UNIVERSITI PUTRA MALAYSIA

ANTI-INFLAMMATORY AND WOUND HEALING POTENTIAL OF Alternanthera sessilis (L.) R.Br. ex DC.'s STEM EXTRACT USING In Vitro APPROACH

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ANTI-INFLAMMATORY AND WOUND HEALING POTENTIAL OF *Alternanthera sessilis* (L.) R.Br. ex DC.'s STEM EXTRACT USING *In Vitro* APPROACH

By

KATYAKYINI A/P MUNIANDY

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirements for the Degree of Master of Science

May 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements of the degree of Master of Science

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Chairman : Associate Professor Suresh Kumar Subbiah, PhD
Faculty : Institute of Bioscience

Impaired wound healing, especially among the diabetic patients has become a major health problem and it is even more frightening with the tremendous increase in the prevalence of diabetic population worldwide. Factors such as the generation of a high amount of oxidative stress and prolonged inflammation than usual results in the delay of the healing process, therefore these factors need to be amended for an effective healing process. Currently, there are many treatments to overcome the wound healing problem, but unfortunately, most of them have some drawbacks such as the development of allergic reactions and high cost of treatment. Therefore, it was an idea to venture into natural product based treatments to enhance the healing rate. In the study conducted, *Alternanthera sessilis*, which was an edible functional food that is abundantly found in tropical countries, was chosen as the candidate to evaluate for its anti-inflammatory mechanism and wound healing activity on keratinocytes and normal fibroblast as well as diabetic human dermal fibroblast. The present study assessed the prepared ethanolic crude extract of stem part of *A. sessilis*. For the anti-inflammatory properties, lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages was used as the best *in vitro* model. As the key inflammatory parameters, including the production of nitric oxide (NO), the cytokines such prostaglandin E2 (PGE_2), interleukin (IL)-6, interleukin (IL)-1β and tumor necrosis factor (TNF)-α as well as the mediators such as cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) were evaluated via Griess reagent, Enzyme-Linked Immunosorbent Assay (ELISA) and Western Blot respectively. The effect of the extract on the activity of Nuclear factor-kappa-B (NF-κB) pathway using immunofluorescence and western blot techniques were examined. Subsequently, the wound healing activity involved the evaluation of cell migration on keratinocytes and fibroblast (normal and diabetic) using a scratch assay and the antioxidant activity was estimated using DPPH scavenging assay. Moreover, hyphenated techniques, Gas chromatography-mass

spectrometry (GC-MS) and Liquid chromatography-mass spectrometry (LC-MS) were used for the identification of the biochemical components present in the extract. From the conducted study, it is proven that crude extract of stem of *A. sessilis* supports wound healing process by exhibiting anti-inflammatory properties by suppressing pro-inflammatory parameters via the NF-κB pathway in a dose-dependent manner.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SIFAT ANTI-RADANG DAN PENYEMBUHAHAN LUKA DARI EKTRAK BATANG *Alternanthera sessilis* (L.) R.Br. ex DC MENGGUNAKAN KAJIAN *In Vitro*

Oleh

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Kemerosotan proses penyembuhan luka, terutamanya dalam kalangan pesakit diabetes telah menjadi suatu masalah kesihatan yang utama, dan perkara ini lebih membimbangkan dengan kadar populasi pesakit diabetes yang kian meningkat di seluruh dunia. Faktor-faktor seperti penjanaan tekanan oksidatif yang tinggi dan keradangan yang berpanjangan pada kadar yang luar biasa, boleh menyebabkan kelewatan proses penyembuhan. Oleh hal yang demikian, ciri-ciri tersebut perlu diseimbangkan untuk menjamin proses penyembuhan yang berkesan. Pada masa ini, terdapat pelbagai jenis rawatan untuk mengatasi masalah penyembuhan luka, malangnya, kebanyakan rawatan yang sedia ada mempunyai kekurangan masing-masing seperti alahan dan kos terapi yang tinggi. Oleh itu, rawatan berasaskan produk semulajadi dipercayai untuk meningkatkan kadar penyembuhan luka. Dalam kajian yang dijalankan, *Alternanthera sessili*s (Kermak putih), sejenis sayuran yang boleh dimakan dan boleh didapati dengan banyak di kawasan tropika telah digunakan. *A. sessilis* telah digunakan sebagai calon uji kaji untuk menilai mekanisma anti-radang dan penyembuhan luka berdasarkan model sel kulit keratinosit, sel fibroblas normal beserta sel fibroblas diabetes. Kajian ini meliputi eksperimen yang menggunakan ekstrak etanol yang disediakan daripada bahagian batang *A. sessilis*. Makrofaj yang diaktifkan menggunakan lipopolisakarida (LPS) telah digunakan sebagai model *in vitro* bagi eksperimen berasaskan sifat anti-radang. Parameter pro-radang yang utama seperti penghasilan nitric oxide (NO), sitokin seperti prostaglandin E2 (PGE_2), interleukin (IL) -6, interleukin (IL) -1β, faktor nekrosis tumor (TNF) -α dan juga pengantara mekanisma keradangan seperti enzim cyclooxygenase (COX-2) dan synthase oxide nitric oxide (iNOS) telah diuji melalui penilaian bahan uji Griess, Asai imunojerapan berpaut (ELISA) dan pemedapan Western. Kami juga telah mengkaji kesan ekstrak terhadap aktiviti laluan mekanisma nuclear faktor-kappa-B (NF-κB) menggunakan teknik imunopendarfluor dan pemedapan Western. Selanjutnya, aktiviti

penyembuhan luka telah dinilai berdasarkan migrasi sel keratinosit dan fibroblas (normal dan diabetes) menggunakan asai calaran manakala aktiviti antipengoksida telah dianggarkan menggunakan asai radikal bebas DPPH. Selain itu, teknik Kromatografi Gas-Spektrometer Jisim (GC-MS) dan Kromatografi Cecair-Spektrometer Jisim (LC-MS) telah digunakan untuk mengenalpasti komponen bioaktif yang terdapat dalam ekstrak ini. Berdasarkan kajian yang dijalankan, keputusan menunjukkan bahawa ekstrak mentah batang *A. sessilis* menyokong proses penyembuhan luka dengan memperlihatkan sifat antiradang dengan penurunan parameter keradangan yang ketara, bersandarkan dos ekstrak melalui mekanisma NF-κB.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
˚C	Degree celcius
$\cdot O^{-2}$	Superoxide
<	Less than
>	More than
×	Dilution/ Times
×g	Times gravity
≥	More than and equal to
µm	Micrometer
µM	Micromolar
A. sessilis	*Alternanthera sessilis*
APS	Ammonium Persulphate
CO^2	Carbon dioxide
COX-2	Cyclooxygenase
DFU	Diabetic Foot Ulcer
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
Eg	Example
FBS	Fetal bovine serum
FITC	Fluorescein
g	Gram
g/L	Gram per liter
G1	Group 1

G2	Group 2
GF	Growth factor
h	Hour
HaCaT	Keratinocytes
HDF-D	Diabetic Human Dermal Fibroblast
ICC	Immunocytochemistry
ICU	Intensive Care Unit
iKB α	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha)
IKKs	Inhibitory kappa B kinases
IL	Interleukin
IL-1 β	Interleukin-1B
IL-6	Interleukin-6
L	Liter
LPS	Lipopolysaccharide
LYM	Lymphocytes
min	Minute
mL	Milliliter
mm	Millimeter
mm^3	Millimeter cube
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	Number
NF-κB	Nuclear factor kappa beta
HDF	Human Dermal Fibroblast
NO	Nitric oxide
PBS	Phosphate buffer saline

PBS	Phosphate Buffer Saline
PGE_2	Prostaglandin E2
ROS	Reactive oxygen species
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WBC	White blood cells
WHO	World Health Organization
SDS	Sodium dodecyl sulfate
TEMED	Tetramethylethylenediamine
PRRs	Pattern recognition receptors
PAMP	Pathogen-associated molecular patterns
DAMPs	Damage-associated molecular patterns
TLRs	Tol-like receptors
Versus	vs

CHAPTER 1

INTRODUCTION

1.1 Background

The wound is defined as the damage occurring on the surface of epithelial and its underlying connective tissues. The rupture of the layer should be amended immediately to prevent the entry of any notorious agents via the broken skin to the body system as in severe cases, it may give a chance for the incidence of systematic inflammation that affects the whole system, which can be fatal. Systemic inflammation is one of the leading cause of mortality all over the world, and in Malaysia alone, according to the Malaysian Registry of Intensive Care, mentioned that sepsis (systemic inflammation) was the most common diagnosis leading to admission to intensive care units (ICU) in Health Ministry hospitals in 2011 (Lum., 2015). The normal physiology of wound healing involved four highly integrated phases, comprised of homeostasis, inflammation, proliferation and remodeling (Nicolaus et al., 2017). Hemostatic involves the process of formation of the clot to avoid any further loss of blood. The second event is the inflammation which the body immune system protects the body from infections from bacteria. Upon the clearance of the foreign substances from the system, the inflammatory process will be regulated into its normal level. This paves the path for the following phase of healing. The following phase, the third phase of wound healing process is the proliferation of cell to form a closure to the wound. Rapid migration of cell accompanied with good collagen formation is vital in this phase to produce good tensile strength on the skin.

An interruption in the normal phases of the physiology of healing process results in the delay in wound healing and it is one of the main challenges faced by the healthcare system. Delayed wound healing is more prominent among the diabetic patients with a statistic displayed that quarter of the diabetic patients are possible of developing foot ulcer (chronic wound healing) (Wardecki et al., 2016). It has become a major health complication with the tremendous increase in the prevalence of diabetic population worldwide, and according to the World Health Organization (2016), it was reported that 422 million adults were living with diabetes in 2014, compared to 108 million in the year 1980 which showed the increase from 4.7% to 8.5% in the adult population around the world (WHO, 2016). Another earlier study by the World Health Organization (2011) mentioned that this metabolic disease is estimated to rise from 171 million to 366 million in the year 2030 (Tam et al., 2011), which means it was predicted to be doubled in 15 years of time. Tactlessly, a current international piece of data showed that the number of diabetic patients achieved earlier than the estimated number for the next 16 years; because in the year 2014 itself the number of diabetic patients has surpassed the estimated number of diabetic patients for the year 2030. When the study is focused on Malaysia, there are about 3.5 million of the population having this metabolic disease (Bernama, 2016).

Some of the major factors that affect the healing ability among the diabetic patients are, the easy colonization of bacteria and the generation of a high amount of oxidative stress that results in the prolonged inflammatory phase than usual (Wardecki et al., 2016), that hinders the healing process to progress to the next healing step which is proliferative phase. Not only diabetic patients, but individuals with no diabetic complication also may experience wound healing problem because high glucose level is not the only systemic factor that affects wound healing. Other factors such as the age, medication, hormone, obesity, nutrition, stress and unhealthy practices such as smoking and alcohol consumption have impacts on wound healing mechanism. However, the systemic factors give impacts on the healing process through the local factors be it infection or lowered angiogenesis that ultimately prolongs the inflammatory process that has the direct impact of the wound (Guo and DiPietro, 2010).

Inflammation is a part of our immune system's process that is essential for the protective purpose of the body system (Kang et al., 2012). At the early stage of inflammation, immune cells are found to be in a high amount as they play a role in safeguarding the wound from contamination by phagocytosis but a high number of them when it is unregulated, give negative influence towards wound repair as their proteases such as elastase degrades most of the extracellular matrix components (Reed & Kita, 2004). Besides, they also produce a large amount of nitric oxide, a free oxygen radical that can generate oxidative stress on the wound which is involved in the pathogenesis of inflammation so the level of inflammatory entities should be reduced for a faster wound healing process (Dunnill et al., 2017). Apart from this, macrophage is the key immune cell in the regulation of inflammation by producing inflammatory cytokines, mediator, as well as growth factors. These products are important to regulate the inflammation to a normal state (Yun et al., 2015) as the overproduction of inflammation delays healing process thus pro-inflammatory cytokines need to be down-regulated as it is an affecting factor for efficient wound healing process. Nuclear Factor-Kappa B (NF-kB) pathway is a key inflammatory signalling pathway that regulates the expression of inflammatory mediators and to repress the pathway is the target for many anti-inflammatory studies (Khan et al., 2014).

Keratinocytes and fibroblast are the major constituents of the main layers of skin that are involved in wound closure mechanism (Wojtowicz et al., 2014). Fibroblast isolated from diabetic type II patient resembles the *in vitro* model of diabetic wound healing. Once the inflammatory is regulated into its original state, the skin cells would undergo the proliferation as well as migration to form a wound closure. This grades the healing process.

1.2 Problem statement

In the current vascular surgery practice, diabetes and chronic wound remain as the leading cause (about 82%) for an individual to necessitate amputation (Johannesson et al., 2009; Pemayun et al., 2015). However, this undesirable circumstance can be

prevented if the wound can be cured, therefore, wound healing has become a significantly important subject of present research. The major problem in a non-healing wound is the lengthy inflammatory phase interrupts the healing cycle by preventing the wound to properly commencing to the next recovery step. The parameters that mark the occurrence of inflammation are the elevated level of pro-inflammatory cytokines and mediators (Monastero & Penthyala., 2017). These entities should be regulated by amending the inflammatory signalling pathway which is the NF-kB, for an effective wound closure process that involves the skin cells proliferation and migration (Song et al., 2017).

Currently, there are many solutions available to overcome wound healing problem, such as antibiotics to kill the bacteria, hyperbaric oxygen therapy to supply and nourish the tissue with sufficient oxygen, and maggot therapy, *Lucilia (Phaenicia) sericata* for debridement purpose to promote rapid healing (Sherman, 2014) and many topical creams such as Santyl, Accuzyme, silver sulfadiazine, fusidic acid and bacitracin-zinc ointment are available (Han et al., 2009; Park et al., 2017). Unfortunately, most of them have their own drawbacks such as the development of resistance by bacteria, high expenses of treatment, unwillingness due to disgust and the occurrence of allergic reactions respectively, that may vary from individuals, attributable to the difference in their sensitivity reactions and health backgrounds, respectively (Roses, 2000).

1.3 Significance of study

In line with that, it was an attempt to venture into natural based therapy yet an assessable source to study its regulatory potential towards the factors affecting the healing process. Therefore, *Alternanthera sessilis* was chosen as the sample in the study. It is commonly eaten as vegetable in many countries around the world. Besides, it has been used as a traditional remedy by folk healers for the past few centuries for the cure of many illnesses, such as headache, snake bites, febrifuge, and made into decoction to be taken orally to treat cough and gastrointestinal problem such as diarrhea. It has an excellent coolant effect that was commonly used for hair, eyes and skin health in general (Walter et al., 2014). Besides, it was also reported to have antibacterial, anti-diabetic, antioxidant, anti-allergic, anti-analgesic as well as anti-inflammatory and wound healing properties (Jalalpure et al., 2008; Tan & Kim., 2012; Phusricom et al., 2013; Rayees et al., 2013; Mondal et al., 2014; Subhashini et al., Jalalpure et al., 2011). The extract was reported to be effective against including bacteria Bacillus *pumillus*, *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, and fungus *Aspergillus fumigatus* that were commonly found in unhealed wound. Besides, the antioxidant capacity of the extract acts as a protectant for the cells against the damage from radicals which is helpful for the healing process.

Moreover, anti-inflammatory related constitutes such as epigallocatechin, catechin, chlorogenic acid, 4-hydroxybenzoic, apigenin, vanillic acid, ferulic acid, ethyl gallate, and daidzein were reported to be found in this plant hence, all these characteristics may validate the usage of this vegetable for this field of study (Othman et al., 2016). For the previous conducted study on the anti-inflammatory and wound healing studies, it involved the usage of leaf part of the plant and it was tested on animal model, thereby leaving a gap of research on the action mechanism of wound healing on different types of skin cell lines and inflammatory parameters associated with wound healing by targeting signaling pathway.

Furthermore, a preliminary result conducted revealed that stem part of *A. sessilis* showed the best wound healing properties compared to leaves and aerial parts of the plant, therefore stem part was chosen as the best candidate to carry out the investigation.. Moreover, edible plants based therapy is also believed to be minimally toxic (Chiang et al., 2017). Therefore, this examination possibly will bridge the therapeutic gap that is important for diabetic wound healing. With the information from previous studies, it is believed that *A. sessilis* is a potential candidate to be further studied for the assessment of wound healing activity along with its ability to suppress pro-inflammatory cytokines and inflammatory mediators, vital for normal skin growth physiology.

1.4 Objectives

1.4.1 General Objective

To determine the anti-inflammatory and wound healing properties of stem extract of *A. sessilis* on RAW 264.7 cells and skin cells of normal and diabetic isolated, respectively.

1.4.2 Specific Objectives

i To evaluate the anti-inflammatory properties of stem extract of *A. sessilis* through *in vitro* based experiments.
ii To investigate the wound healing activities of stem extract of *A. sessilis* on skin cells; keratinocytes, normal dermal fibroblast and diabetic human dermal fibroblast cells.

1.5 Hypothesis

1.5.1 Null Hypothesis

Stem extract of *A. sessilis* has no significant role in anti-inflammatory and wound healing properties.

1.5.2 Alternative Hypothesis

Stem extract of *A. sessilis* has significant role in anti-inflammatory and wound healing properties. These properties will provide strong rationale to identify the responsible bioactive compounds for diabetic wound healing.

REFERENCES

Adan A., Kiraz, Y., & Baran, Y. (2016). Cell proliferation and cytotoxicity assays. *Curr Pharm Biotechnol, 17*(14), 1213-1221.

Agyare, C., Lechtenberg, M., Deters, A., Petereit, F., & Hensel, A. (2011). Ellagitannins from *Phyllanthus muellerianus* (Kuntze) Exell. : Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. *Phytomedicine*, *18*(7), 617–624. DOI. 10.1016/j.phymed.2010.08.020.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2012). Epidermis and its renewal by stem cells. *Molecular Biology of the Cell*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK26865/.

Alexander, C., & Rietschel, E.T. (2001). Bacterial lipopolysaccharides and innate immunity. *J Endotoxin Res, 7*(3), 167-202.

Ambiga, S., Narayanan, R., Gowri, D., Sukumar, D., & Madhavan, S. (2007). Evaluation of wound healing activity of flavonoids from *Ipomoea carnea* Jacq. *Anc Sci Life, 26*(3), 45-51.

Aquino, D. F. D.S., Piccinelli, A.C., Soares, F.L.P., Arena, A.C., Salvador, M.J., & Kassuya, C.A.L. (2015). Anti-hyperalgesic and anti-inflammatory activity of Alternanthera Maritima extract and 2″-O-α-l-rhamnopyranosylvitexin in mice. *Inflammation, 38*(6), 2057–2066. DOI. 10.1007/s10753-015-0187-0.

Armstrong, D.G., Wrobel, J., & Robbins, J.M. (2007). Guest Editorial: are diabetes-related wounds and amputations worse than cancer? *Int Wound J, 4*(4), 286-287. DOI. 10.1111/j.1742-481X.2007.00392.x.

Arulselvan, P., Tan, W.S., Gothai, S., Muniandy, K., Fakurazi, S., Esa, N.M., Alarfaj, A.A.,& Kumar, S.S. (2016). Anti-Inflammatory potential of ethyl acetate fraction of Moringa oleifera in downregulating the NF-κB signaling pathway in lipopolysaccharide-stimulated macrophages. *Molecules*, 21, pii: E1452. DOI. 10.3390/molecules21111452.

Atta, A., Soufy, H., Nasr, S.M., Soliman, A.M., Nassar, S.A., Maweri, A.A., El-Aziz, T.H.A., Desouky, H.M., & Abdalla, A.M. (2017). Hepatoprotective and antioxidant effects of methanol extract of *Moringa oleifera* leaves in rats. *Wulfenia*, *24*(3), 249-268.

Bernama 2016, 'Hilmi: Alarming increase in diabetes among Malaysians', *The Star Online*, 8 April, viewed 27 April 2017, Retrieved from <http://www.thestar.com.my/news/nation/2016/04/08/hilmi-alarming-increase-in-diabetes-among-malaysians/>.

Bessa, L., Fazii, P., Di Giulio, M., & Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. *Int Wound J*, *12*(1), 47-52. DOI. 10.1111/iwj.12049.

Bikle, D.D. (2012). Vitamin D and the skin: physiology and pathophysiology. *Rev Endocr Metab Disord*, *13*(1), 3-19. DOI. 10.1007/s11154-011-9194-0.

Borah, A., Yadav, R.N.S., & Unni, B.G. (2011). *In vitro* antioxidant and free radical scavenging activity of *Alternanthera sessilis. IJPSR*, *2*(6), 1502-1506.

Brem, H., & Tomic-Canic.M. (2007). Cellular and molecular basis of wound healing in diabetes. *J Clin Invest*, *117*(5), 1219-1222. DOI. 10.1172/JCI32169.

Brenner, M., & Hearing, V.J. (2008). The protective role of melanin against UV damage in human skin. *Photochem Photobiol*, *84*(3), 539–549. DOI. 10.1111/j.1751-1097.2007.00226.x.

Brown, G.C. (2001). Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. *Biochim Biophys Acta*, *1504*(1), 46-57. DOI. 10.1016/S0005-2728(00)00238-3.

Cecotti, R., Carpana, E., Bergomi, P., & Tava, A. (2013). Volatile constituents of *Trifolium pratense* spp. nivale quantified at different growth stages, and evaluation of their antimicrobial activity. *Nat Prod Commun*, *8*(11), 1625-1628.

Chandel, E., & Kumar, B. (2015). Antimicrobial activity and phytochemical analysis of *Cynodon dactylon*: A review. *World J Pharm Sci*, *4*(1), 515-530.

Chandrasekaran, M., Senthilkumar, A., & Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *Eur Rev Med Pharmacol Sci*,*15*(7), 775-780.

Chang, A.C., Liu, B.H., Shao, P.L., & Liao, J.D. (2017). Structure-dependent behaviours of skin layers studied by atomic force microscopy. *J Microsc*, *267*(3), 265-271. DOI 10.1111/jmi.12562.

Chen, L., Du, Y., & Xiao, L. (2002). Relationship between molecular structure and moisture-retention ability of carboxymethyl chitin and chitosan. *J Appl Polym Sci*, *83*(6), 1233–1241. DOI. 10.1002/app.2290.

Chen, L., Nagaraja, S., Zhou, J., Zhao, Y., Fine, D., Mitrophanov, A.Y., Reifman, J., & DiPietro, L.A. (2017). Wound healing in Mac-1 deficient mice. *Wound Repair Regen*, *25*(3), 366-376. DOI. 10.1111/wrr.12531.

Chen, Y.F., Wang, K, Zhang, Y.Z, Zheng, Y.F, & Hu, F.L. (2016). *In vitro* anti-inflammatory effects of three fatty acids from royal jelly. *Mediators Inflamm 2016*:3583684. DOI. 10.1155/2016/3583684.

Chiang, M., Kurmoo, Y. & Khoo, T. (2017). Chemical- and cell-based antioxidant capacity of methanolic extracts of three commonly edible plants from Zingiberaceae family. *Free Radicals and Antioxidants*, *7*(1), 57-62. DOI. 10.5530/fra.2017.1.9.

Choi, G.J., Jang, K.S., Choi, Y.H., Yu, J.H., & Kim, J.C. (2010). Antifungal activity of lower alkyl fatty acid esters against powdery mildews. *Plant Pathol J, 26,* 360-366. DOI. 10.5423/PPJ.2010.26.4.

Chou, C.C., & Buehler, M.J. (2012). Structure and mechanical properties of human trichocyte keratin intermediate filament protein. *Biomacromolecules*, *13*(11), 3522-3532. DOI. 10.1021/bm301254u.

Citron, D.M., Goldstein, E.J., Merriam, C.V., Lipsky, B.A., & Abramson, M.A. (2007). Bacteriology of moderate-to-severe diabetic foot infections and *in vitro* activity of antimicrobial agents. *J Clin Microbiol*, *45*(9), 2819-2828. DOI. 10.1128/JCM.00551-07.

Corderi, S., & González, B. (2012). Ethanol extraction from its azeotropic mixture with hexane employing different ionic liquids as solvents. *J Chem Thermodyn*, *55*,138-143. DOI. 10.1016/j.jct.2012.06.028.

Dallet, P., Labat, L., Kummer, E., & Dubost, J.P. (2000). Determination of urea, allantoin and lysine pyroglutamate in cosmetic samples by hydrophilic interaction chromatography. *J Chromatogr B Biomed Sci Appl*, *742*(2), 447-452. DOI. 10.1016/S0378-4347(00)00196-1.

Darby, I.A., Laverdet, B., Bonté, F., & Desmoulière, A. (2014). Fibroblasts and myofibroblasts in wound healing. *Clin Cosmet Investig Dermatol*, *7*, 301-311. DOI. 10.2147/CCID.S50046.

Das, M., Kumar, A., Mastanaiah, K., & Das, A. (2015). Evaluation of anti-diabetic activity of ethanolic extract of *Alternanthera sessilis* Linn. in streptozotocin-induced diabetic rats. *IJPSR*, *6*(7), 1027-1032.

Davis, R., & Maro, N.P. (1989). Aloe vera of Australia products. *Journal of the American Podiatric Medical Association, 79*(1), 1-4.

Doron, S., & Snydman, D. R. (2015). Risk and Safety of Probiotics. *Clin Infect Dis*, 60(Suppl 2), S129–S134. DOI. 10.1093/cid/civ085.

Dunnill, C., Patton, T., Brennan, J., Barrett, J., Dryden, M., Cooke, J., Leaper, D., & Georgopoulos, N.T. (2017). Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *Int Wound J*, *14*(1), 89-96. DOI. 10.1111/iwj.12557.

Dutta, S., Ray, S., & Nagarajan, K. (2013). Glutamic acid as anticancer agent: An overview. *Saudi Pharm J*, *21*(4), 337–343. DOI. 10.1016/j.jsps.2012.12.007.

Eldeen, A.M., Kawashty, S.A., Ibrahim, L.F., Shabanas, M.M., & Negoumy, S.I. (2004). Evaluation of antioxidant, anti-inflammatory, and antinociceptive properties of aerial parts of *Vicia sativa* and its flavonoids. *Journal of Natural Remedies, 4*(1), 81-96. DOI. 10.18311/jnr/2004/388

Escandón, R.A., Campo, M.D., López-Solis, R., Obreque-Slier, E., & Toledo, H. (2016). Antibacterial effect of kaempferol and (−)-epicatechin on Helicobacter pylori. *Eur Food Res Technol, 242*(9), 1495-1502. DOI. 10.1007/s00217-016-2650-z.

Eyre, D.R., Weis, M.A., & Wu, J. (2008). Advances in collagen cross-link analysis. *Methods, 45*(1), 65–74. DOI. 10.1016/j.ymeth.2008.01.002.

Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis Res Ther, 8*(Suppl 2), S3. DOI. 10.1186/ar1917.

Gamaniel, K., Samuel, B.B., Kapu, D.S., Amos, S., Wagner, H., Okogun, J.I., & Wambebe, C. (2000). Anti-sickling, analgesic and anti-inflammatory properties of 3,5-Dimethoxy-4-Hydroxy Benzoic Acid and 2,3,4-Trihydroxyacetophenone. *Phytomedicine, 7*(2), 105-110. DOI. 10.1016/S0944-7113(00)80081-1.

Gilliver, S.C., Ruckshanthi, J.P., Atkinson, S.J., & Ashcroft, G.S. (2007). Androgens influence expression of matrix proteins and proteolytic factors during cutaneous wound healing. *Lab Invest, 87*(9), 871-881. DOI. 10.1038/labinvest.3700627.

Global report on diabetes. Geneva, World Health Organization, (2016). Retrieved from http://www.who.int/mediacentre/factsheets/fs312/en/.

Gołezbiowski, M., Cerkowniak, M., Urbanek, A., Dawgula, M., Kamysz, W., Bogu, M.I., Sosnowska, D., & Stepnowski, P. (2014). Antimicrobial activity of untypical lipid compounds in the cuticular and internal lipids of four fly species. *J Appl Microbiol*, *116*(2), 269-87. DOI. 10.1111/jam.12370.

Gopalakrishnan, K., & Udayakumar, R. (2014). GC-MS analysis of phytocompounds of leaf and stem of *Marsilea quadrifolia* (L.). *Int J Biochem Res Rev*, *4*, 517-526. DOI. 10.9734/IJBCRR/2014/11350.

Guo, S., & DiPietro, L. A. (2010). Factors affecting wound healing. *J Dent Res*, *89*(3), 219–229. DOI. 10.1177/0022034509359125.

Gupta, R., & Singh, H.K. (2012). Nootropic potential of *Alternanthera sessilis* and *Clerodendrum infortunatum* leaves on mice. *Asian Pac J Trop Biomed*, *2*(1), S465-S470. DOI. 10.1016/S2222-1808(12)60204-7.

Gupta, R., & Singh, H.K. (2014). Antidepressant like effects of *Alternanthera sessilis* and *Clerodendrum infortunatum* leaves extract in immobility models. *J Nat Prod*, *4*(1), 33-37. DOI. 10.2174/2210315504666140515004826.

Gutiérrez-Fernández, A., Inada, M., Balbin, M., Fueyo, A., Pitiot, A.S., Astudillo, A., Hirose, K., Hirata, M., Shapiro, S.D., Noel, A., Werb, Z., Krane, S.M., López-Otín, C., & Puente, X.S. (2007). Increased inflammation delays wound healing in mice deficient in collagenase-2 (MMP-8). *FASEB J, 21*(10), 2580-91. DOI: 10.1096/fj.06-7860com.

Halprin, K.M. (2006). Epidermal "Turnover time"- A re-examination. *Br J Derm, 86*(1), 14-19. DOI. 10.1111/j.1365-2133.

Hameed, E.S., Bazais, S.A., & Salman, M.S. (2013). Characterization of the phytochemical constituents of Taif rose and its antioxidant and anticancer activities. *Biomed Res Int*, 2013, 345465. DOI. 10.1155/2013/345465.

Han, D.O., Lee, H.J., & Hahm, D.H. (2009). Wound-healing activity of Astragali Radix in rats. *Methods Find Exp Clin Pharmacol*, *31*(2), 95-100. DOI. 10.1358/mf.2009.31.2.1353846.

Healy, B., & Freedman, A. (2006). Infections. *BMJ*, *332*(7545), 838-841. DOI. 10.1136/bmj.332.7545.838.

Heleno, S.A., Ferreira, I.C.F.R., Esteves, A.P., Glamočlija, A.J., Martins, A., Soković, M., & Queiroz, M.J.R.P. (2013). Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food Chem Toxicol*, *58*, 95-100. DOI. 10.1016/j.fct.2013.04.025.

Hirobe, T. (2014). Keratinocytes regulate the function of melanocytes. *DSI*, *32*(4): 200-204. DOI. 10.1016/j.dsi.2014.05.002.

Hudson, D.M., Weis, M., Raj, J., Joeng, K.S., Dimori, M., Lee, B.H., Morello,R., & Eyre, D.R. (2017). P3h3-null and Sc65-null mice phenocopy the collagen lysine under-hydroxylation and cross-linking abnormality of Ehlers-danlos syndrome type VIA. *J Biol Chem*, *292*(9), 3877-3887. DOI. 10.1074/jbc.M116.762245.

Hwang, Y.J., Lee, E.J., Kim, H.R., & Hwang, K.A. (2013). *In vitro* antioxidant and anticancer effects of solvent fractions from *Prunella vulgaris* var. lilacina. *BMC Complement Altern Med* 2013, 13, 310. DOI. 10.1186/1472-6882-13-310.

Igarashi, T., Nishino, K. & Nayar, S.K. (2005). The appearance of human skin. Retrieved from http://www1.cs.columbia.edu/CAVE/publications/pdfs/Igarashi_CUTR05.pdf.

Igarashi, T., Nishino, K. and Nayar, S.K. (2007). The appearance of human skin: A survey. *Foundations and TrendsR in Computer Graphics and Vision*, *3*(1), 1-95. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.138.2303&rep=rep1&type=pdf.

Inoue, H., Takamori, M., Shimoyama, Y., Ishibashi, H., Yamamoto, S., & Koshihara, Y. (2002). Regulation by PGE_2 of the production of interleukin-6, macrophage colony stimulating factor, and vascular endothelial growth factor in human synovial fibroblasts. *Br J Pharmacol*, *136*(2), 287-295. DOI. 10.1038/sj.bjp.0704705.

Intayoung, P., Limtrakul, P.,& Yodkeeree, S. (2016). Antiinflammatory activities of crebanine by inhibition of NF-Kb and AP-1 activation through suppressing MAPKs and Akt signaling in LPS-induced RAW264.7 macrophages. *Biol Pharm Bull*, *39*, 54–61, DOI. 10.1248/bpb.b15-00479.

Isaac, C., Paggiaro, A.O., Conduta, J.L., Aldunate, B., Herson, M.R., Altran, S.C., Mathor, M.B., & Ferreira. (2011). Role of keratinocytes in wound contraction: an impact assessment using a model of collagen matrix populated with fibroblasts. *Rev Bras Cir Plást*, *26*(3), 402-406.

Jalalpure, S.S., Agrawal, N., Patil, M.B., Chimkode, R., Tripathi, A. (2008). Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis* Linn. *Int. J. Green Pharm*, 2(3), 141-144.

Jiang, S., Lai, P., & Wang, G. (2012). Antioxidant activities and essential oil composition of *Herba Artemisiae Scopariae* from China. *J Oleo Sci*, *61*(5), 291-295. DOI. 10.5650/jos.61.291

Johannesson, A., Larsson, G.U., Ramstrand, N., Turkiewicz, A., Wirehn, A.B., & Atroshi, I. (2009). Incidence of lower-limb amputation in the diabetic and nondiabetic general population: a 10-year population-based cohort study of initial unilateral and contralateral amputations and reamputations. *Diabetes Care*, *32*(2), 275-280. DOI. 10.2337/dc08-1639.

Jones, C., & Ehrlich, H.P. (2011). Fibroblast expression of α-smooth muscle actin, α2β1 integrin and αvβ3 integrin: influence of surface rigidity. *Exp Mol Pathol*, *91*(1), 394-399. DOI. 10.1016/j.yexmp.2011.04.007.

Joseph, B., Dhas, B., Hena, V., & Raj, J. (2013). Bacteriocin from *Bacillus subtilis* as a novel drug against diabetic foot ulcer bacterial pathogens. *Asian Pac J Trop Biomed*, *3*(12), 942-946. DOI. 10.1016/S2221-1691(13)60183-5.

Jung, K.W., Yoo, S.K., Moon, S.K., & Lee, Ung.S. (2007). Furfural from pine needle extract inhibits the growth of a plant pathogenic fungus, *Alternaria mali*. *Mycology*, *35*(1), 9–43. DOI. 10.4489/MYCO.2007.35.1.039.

Kang, J., Zhang, Y., Cao, X., Fan, J., Li, G., Wang, Q., Diao, Y., Zhao, Z., Luo, L., & Yin, Z. (2012). Lycorine inhibits lipopolysaccharide-induced iNOS and COX-2 up-regulation in RAW264.7 cells through suppressing P38 and STATs activation and increases the survival rate of mice after LPS challenge. *Int Immunopharmacol*, *12*(1), 249–256. DOI. 10.1016/j.intimp.2011.11.018.

Khan, S., Shehzad, O., Lee, K.J., Tosun, A., & Kim, Y.S. (2014). Anti-inflammatory properties of samidin from *Seseli resinosum* through suppression of NF-kB and AP-1 mediated-genes in LPS-stimulated RAW 264.7 cells. *Arch Pharm Res, 37*(11), 496-1503. DOI. 10.1007/a12272-014-0346-0.

Kiecolt-Glaser, J.K., Loving, T.J., Stowell, J.r., Malarkey, W.B., Lemeshow, S., Dickinson, S.L., & Glaser, R. (2005). Hostile marital interactions, proinflammatory cytokine production, and wound healing. *Arch Gen Psychiatry*, *62*(12), 1377-1384. DOI. 10.1001/archpsyc.62.12.1377.

Kim, D.S., Shin, M.R., Kim, Y.S., Bae, W.J., Roh, D.H., Hwang, Y.S., & Kim, E.C. (2015). Anti-inflammatory effects of glutamine on LPS-stimulated human dental pulp cells correlate with activation of MKP-1 and attenuation of the MAPK and NF-κB pathways. *Int Endod J*, *48*(3), 220-228. DOI. 10.1111/iej.12303.

Kim, K., Park, J., & Rhee, S. (2007). Structural and functional basis for (S)-Allantoin formation in the ureide pathway. *J Biol Chem*, *282*, 23457–23464. DOI. 10.1074/jbc.M703211200.

Klein, A.M., Nikolaidou-Neokosmidou, V., Doupé, D.P., Jones, P.H., & Simons, B.D. (2011). Patterning as a signature of human epidermal stem cell regulation. *J R Soc Interface*, *8*(65), 1815–1824. DOI. 10.1098/rsif.2011.0240.

Knöfler, M., Kiss, H., Mösl, B., Egarter, C., & Husslein, P. (1997). Interleukin-1 stimulates tumor necrosis factor-alpha (TNF-alpha) release from cytotrophoblastic BeWo cells independently of induction of the TNF-alpha mRNA. *FEBS Lett*, *405*(2), 213-218. DOI. 10.1016/S0014-5793(97)00190-7.

Koech, D. K., Herman, J.A., Kofi-Tsekpo, M.W., & Herman, S.D. (1994). Trioxolanes: A new generation of compounds with wide-ranging activities. *Afr J Health Sci*, *1*(4), 147-150.

Koh, T.J., & DiPietro, L.A. (2011). Inflammation and wound healing: The role of the macrophage. *Expert Rev Mol Med*, 13, e23. DOI. 10.1017/S1462399411001943.

Kota, S., Govada, V.R., Anantha, R.K.,& Verma, M.K.(2017). An investigation into phytochemical constituents, antioxidant, antibacterial and anti-cataract activity of *Alternanthera sessilis*, a predominant wild leafy vegetable of South India. *Biocatal Agric Biotechnol*, *10*, 197-203. DOI. 10.1016/j.bcab.2017.03.008.

Kurashov, E., Fedorova, E.V., Krylova, J.V., & Mitrukoval, G.G. (2016). Assessment of the potential biological activity of low molecular weight metabolites of freshwater macrophytes with QSAR. *Scientifica*, Article ID 1205680, 9 pages. DOI. 10.1155/2016/1205680.

Larson, B.J., Lomgaker, M.T., & Lorenz, H.P. (2010). Scarless fetal wound healing: a basic science review. *Plast Reconstr Surg*, *126*(4), 1172-1180. DOI. 10.1097/PRS.0b013e3181eae781

Li, B., & Wang, J.H.C. (2011). Fibroblasts and myofibroblasts in wound healing: force generation and measurement. *J Tissue Viability*, *20*(4), 108–120. DOI. 10.1016/j.jtv.2009.11.004.

Li, H., Pan, L., Ke, Y., Batnasan, E., Jin, X., Liu, Z., & Ba, X. (2014). Daidzein suppresses pro-inflammatory chemokine Cxcl2 transcription in TNF-α-stimulated murine lung epithelial cells via depressing PARP-1 activity. *Acta Pharmacol Sin*, *35*(4), 496–503. DOI. 10.1038/aps.2013.191.

Li, H., Song, F., Xing, J., Tsao, R., Liu, Z., & Liu, S. (2009). Screening and structural characterization of α-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MSn and SORI-CID FTICR MS. *J Am Soc Mass Spectrom*, *20*(8), 1496-1503. DOI. 10.1016/j.jasms.2009.04.003.

Liang, C., Park, A.Y., & Guan, J. (2007). *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*. *Nature Protocols*, *2*(2), 340–342. DOI.10.1038/nprot.2007.30.

Liao, P.C., Chien, S.C., Ho, C.L., Wang, E.I.C., Lee, S.C., Kuo, Y.H., Jeyashoke, N., Chen, J., Dong, W.C., Chao, L.K., & Hua, K.F. (2010). Osthole regulates inflammatory mediator expression through modulating NF-KB, mitogen-activated protein kinases, protein kinase C, and reactive oxygen species. *J Agric Food Chem*, *58*(19), 10445–1045. DOI. 10.1021/jf102812t.

Limtrakul, O., Yodheeree, S., Pitchakarn, P. & Punfa, W. (2015). Suppression of inflammatory responses by black rice extract in RAW 264.7 macrophages cells via downregulation of NF-κB and AP-1 signaling pathways. *Asian Pacific Journal of Cancer Prevention*, *16*(10), 4277-4283.

Liu, C., Chen, C., Mo, H., Ma, H., Yuan, E., Li, Q. (2014). Characterization and DPPH radical scavenging activity of gallic acid-lecithin complex. *Trop J Pharm Res*, *13* (8): 1333-1338.

Liu, D., Ahmet, A., Ward, L., Krishnamoorthy, P., Mandelcorn, E.D., Leigh, R., Brown, J.P., Cohen, A., & Kim, H. (2013). A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy Asthma Clin Immunol*, *9*(1), 30. DOI. 10.1186/1710-1492-9-30.

Liu, W, Liu, J., Yin, D., & Zhao, X. (2015). Influence of ecological factors on the production of active substances in the anti-cancer plant *Sinopodophyllum hexandrum* (Royle) T.S. Ying. *PLoS One*, *10*(4), e0122981.

López-García, J., Lehocký, M., Humpolíček, P., & Saha, P. (2014). HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. *J Funct Biomater*, *5*(2), 43–57.

Lum, M. (2015). When sepsis strikes. Retrieved from https://www.thestar.com.my/lifestyle/health/2015/04/12/when-sepsis-strikes/ [12 April 2015]

MacMicking, J., Xie, Q.W., & Nathan, C. (1997). Nitric oxide and macrophage function. *Annu Rev Immunol*, 15, 323-350. DOI. 10.1146/annurev.immunol.15.1.323.

Manca, M.L., Matricardi, P., Cencetti, C., Peris, J.E., Melis, V., Carbone, C., Escribano, E., Zaru, M., Fadda, A.M., & Manconi, M. (2016). Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery. *Int J Pharm*, *505*(1-2), 204-211. DOI. 10.1016/j.ijpharm.2016.04.008.

Mandal, A., & Reddy, J.M. (2017). A review of phytochemical, pharmacological and potential therapeutic uses of *Phyllanthus emblica*, *World J Pharm Res Int*, *6*(7), 817-830. DOI. 10.20959/wjpr20177-8869.

Martin, R., Miquel, S., Ulmer, J., Kechaou, N., Langella, P., & Bermúdez-Humarán, L.G. (2013). Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. *Microb Cell Fact*, *21*(71), 1-11. DOI. 10.1186/1475-2859-12-71.

McDougall, S., Dallon, J., Sherratt, J., & Maini, P. (2006). Fibroblast migration and collagen deposition during dermal wound healing: mathematical modelling and clinical implications. *Phil. Trans. R. Soc. A*, *364*(1843), 1385–1405. DOI. 10.1098/rsta.2006.1773.

Membrez, M., Chou, C.J., Raymond, F., Mansourian, R., Moser, M., Monnard, I., Ammon-Zufferey, C., Mace, K., Mingrone, G., & Binnert, C. (2010). Six weeks' sebacic acid supplementation improves fasting plasma glucose, HbA1c and glucose tolerance in db/db mice. *Diabetes Obes Metab*, *12*(12), 1120–1126. DOI. 10.1111/j.1463-1326.2010.01308.x.

Mempel, M., Schmidt, T., Boeck, K., Brockow, K., Stachowitz, S., Fesq, H., Schäfer, T., Thomsen, S., Schnopp, C., Ring, J., Probst, R., Luppa, P., & Abeck, D. (2000). Changes in collagen I and collagen III metabolism in patients with generalized atopic eczema undergoing medium-dose ultraviolet A1 phototherapy. *Br J Dermatol*, *142*(3), 473-480. DOI. 10.1046/j.1365-2133.2000.03359.x.

Méndez-Lagunas, L., Rodríguez-Ramírez, J., Cruz-Gracida, M., Sandoval-Torres, S., & Barriada-Bernal., G. (2017). Convective drying kinetics of strawberry (Fragaria ananassa): Effects on antioxidant activity, anthocyanins and total phenolic content. *Food Chem*, *230*, 174–181. DOI. 10.1016/j.foodchem.2017.03.010.

Menon, S.N., Flegg, J.A., McCue, S.W., Schugart, R.C., Dawson, R.A., & MecElwain, D.L.S. (2012). Modelling the interaction of keratinocytes and fibroblasts during normal and abnormal wound healing processes. *Proc. R. Soc. B*, *279*(1741), 3329-3338. DOI. 10.1098/rspb.2012.0319.

Michailidou, Z., Turban, S., Miller, E., Zou, X., Schrader, J., Ratcliffe, P.J., Hadoke, P.W., Walker, B.R., Iredale, J.P., Morton, N.M., & Seckl, J.R. (2012). Increased angiogenesis protects against adipose hypoxia and fibrosis in metabolic disease-resistant 11β-hydroxysteroid dehydrogenase type 1 (HSD1)-deficient mice. *J Biol Chem*, *287*(6), 4188-4197. DOI. 10.1074/jbc.M111.259325.

Mills, C.D., Kincaid, K., Alt J.M., Heilman, M.J., & Hill, A.M. (2000). M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol*, *164*(12), 6166-6673. DOI. 10.4049/jimmunol.164.12.6166.

Monastero, R.N., & Penthyala, S. (2017). Cytokines as biomarkers and their respective clinical cutoff levels. *Int J Inflam*, *2017*, 4309485. DOI. 10.1155/2017/4309485.

Mondal, H., Saha, S., Awang, K., Hossain, H., Ablat, A., Islam, M.K., Jahan, I.A., Sadhu, S.K., Hossain, M.G., Shilpi, J.A., & Uddin, S.J. (2014). Central-stimulating and analgesic activity of the ethanolic extract of *Alternanthera sessilis* in mice. *BMC Complement Altern Med*, *14*, 398. DOI 10.1186/1472-6882-14-398.

Moon, N.R., Kang, S., & Park, S. (2017). Consumption of ellagic acid and dihydromyricetin synergistically protects against UV-B induced photoaging, possibly by activating both TGF-β1 and wnt signaling pathways. *J Photochem Photobiol B*, *178*, 92-100. DOI. 10.1016/j.jphotobiol.2017.11.004.

Negria, G., Matteia, R., & Mendesb, F.R. (2014). Antinociceptive activity of the HPLC- and MS-standardized hydroethanolic extract of *Pterodon emarginatus* Vogel leaves. *Phytomedicine*, *21*(8-9), 1062-1069. DOI. 10.1016/j.phymed.2014.04.009.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K., & Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*, *113*(9), 1271–1276. DOI. 10.1172/JCI20945.

Newman, D.J., & Cragg, G.M. (2016). Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod*, *79*(3), 629−661. DOI. 10.1021/acs.jnatprod.5b01055.

Nicolaus, C., Junghanns, S., Hartmann, A., Murillo, R., Ganzera, M., & Merfort, I. (2017). *In vitro* studies to evaluate the wound healing properties of *Calendula officinalis* extracts. *J. Ethnopharmacol*, *196*, 94-103. DOI. 10.1016/j.jep.2016.12.006.

Niyonsaba, F., & Ogawa, H. (2005). Protective roles of the skin against infection: implication of naturally occurring human antimicrobial agents beta-defensins, cathelicidin LL-37 and lysozyme. *J Dermatol Sci*, *40*(3), 157-168. DOI. 10.1016/j.jdermsci.2005.07.009.

O'Donnell, M.J., & Donini, A. (2017) Nitrogen excretion and metabolism in insects. *Acid-Base Balance and Nitrogen Excretion in Invertebrates.* Retrieved from: https://link.springer.com/chapter/10.1007/978-3-319-39617-0_4#citeas.

O'Loughlin, A., & O'Brien, T. (2011). Topical stem and progenitor cell therapy for diabetic foot ulcers. *Stem Cells in Clinic and Research*. DOI. 10.5772/19070.

Oskoueian, E., Abdullah, N., Ahmad, S., Saas, W.Z., Omar, A.R., & Ho, Y. W. (2011). Bioactive compounds and biological activities of *Jatropha curcas* L. kernel meal extract. *Int J Mol Sci*, *12*(9), 5955–5970. DOI. 10.3390/ijms12095955.

Othman, A., Ismail, A., Hassan, F.A., Yusof, B.N.M., & Khatib, A. (2016). Comparative evaluation of nutritional compositions, antioxidant capacities, and phenolic compounds of red and green sessile joyweed (*Alternanthera sessilis*). *J Funct Foods*, *21*, 263–271. DOI. 10.1016/j.jff.2015.12.014.

Park, B., Hwang, E., Seo, S.A., Cho, Jin-Gyeong., Yang, Jun-Eun., & Yi, Tae-Hoo. (2018). Eucalyptus globulus extract protects against UVB-induced photoaging by enhancing collagen synthesis via regulation of TGF-β/Smad signals and attenuation of AP-1. *Arch Biochem and Biophys*, *637*, 31-39. DOI. 10.1016/j.abb.2017.11.007.

Park, J.Y., Kawak, J.H., Kang, K.S., Jung, E.B., Lee, D.S., Lee, S., Jung, Y., Kim, K. H., Hwang, G. S., Lee, H. L., Yamabe, N., & Kim, S.N. (2017). Wound healing effects of deoxyshikonin isolated from Jawoongo: In vitro and in vivo studies. J Ethnopharmacol, 199: 128-137. DOI. 10.1016/j.jep.2016.10.031.

Parkinson, E.K. (1992). Epidermal keratinocyte stem cells: their maintenance and regulation. *Semin Cell Biol*, *3*(6), 435-444. DOI. 10.1016/1043-4682(92)90014-M.

Pastar, I., Stojadinovic, O., Yin, N.C., Ramirez, H., Nusbaum, A.G., Sawaya, A., Patel, S.B., Khalid, L., Isseroff, R.R., & Canic, M.T. (2014). Epithelialization in wound healing: A comprehensive review. *Adv Wound Care (New Rochelle)*, *3*(7), 445-464. DOI. 10.1089/wound.2013.0473.

Patra, M., Salonen, E., Terama, E., Vattulainen, I., Faller, R., Lee, B.W., Holopainen, J., & Karttunen, M. (2006). Under the influence of alcohol: The effect of ethanol and methanol on lipid bilayers. *Biophys J, 90*(4), 1121–1135. DOI. 10.1529/biophysj.105.062364.

Pemayun, T.G.D., Naibaho, R.M., Novitasari, D., Amin, N., & Minuljo, T.T. (2015). Risk factors for lower extremity amputation in patients with diabetic foot ulcers: a hospital-based case–control study. *Diabet Foot Ankle, 6.* DOI. 10.3402/dfa.v6.29629.

Peshkova, A.D., Minh, G.L., Tutwiler, V., Andrianova, I.A., Weisel, J.W., & Litvinov, R.I. (2017). Activated monocytes enhance platelet-driven contraction of blood clots via tissue factor expression. *Sci Rep, 7*(1), 5149. DOI. 10.1038/s41598-017-05601-9.

Pham-Huy, L.H.A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *Int J Biomed Sci, 4*(2), 89-96.

Phusricom, S., Chatuphonprasert, W., Monthakantirat, O., Pearaksa, P., & Jarukamjorn, K. (2013). *Alternanthera sessilis* and *Alternanthera bettzickiana* improved superoxide dismutase and catalase activities in the livers of ovariectomized mice. *J Appl Biopharm. Pharmacokinet, 1*(2), 64-71.

Priya, V., Jananie, K., & Vijayalakshmi, K. (2011). GC/MS determination of bioactive components of *Trigonella foenum grecum*. *J Chem Pharm Res, 3*(5), 35-40.

Provenzano, P.P., Inman, D.R., Eliceiri, K.W., Trier, S.M., & Keely, P.J. (2008). Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys J, 95*(11), 5374–5384. DOI. 10.1529/biophysj.108.133116.

Raman, V., Samuel, L. A., Pardha, S.M., Narashimha, R.B., Naga, V.K.A., Sudhakar, M., & Radhakrishnan, T.M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian J Pharm Clin Res, 5*, 99-106.

Rashaduz, Z., Mohammed, P., Md, J., & Abu, S.M. (2015). In-vitro cytotoxic and thrombolytic potential of methanolic extract of *Podocarpus neriifolius* D. Don leaves. *IJPSR, 6*, 273-277.

Ravi, A., Gupta, M., Rao, M.R.K., Kalaivani., Kalaiselvi, V.S., Prabhu, K., Dinakar, S.,& Rao, G.V. (2015). GC MS analysis of an Ayurvedic medicine "Ashokarishtam". *Der Pharmacia Lettre, 7*(12), 45-52.

Rayees, S., Kumar, A., Rasool, S., Kaiser, P., Satti, N.K., Sangwan, P.L., Singh, S., Johri, R.K., & Singh, G. (2013). Ethanolic extract of *Alternanthera sessilis* (AS-1) inhibits IgE-mediated allergic response in RBL-2H3 cells. *Immunol Invest, 42*(6), 470-480. DOI. 10.3109/08820139.

Reed, C.A., & Kita, H. (2004). The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immuno*, *114*(5), 997-1008. DOI: 10.1016/j.jaci.2004.07.060.

Robertson, K., & Rees, J.L. (2010). Variation in epidermal morphology in human skin at different body sites as measured by reflectance confocal microscopy. *Acta Derm Venereol*, *90*(4), 368–373. DOI. 10.2340/00015555-0875.

Roses, A.D. (2000). Pharmacogenectis and future drug development and delivery. *Lancet*, *355*(9212), 1358-1361. DOI. 10.1016/S0140-6736(00)02126-7.

Saeed, N.M., El-Demerdash, E., Abdel-Rahman, H.M., Algandaby, M.M., Al-Abbasi, F.A., Abdel-Naim, A.B. (2012). Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. *Toxicol Appl Pharmacol*, *264*(1), 84-93. DOI. 10.1016/j.taap.2012.07.020.

Sayed, A.M.E., Ezzat, S.M., Naggar, M.M.E., & Hawary, S.S.E. (2016). In vivo diabetic wound healing effect and HPLC–DAD–ESI–MS/MS profiling of the methanol extracts of eight Aloe species. *Rev Bras Farmacog*, *26*(3), 352-362. DOI. 10.1016/j.bjp.2016.01.009

Scarpioni, R., Ricardi, M.,& Albertazzi, V. (2016). Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage. *World J Nephrol*, *5*(1), 66–75. DOI. 10.5527/wjn.v5.i1.66.

Schulte, W., Bernhagen, J., & Bucala, R. (2013). Cytokines in sepsis: potent immunoregulators and potential therapeutic targets-an updated view. *Mediators Inflamm*, *2013*, 165974. DOI.10.1155/2013/165974.

Sen, C.K. (2009). Wound healing essentials: Let there be oxygen. *Wound Repair Regen*, *17*(1), 1-18. DOI. 10.1111/j.1524-475X.2008.00436.x.

Serra, M, B., Barroso, W.A., daSilva, N.N., Silva, S.N., Borges, A.C.R., Abreu, I.C., & Borges, O.R. (2017). From inflammation to current and alternative therapies involved in wound healing. *Int J Inflam*, *2017*, 3406215. DOI. 10.1155/2017/3406215.

Sgonc, R., & Gruber, J. (2013). Age-related aspects of cutaneous wound healing: a mini-review. *Gerontology*, *59*(2), 159-164. DOI. 10.1159/000342344.

Sharma, P., Jhan, A.B., Dubey, R.S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot*, *2012*, 26 pages. DOI. 10.1155/2012/217037.

Sherman, R.A. (2014). Mechanisms of maggot-induced wound healing: What do we know, and where do we go from here? *Evid Based Complement Alternat Med*, *2014*, 592419. DOI. 10.1155/2014/592419.

Shimizu, M., & Tomoo, T. (1994). Anti-inflammatory constituents of topically applied crude drugs. V. constituents and anti-inflammatory effect of Aoki, *Aucuba japonica* Thunb. *Biol Pharm Bull*, *17*(5), 665-667.

Shin, S., Kim, C., Go, R., & C.K. (2016). Effect of benzophenone-1 and octylphenol on the regulation of epithelial-mesenchymal transition via an estrogen receptor-dependent pathway in estrogen receptor expressing ovarian cancer cells. *Food Chem Toxicol*, *93*, 58-65. DOI. 10.1016/j.fct.2016.04.026

Shindo, Y., Witt, E., Han, D., Epstein, W., & Packer, L. (1994). Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J Invest Dermatol*, *102*(1):122-124. DOI. 10.1111/1523-1747.ep12371744.

Sivamani, R.K., Ma, B.R., & Maverakis, E. (2012). Phytochemicals and naturally derived substances for wound healing. *Adv Wound Care (New Rochelle)*, *1*(5), 213-217. DOI: 10.1089/wound.2011.0330.

Song, Q., Gou, Q., Xie, Y., Zhang, Z., & Fu, C. (2017). *Periplaneta americana* extracts promote skin wound healing via Nuclear Factor Kappa β canonical pathway and extracellular signal-regulated kinase signaling. *Evid Based Complement Alternat Med*, *2017*, 5821706. DOI. 10.1155/2017/5821706.

Song, Y., Zen, R., Hu, L., Maffucci, K.G., Ren, X., & Qu, Y. (2017). In vivo wound healing and *in vitro* antioxidant activities of *Bletilla striata* phenolic extracts. *Biomed Pharmacother*, *93*, 451-461. DOI. 10.1016/j.biopha.2017.06.079.

Sprague, A.H., & Khalil, R.A. (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol*, *78*(6): 539-552. DOI. 10.1016/j.bcp.2009.04.029.

Staiger, C. (2007). Comfrey: ancient and modern uses. *Pharm J*, *279*. Retrieved from https://www.pharmaceutical-journal.com/news-and-analysis/news/comfrey-ancient-and-modern-uses/10005729.article [20th October 2017]

Staiger, C. (2012). Comfrey: A clinical overview. *Phytother Res*, *26*(10), 1441–1448. DOI. 10.1002/ptr.4612.

Subhashini, T., Krishnaveni, B., & Reddy, A. (2010). Anti- Inflammatory activity of leaf extracts of *Alternanthera sessilis*. *Hygeia J D Med*, *2*(1), 54-56.

Sunmathi, D., Svakumar, R., & Ravikumar, K. (2016). *In vitro* anti-inflammatory and antiarthritic activity of ethanolic leaf extract of *Alternanthera sessilis* (L.) R.Br. ex DC and *Alternanthera philoxeroides* (Mart.) Griseb. 5(2), 1009-115.

Sversut, R.A., Vieira, J.C., Rosa, A.M., Singh, A.K., Amaral, M.S., & Kassab. N.M. (2015). Improved UV spectrophotometric method for precise, efficient and selective determination of Dexamethasone in pharmaceutical dosage forms. *Orbital: Electron J. Chem 7*(1), 5-7.

Szkudelska, K., Szkudelski, T., & Nogowski, L. (2002). Daidzein, coumestrol and zearalenone affect lipogenesis and lipolysis in rat adipocytes. *Phytomedicine*, *9*(4), 338-345. DOI. 10.1078/0944-7113-00148

Tam, J.C.W., Lau, K.M., Liu, C.L., To, M.H., Kwok, H.F., Lai, K.K., Lau, C.P., Ko, C.H., Leung, P.C., Fung, K.P., & Lau, C.B.S. (2011). The in vivo ad *in vitro* diabetic wound healing effects of a 2-herb formula and its mechanism of action. *J Ethnopharmacology*, *134*(3), 831-838. DOI. 10.1016/j.jep.2011.01.032.

Tan, K.K., & Kim, K.H. (2013). *Alternanthera sessilis* red ethyl acetate fraction exhibits antidiabetic potential on obese type 2 diabetic rats. *Evid Based Complement Alternat Med*, *2013*, 845172. DOI. 10.1155/2013/845172.

Tanabe, K., Matsushima, N.R., Yamaguchi, S., Lida, H., Dohi, S., & Kozawa, O. (2010). Mechanisms of tumor necrosis factor-alpha-induced interleukin-6 synthesis in glioma cells. *J Neuroinflammation*, *6*. DOI. 10.1186/1742-2094-7-16.

Tandara, A.A., & Mustoe, T.A. (2004). Oxygen in wound healing— more than a nutrient. *World J Surg*, *28*(3), 294–300. DOI. 10.1007/s00268-003-7400-2.

Tang, C., Xue, H.L., Bai, C.L., & Fu, R. (2011). Regulation of adhesion molecules expression in TNF-α-stimulated brain microvascular endothelial cells by tanshinone IIA: involvement of NF-κB and ROS generation. *Phytother Res*, *25*(3):376-380. DOI. 10.1002/ptr.3278.

Thanga, K.K.S., Muthukumarasamy, S., & Mohan, V.R. (2012). GC-MS analysis of ethanol extract of *Sarcostemma secamone* (L) Bennet (Asclepiadaceae). *Sci Res*, *2*(3), 187-191.

Thomas, J.G., & Percival, S.L. (2009). Indigenous microbiota and association with the host. *Microbiology and Aging: Clinical Manifestations.* Retrieved from https://doi.org/10.1007/978-1-59745-327-1_2

Tornatore, L., Thotakura, A.K., Bennett, J., & Franzoso, G. (2012). The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. *Trends Cell Biol*, *22*(11), 557-566. DOI. 10.1016/j.tcb.2012.08.001.

Tschumperlin, D.J. (2013). Fibroblasts and the ground they walk on. *Physiology (Bethesda)*, *28*(6), 380-390. DOI. 10.1152/physiol.00024.2013.

Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A., & Bornstein, S.R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *Biomed Res Int*, 2013, 385641. DOI. 10.1155/2013/385641.

Turner, J.D. & Muller, C.P. (2005). Structure of the glucocorticoid receptor (NR3C1) gene 5' untranslated region: identification, and tissue distribution of multiple

new human exon 1. *J Mol Endocrinol*, *35*(2), 283-292. DOI. 10.1677/jme.1.01822

Uchechi, O., Ogbonna, D.N., & Attama, A.A. (2014). Nanoparticles for dermal and transdermal drug delivery. *Application of Nanotechnology in Drug Delivery*. DOI. 10.5772/58672.

Valencia, A., & Kochevar, I.E. (2007). Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. *J Investig Dermatol*, *128*(1), 214-222. DOI. 10.1038/sj.jid.5700960.

Voytena, A.P.L., Affonso, R.C.L., Pitz, H.S., Ramlov, F., Albert, T., Coelho, D.S., Pereira, A., Navarro, B.B., Fanan, S., Casagrande, M., Ribeiro-do-Valle, R.M., & Maraschin, M. (2018). Phytochemical profile and *in vitro* assessment of the cytotoxicity of green and roasted coffee oils (*Coffea arabica* L.) and their polar fractions. *Rec Nat Prod, 12*(2), 169-174.

Wallecham, A., Wood, L., Pan, Z-K., Maciag, P.C., Shahabi, V., & Paterson, Y. (2013). Listeria monocytogenes-derived Listeriolysin O has pathogen-associated molecular pattern-like properties independent of its hemolytic ability. *Clin Vaccine Immunol*, *20*(1), 77–84. DOI. 10.1128/CVI.00488-12.

Walter, T.M., Merish, S., & Tamizhamuthu, M. (2014). Review of *Alternanthera sessilis* with reference to traditional Siddha. *J Pharmacognosy Phytochem*, *6*(2), 249-254.

Wang, L.H., Zhou, S.X., Li, R.C., Zheng, L.R., Zhu, J.H., Hu, S.J., & Sun, Y.L. (2012). Serum levels of calcitonin gene-related peptide and substance P are decreased in patients with diabetes mellitus and coronary artery disease. *J Int Med Res*, *40*(1), 134-140. DOI: 10.1177/147323001204000114.

Wang, Z., Wang, Y., Farhangfar, F., Zimmer, M., & Zhang, Y. (2012). Enhanced keratinocyte proliferation and migration in co-culture with fibroblasts. *PLoS One*, 7, e40951. DOI. 10.1371/journal.pone.0040951.

Wardecki, T., Werner, P., Thomas, M., Templin, M.F., Schmidt, G., Brandner, J.M., & Merfort, I. (2016). Influence of birch bark triterpenes on keratinocytes and fibroblasts from diabetic and nondiabetic donors. *J Nat Prod*, *79*(4), 1112–1123. DOI. 10.1021/acs.jnatprod.6b00027.

Werner, S., Krieg, T., & Smola, H. (2007). Keratinocyte-fibroblast interactions in wound healing. *J Invest Dermatol*, *127*(5), 998-1008. DOI. 10.1038/sj.jid.5700786.

Wink, M. (2015). Modes of action of herbal medicines and plant secondary metabolites. *Medicines (Basel)*, *2*(3), 251-286. DOI. 10.3390/medicines2030251.

Winter, A.N., Brenner, M.C., Punessen, N., Snodgrass, M., Byars, C., Arora, Y., & Linseman, D.A. (2017). Comparison of the neuroprotective and anti-inflammatory effects of the anthocyanin metabolites, protocatechuic acid and 4-hydroxybenzoic acid. *Oxidative medicine and cellular longevity, 2017*, 13 pages. DOI. 10.1155/2017/6297080.

Wojtowicz, A.M., Oliveira, S., Carlson, M.W., Zawadzka, A., Rousseau, C.F., & Baksh, D. (2014). The importance of both fibroblasts and keratinocytes in a bilayered living cellular construct used in wound healing. *Wound Repair Regen, 22*(2), 246-255. DOI. 10.1111/wrr.12154.

Wong. S.L., Chang, H.S., Wang, G.J., Chiang, M.Y., Huang, H.Y., Chen, C.H., Tsai, S.C., Lin, C.H., & Chen. I.S. (2011). Secondary metabolites from the roots of *Neolitsea daibuensis* and their anti-inflammatory activity. *J Nat Prod*, *74*(12), 2489–2496. DOI. 10.1021/np100874f.

Wu, E.L., Engstrom, O., Jo, S., Stuhlsatz, D., Yeom, M.S., Klauda, J.B., Widmalm, G., & Im, W. (2013). Molecular dynamics and NMR spectroscopy studies of *E. coli* lipopolysaccharide structure and dynamics. *Biophys J, 105*(6), 1444-1455. DOI. 10.1016/j.bpj.2013.08.002.

Xiang, P., Chen, T., Mou, Y., Wu, H., Xie, P., Lu, G., Gong, X., Hu, Y., Zhang, Y., & Ji, H. (2015). NZ suppresses TLR4/NF-kβ signaling and NLRP3 inflammasome activation in LPS-induced RAW264.7 macrophages. *Inflamm Res*, *64*(10), 799-808. DOI. 10.1007/s00011-015-0863-4.

Xiao, J., Capanoglu, E., Jassbi, A.R., & Miron, A. (2016). Advance on the flavonoid c-glycosides and health benefits. *Crit Rev Food Sci Nutr, 56*(Suppl 1), S29-S45. DOI. 10.1080/10408398.2015.1067595.

Yamaguchi, Y., Takahashi, K., Zmudzka, B.Z., Kormhauser, A., Miller, S.A., Tadokoro, T., Berens, W., Beer, J.Z., & Hearing, V.J. (2006). Human skin responses to UV radiation: pigment in the upper epidermis protects against DNA damage in the lower epidermis and facilitates apoptosis. *FASEB J, 20*(9), 1486-1488. DOI. 10.1096/fj.06-5725fje.

Yang, J.H., Yoo, J.M., Lee, E., Lee, B.H., C, W., Park, K., & Ma, J.Y. (2018). Anti-inflammatory effects of Perillae Herba ethanolic extract against TNF-α/IFN-γ-stimulated human keratinocyte HaCaT cells. *J Ethnopharmacol, 211*, 217-223. DOI. 10.1016/j.jep.2017.09.041.

Yang, Y.N, Huang, X.Y, Feng, Z. M., Jiang, J.S., & Zhang, P.C. (2015). New butyrolactone type lignans from *Arctii fructus* and their anti-inflammatory activities. *J Agric Food Chem, 63*(36), 7958-7966. DOI. 10.1021/acs.jafc.5b02838.

Yu, X., Wang, Y., & Zhang, Y. (2018). Transmural variation in elastin fiber orientation distribution in the arterial wall. *J Mech Behav Biomed Mater, 77*, 745-753. DOI. 10.1016/j.jmbbm.2017.08.002.

Yun, C.H., Jang, E.J., Kwon, S.C., Lee, M-Y., Lee, S., Oh, S-R., Lee, H-K., Ahn, K-S., & Lee, H-J. (2015). A novel synthetic compound, YH-1118, inhibited LPS-indices inflammatory response by suppressing ikB kinase/ NF-kB pathway in RAW 264.7 cells. *J Microbiol Biotechnol, 25*(7), 1047-1055. DOI. 10.4014/jmb.1504.04027.