FRACTIONATION OF *FICUS DELTOIDEA* LEAVES EXTRACT USING SOLID PHASE EXTRACTION ON ANTI-AGEING ACTIVITY IN VITRO

FATIN HUSNA BINTI ZULIKRAM

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

School of Chemical and Energy Engineering
Faculty of Engineering
Universiti Teknologi Malaysia

APRIL 2019

ABSTRACT

Ficus deltoidea (*F. deltoidea*) belongs to moraceae. Vitexin and isovitexin that have high antioxidant property are considered as the chemical markers of *F. deltoidea* leaves. The objective of this research was to investigate the fractionation of *F. deltoidea* leaves using solid phase extraction on anti-ageing activity *in vitro*. The leaves of *F. deltoidea* were extracted by methanol by using ultrasonic extraction method and further fractionated using different concentration of methanol. Antioxidant activity of *F. deltoidea* leaves fractions and extract was analyzed through 2,2-diphenyl-1-picrylhrazyl (DPPH) scavenging ability test and total flavanoid content (TFC) activity. The result shows that 80% methanol 20% water fraction (F4) have higher percentage of scavenging activity and flavonoid content with 87.16% and 237.57 mg, respectively. The quantification of biomakers (vitexin and isovitexin) was performed using high performance liquid chromatography (HPLC) and the result showed that F4 fraction have higher percentage of vitexin (11.02%) and isovitexin (0.49%) compared to other fractions and extract. Moreover, cytotoxicity study on human skin fibroblasts cell (HSF 1184) also demonstrated that F4 has higher percentage of cell viability with value 175.29% at 100 µg/mL. The anti-ageing activity of *F. deltoidea* leaves fractions and extract were further evaluated using sircol collagen assay, inhibition of elastase assay, hyaluronidase assay and lipoxygenase assay, where F4 also showed the strongest activities compared to other fractions and extract for all assays with the value of 8.9 µg collagen concentration, 83%, 97.82%, and 88.56%, respectively. Taken together, as F4 contains high amount of vitexin and isovitexin, therefore these compounds have the potential to be further developed as anti-ageing agent.

ABSTRAK

Ficus deltoidea (*F. deltoidea*) tergolong dalam moraceae. Viteksin dan isoviteksin yang mempunyai antioksida yang tinggi merupakan penanda kimia yang terdapat dalam daun *F. deltoidea*. Objektif kajian ini dilakukan untuk pemeringkatan daun *F. deltoidea* menggunakan pengekstrakan fasa pepejal secara in vitro untuk aktiviti anti-penuaan. Daun *F. deltoidea* diekstrak menggunakan metanol dan pemeringkatan dijalankan menggunakan kepekatan metanol dengan kaedah ultrasonik yang berbeza. Aktiviti antioksida pecahan dan daun mentah *F. deltoidea* dianalisis melalui ujian aktiviti pemerangkapan 2,2-diphenil-1-picrilhrazil dan jumlah kandungan flavanoid. Keputusan yang diperolehi menunjukkan campuran 80% metanol : 20% air (F4) mempunyai peratusan yang tertinggi bagi aktiviti memerangkap dan kandungan flavanoid masing-masing dengan nilai 87.16% dan 237.57 mg. Pengukuran biomarker (viteksin dan isoviteksin) ditentukan menggunakan kromatografi cecair prestasi tinggi dan keputusan menunjukkan pecahan F4 mempunyai peratusan viteksin (11.02%) dan isoviteksin (0.49%) yang tinggi berbanding ekstak dan pecahan yang lain. Tambahan pula, kajian sitotoksik terhadap kulit manusia dijalankan menggunakan sel fibroblast (HSF 1184) menunjukkan F4 juga mempunyai peratusan daya maju sel tertinggi iaitu 175.29% pada kepekatan 100 µg/mL. Kajian aktiviti anti-penuaan terhadap pecahan dan ekstrak daun *F. deltoidea* seterusnya dikaji melalui kaedah kolagen sirkol, perencatan elastase, hialuronidase dan liposiginase mendapati F4 juga menunjukkan aktiviti yang terkuat berbanding pecahan dan ekstrak keatas kesemua aktiviti masing-masing dengan nilai 8.9 µg , 97.82%, 97.82% dan 88.56%. Secara keseluruhannya, F4 mempunyai kandugan viteksin dan isovteksin yang tinggi di mana sebatian ini berpotensi dibangunkan sebagai agen anti-penuaan.

ACKNOWLEDGEMENT

First of all, praise to Allah for giving me strength and good health in completing my research project.

I would like the express my sincere gratitude to my supervisors, Dr Rosnani Hasham for her constant guidance, kind assistance and precious time for discussion throughout of this research project. I would like to thank all the lab mates for their support and encouragement.

Besides, I would like to record my special thanks to my lovely husband Mohammad Khaidree for his support physically, mentally and financially and encouragement in completing this research. I would like to extend my thanks to my parents Zulikram Mustapa and Roselina Mustaffa for their encouragement, love and advice throughout in completing this research project. Not to forget also, to my two cutie babies Luqman Hakim and Hanna Humaira for behaving well and let your mummy completing her master research.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURE

LIST OF ABBREVIATION

UV	Ultra violet
ROS	Reactive Oxygen Species
AP-1	Activator protein-1
MMP	Matrix metalloproteinase
ECM	Extracellular matrx
SPE	Solid phase extraction
MMP-1	Matrix metalloproteinase-1
MMP-9	Matrix metalloproteinase-9
MMP-3	Matrix metalloproteinase-3
HA	Hyaluronic acid
GAG	Glycosaminoglycan
HYAL	Hyaluronoglucosaminidase
DFT	Density Functional theory
iNOS	inducible nitric oxide synthase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
UAE	Ulrasound-assisted extraction
TFC	Total flavonoid content
MTT	3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
LOX	Lipoxygenase
HPLC	High performance liquid chromatography
UPM	Universiti Putra Malaysia
MeOH	Methanol
F1	Fraction 1
F2	Fraction 2
F3	Fraction 3

F3	Fraction3
F4	Fraction4
F5	Fraction5
TFA	Trifloroaceticacid
DMEM	Dulbecco's modified essential medium
FBS	Fetal Bovine Serum
CO2	Carbon dioxide
HSF	Human skin fibroblast
DMSO	Dimethyl sulfoxide
SCA	Sircol collagen assay
HCL	Hydrochloric acid
SANA	5 mM N-Succinyl-Ala-Ala-Ala-nitroanilide
HNE	human neutrophil elastase
$Na_2H_2PO_4$	Sodium phosphate
NDGA	Nordihydroguaiaretic acid
SPSS	Statistical Package Social Science
ANOVA	Analysis of variance
UAE	Ultrasonic assisted extraction

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ageing is a natural phenomenon, a physiological change that is certain to be experienced by each living organism. It is a complex metabolic process that occurs over a period of time through growth, development and maturity. The ageing process is said to happen when the energy to maintain the structural and functional molecules that are synthesized in our life is no longer accessible (Hayflick, 2004). As the bodies reach maturity, the skin appearance and characteristics change. In general, ageing is more prominently seen on skin where thin, dry, unblemished, elasticity-depleted texture and fine wrinkles are the common indicators (Rogers *et al.*, 2008).

Skin is the largest organ of the body with multiple cell types and structures that exhibits multiple functions, among them is a protective barrier between internal organs and outer environment (Fearnley, 2009). The outer part of skin is composed of fibro, protein, collagen and elastin. Collagen is one of the major building block of skin which responsible for elasticity and strength of the skin.

Skin ageing can be divided into intrinsic and extrinsic ageing (Bennett & Cooper, 2009). Intrinsic ageing is determined primarily by oxidative metabolism,

genetic and hormonal factors while extrinsic ageing emphasize on the exposure to the solar ultraviolet (UV) radiation. This UV radiation will induce free radical damage and increase Reactive Oxygen Species (ROS), which responsible for oxidative stresses and inflammatory responses in the dermal or epidermal layer by destructing the connective tissues fibers. Free radical damage will bring about DNA damage, protein and gene modifications. High level of ROS will induce the transcription of Activator protein 1 (AP-1). AP-1 is responsible in regulation of cell growth and differentiation. AP-1 strongly regulated the transcription of Matrix metalloproteinase (MMP), which causes further degradation of mature fibrillar collagen in the skin which contributes to skin ageing (Yin, 2014). Bissett *et al* (1987) reported that the decrease of skin elasticity with premature ageing is significantly correlated with increased elastase and hyaluronidase activity. Therefore, the inhibition of these enzymes may be the most effective therapy to improve the structure of collagen in the extracellular matrix (ECM) and control its metabolism (Mukherjee *et al.*, 2011).

The widespread awareness on depletion of ozone layer and the danger of UV radiation reaching directly to the surface of the Earth has made the society at large become more alert regarding the effect of this harmful ray on their skin. Nevertheless, skin care is not just a matter of health but an affair of beauty as well, which lead to the ever growing skin-based research conducted by various interested parties. In the turn of the decade, high accessibility of information regarding skin care products by consumer demands a greater need for anti-ageing products that are scientifically-proven in its efficiency (Rogers *et al.*, 2008).

Consuming proper food choice might be the larger part of what makes skin age gracefully while remaining healthy, strong and disease-free. Powerful anti-ageing benefits can be obtained from wide range of natural food, spices and herbs that carry high incidence of antioxidant properties. Traditional herbs have been proved to be safe and effective for ageing-related problem and very much often attracted the growing industry of skin-care product with niche in herb-based medicine (Hoffmann, 2013). Treatment of the skin with products containing plant-

derived antioxidant ingredients has been proven useful for the prevention of UV-mediated cutaneous damage. Various parts of the plant are available to be processed and consumed in the form of powder, tablets and extract.

This study attempts to focus on *Ficus deltoidea,* (*F. deltoidea*) a traditional herb from the genus *Ficus* of Moraceae family. *F. deltoidea,* locally known as 'Mas Cotek' is widely distributed in Peninsular Malaysia and a popular medicinal herb among Malay. This miraculous herb has been used traditionally to treat wounds and sore, used as an antidiabetic treatment and an after-birth tonic to contract the uterus and vaginal muscle (Bunawan *et al.*, 2014). *F. deltoidea* also reported to possess powerful antioxidant activity which is good for anti-ageing (Mohd *et al.*, 2015).

Bunawan *et al.* (2014) reported the biological properties of *F. deltoidea* which are antioxidant, antidiabetic, anti-inflammatory, anti-ulcerogenic, wound healing activity, anti-bacterial activity and anticancer activity. Wide ranges of chemical compound have been identified from leaves of *F. deltoidea.* Some of the compound that is stated to be present in the *F. deltoidea* is flavonoids such as isovitexin, vitexin, proanthocynidins, flavan-3-ol monomers and flavones glycosides (Misbah *et al.*, 2013). The volatile compound identified is mainly product of shikimic acid pathway, terpenoids and aliphatic groups. Vitexin and isovitexin has been reported can be effective for the prevention of free radical scavenging (Kim *et al.*, 2005). Isolation and purification process are important to identify the bioactive compound in *F. deltoidea* leaves.

Fractionation is a separation process in which a certain quantity of a mixture is divided into smaller quantities in which of the composition vary according to the gradient (Baynes, 2017). The use of fractionation could isolate the structural and morphology of identifiable entity for subsequent analysis, the emphasis being on purity at the expense of yield. During Solid phase extraction (SPE), the target analyte and structurally related compounds are adsorbed onto a solid, stationary phase. The solid phase is then washed with selective eluents in order to eliminate any interfering

substances and to reduce the complexity of the matrix. Finally, the bound target analyte is recovered by an elution step (Sigma, 1998). The solvent should enable rapid elution of the analyte from the solid phase. The recovery of organic compounds by SPE is highly dependent on the polarity of the eluents. A study conducted by Barbosa *et al* (2013) proved that purification obtain from the fractionation process yield higher antioxidant activity than the crude extract. Therefore, by applying this approach, reliable information about the potential of chemical compound in the *F. deltoidea* extracts on anti-ageing activity could be well understood and ultimately, a product which can slow the ageing process and make for a younger-looking skin could be produced.

1.2 Problem Statement

Exposure of the skin to UV occurs from both natural and artificial sources. The sun is the main source of UV radiation on human while the depletion of the ozone layer would intensifies the harmful exposure to humankind and the environment. As stated by Sudel *et al.* (2005), people without natural protection are estimated to account up to 90% visible skin ageing due to the effect of sunlight on the skin. As people age, their concern towards outward appearance increases profoundly and the concept of delaying the process of ageing seems appealing to most person. Today's anti-ageing market is expanding to incorporate diverse consumer concerns.

Recently, many herbs and natural products has been receiving public interest as complement and alternative medicine. Herbs which contain antioxidant and anti-inflammatory are used in cosmetic and dermatological products to improve signs of extrinsic ageing (Ho *et al.*, 2010). *F. deltoidea* extracts have been found to have photo-protective effects on epidermal cell line. A study reported by Mohd *et al.* (2015) stated that *F. deltoidea* has very strong antioxidant properties. Antioxidant can neutralize and stimulate the production of collagen and restore skin elasticity, thus can slow down the ageing process (Watson, 2013). Moreover, Zino *et al.* (1997)

reported the ability of *F. deltoidea* antioxidant that can delay some effects of ageing. A study conducted by Hasham *et al.* (2013) reported that skin ageing was strongly related to the inflammatory process. Leaves of *F. deltoidea* also have been confirmed devoid any toxic elements as reported by Shafei *et al.* (2011) and Farsi *et al.* (2013) also showed that *F. deltoidea* do not have any potential to induce mutation.

Flavanoids are a class of secondary plant phenolics with significant antioxidant and chelating properties (Seawan & Jimtaisong, 2013). Furthermore, flavonoids protect plants from solar UV radiation and scavenge UV generated ROS (Shirley, 1996). Therefore, flavonoids have three different photoprotection effects including UV absorption, direct and indirect antioxidant properties, and modulating several signaling pathways. Vitexin and isovitexin are flavones, which is one kind of flavanoid. These compound have been drawing more attention antioxidant activity and anti-inflammatory activity that can help in slowing the ageing process (He *et al.*, 2016). In order to isolate the bioactive compound in *F. deltoidea* leaves, fractionation method by using Solid Phase Extraction (SPE) was used in this study. Therefore, the proposed project is expected to yield novel insight on *F. deltoidea* leaves fractions on anti-ageing effects and lead to better understanding of anti-ageing properties of *F. deltoidea* leaves which is important and can give benefits to pharmaceutical and cosmeceutical industry.

1.3 Objective

The objective of this research was to investigate the fractionation of *Ficus deltoidea* leaves using solid phase extraction on anti-ageing activity *in vitro*.

1.4 Scope of Research

The scopes of research were:

1. Fractionation of *F. deltoidea* leaves extract by using solid phase extraction (SPE) method.
2. Determination of antioxidant properties of *F. deltoidea* leaves fractions using DPPH free radicals scavenging assay and total flavanoid content.
3. Evaluation of vitexin and isovitexin in *F. deltoidea* leaves fractions using HPLC.
4. Observation and investigation of anti-ageing effects caused by UVB irradiation of *F. deltoidea* leaves fractions on fibroblast cell line.
5. Evaluation of anti-ageing properties of *F. deltoidea* leaves fractions by using sircol collagen assay, MTT assay, elastase assay, hyaluronidase assay, and lipoxygenase assay.

1.5 Significant of Study

The finding of this study will rebound the benefit to pharmaceutical and cosmeceutical industry. From the result, fractionation of bioactive compound by using SPE method can isolate vitexin and isovitexin at mixture of 80% methanol : 20% water. This fraction shows the highest ability to slow down the ageing problem by ability to proliferate high percentage of fibroblast cells, produce more collagen content, inhibit elastase activity lipoxygenase and hyaluronidase assay.

REFRENCES

Abdulla, M. A., Ahmed, K. A., Abu-Luhoom, F. M., and Muhanid, M. (2010). Role of *Ficus deltoidea* extract in the enhancement of wound healing in experimental rats. Biomedical Research. 2(3): 241-245

Abdullah, Z., Khalid, H., Zhari, I and Rasadah, M. A., (2009). Anti-inflammatory activity of standardized extracts of leaves of three varieties of *Ficus deltoidea*. International Journal Pharmaceutical.

Adam, Z., Hamid, M., Ismail, A. and Khamis, S. (2009). Effect of *Ficus deltoidea* Aqueous Extract on Blood Glucose Level in Normal and Mild Diabetic Rats. *Malaysian Journal of Health Science*. 5(2): 9-16.

Andrade-Eiroa, A., Shahla, R., Romanías, M. N., & Dagaut, P. (2014). An alternative to trial and error methodology in solid phase extraction: An original automated solid phase extraction procedure for analysing PAHs and PAH-derivatives in soot. *RSC Adv., 4*(63), 33636-33644.

Anwar, F., & Przybylski, R. (2012). Effect Of Solvents Extraction On Total Phenolics And Antioxidant Activity of Extracts(LINUM USITATISSIMUM L.). *Acta Sci. Pol., Technol. Aliment, 11*(3).

Aris, S. R. S., Mustafa, S., Ahmat, N., Jaafar, F. M and Ahmad, R. (2009). Phenolic content and antioxidant activity of fruits of Ficus deltoidea var angustifolia sp. Malays. Journal of Analytical Science. 13(2): 146-150.

Armaghan, S., Eilham, F., Khadeer, A., and Siddiqui, A. (2011). Evaluation of Toxicological and Standardization Parameters and Phytochemical Investigation of Ficus deltoidea Leaves. *American Journal of Biochemistry and Molecular Biology.* 1(3): 237-243

Alim, N. A.S., Sulaiman, A. Z., and Ajit. A. (2016). Application of ultrasound on the extraction of vitexin from *Ficus deltoidea* leaves. Journal of Engineering and Applied Science. 11(4).

Barbosa-Pereira, L., Angulo, I., Paseiro-Losada, P., & Cruz, J. M. (2013). Phenolic profile and antioxidant properties of a crude extract obtained from a brewery waste stream. *Food Research International, 51*(2), 663-669.

Bennett, M. F., & Cooper, K. D. (2009). Photoaging in Skin of Color. *Light-Based Therapies for Skin of Color,* 45-81.

Buhler, D. and Cristobal, M (2010). Antioxidant Activity of Flavanoid. http://lpi.oregonstate.edu/research-newsletter [6 January 2016)

Bunawan, H., Amin, N. M., Bunawan, S. N., Baharum, S. N., & Noor, N. M. (2014). Ficus deltoideaJack: A Review on Its Phytochemical and Pharmacological Importance. *Evidence-Based Complementary and Alternative Medicine, 2014*, 1-8

Callaghan, T. M., & Wilhelm, K. (2008). A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part 2: Clinical perspectives and clinical methods in the evaluation of ageing skin. *International Journal of Cosmetic Science, 30*(5), 323-332.

Choo, C., Sulong, N., Man, F., & Wong, T. (2012). Vitexin and isovitexin from the Leaves of Ficus deltoidea with in-vivo α-glucosidase inhibition. *Journal of Ethnopharmacology, 142*(3), 776-781.

Chowdhury, Z., & Watson, R. (2013). Pycnogenol® and Antioxidant Activity in Health Promotion. *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases,* 405-411.

Cushnie, T. P. T., and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *Journal of Antimicrob Agents*. 26 (5): 343-56

Dong, K., Damaghi, N., Picart, S., Markova, N., Obayashi, K., Okano, Y., Masaki, H., Grether-Beck, S., Krutmann, J., Smiles, K. and Yarosh, D. (2008). UV-induced DNA damage initiates release of MMP-1 in human skin. *Experimental Dermatology*, 17(12), pp.1037-1044.

Fan. P and He. C. H (2006). "Simultaneous quantification of three major bioactive triterpene acids in the leaves of Diospyros kaki by high-performance liquid chromatography method," *Journal of Pharmaceutical and Biomedical Analysis*, 41(3): 950–956.

Farsi, E., Shafaei, A., Hor, S., Ahamed, M., Yam, M., Asmawi, M., & Ismail, Z. (2013). Genotoxicity and acute and subchronic toxicity studies of a

standardized methanolic extract of Ficus deltoidea leaves. *Clinics, 68*(6), 865-875.

Fearnley, K. (2009). The Science inside Skin. The American Association for The Advancement of The Science.

Fisher, G. J., Quan, T., Purohit, T., Shao, Y., Cho, M. K., He, T., Voorhees, J. J. (2009). Collagen Fragmentation Promotes Oxidative Stress and Elevates Matrix Metalloproteinase-1 in Fibroblasts in Aged Human Skin. *The American Journal of Pathology, 174*(1), 101-114.

Gasmalla, M. A., Yang. R., and Hua. X (2015). Extraction of rebaudioside-A by sonication from Stevia rebaudiana Bertoni leaf and decolorization of the extract by polymers. *Journal of Food Science and Technology*. 52 (9): 5946-53.

Giri. D. (2015). High Performance Liquid Chromatography (HPLC) : Principle, Types, Instrumentation and Applications | LaboratoryInfo.com. Retrieved March 28, 2016, from http://laboratoryinfo.com/hplc/

Harbone, J. B., and Williams, C. A. (2000). Advances in flavonoid research since 1992. *Journal of Phytochemistry*. 55(6): 481-504

Hasham, R., Park, C. S., and Sarmidi, R (2011). Use of Sep-pak C18 to cleanup *Ficus deltoidea* extract enhanced the effect of anti-photoageing activity in UVB-induced skin cells. *Korea Biotechnology Congress Spring Conference.* 4:270-270.

Hayflick, L. (1999). Aging and the Genome. *Science, 283*(5410).

Hayflick, L. (2004). Aging: The Reality: "Anti-Aging" Is an Oxymoron. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 59*(6).

Igarashi, T., Nishino, K. and Nayar, S. (2007). The Appearance of Human Skin: A Survey. Foundations and Trends® in Computer Graphics and Vision, 3(1), 1-95.

Jinno, M., Tamura, H., & Yonei, Y. (2012). Anti-Aging Medicine and Reproductive Health. *Anti-Aging Med ANTI-AGING MEDICINE, 9*(1), 6-13.

Kanatakis. J (2002). Anatomy, histology and immunohistochemistry of normal human skin. *European Journal of Dermatology*. 12 (4): 390-9.

Katiyar, S. K. (2005). Silymarin and skin cancer prevention: anti-inflammatory, antioxidant and immunmodulatory effects (review). Internal Journal of Oncology. 26 (1): 169-76.

Katoch, N., Kaur, P., & Kashyap, P. (2013). Role of Oxidative Stress in Cardiovascular Diseases. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4*(3), 870-881.

Kim, S. R., Jung, Y. R., An, H. J., Kim, D. H., Jang, E. J., Choi, Y. J., Chung, H. Y. (2013). Anti-Wrinkle and Anti-Inflammatory Effects of Active Garlic Components and the Inhibition of MMPs via NF-κB Signaling. *PLoS ONE, 8*(9)

Kirkwood, T. B. (2005). Understanding the odd science of aging. Cell press open access. 120 (4): 437-47.

Kochummen, K. M., (1978). Moraceae. In: Ng FSP, editor. Tree Flora of Malaya (A Manual of Foresters), Vol. 3. Petaling Jaya, Malaysia: Longman Malaysia Sdn. Bhd., pp. 119–168.

Korpi, J. (2010). *Collagenase-2 (matrix metalloproteinase-8) in tongue squamous cell carcinoma, bone osteosarcoma, and wound repair*. Oulu: Oulun yliopisto.

Lucci, P., Pacetti, D., Nunez, C. and Frega , N. G. (2011). Current Trends in Sample Treatment Techniques for Environmental and Food Analysis, Chromatography Leonardo Calderon, IntechOpen, DOI: 10.5772/47736. Available from: https://www.intechopen.com/books/chromatography-the-most-versatile-method-of-chemical-analysis/current-trends-in-sample-treatment-techniques-for-environmental-and-food-analysis

Mansor, H. and Mahmood, M. (2009). Non Enzymatic and Enzymatic Antioxidant Activities in Aqueous Extract of Different *Ficus deltoidea* Accesions. *Journal of Medicinal Plants Research.* 3(3):120-131

Marwah, R. G., Fatope, M. O., Mahrooqi, R. A., Varma, G. B., Abadi, H. A., & Al-Burtamani, S. K. (2007). Antioxidant capacity of some edible and wound healing plants in Oman. *Food Chemistry, 101*(2), 465-470

Matsumara. Y and Ananthaswamy, H. N. (2004). Toxic effects of ultraviolet radiation on skin. *Journal of Toxicol Applied Pharmacology*. 195 (3): 298-308

Meotti, F. C. (2005). Analysis of the Antinociceptive Effect of the Flavonoid Myricitrin: Evidence for a Role of the L-Arginine-Nitric Oxide and Protein Kinase C Pathways. *Journal of Pharmacology and Experimental Therapeutics, 316*(2), 789-796.

Meyer, L. J., and Stern, R. (1994). Age-Dependent Changes of Hyaluronan in Human Skin. *Journal of Investigative Dermatology, 102*(3), 385-389.

Misbah, H., Aziz, A., & Aminudin, N. (2013). Antidiabetic and antioxidant properties of Ficus deltoidea fruit extracts and fractions. *BMC Complementary and Alternative Medicine BMC Complement Altern Med, 13*(1), 118.

Mohd, K., Azemin, A., Rosli, A., Mat, N., Ali, A., & Ismail, Z. (2013). Standardization and Bioassays Characterization on Malaysian herb, Ficus deltoidea Jack. *Planta Med Planta Medica, 79*(13).

Mukherjee, P., Maity, N., Nema, N. and Sarkar, B. (2011). Bioactive compounds from natural resources against skin aging. *Phytomedicine*, 19(1), pp.64-73.

Musa, Y., & Lip, J. M. (2007). Mas cotek (Ficus deltoidea): A new potential medicinal plant in Malaysia. *Planta Med Planta Medica, 73*(09).

Musa, Y., & Lip, J. M. (2007). Mas cotek (Ficus deltoidea): A new potential medicinal plant in Malaysia. *Planta Med Planta Medica, 73*(09)

Nakanishi. K., Minakuchi, H., and Soga, N. (1998). Structure design of double pore silica and its application to HPLC. J. Sol-Gel Sci. Technol., 13(1- 3): 163-169.

Norra Ismail. (2011). Free radical scarvenging activity and phenolic content of Ficus deltoidea accessions MFD4 and MFD6 leaves. Journal of Tropical Agriculture and Food Science. 39(1): 000-000.

Organization, W. H. (2003). INTERSUN: the Global UV Project: a guide and compendium.

P. Fan and C. H. He (2006). "Simultaneous quantification of three major bioactive triterpene acids in the leaves of Diospyros kaki by high-performance liquid chromatography method," Journal of Pharmaceutical and Biomedical Analysis, 41(3): 950–956.

Papakonstantinou. E., Roth. M., and Karakiulakis. G. (2012). Hyaluronic acid: A key molecule in skin aging. *Journal of Dermatoendoctinol*. 4(3): 253-258.

Park, H. M., Moon, E., Kim, A. J., Lee. S., Park, Y. K., Jung, H. S., Kim, Y. B and Kim, S. Y. (2010). Extract of Punica granatum inhibits skin photoaging induced by UVB radiation. *International Journal of Dermatology*. 49 (3): 276-82

Petelinc, T., Polak, T., Demsar, L., and Jamnik, P. (2013). Fractionation of Phenolic Compounds Extracted from Propolis and Their Activity in the Yeast Saccharomyces cerevisiae. Plos ONE. 8(2).

Poljšak, B., & Dahmane, R. (2012). Free Radicals and Extrinsic Skin Aging. *Dermatology Research and Practice, 2012*, 1-4.

Pyla, R., Kim. T. J., Silva. J.L., and Jung. Y. S (2009). Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant. *International Journal of Food Microbiology*. 137 (2-3); 154-160.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J., Bailleul, F. and Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. *Journal of Ethnopharmacology*, 72(1-2): 35-42.

Ramamurthy, S., Kumarappan, C., Dharmalingam, S. R. and Sangeh, J. K. 2014. Phytochemical, Pharmacological and Toxicological Properties of *Ficus deltoidea*: A Review of a Recent Research. *Annual Research & Review in Biology*. 4(14):2357-2371.

Röck, K., Grandoch, M., Majora, M., Krutmann, J., and Fischer, J. W. (2011) Collagen fragments inhibit hyaluronan synthesis in skin fibroblasts in response to ultraviolet B (UVB): new insights into mechanisms of matrix remodeling. *Journal of Biol Chemical*, 286 (182): 68–76

Rogers, S. A., Vlassopoulos, D., & Callaghan, P. T. (2008). Aging, Yielding, and Shear Banding in Soft Colloidal Glasses. *Phys. Rev. Lett. Physical Review Letters, 100*(12).

Seawan. N and Jimtaisong. A. (2013). Photoprotection of natural flavonoids. *Journal of applied pharmaceutical science*. 3 (9) : 129-141.

Sethi, C. S. (2000). Matrix metalloproteinase biology applied to vitreoretinal disorders. *British Journal of Ophthalmology, 84*(6), 654-666.

Shafaei, A., Muslim, N., Nassar, Z., Aisha, A., Majid, A., & Ismail, Z. (2014). Antiangiogenic Effect of Ficus deltoidea Jack Standardised Leaf Extracts.

Tropical Journal of Pharmaceutical Research Trop. J. Pharm Res, 13(5), 761.

Sjerobabski-Masnec, I., & Šitum, M. (2010). Skin Aging. *Acta Clin Croat, 49*, 515-519.

Stern, R., & Maibach, H. I. (2008). Hyaluronan in skin: Aspects of aging and its pharmacologic modulation. *Clinics in Dermatology,26*(2), 106-122.

Südel, K. M., Venzke, K., Mielke, H., Breitenbach, U., Mundt, C., Jaspers, S., and Gallinat, S. (2005). Novel Aspects of Intrinsic and Extrinsic Aging of Human Skin: Beneficial Effects of Soy Extract. *Photochemistry and Photobiology Photochem Photobiol, 81*(3), 581.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules, 14*(6), 2167-2180.

Sultana, N. and Lee, N. (2007). Antielastase and free radical scavenging activities of compounds from the stems of *Cornus kousa*. Phytotherapy Research, 21(12), pp.1171-1176.

Svobodova. A., Walterova. D., and Vostalova. J. (2006). Ultraviolet light induced alteration to the skin. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 150(1):25–38

Takeda, K., Gosiewska, A., & Peterkofsky, B. (1992). Similar, but not identical, modulation of expression of extracellular matrix components during in vitro and in vivo aging of human skin fibroblasts. *J. Cell. Physiol. Journal of Cellular Physiology, 153*(3), 450-459.

Tzellos, T., Klagas, I., Vahtsevanos, K., Triaridis, S., Printza, A., Kyrgidis, A., Karakiulakis, G., Zouboulis, C. and Papakonstantinou, E. (2009). Extrinsic ageing in the human skin is associated with alterations in the expression of hyaluronic acid and its metabolizing enzymes. *Experimental Dermatology*. 18(12), pp.1028-1035.

Uitto. J. (2008). The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. *Journal of Drug Dermatology*. 7(2): 12-6.

USDA (2007). ARS, National Genetic Resources Program, Germplasm Resources Information Network – (GRIN) Database. Beltsville, MD, USA: National Germplasm Resources Laboratory. [accessed 3 March 2016].

Watson, R & Chowdhury, Z. (2013). Pycnogenol® and Antioxidant Activity in Health Promotion. *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases,* 405-411.

Wilson, I. D., & Brinkman, U. A. (2007). Hype and hypernation: Multiple hyphenation of column liquid chromatography and spectroscopy. *TrAC Trends in Analytical Chemistry, 26*(9), 847-854.

Xiao, J., Capanoglu, E., Jassbi, A. and Miron, A. (2016). Advance on the FlavonoidC-glycosides and Health Benefits. *Critical Reviews in Food Science and Nutrition*, 56(1), pp.29-45.

Zakaria, Z. A., Hussain, M. K., Mohamad, A. S., Abdullah, F. C., and Sulaiman, M. R., (2012). Anti-Inflammatory Activity of the Aqueous Extract of Ficus Deltoidea. Biological Research for Nursing .

Zimniak, P. (2011). Relationship of electrophilic stress to aging. Journal of Biomedical. 51 (10): 1087–1105

Zino, S., Skeaff, M., Williams, S., & Mann, J. (1997). Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids

Zwir-Ferenc, A. and Biziuk, M. (2006). Solid phase extraction Technique-Trend, opportunities and Applications. Polish Journal of Environmental Studies. 15(5): 667-690