkhäuser.
Mathew, C., Tesfaye, W., Rasmussen, P., Peterson, G. M., Bartholomaeus, A., Sharma, M., & Thomas, J. (2020). Mānuka Oil-A Review of Antimicrobial and Other Medicinal Properties. *Pharmaceuticals* (Basel, Switzerland), 13(11), 343. https://doi.org/10.3390/ph13110343
Mazutti da Silva, S. et al. (2018) "Wound healing effect of essential oil extracted from Eugenia dysenterica DC (Myrtaceae) leaves," *Molecules*, 24(1), p. 2. Available at: https://doi.org/10.3390/molecules24010002.
Molinie, N., & Gautreau, A. (2018). Directional Collective Migration in Wound Healing Assays. *Cell Migration*, 11–19. doi:10.1007/978-1-4939-7701-7_2
Nikoletopoulou, V., Markaki, M., Palikaras, K., & Tavernarakis, N. (2013). Crosstalk between apoptosis, necrosis and autophagy. *Biochimica et Biophysica Acta - Molecular Cell Research*. https://doi.org/10.1016/j.bbamcr.2013.06.001
Negut, I., Grumezescu, V., & Grumezescu, A. M. (2018). Treatment strategies for infected wounds. *Molecules*. MDPI AG. https://doi.org/10.3390/molecules23092392
Old, N. (2013). The Medicine of the Manuka: An Investigation of the Usages and Methods for Utilization of Honey Derived From the Pollen of Leptospermum scoparium in Holistic Nursing Practice. *Journal of Holistic Nursing*, 31(3), 200–203. https://doi.org/10.1177/0898010113481405
Orchard, A., Kamatou, G., Viljoen, A. M., Patel, N., Mawela, P., & Vuuren, S. F. V. (2019). The influence of carrier oils on the antimicrobial activity and cytotoxicity of essential oils. *Evidence-Based Complementary and Alternative Medicine*, 2019. https://doi.org/10.1155/2019/6981305
Pavithra, P. S., Mehta, A., & Verma, R. S. (2017). Induction of apoptosis by essential oil from P.missionis in skin epidermoid cancer cells. *Phytomedicine*. doi:10.1016/j.phymed.2017.11.004
Porter, G.C. et al. (2021) "Formulation of a semisolid emulsion containing Leptospermum scoparium essential oil and evaluation of in vitro antimicrobial and antibiofilm efficacy," *Planta Medica*, 87(03), pp. 253–266. Available at: https://doi.org/10.1055/a-1330-8765.
Rathinamoorthy, R., & Sasikala, L. (2019). In vivo - Wound Healing Studies of Leptospermum scoparium Honey loaded Chitosan Bioactive Wound dressing. Wound Medicine, 100162. doi:10.1016/j.wndm.2019.100162.
Segeritz, C. P., & Vallier, L. (2017). Cell Culture: Growing Cells as Model Systems In Vitro. *Basic Science Methods for Clinical Researchers,* 151–172. https://doi.org/10.1016/B978-0-12-803077-6.00009-6
Sharmeen, J. B., Mahomoodally, F. M., Zengin, G., & Maggi, F. (2021). Essential Oils as Natural Sources of Fragrance Compounds for Cosmetics and Cosmeceuticals. *Molecules* (Basel, Switzerland), 26(3), 666. https://doi.org/10.3390/molecules26030666
Shellman, Y. G., Ribble, D., Yi, M., Pacheco, T., Hensley, M., Finch, D., Kreith, F., Mahajan, R. L., & Norris, D. A. (2004). Fast response temperature measurement and highly reproducible heating methods for 96-well plates. *BioTechniques*, 36(6), 968–976. https://doi.org/10.2144/04366ST01
Sieuwerts, A. M., Klijn, J. G. M., Peters, H. A., & Foekens, J. A. (1995). The MTT Tetrazolium Salt Assay Scrutinized: How to Use this Assay Reliably to Measure Metabolic Activity of Cell Cultures in vitro for the Assessment of Growth Characteristics, IC50-Values and Cell Survival. *Clinical Chemistry and Laboratory Medicine*, 33(11), 813–824. https://doi.org/10.1515/cclm.1995.33.11.813

Šitum, M., & Kolić, M. (2012). Atypical wounds: Definition and classification. *Acta Medica Croatica*, 66(SUPPL.1), 5–11. Retrieved from http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L369115695

Silva, A. B. atista da, & Peniche, A. de C. G. iani. (2014, October 1). Perioperative hypothermia and incidence of surgical wound infection: a bibliographic study. *Einstein* (São Paulo, Brazil). https://doi.org/10.1590/S1679-45082014RW2398

Süntar, I. et al. (2011) "A novel wound healing ointment: A formulation of Hypericum perforatum oil and sage and oregano essential oils based on traditional Turkish knowledge," *Journal of Ethnopharmacology*, 134(1), pp. 89–96. Available at: https://doi.org/10.1016/j.jep.2010.11.061.

Suarez-Arnedo, A., Figueroa, F. T., Clavijo, C., Arbeláez, P., Cruz, J. C., & Muñoz-Camargo, C. (2020). An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays. *PLoS ONE*, 15(7 July). https://doi.org/10.1371/journal.pone.0232565

Stephens, J. M. C., Molan, P. C., & Clarkson, B. D. (2005). A review of leptospermum scoparium (Myrtaceae) in New Zealand. *New Zealand Journal of Botany*. https://doi.org/10.1080/0028825X.2005.9512966

Stockert, J. C., Blázquez-Castro, A., Cañete, M., Horobin, R. W., & Villanueva, Á. (2012). MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta Histochemica*, 114(8), 785–796. doi:10.1016/j.acthis.2012.01.006

Thrimawithana, A. H., Jones, D., Hilario, E., Grierson, E., Ngo, H. M., Liachko, I., … Schwinn, K. E. (2019). A whole genome assembly of Leptospermum scoparium (Myrtaceae) for mānuka research. *New Zealand Journal of Crop and Horticultural Science,* 47(4), 233–260. https://doi.org/10.1080/01140671.2019.1657911

Tonnesen, M.G., Feng, X. and Clark, R.A.F. (2000) "Angiogenesis in wound healing," *Journal of Investigative Dermatology Symposium Proceedings*, 5(1), pp. 40–46. Available at: https://doi.org/10.1046/j.1087-0024.2000.00014.x.

Vivcharenko, V., & Przekora, A. (2021). Modifications of wound dressings with bioactive agents to achieve improved pro-healing properties. *Applied Sciences* (Switzerland), 11(9). https://doi.org/10.3390/app11094114

Vaupel, P. (2022). Water-filtered infrared a Wira irradiation: From research to clinical settings. *SPRINGER*.

Wang, P.-H. et al. (2018) "Wound healing," *Journal of the Chinese Medical Association,* 81(2), pp. 94–101. Available at: https://doi.org/10.1016/j.jcma.2017.11.002.

Wicaksono, W. A., Jones, E. E., Monk, J., & Ridgway, H. J. (2016). The bacterial signature of Leptospermum scoparium (Mānuka) reveals core and accessory communities with bioactive properties. *PLoS ONE*, 11(9). https://doi.org/10.1371/journal.pone.0163717

Wilkinson, H.N. and Hardman, M.J. (2020) "Wound healing: Cellular mechanisms and pathological outcomes," *Open Biology*, 10(9). Available at: https://doi.org/10.1098/rsob.200223.

Winkel, R., Hoffmann, G., & Hoffmann, R. (2014). Water-filtered infrared-A (wIRA) promotes wound healing. *Chirurg*, 85(11), 980–992. https://doi.org/10.1007/s00104-014-2809-