POTENTIAL ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF *Erythroxylum cuneatum* (Miq.) Kurz LEAF EXTRACT AGAINST OXIDISED LOW-DENSITY LIPOPROTEIN IN HUMAN AORTIC ENDOTHELIAL CELL

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Oxidative stress and inflammation are known to be associated with the pathogenesis of most chronic diseases such as atherosclerosis, cancer and diabetes. Medications like non-steroidal anti-inflammatory drugs are commonly used to treat the diseases but are accompanied by adverse effects. *Erythroxylum cuneatum* (EC), also known locally as "Chinta mula", belongs to the *Erythroxylaceae* family. Scientific evidence for the medicinal properties of the plant is still limited. Therefore, this study aims to determine the antioxidant and anti-inflammatory properties of EC leaf extract for the prevention of atherosclerosis *in vitro*. The study was divided into two phases. The first phase is screening of EC leaf extract using four solvents, namely acetone, water, hexane and ethanol. The four different types of EC leaf extracts were analysed for preliminary phytochemical screening individually. The antioxidant activity was tested by total phenolic content (TPC), *2,2-diphenyl-1-picrylhydrazyl* (DPPH) and hydrogen peroxide (H_2O_2)-scavenging activity. Based on Phase 1 results, acetone and ethanol extracts were chosen to test the antioxidant and anti-inflammatory properties *in vitro* with oxidised low-density lipoprotein (oxLDL)-induced human aortic endothelial cells (HAoEC). Cell viability assay of EC leaf extract was conducted to determine the number of viable cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Antioxidant activity was determined by thiobarbituric acid reactive substances (TBARS) assay, reactive oxygen species (ROS) assay and nitric oxide (NO) production assay. The anti-inflammatory effects of EC leaf extract in HAoEC were determined by U937 cell monocyte adhesion and migration assay. The expression of adhesion molecules, namely human soluble intracellular adhesion molecule-1 (ICAM-1) and human soluble vascular cell adhesion molecule-1 (VCAM-1) were quantified using ELISA kit. Phase 1 results showed the presence of alkaloids, flavonoids and tannins in the acetone and ethanol extract. Phenols were found only in acetone extract while saponins were detected only in water extract. Additionally, acetone extracts exhibited the highest TPC and DPPH-

scavenging activity, while ethanol extract showed the highest H_2O_2-scavenging activity. Both extracts in Phase 2 inhibited lipid peroxidation and ROS production. They were also able to increase NO production indicating their antioxidant activity. Acetone extract was able to inhibit lipid peroxidation, ROS production and increase NO production better than ethanol extract at 80 µg/ml. Both extracts showed anti-inflammatory activities by reducing monocyte adhesion and migration and expression of ICAM-1 and VCAM-1. Acetone extract was able to inhibit monocyte adhesion and expression of ICAM-1 better than ethanol extract. While, ethanol extract showed significantly better inhibition of monocyte migration and expression of VCAM-1 than acetone extract. This study showed that EC acetone and ethanol extracts have high antioxidant activity among the four extracts. Both extracts showed antioxidant and anti-inflammatory activity in HAoEC-induced with oxLDL. Generally, acetone extract at 80 µg/ml showed better antioxidant and anti-inflammatory activities than ethanol extract.

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

POTENSI KESAN ANTIOKSIDAN DAN ANTI-RADANG EKSTRAK DAUN *Erythroxylum cuneatum* (Miq.) Kurz TERHADAP LIPOPROTEIN KETUMPATAN RENDAH TEROKSIDA DALAM SEL ENDOTEL AORTA MANUSIA

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Inflamasi dan tekanan oksidatif sering dikaitkan dengan patogenesis penyakit kronik seperti aterosklerosis, kanser, dan diabetes. Ubat-ubatan seperti ubat anti-radang bukan steroid biasanya digunakan untuk merawat penyakit tetapi kerap menyebabkan kesan buruk. *Erythroxylum cuneatum* (EC) juga dikenali sebagai "Chinta Mula" tergolong dalam keluarga *Erythroxylaceae*. Bukti saintifik bagi ciri-ciri perubatan tumbuhan ini adalah terhad. Oleh itu, penyelidikan ini bertujuan untuk mengkaji sifat antioksidan dan anti-radang ekstrak daun EC bagi mencegah aterosklerosis secara *in vitro*. Kajian ini dibahagikan kepada dua fasa. Fasa 1 adalah ujian saringan ekstrak daun EC dengan menggunakan empat pelarut iaitu aseton, air, heksana dan etanol. Empat jenis ekstrak daun EC dianalisis untuk pemeriksaan fitokimia secara individu. Aktiviti antioksidan telah diuji dengan menggunakan jumlah kandungan fenolik, serta aktiviti memerangkap *2,2-diphenyl-1-picrylhydrazyl* (DPPH) dan hidrogen peroksida (H_2O_2). Berdasarkan keputusan daripada Fasa 1, ekstrak aseton dan etanol telah dipilih untuk diuji kesan antioksidan dan anti-radang *in vitro* dengan menggunakan lipoprotein ketumpatan rendah (oxLDL) teroksida dalam sel endothelium aorta manusia (HAoEC). Ujian ketoksikan ekstrak daun EC telah dijalankan untuk mengesan sel yang hidup menggunakan ujian 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Aktiviti antioksidan telah ditentukan melalui ujian bahan reaktif asid thiobarbiturik (TBARS), spesies oksigen reaktif (ROS) dan penghasilan nitrik oksida (NO). Kesan anti-radang ekstrak daun EC di HAoEC pula telah ditentukan dengan menggunakan ujian lekatan dan migrasi sel monosit U937. Pengekspresan molekul lekatan seperti molekul melekat intrasel-1 (ICAM-1) manusia dan molekul melekat sel vaskular-1 (VCAM-1) manusia telah diukur menggunakan kit ELISA. Keputusan Fasa 1 menunjukkan kewujudan alkaloid, flavonoid dan tanin dalam ekstrak aseton dan etanol. Fenol ditemui dalam ekstrak aseton sahaja dan saponin hanya dikesan dalam ekstrak air sahaja. Tambahan pula, ekstrak aseton mempamerkan TPC dan aktiviti memerangkap DPPH yang tertinggi, manakala ekstrak etanol menunjukkan aktiviti H_2O_2

yang tertinggi. Kedua-dua ekstrak dalam Fasa 2 telah menghalang peroksida lipid dan penghasilan ROS. Kedua-kedua ekstrak boleh meningkatkan penghasilan NO yang menunjukkan aktiviti oksidan ekstrak tersebut. Ekstrak aseton berupaya untuk menghalang peroksida lipid, penghasilan ROS dan meningkatkan penghasilan NO lebih baik berbanding ekstrak etanol pada 80 µg/ml. Kedua-dua ekstrak menunjukkan aktivi anti-radang dengan mengurangkan lekatan dan migrasi monosit, serta pengekspresan ICAM-1 dan VCAM-1. Ekstrak aseton berupaya untuk merencat lekatan monosit dan ekspresi ICAM-1 lebih baik berbanding ekstrak etanol. Manakala ekstrak etanol pula menunjukkan perencatan migrasi monosit dan ekspresi VCAM-1 lebih baik secara signifikan berbanding ekstrak aseton. Kajian ini menunjukkan bahawa ekstrak aseton dan etanol daun EC mempunyai aktiviti antioksidan yang tinggi di antara empat ekstrak. Kedua-dua ekstrak mempamerkan aktiviti antioksidan dan anti-radang dalam HAoEC dirangsang oleh oxLDL. Secara umum, ekstrak aseton menunjukkan aktiviti antioksidan dan anti-radang yang lebih baik daripada ekstrak etanol pada 80 µg/ml.

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis
ANOVA	Analysis of variance
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CVD	Cardiovascular disease
DCF	Dichlorofluorescin
DCFH-DA	2, 7-dichlorodihydrofluorescein diacetate
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	*Erythroxylum cuneatum*
ELISA	Enzyme-linked immunosorbent assay
H_2O_2	Hydrogen peroxide
HAoEC	Human aortic endothelial cell
HCl	Hydrochloric acid
HDL	High density lipoprotein
HepG2	Liver hepatocellular cells
HUVEC	Human umbilical vein endothelial cells
IC_{50}	Half maximal inhibitory concentration
ICAM	Intracellular adhesion molecule
kDa	Kilodalton
LDL	Low-density lipoprotein
MDA	Malondialdehyde

MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
Nm	Nanometer
NO	Nitric oxide
OH	Hydroxyl radical
OxLDL	Oxidized low-density lipoprotein
PBS	Phosphate-buffered saline
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Standard deviation
SMC	Smooth muscle cells
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptor
SPSS	Statistical Package for the Social Sciences
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tertiary butyl hydroquinone
TCA	Trichloroacetic acid
TNFα	Tumour necrosis factor alpha
TPC	Total phenolic content
VCAM	Vascular cell adhesion molecule
WHO	World Health Organization

WST-1	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt
XTT	2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-carboxanilide-2H-tetrazolium

CHAPTER 1

INTRODUCTION

1.1 Background

Cardiovascular diseases (CVD) remain as a major cause of global deaths. As the World Health Organization (WHO) has indicated, nearly 32% of global deaths in 2019 were caused by CVD, which is 17.9 million deaths annually (WHO, 2020). About 85% of the deaths were caused by heart attacks and strokes triggered by blockage of blood vessels (WHO, 2020). Malaysia, as one of the developing countries experienced CVD epidemic and likewise a primary cause of death at 35% (WHO, 2018).

The most prevalent CVDs are associated with cardiac and vascular system, heart attack, stroke and peripheral arterial disease. It is a chronic vascular inflammatory disease associated with oxidative stress, endothelial dysfunction, oxidative damage, inflammation and platelet-endothelium interactions (Marchio et. al., 2019). Atherosclerosis is one of the leading causes of CVD, which occurs when there is hardening accompanied by narrowing of arteries that ultimately reduces blood flow throughout the body (Cervantes Gracia, Llanas-Cornejo & Husi, 2017). Despite the development of atherosclerosis therapy, the mortality rate remains high (Saleh, Iratni, & Eid, 2015).

Treatment using synthetic drugs are available for oxidative damage and inflammation, but they are hampered with complicacy. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) can be toxic and are possibly carcinogenic (Anbudhasan, Surendraraj, Karkuzhali, & Sathishkumaran, 2014). Non-steroidal anti-inflammatory drugs such as aspirin, diclofenac and celecoxib are widely used for pain and inflammation, yet they are known for their complications (Pirmohamed et. al., 2004).

Plants have been an important source of traditional medicine used by about 60% of the world's population for centuries, known to be safer than synthetic drugs (Zhang & WHO, 2000). In Malaysia, *Erythroxylum cuneatum (*EC) aqueous leaf extract has been reported to have anti-inflammatory and antioxidant activities (Saleh, Hasan, Said, Adenan & Adam, 2012). However, its effectiveness and related mechanisms in relation to atherosclerosis are still unexplored. Therefore, this research was conducted to identify the antioxidant and anti-inflammatory activities of EC leaf extracts *in vitro*.

1.2 Hypothesis

EC leaf extract contains phytochemicals which possess antioxidant activities such as high total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activity and hydrogen peroxide (H_2O_2)-scavenging activity. The extracts do not cause toxicity to human aortic endothelial cell (HAoEC). With high antioxidant activity, EC leaf extract reduces the production of malondialdehyde (MDA), reactive oxygen species (ROS) while increasing nitric oxide (NO) in oxidised low-density lipoprotein (oxLDL)-induced HAoEC. Moreover, the extract is able to suppress the inflammatory response by reducing monocyte adhesion, monocyte migration and adhesion molecule expression in oxLDL-induced HAoEC.

1.3 Objectives

1.3.1 General objective

To determine the antioxidant and anti-inflammatory effects of EC leaf extracts on preventing atherosclerosis *in vitro*.

1.3.2 Specific objectives

1. To identify the presence of various phytochemicals in the ethanol, acetone, water and hexane extracts of EC leaf.
2. To determine the antioxidant activity of EC leaf extracts based on TPC, DPPH-scavenging activity and H_2O_2-scavenging activities.
3. To determine the cytotoxicity level of EC leaf extract in HAoEC.
4. To determine the antioxidant effects of EC leaf extract in oxLDL-induced HAoEC.
5. To investigate the anti-inflammatory effects of EC leaf extract against oxLDL-induced inflammation in HAoEC.

REFERENCES

Aadesariya, M. K., Ram, V. R., & Dave, P. N. (2017). Evaluation of antioxidant activities by use of various extracts from Abutilon pannosum and Grewia tenax in the Kachchh Region. *MOJ Food Process and Technology*, *5*(1), 00116.

Adjimani, J. P., & Asare, P. (2015). Antioxidant and free radical scavenging activity of iron chelators. *Toxicology reports*, *2*, 721-728.

Akeson, A. L., & Woods, C. W. (1993). A fluorometric assay for the quantitation of cell adherence to endothelial cells. *Journal of immunological methods*, *163*(2), 181-185.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Scientific Research and Essays*, *2*(5), 163-166.

Alabri, T. H. A., Al Musalami, A. H. S., Hossain, M. A., Weli, A. M., & Al-Riyami, Q. (2014). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L. *Journal of King Saud University-Science*, *26*(3), 237-243.

Al-Farsi, M. A., & Lee, C. Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*, *108*(3), 977-985.

Alihosseini, F. (2016). Plant-based compounds for antimicrobial textiles. In *Antimicrobial Textiles* (pp. 155-195). Woodhead Publishing.

Amari, N. O., Bouzouina, M., Berkani, A., & Lotmani, B. (2014). Phytochemical screening and antioxidant capacity of the aerial parts of Thymelaea hirsuta L. *Asian Pacific Journal of Tropical Disease*, *4*(2), 104-109.

Anbudhasan, P., Surendraraj, A., Karkuzhali, S., & Sathishkumaran, S. (2014). Natural antioxidants and its benefits. *International Journal of Food and Nutritional Sciences*, *3*(3), 225-232.

Aziz, M., & Yadav, K. S. (2016). Pathogenesis of atherosclerosis. *Medical and Clinical Review, 2*(3).

Babu, P. V. A., Si, H., Fu, Z., Zhen, W., & Liu, D. (2012). Genistein Prevents Hyperglycemia-Induced Monocyte Adhesion to Human Aortic Endothelial Cells through Preservation of the cAMP Signaling Pathway and Ameliorates Vascular Inflammation in Obese Diabetic Mice–3. *The Journal of nutrition*, *142*(4), 724-730.

Bai, L., Wang, L., Zhao, M., Toki, A., Hasegawa, T., Ogura, H., ... & Ando, M. (2007). Bioactive pregnanes from Nerium oleander. *Journal of natural products*, *70*(1), 14-18.

Baskar, R., Rajeswari, V., & Kumar, T. S. (2007). In vitro antioxidant studies in leaves of Annona species.

Belščak, A., Komes, D., Horžić, D., Ganić, K. K., & Karlović, D. (2009). Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Research International*, *42*(5-6), 707-716.

Bentzon, J. F., Otsuka, F., Virmani, R., & Falk, E. (2014). Mechanisms of plaque formation and rupture. *Circulation research*, *114*(12), 1852-1866.

Bergheanu, S. C., Bodde, M. C., & Jukema, J. W. (2017). Pathophysiology and treatment of atherosclerosis. *Netherlands Heart Journal*, *25*(4), 231-242.

Bhandary, S. K., Bhat, V. S., Sharmila, K. P., & Bekal, M. P. (2012). Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. *Journal of Health and Allied Sciences NU*, *2*(04), 34-38.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Boamponsem, A. G., & Boamponsem, L. K. (2011). The role of inflammation in atherosclerosis. *AASRFC. ISSN*, *978610*, 194-207.

Borenfreund, E., Babich, H., & Martin-Alguacil, N. (1988). Comparisons of two in vitro cytotoxicity assays—the neutral red (NR) and tetrazolium MTT tests. *Toxicology in vitro*, *2*(1), 1-6.

Bribi, N., Algieri, F., Rodriguez-Nogales, A., Garrido-Mesa, J., Vezza, T., Maiza, F., ... & Galvez, J. (2015). Antinociceptive and anti-inflammatory effects of total alkaloid extract from Fumaria capreolata. *Evidence-based Complementary and Alternative Medicine*, *2015*.

Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310). Academic Press.

Cervantes Gracia, K., Llanas-Cornejo, D., & Husi, H. (2017). CVD and oxidative stress. *Journal of Clinical Medicine, 6*(2), 22.

Charo, I. F., & Taub, R. (2011). Anti-inflammatory therapeutics for the treatment of atherosclerosis. *Nature reviews Drug discovery*, *10*(5), 365.

Chávez-Sánchez, L., Chávez-Rueda, K., Legorreta-Haquet, M. V., Montoya-Díaz, E., & Blanco-Favela, F. (2012). The Innate Immune Response Mediated by TLRs in

Atherosclerosis. In *Inflammation, Chronic Diseases and Cancer-Cell and Molecular Biology, Immunology and Clinical Bases*. IntechOpen.

Chuakul, W., Saralamp, P., & Boonpleng, A. (2002). Medicinal plants used in the Kutchum district, Yasothon Province, Thailand.

Chung, R. C. K. (2010). *Erythroxylaceae. Flora of Penisular Malaysia* (Vol. 8, pp. 133–134).

Chung, R.C.K. (2006). The Angiosperm Flora of Singapore: Erythroxylaceae. Singapore University Press.

Consigny, P. M. (1995). Pathogenesis of atherosclerosis. *AJR. American journal of roentgenology*, *164*(3), 553-558.

Coruh, I., Gormez, A., Ercisli, S., & Sengul, M. (2008). Total phenolic content, antioxidant, and antibacterial activity of Rumex crispus grown wild in Turkey. *Pharmaceutical biology*, *46*(9), 634-638.

da Costa Cordeiro, B. M. P., de Lima Santos, N. D., Ferreira, M. R. A., de Araújo, L. C. C., Junior, A. R. C., da Conceição Santos, A. D., ... & Paiva, P. M. G. (2018). Hexane extract from Spondias tuberosa (Anacardiaceae) leaves has antioxidant activity and is an anti-Candida agent by causing mitochondrial and lysosomal damages. *BMC complementary and alternative medicine*, *18*(1), 1-10.

Daniel, A. E., & Van Buul, J. D. (2013). Endothelial junction regulation: a prerequisite for leukocytes crossing the vessel wall. *Journal of innate immunity*, *5*(4), 324-335.

de Boer, H. J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I., & Levenfors, J. J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of ethnopharmacology*, *96*(3), 461-469.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of food and drug analysis*, *22*(3), 296-302.

Donnini, D., Perrella, G., Stel, G., Ambesi-Impiombato, F. S., & Curcio, F. (2000). A new model of human aortic endothelial cells in vitro. *Biochimie*, *82*(12), 1107-1114.

Douglas, G., & Channon, K. M. (2014). The pathogenesis of atherosclerosis. *Medicine*, *42*(9), 480-484.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews*, *82*(1), 47-95.

El-Imam, Y. M. A., Evans, W. C., & Grout, R. J. (1988). Alkaloids of *Erythroxylum cuneatum, E. ecarinatum* and *E. austral*. *Phytochemistry,27*, 2181-2184.

Feingold, K. R., & Grunfeld, C. (2018). Introduction to lipids and lipoproteins. In *Endotext [Internet]*. MDText. com, Inc..

Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation. *Journal of Agricultural and Food Chemistry*, *48*(5), 1485-1490.

Harwood, H. J., Chandler, C. E., Pellarin, L. D., Bangerter, F. W., Wilkins, R. W., Long, C. A., ... & Pettini, J. L. (1993). Pharmacologic consequences of cholesterol absorption inhibition: alteration in cholesterol metabolism and reduction in plasma cholesterol concentration induced by the synthetic saponin beta-tigogenin cellobioside (CP-88818; tiqueside). *Journal of Lipid Research*, *34*(3), 377-395.

Haslam, E., Lilley, T. H., Cai, Y., Martin, R., & Mangnolato, D. (1989). Traditional herbal medicines-the role of polyphenols. *Planta medica*, *55*(01), 1-8.

Hässig, A., Linag, W. X., Schwabl, H., & Stampfli, K. (1999). Flavonoids and tannins: plant-based antioxidants with vitamin character. *Medical hypotheses*, *52*(5), 479-481.

Honda, T., Gribble, G. W., Suh, N., Finlay, H. J., Rounds, B. V., Bore, L., ... & Sporn, M. B. (2000). Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages. *Journal of medicinal chemistry*, *43*(9), 1866-1877.

Hong, C. Y., Wang, C. P., Huang, S. S., & Hsu, F. L. (1995). The inhibitory effect of tannins on lipid peroxidation of rat heart mitochondria. *Journal of Pharmacy and Pharmacology*, *47*(2), 138-142.

Hostettmann, K., & Marston, A. (2005). *Saponins*. Cambridge University Press.

Huang, C. S., Lin, A. H., Liu, C. T., Tsai, C. W., Chang, I. S., Chen, H. W., & Lii, C. K. (2013). Isothiocyanates protect against oxidized LDL-induced endothelial dysfunction by upregulating Nrf2-dependent antioxidation and suppressing NFκB activation. *Molecular Nutrition and Food Research*, *57*(11), 1918-1930.

Iqbal, M., Sharma, S. D., & Okada, S. (2004). Probucol as a potent inhibitor of oxygen radical-induced lipid peroxidation and DNA damage: in vitro studies. *Redox report*, *9*(3), 167-172.

Jadhav, A. P., Kareparamban, J. A., Nikam, P. H., & Kadam, V. J. (2012). Spectrophotometric estimation of ferulic acid from Ferula asafoetida by Folin-ciocalteu's reagent. *Der Pharmacia Sinica*, *3*(6), 680-684.

Jain, P. K., Kharya, M. D., Gajbhiye, A., Sara, U. V. S., & Sharma, V. K. (2010). Flavonoids as nutraceuticals. A review. *Herba Polonica*, *56*(2), 105-17.

Jayaraman, S., Gantz, D. L., & Gursky, O. (2007). Effects of oxidation on the structure and stability of human low-density lipoprotein. *Biochemistry*, *46*(19), 5790-5797.

Jesch, E. D., & Carr, T. P. (2017). Food ingredients that inhibit cholesterol absorption. *Preventive Nutrition and Food Science*, *22*(2), 67.

Katavic, P. L. (2005). Chemical investigations of the alkaloids from the plants of the family Elaeocarpaceae. *Natural Product Discovery (NPD). Faculty of Science, Griffith University. Australia*.

Katib, S., & Ruangrungsi, N. (2020). Macroscopic-microscopic characteristics and AFLP fingerprint for identification of Erythroxylum novogranatense, E. cambodianum and E. cuneatum endemic to Thailand. *International Journal of Research in Pharmaceutical Sciences, 11*(4), 6144-6154.

Keser, S., Celik, S., Turkoglu, S., Yilmaz, Ö., & Turkoglu, I. (2012). Hydrogen peroxide radical scavenging and total antioxidant activity of Hawthorn. *Chemistry Journal*, *2*(1), 9-12.

Ko, Y. S., Nash, O., Choi, S., & Kim, H. J. (2019). Methanolic extract of Kigelia africana exhibits antiatherosclerotic effects in endothelial cells by downregulating RAGE and adhesion molecules. *Tropical Biomedicine*, *36*(1), 172-182.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L., & Opletal, L. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini reviews in medicinal chemistry*, *8*(5), 436-447.

Kumar, R. S., Rajkapoor, B., & Perumal, P. (2012). Antioxidant activities of Indigofera cassioides Rottl. Ex. DC. using various in vitro assay models. *Asian Pacific Journal of Tropical Biomedicine*, *2*(4), 256-261.

Lee, Y. W., Liao, J. W., Kuo, Y. T., Huang, Y. T., Chen, Y. C., & Lee, H. Y. (2006). The expression profiles of lysophospholipid receptors (LPLRs) in different endothelial cells. *Taiwania*, *51*(1), 11-24.

Leick, M., Azcutia, V., Newton, G., & Luscinskas, F. W. (2014). Leukocyte recruitment in inflammation: basic concepts and new mechanistic insights based on new models and microscopic imaging technologies. *Cell and Tissue Research*, *355*(3), 647-656.

Leiva, E., Wehinger, S., Guzmán, L., & Orrego, R. (2015). Role of Oxidized LDL in Atherosclerosis.

Li, L. S., Chiroma, S. M., Hashim, T., Adam, S. K., Moklas, M. A. M., Yusuf, Z., & Rahman, S. A. (2020). Antioxidant and anti-inflammatory properties of Erythroxylum cuneatum alkaloid leaf extract. *Heliyon*, *6*(6), e04141.

Libby, P. (2006). Inflammation and cardiovascular disease mechanisms–. *The American Journal of Clinical Nutrition*, *83*(2), 456S-460S.

Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature*, *473*(7347), 317-325.

Lusis, A. (2000). Atherosclerosis. *Nature*, *407*, 233-241.

Majinda, R. R. (2012). Extraction and isolation of saponins. In *Natural Products Isolation* (pp. 415-426). Humana Press.

Marchio, P., Guerra-Ojeda, S., Vila, J. M., Aldasoro, M., Victor, V. M., & Mauricio, M. D. (2019). Targeting Early Atherosclerosis: A Focus on Oxidative Stress and Inflammation. *Oxidative Medicine and Cellular Longevity*, *2019*.

Massoudy, P., Becker, B. F., & Gerlach, E. (1995). Nitric oxide accounts for postischemic cardioprotection resulting from angiotensin-converting enzyme inhibition: indirect evidence for a radical scavenger effect in isolated guinea pig heart. *Journal of Cardiovascular Pharmacology*, *25*(3), 440-447.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal Science Technology*, *26*(2), 211-219.

Morris, T. E., Mattox, P. A., Shipley, G. D., Wagner, C. R., & Hosenpud, J. D. (1993). The pattern of cytokine messenger RNA expression in human aortic endothelial cells is different from that of human umbilical vein endothelial cells. *Transplant Immunology*, *1*(2), 137-142.

Naito, Y., Shimozawa, M., Manabe, H., Kuroda, M., Tomatsuri, N., Uchiyama, K., ... & Yoshikawa, T. (2004). Inhibitory effects of red wine extracts on endothelial-dependent adhesive interactions with monocytes induced by oxysterols. *Biological Research*, *37*(2), 231-238.

Navab, M., Fogelman, A. M., Berliner, J. A., Territo, M. C., Demer, L. L., Frank, J. S., ... & Lusis, A. J. (1995). Pathogenesis of atherosclerosis. *The American Journal of Cardiology*, *76*(9), 18C-23C.

Negi, J. S., Negi, P. S., Pant, G. J., Rawat, M. S. M., & Negi, S. K. (2013). Naturally occurring saponins: chemistry and biology. *Journal of Poisonous and Medicinal Plant Research 1*(1), 1-6.

Nègre-Salvayre, A., & Salvayre, R. (1992). Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. *Free Radical Biology and Medicine*, *12*(2), 101-106.

Ng, T. B., Liu, F., & Wang, Z. T. (2000). Antioxidative activity of natural products from plants. *Life Sciences*, *66*(8), 709-723.

Nita, M., & Grzybowski, A. (2016). The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxidative Medicine and Cellular Longevity*, *2016*.

Ou, H. C., Hsieh, Y. L., Yang, N. C., Tsai, K. L., Chen, K. L., Tsai, C. S., ... & Lee, S. D. (2012). Ginkgo biloba extract attenuates oxLDL-induced endothelial dysfunction via an AMPK-dependent mechanism. *Journal of Applied Physiology*, *114*(2), 274-285.

Özen, T., & Taş, M. (2009). Screening and evaluation of antioxidant activity of some amido-carbonyl oxime derivatives and their radical scavenging activities. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(5), 1141-1147.

Palma, P. F., Baggio, G. L., Spada, C., Silva, R. D., Ferreira, S. I. A., & Treitinger, A. (2008). Evaluation of annexin V and Calcein-AM as markers of mononuclear cell apoptosis during human immunodeficiency virus infection. *Brazilian Journal of Infectious Diseases*, *12*(2), 108-114.

Park, S. H., Koo, H. J., Sung, Y. Y., & Kim, H. K. (2013). The protective effect of Prunella vulgaris ethanol extract against vascular inflammation in TNF-α-stimulated human aortic smooth muscle cells. *BMB reports, 46*(7), 352-357. doi:10.5483/bmbrep.2013.46.7.214.

Payens, J. P. D. W. (1955). Erythroxylaceae. *Flora Malesiana-Series 1, Spermatophyta*, *5*(1), 543-552.

Pehlivan, F. E. (2017). Vitamin C: An antioxidant agent. *Vitamin C*, *2*, 23-35.

Piazza, S., Pacchetti, B., Fumagalli, M., Bonacina, F., Dell'Agli, M., & Sangiovanni, E. (2019). Comparison of Two Ginkgo biloba L. Extracts on Oxidative Stress and Inflammation Markers in Human Endothelial Cells. *Mediators of Inflammation*, *2019*.

Pirmohamed, M., James, S., Meakin, S., Green, C., Scott, A. K., Walley, T. J., ... & Breckenridge, A. M. (2004). Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ*, *329*(7456), 15-19.

Plowman, T., & Rivier, L. (1983). Cocaine and cinnamoylcocaine content of Erythroxylum species. *Annals of Botany*, *51*(5), 641-659.

Prayong, P., Barusrux, S., & Weerapreeyakul, N. (2008). Cytotoxic activity screening of some indigenous Thai plants. *Fitoterapia*, *79*(7-8), 598-601.Kanchanapoom, T., Sirikatitham, A., Otsuka, H., & Ruchirawat, S. 2006. Cuneatoside, a new megastigmane diglycoside from Erythroxylum cuneatum Blume: Note. *Journal of Asian Natural Products Research*, *8*(8), 747-51.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302.

Proestos, C., Bakogiannis, A., Psarianos, C., Koutinas, A. A., Kanellaki, M., & Komaitis, M. (2005). High performance liquid chromatography analysis of phenolic substances in Greek wines. *Food Control*, *16*(4), 319-323.

Rafiee, L., Hajhashemi, V., & Javanmard, S. H. (2016). Fluvoxamine inhibits some inflammatory genes expression in LPS/stimulated human endothelial cells, U937 macrophages, and carrageenan-induced paw edema in rat. *Iranian Journal of Basic Medical Sciences*, *19*(9), 977.

Rajendran, P., Rengarajan, T., Thangavel, J., Nishigaki, Y., Sakthisekaran, D., Sethi, G., & Nishigaki, I. (2013). The vascular endothelium and human diseases. *International Journal of Biological Sciences*, *9*(10), 1057.

Ramirez, S. H., Heilman, D., Morsey, B., Potula, R., Haorah, J., & Persidsky, Y. (2008). Activation of peroxisome proliferator-activated receptor γ (PPARγ) suppresses Rho GTPases in human brain microvascular endothelial cells and inhibits adhesion and transendothelial migration of HIV-1 infected monocytes. *The Journal of Immunology*, *180*(3), 1854-1865.

Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: how are they linked?. *Free Radical Biology and Medicine*, *49*(11), 1603-1616.

Ribeiro, G., de Amorim, L. L., & Guimarães, S. S. (2015). Antioxidant Activity and Phytochemical Screening of Extracts of Erythroxylum suberosum A. St.-Hil (Erythroxylaceae). *Research Journal of Phytochemistry*, *9*(2), 68-78.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2*(4), 152-159.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*(6), 1003-1008.

Saba, A., & Oridupa, O. (2012). Lipoproteins and cardiovascular diseases. In *Lipoproteins-role in Health and Diseases*. InTech.

Saleh, S. R., Hasan, M. H., Said, M. I. M., Adenan, M. I., & Adam, A. (2012, June). Antioxidant, anti-inflammatory and antinociceptive activities of Mitragyna speciosa and Erythroxylum cuneatum. In *2012 IEEE Symposium on Humanities, Science and Engineering Research* (pp. 1087-1091). IEEE.

Saleh, T., Iratni, R., & Eid, A. (2015). Anti-atherosclerotic plants which modulate the phenotype of vascular smooth muscle cells. *Phytomedicine*. doi:10.1016/j.phymed.2015.10.016.

Sastrahidayat, I. R. (2016). *Penyakit pada tumbuhan obat-obatan, rempah-bumbu dan stimulan*. Universitas Brawijaya Press.

Savage, G. P. (2003). Chemical and Physical properties of saponins. Encyclopedia of Food Sciences and Nutrition.

Sayhan, H., Beyaz, S. G., & Çeliktaş, A. (2017). The local anesthetic and pain relief activity of alkaloids. *Alkaloids: Alternatives in Synthesis, Modification and Application*, 57.

Singh, G. B., Singh, S., Bani, S., Gupta, B. D., & Banerjee, S. K. (1992). Anti-inflammatory activity of oleanolic acid in rats and mice. *Journal of Pharmacy and Pharmacology*, *44*(5), 456-458.

Singh, R. B., Mengi, S. A., Xu, Y. J., Arneja, A. S., & Dhalla, N. S. (2002). Pathogenesis of atherosclerosis: A multifactorial process. *Experimental and Clinical Cardiology, 7*(1), 40.

Singh, S. (2019). Herbal Approach for Management of Atherosclerosis: a Review. *Current Atherosclerosis Reports*, *21*(4), 12.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, *299*, 152-178.

Sousa, J. C., Marques, F., Dias-Ferreira, E., Cerqueira, J. J., Sousa, N., & Palha, J. A. (2007). Transthyretin influences spatial reference memory. *Neurobiology of Learning and Memory*, *88*(3), 381-385.

Stanković, M., Topuzović, M., Marković, A., Pavlović, D., Solujić, S., Nićiforović, N., & Mihailović, V. (2010). Antioxidant activity, phenol and flavonoid contents of

different Teucrium chamaedrys L. exstracts. *Biotechnology and Biotechnological Equipment*, *24*(sup1), 82-86.

Stoddart, M. J. (2011). Cell viability assays: introduction. In *Mammalian Cell Viability* (pp. 1-6). Humana Press.

Stroka, K. M., Levitan, I., & Aranda-Espinoza, H. (2012). OxLDL and substrate stiffness promote neutrophil transmigration by enhanced endothelial cell contractility and ICAM-1. *Journal of Biomechanics*, *45*(10), 1828-1834.

Suliman, N. A., Mohd Moklas, M. A., Mat Taib, C. N., Adenan, M. I., Hidayat Baharuldin, M. T., Basir, R., & Amom, Z. (2016). Morphine Antidependence

Sur, P., Chaudhuri, T., Vedasiromoni, J. R., Gomes, A., & Ganguly, D. K. (2001). Antiinflammatory and antioxidant property of saponins of tea [Camellia sinensis (L) O. Kuntze] root extract. *Phytotherapy Research*, *15*(2), 174-176.

Terblanche, U., Semakalu, S. C. C., Mtunzi, F., & Pillay, M. (2017). Screening of variables influencing extraction yield of Cotyledon orbiculata: 23 full factorial design. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(3), 303-312.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98-106.

Tua-Ngam, P., Jira-Anankul, N., Anuwongnukroh, N., Dechkunakorn, S., & Laokijcharoen, P. (2017). Cytotoxicity of three commercial orthodontic elastomeric ligature brands. In *MATEC Web of Conferences* (Vol. 108, p. 06004). EDP Sciences.

Tucker W. D., Bhimji S. S. (2018). *Anatomy, Blood Vessels*. Treasure Island, FL: StatPearls.

Vadivu, R. (2011). Pharmacognostical Phytochemical and Pharmacological evaluation of the leaves of Symplocos cochinchinensis Lour S Moore Ssp Laurina Retz Nooteb Symplocaceae.

Van Thiel, B., Van der Pluijm, I., Kanaar, R., Danser, A., & Essers, J. (2017). Structure and cell biology of the vessel wall: ESC Textbook of Vascular Biology. Oxford University Press.

Vogel, M. E., Idelman, G., Konaniah, E. S., & Zucker, S. D. (2017). Bilirubin prevents atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice by inhibiting endothelial VCAM-1 and ICAM-1 signaling. *Journal of the American Heart Association*, *6*(4), e004820.

Wang, H. S., Li, F., Runge, M. S., & Chaikof, E. L. (1997). Endothelial cells exhibit differential chemokinetic and mitogenic responsiveness to α-thrombin. *Journal of Surgical Research*, *68*(2), 139-144.

Wang, T., & Butany, J. (2017). Pathogenesis of atherosclerosis. *Diagnostic Histopathology*, *23*(11), 473-478.

Wang, T., Palucci, D., Law, K., Yanagawa, B., Yam, J., & Butany, J. (2012). Atherosclerosis: pathogenesis and pathology. *Diagnostic Histopathology, 18*(11), 461-467. doi:10.1016/j.mpdhp.2012.09.004.

Wesam, R. K., Ghanya, A. N., Mizaton, H. H., ILham, M., & Aishah, A. (2013). Assessment of genotoxicity and cytotoxicity of standardized aqueous extract from leaves of Erythroxylum cuneatum in human HepG2 and WRL68 cells line. *Asian Pacific Journal of Tropical Medicine*, *6*(10), 811-816.

Wettasinghe, M., & Shahidi, F. (1999). Evening primrose meal: a source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *Journal of Agricultural and Food Chemistry*, *47*(5), 1801-1812.

Wong, T. M. (2002). A Dictionary of Malaysian Timbers. Revised by Lim, S. C & Chung, RCK Malayan Forest Records No. 30. *Forest Research Institute Malaysia, Kepong*.

World Health Organization. (2018). Noncommunicable diseases country profiles 2018: Malaysia.

World Health Organization. (2020). Cardiovascular diseases (CVDs). Retrieved from http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

Yamashita, S., & Matsuzawa, Y. (2009). Where are we with probucol: a new life for an old drug?. *Atherosclerosis*, *207*(1), 16-23.

Yang, T. L., Lin, F. Y., Chen, Y. H., Chiu, J. J., Shiao, M. S., Tsai, C. S., ... & Chen, Y. L. (2011). Salvianolic acid B inhibits low-density lipoprotein oxidation and neointimal hyperplasia in endothelium-denuded hypercholesterolaemic rabbits. *Journal of the Science of Food and Agriculture*, *91*(1), 134-141.

Yusnawan, E. (2013). The effectiveness of polar and non polar fractions of Ageratum conyzoides l. to control peanut rust disease and phytochemical screenings of secondary metabolites. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *13*(2), 159-166.

Zapolska-Downar, D., Zapolski-Downar, A., Markiewski, M., Ciechanowicz, A., Kaczmarczyk, M., & Naruszewicz, M. (2001). Selective inhibition by probucol

of vascular cell adhesion molecule-1 (VCAM-1) expression in human vascular endothelial cells. *Atherosclerosis*, *155*(1), 123-130.

Zhang, X., & World Health Organization (WHO). (2000). General guidelines for methodologies on research and evaluation of traditional medicine. *World Health Organization*, *1*, 71.

Zhao, Y., Vanhoutte, P. M., & Leung, S. W. (2015). Vascular nitric oxide: Beyond eNOS. *Journal of Pharmacological Sciences*, *129*(2), 83-94.

Zhu, M., Chang, Q., Wong, L. K., Chong, F. S., & Li, R. C. (1999). Triterpene antioxidants from Ganoderma lucidum. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *13*(6), 529-531.