CHEMICAL CONSTITUENTS AND BIOACTIVITY STUDIES OF *Artocarpus fulvicortex* F. M. JARRET, *Artocarpus integer* var. *silvestris* CORNER AND *Artocarpus rigidus* BLUME

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

MARCH 2019

This is for you, my supermom Maziah,
for your endless supports and prayers.

To my beloved dad Kamaruddin Shah and my brother Firuz Shah
how I wish you could read this. I miss both of you so much

To all my sisters that always there for me no matter how emotionally unstable I am at the time. Thanks for having me!

To all my friends that have been waiting for me to officially be "Dr. Surie"
Even though it is sounds impossible initially but finally I am almost there!

To whom who is always be the driven force for me to finish this thesis
This is for you

ACKNOWLEDGEMENT

Praise to our Almighty God for giving endless blessings throughout this academic programme. I would like to express my deepest appreciation to Prof. Dr. Hasnah Mohd. Sirat for her willingness to support of my research and writing, for her constant encouragement, for her valuable sharing of knowledge, positivity and guidance to help me fulfil the requirement in this PhD course. It was a great honour to finish this work under her supervision. A special thanks to my supervisor, Assoc. Prof. Dr. Shajarahtunnur Jamil for her care and supports.

Through sweat and tears, yet by my own desire to complete this research and it has been wonderful as I gain unconditional love and support from many people especially my family and friends. I want to take this opportunity to express deep sense of gratitude to my family and friends for the greatest moral supports along my PhD's journey.

My appreciation also extends to all academic staffs of Department of Chemistry, Faculty of Science and postgraduate students for the helps and contribution to ease this study. Lastly, I would like to thanks to MyBrain15, Ministry of Higher Education for the funding throughout this doctor of philosophy research programme and make my dream come through.

PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between September 2011 and September 2015 under supervision of Prof. Dr. Hasnah Mohd Sirat and Prof. Madya Dr. Shajarahtunnur Jamil. Part of my work described in this thesis have been reported in the following publications.

1 Shah, M. K. K., Sirat, H. M., and Jamil, S. (2016). Cholinesterase inhibitors from heartwood of *Artocarpus fulvicortex* F. M. Jarret (Moraceae). *Jurnal Teknologi*. 78(6), 185-189.

2 Shah, M. K. K., Sirat, H. M., Jamil, S., and Jalil, J. (2016). Flavonoids from the bark of *Artocarpus integer* var. *silvestris* and their anti-inflammatory properties. *Natural Product Communications*. 11(9), 1275-1278.

ABSTRACT

Investigations on the chemical constituents of *A. fulvicortex, A. integer* var. *silvestris* and *A. rigidus* have been carried out. All the extracts were obtained from maceration method by successive extractions using petroleum ether, dichloromethane, ethyl acetate and methanol as solvents. A total of sixteen compounds were successfully isolated; nine from *A. fulvicortex*, five from *A. integer* var. *silvestris* and two from *A. rigidus*. Purification of the extract from heartwood of *A. fulvicortex* led to the isolation of catechin, oxyresveratrol, lupeol-3-acetate and fridelin. 5-Hydroxy-(6:7,3':4')-di(2,2-dimethylpyrano)flavone, carpachromene, norartoarpetin, cycloartocarpesin and fridelin were isolated from the leaves of *A. fulvicortex*. Purification on the barks extract of *A. integer* var. *silvestris* afforded one new pyranoflavone class named as methoxycyclocommunol along with four known flavonoids, heteroflavanone A, artonin F, cudraflavone C and cyclocommunol. Recrystallisation of a fraction from the ethyl acetate barks extract of *A. rigidus* gave artonin E while purification of the dichloromethane extract yielded cyclorigidol. All the pure compounds except cycloartocarpesin and cyclocommunol were tested for their anti-inflammatory properties using radioimmunoassay method on human whole blood. Compounds that inhibited prostaglandin E_2 (PGE_2) production greater than 55% were tested in serial concentration to determine the IC_{50} values. Cudraflavone C exhibited the strongest inhibition toward vasoactive PGE_2 with IC_{50} value of 0.03 μg/mL which is higher than positive control, indomethacin that gave IC_{50} value of 0.06 μg/mL. Catechin and oxyresveratrol showed significant values of inhibition against butyrylcholinesterase enzyme in dose dependent manner. The IC_{50} values of catechin and oxyresveratrol are 25.0 mM and 3.13 mM, respectively.

ABSTRAK

Penyelidikan mengenai komposisi kimia ke atas *A. fulvicortex, A. integer* var. *silvestris* dan *A. rigidus* telah dijalankan. Semua ekstrak diperoleh dengan kaedah rendaman secara berturutan menggunakan petroleum eter, diklorometana, etil asetat dan metanol sebagai pelarut. Sejumlah enam belas sebatian kimia telah berjaya diasingkan iaitu sembilan daripada *A. fulvicortex,* lima daripada *A. integer* var. *silvestris* dan dua daripada *A. rigidus.* Penulenan ekstrak kulit batang *A. fulvicortex* telah memberikan katekin, oksiresveratrol, lupeol-3-asetat dan fridelin. 5-Hidroksi-(6:7,3':4')-di(2,2-dimetilpirano)flavon, karpakromena, norartokarpetin, sikloartokarpesin dan fridelin telah diasingkan daripada ekstrak daun *A. fulvicortex*. Penulenan ke atas ekstrak batang *A. integer* var. *silvestris* telah menghasilkan satu kelas piranoflavon baharu dinamakan sebagai metoksisiklokomunol dan empat flavonoid sedia ada iaitu heteroflavanon A, artonin F, kudraflavon C dan siklokomunol. Penghabluran semula fraksi daripada ekstrak etil asetat batang *A. rigidus* memberikan artonin E manakala penulenan ekstrak diklorometana memberikan siklorigidol. Semua sebatian tulen kecuali sikloartokarpesin dan siklokomunol telah dikaji sifat anti-inflamasi menggunakan kaedah radioimunocerakinan ke atas darah manusia. Sebatian yang merencatkan penghasilan prostaglandin E_2 (PGE_2) melebihi 55% telah diuji menggunakan kepekatan bersiri untuk menentukan nilai IC_{50}. Kudraflavon C mempamerkan rencatan tertinggi ke atas vasoaktif PGE_2 dengan nilai IC_{50} 0.03 µg/mL melebihi nilai IC_{50} 0.06 µg/mL kawalan positif indometasin. Katekin dan oksiresveratrol menunjukkan nilai perencatan yang signifikan terhadap enzim butirilkolinesterase dengan kebergantungan kepada dos. Nilai IC_{50} katekin dan oksiresveratrol masing-masing ialah 25.0 mM dan 3.13 mM.

TABLE OF CONTENT

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
1D	1 dimensional
2D	2 dimensional
Abs	Absorbance
AChe	Acetylcholinesterase
β	Beta
br	Broad
BuChe	Butyrylcholinesterase
^{13}C	Carbon-13
cm	centimeter
δ	Chemical shift
$CHCl_3$	Choroform
CC	Column Chromatography
COSY	Correlation Spectroscopy
J	Coupling constant
COX	Cyclooxygenase
DEPT	Distortionless Enhancement by Polarization Transfer
DEPTQ	Distortionless Enhancement by Polarization Transfer Including the Detection of Quaternary Nuclei
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
EI	Electron Impact
ELISA	Enzyme Linked Immunosorbent Assay
EtOAc	Ethyl acetate
EDTA	Ethylene Diamine Triacetic Acid
FTIR	Fourier Transform Infrared

γ	Gamma
IC_{50}	Half maximal inhibitory concentration
Hz	Hertz
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HSQC	Heteronuclear Single Quantum Coherence
IR	Infrared
λ	lamda
LPS	Lipopolysaccharide
LOX	Lipoxygenase
LSC	Liquid Scintillation Counter
L	Liter
MS	Mass Spectrometry
m/z	Mass to change ratio
MHz	megahertz
m.p	Melting point
MeOH	Methanol
μ	micro
mm	milimeter
mg	milligram
mL	Millilitre
M	molar
M^+	Molecular ion
m	multiplet
nm	Nanometer
NMR	Nuclear Magnetic Resonance
ppm	Part per million
PE	Petroleum ether
PG	Prostaglandin
^1H	Proton
QSAR	Quantitative Structure Activity Relationship
q	quartet
RIA	Radioimmunoassay

ROS	Reactive Oxygen Species
R_f	Retention factor
RDA	Retro-Diels-Alder
s	singlet
SD	Standard deviation
TLC	Thin Layer Chromatography
t	triplet
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 General Information

Medicinal plants are generally known as "Chemical Goldmines" as they contain natural bioactive chemicals that could give benefits to their users by acting as tools for maintaining overall health and vitality [1]. The bioactive chemicals which are acceptable to human and animal systems also work as the secondary metabolites within the plants. Like the human beings, plants are complete organisms that adapted and survived by developing their own natural defences against their enemies [2].

The natural production of the precious bioactive chemicals by medicinal plants creates the intention of many scientists to study it thoroughly. The study of plant is called ethnopharmacology or frequently known as ethno-medicine [3]. Over 250,000 plants species on earth, more than 80,000 are potential to become the herbal medicine. Approximately, 5000 species are extensively used as phytomedicine or the practice of using any parts of the plant and were studied in details [1, 2].

Generally, natural occurring compounds can be divided into three broad categories. First category is related to primary metabolites in which the compounds play important roles in metabolism and cells production. Another two categories are high molecular weight polymeric materials and secondary metabolites. The most attractive part to be discovered is the secondary metabolites because they can be only characterised in limited range of species [4]. Our nature which is extremely complex

and more challenging enhanced the scientists to reveal one by one of the special properties of the secondary metabolites.

Initially, the scientists and doctors began to make a connection between chemistry and diseases by focusing on phytochemical studies of secondary metabolites that may lead to the discovery of new drug candidates. The phytochemicals can be used unmodified as drugs, as precursor for the partial synthesis of drugs or as total synthesis of the new drugs [5]. As the medicinal field developed, many sophisticated and powerful weapons were invented to help people against unpredictable diseases, example the radiotherapy, chemotherapy, steroidal painkillers and antibiotics [2]. More or less the inventions could give bad effects towards the consumers.

As the result, many people prefer traditional alternative and complementary medicines especially herbal medicine, as it is safe for human consumption to boost the immune systems. Nowadays, the research approaches are focusing on the development of herbal medicines as the new pharmaceutical drugs. This creates high demand for novel bioactive compounds from medicinal plants for various purposes [6].

However, even though the utilisation of natural ingredients in product formulations is a popular trend amongst the manufacturers, the terms conventional drugs and herbal medicine usually are misunderstood. Although many of the prescriptions and over-the-counter drugs consumed today are originally come from the plant, conventional drugs are synthetic chemicals which must be approved by the Food and Drug Administration (FDA) for their specific uses and to provide a desired response when it is consumed.

In the other words, these pharmaceutical products are based on the single isolated compound of the plants. Meanwhile, herbal medications are taken from the natural chemicals within a plant either the extract is taken in its original form, combined with other herbal extracts, or in its purified form. This is due to plants comprise of myriad active components which work together to develop the medicinal actions [2].

The phytochemical study of plant generally is the preliminary study to identify the active compounds that may contribute to certain specific properties such as anti-microbial, anti-oxidant, anti-inflammatory, anti-cancer and many more. In this research, *Artocarpus fulvicortex* F. M. Jarret, *A.integer* var. *silvestris* Corner, and *A. rigidus* Blume belong to Moraceae family were chosen as the samples.

1.2 Problem Statements

Besides the appreciable importance as a source of edible fruit, *Artocarpus* species have been reported to have abundance of fascinating and variety of chemical constituents. Some of the metabolites possess remarkable bioactivities that might be considered as the potential medicinal drugs in the future. There is a great opportunity and need to study further on this genus and identify the bioactive compounds from selected three species which are *A. fulvicortex, A.integer* var. *silvestris* and *A. rigidus.*

Phytochemical study on first species, *A. fulvicortex* collected from different locality with the previous study was performed on two parts, leaves and heartwood [7]. The second species, *A. integer* var. *silvestris* which was believed to possess interesting secondary metabolites as this species considered as underutilised species and the study on its main variety revealed the isolation of abundance phenolic isolates [8]. Since there are no publication regarding the phytochemicals and bioactivities on *A. rigidus* collected from Malaysia it was chosen to be investigated in this research, since different locality could effects the production of the secondary metabolites.

1.3 Objectives

The objectives of the research are:

1. To extract, isolate and identify the phytochemicals of *A. fulvicortex, A.integer* var. *silvestris* and *A. rigidus*.

2. To elucidate the structure of isolated compounds using spectroscopic technique including infrared, nuclear magnetic resonance, ultraviolet spectroscopies and mass spectrometry.
3. To screen and evaluate the ability of the phytochemicals as well as the crude extracts as anti-inflammatory agents and enzyme inhibitors.

1.4 Scope of Study

Studies were carried out to identify the phytochemicals from three *Artocarpus* species and their biological activities. The crude extracts were obtained by cold extraction method. Then, the purification was carried out by successive column chromatography after being fractionated by vacuum liquid chromatographic technique. The bioactivity studies of pure compounds focused on anti-inflammatory activity using several assays in expression of enzyme inhibitory such as cyclooxygenase, lipoxygenase and cholinesterase.

1.5 Significance of Study

The importance of this research is to isolate the bioactive compounds that may lead to the production of new nature-based products. Begins with the isolation and identification of the chemical constituents in the *Artocarpus* species, then chemistry of the compounds can be studied for the evaluation of their bioactivities. This research may reveal the secondary metabolites that might be useful for human being. Those secondary metabolites can also be synthesised chemically or biologically. The bioactive constituents obtained will be used as the basis for product formulation in the agro-based industries. This research can also contribute to the database of secondary metabolites of the plants.

REFERENCES

1. Joy, P. P., Thomas, J., Mathew, S., and Skaria, B. P. (1998). *Zingiberaceous Medicinal and Aromatic Plants*. India: Aromatic and Medicinal Plants Research Station.
2. Connors, M. S., and Altshuler, L. (2009). *Guide to Herbal Remedies*. USA: Advantage Quest Publications. 1-17.
3. Ahmed, Z., and Dar, A. (2008). *Advances in Natural Products Importance in Health and Economy*. Delhi: Daya Publishing House. 213-218.
4. Hanson, J. R. (2003). *Natural Products the Secondary Metabolites*. Cambridge, UK: The Royal Society of Chemistry. 1-30.
5. Jantan, I. (2004). Medicinal Plant Research in Malaysia: Scientific Interests and Advances. *Jurnal Sains Kesihatan Malaysia*. 2 (2), 27-46.
6. Mailina, J., Nor Azah, M. A., Mastura, M., Mazura, P., Fadzureena, J., and Siti Humeirah, A. G. *Utilisation of Phytoextracts and Essential Oils from Malaysian Zingiberaceae and Annonaceae for The Development of Cosmeceutical Products*. Forest Research Institute Malaysia (FRIM).
7. Jamil, S., Taher, M., Sirat, H. M. and Othman, N. A. 2012. Flavonoids and Triterpenes from the leaves of *Artocarpus fulvicortex*. *Nat Prod Commun*. 7(12). 1587-1588.
8. Wang, M., and Zerega, N. (2014). Origin and diversity of an underutilized tropical fruit tree, Cempedak (*Artocarpus integer*, Moraceae), and its putative wild relative (*Artocarpus integer* var. *silvestris*). Oral Session: *Botany 2014*. Boise, ID, USA.
9. Jones, A. M. P., Ragone, D., Tavana, N. G., Bernotas, D. W., Murch, S. J. (2011). Beyond the Bounty: Breadfruit (*Artocarpus altilis*) for food security and novel foods in the 21st Century. *Ethnobot Res Appl*. 9, 129-150.
10. Jagtap, U. B., Bapat, V. A. (2010). *Artocarpus*: A review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol*. 129. 142-166.

11. Whitmore, T. C. (1978) *Tree Flora of Malaya* (Volume 3). Longman Malaysia Sdn. Bhd. Kuala Lumpur.
12. Soepadmo, E., and Saw, L. G. (2000) *Tree Flora of Sabah and Sarawak* (Volume 3). Ampang Press Sdn. Bhd. Kuala Lumpur.
13. Wyatt-Smith, J. (1999) *Pocket Check List of Timber Trees* (3rd revision). Forest Research Institute Malaysia. Malaysia.
14. Ribera, A. E., and Zuniga, G. (2012). Induced plant secondary metabolites for phytopatogenic fungi control: a review. *J. Soil Sci. Plant Nutr.* 2 (4), 893-911.
15. Rao, V. (2012). *Phytochemicals - A Global Perspective of Their Role in Nutrition and Health.* InTech. Croatia.
16. Kodan, A., Kuroda, H., and Sakai, F. (2002). A stilbene synthase from Japanese red pine (Pinus densiflora): Implications for phytoalexin accumulation and down-regulation of flavonoid biosynthesis. *Proc Natl Acad Sci.* 99(5). 3335-3339.
17. Hakim, A. (2010). Diversity of secondary metabolites from Genus *Artocarpus* (Moraceae). *Nusantara Bioscie.* 2 (3), 146-156.
18. Shen, G., Huhman, D., Lei, X., Snyder, J., Sumner, L. W., and Dixon, R. A. (2012). Characterization of an Isoflavonoid-Specific Prenyltransferase from *Lupinus albus*. *Plant Physiol.* 159, 70-80.
19. Venkataraman, K. (1972). Wood Phenolics in the Chemotaxonomy of the Moraceae. *Phytochemistry.* 11, 1571-1586.
20. Botta, B., Vitalib, A., Menendezc, P., Misitia, D., and Monachea, G. D. (2005). Prenylated Flavonoids: Pharmacology and Biotechnology. *Curr Med Chem.* 12, 713-739.
21. Lathiff, S. M. A., Jemaon, N., Abdullah, S. A. and Jamil, S. (2015). Flavonoids from *Artocarpus anisophyllus* and their Bioactivities. *Nat Pro Commun.* 10(3), 393-396.
22. Shamaun, S. S., Rahmani, M., Hashim, N. H., Ismail, H. B. M., Sukari, M. A., Lian. G. E. C., and Go, R. (2010). Prenylated flavones from *Artocarpus altilis*. *J Nat Med.* 64, 478–481.
23. Zong-Ping, Z., Yang, X., Chuan, Q., Shuang, Z., Xiaohong, G., Yingying, L., Guobin, X., Mingfu, W., and Jie, C. (2014). Characterization of Antiproliferative Activity Constituents from *Artocarpus heterophyllus*. *J Agric Food Chem.* 62, 5519-5527.

24. Wen-Chun, L., Cheng-Wei, T., Chun-Ching, L., Feng-Lin, Y., and Horng-Huey, K. (2013). Prenylated flavonoids from *Artocarpus altilis*: Antioxidant activities and inhibitory effects on melanin production. *Phytochemistry*. 8, 78–88.

25. Nguyen, N. T., Nguyen, M. H. K., Nguyen, H. X., Bui, N. K. N., and Nguyen, M. T. T. (2012). Tyrosinase Inhibitors from the Wood of *Artocarpus heterophyllus*. *J Nat Prod*. 75, 1951−1955.

26. Arung, E. T., Shimizu, K., Tanaka, H., Kondo, R. (2010). 3-Prenyl luteolin, a new prenylated flavone with melanin biosynthesis inhibitory activity from wood of *Artocarpus heterophyllus*. *Fitoterapia*. 81, 640–643.

27. Dej-adisai, S., Meechai, I., Puripattanavong, J., and Kummee, S. (2013). Antityrosinase and antimicrobial activities from Thai medicinal plants. *Arch Pharm Res*. 37(4), 473-483.

28. Minakawa, T., Toume, K., Arai, M. A., Koyano, T., Kowithayakorn, T., and Ishibashi, M. (2013). Prenylflavonoids isolated from *Artocarpus champeden* with TRAIL-resistance overcoming activity. *Phytochemistry*. 96. 299–304.

29. Panthong, K., Tohdee, K., Hutadilok-Towatana, N., Voravuthikunchaib, S. P., and Chusri, S. (2013) Prenylated Flavone from Roots of a Hybrid between *Artocarpus heterophyllus* and *Artocarpus integer* and its Biological Activities. *J Braz Chem Soc*. 24(10), 1656-1661.

30. Mei-Hua, Y., Ting, Z., Gui-Rui, Y., Hong-Xun, Y., He-Yao, W., and Ai-Jun, H. (2012). New Isoprenylated Flavones and Stilbene Derivative from *Artocarpus hypargyreus*. *Chem Biodivers*. 9(2), 394-402.

31. Pethakamsetty, L., Ganapaty, S. and Bharathi, K. M. (2013). Phytochemical and Biological Examination of the Root Extract of *Artocarpus Hirsuta*, Lam. *Int J Bioassays*. 2(4), 735-738.

32. Bourjot, M., Apell, C., Martin, M., Grellier, P., Nguyen, V. H., Guéritte, F., and Litaudon, M. (2010). Antiplasmodial, Antitrypanosomal, and Cytotoxic Activities of Prenylated Flavonoids Isolated from the Stem Bark of *Artocarpus styracifolius*. *Planta Med*. 76, 1600-1604.

33. Ren, Y., Kardono, L. B. C., Riswan, S., Chai, H., Farnsworth, N. R., Soejarto, D. D., Blanco, E. J. C., and Kinghorn, A. D. (2010) Cytotoxic and NF-KB Inhibitory Constituents of *Artocarpus rigida*. *J Nat Prod*. 73, 949-955.

34. Maneechai, S., De-Eknamkul, W., Umehara, K., Noguchi, H., and Likhitwitayawuid, K. (2012). Flavonoid and stilbenoid production in callus cultures of *Artocarpus lakoocha*. *Phytochemistry*. 81, 42-49.

35. Chin-Lin, H., Ming-Huan, S., Jer-An, L., Gow-Chin, Y., and Song-Chwan, F. (2011). Cytotoxic effects of geranyl flavonoid derivatives from the fruit of *Artocarpus communis* in SK-Hep-1 human hepatocellular carcinoma cells. *Food Chem*. 127(1), 127–134

36. Mai, N. T. T., Hai, N. X., Phu, D. H., Trong, P. N. H., and Nhan, N. T. (2012). Three new geranyl aurones from the leaves of *Artocarpus altilis*. *Phytochem Lett*. 5(3), 647-650.

37. Gang, R., Heng-Yun, X., Zhi-Cheng, H., Rong-Hua, L., Zhi-Wang, Z., Hui-Lian, H., Feng, S., and Ming, Y. (2013). A new isoprenylated flavone from the root bark of *Artocarpus styracifolius*. *Biochem Syst Ecol*. 46, 97-100.

38. Huihui, T., Ping, W., Lidong, L., and Xiaoyi, W. (2011). Stilbenes and flavonoids from *Artocarpus nitidus* subsp. *lingnanensis*. *Fitoterapia*. 82, 662-665.

39. Jer-An, L., Song-Chwan, F., Chi-Hao, W., Shang-Ming, H., and Gow-Chin, Y. (2011). Anti-inflammatory Effect of the 5,7,40-Trihydroxy-6-geranylflavanone Isolated from the Fruit of *Artocarpus communis* in S100B-Induced Human Monocytes. *J Agric Food Chem*. 59, 105-111.

40. Hongjie, Z., Minghua, Q., Yegao, C., Jinxiong, C., Yun, S., Cuifang, W. and Harry, H. S. F. (2011). *Plant Terpenes*. Cock, E. I. (Ed.) *The Encyclopedia of Life Support Systems*. India: EManuscript Publishing Services.

41. Ramli, F., Rahmani, M., Kassim, N. K., Hashim, N. H., Sukari, M. A., Akim, M. A., and Go, R. (2013). New diprenylated dihydrochalcones from leaves of *Artocarpus elasticus*. *Phytochem Lett*. 6, 582-585.

42. Gang, R., Zhi, C. H., Heng, Y. X., Jia-Bing, P., Rong-Hua, L., Hui-Lian, H., and Feng, S. (2013). Chemical constituents from the fruiting branches of *Artocarpus nanchuanensis* endemic to China. *Biochem Syst Ecol*. 51, 98-100.

43. Iverson, C. D., Zahid, S., Yimeng, L., Shoqafi, A. H., Ata, A., and Samarasekera, R. (2010). Glutathione S-transferase inhibitory, free radical scavenging, and anti-leishmanial activities of chemical constituents of *Artocarpus nobilis* and *Matricaria chamomilla*. *Phytochem Lett*. 3, 207-211.

44. Gorham, J. (1980). The stilbenoids. *In progress in phytochemistry*. 6, 203-252.

45. Namdaung, U., Athipornchai, A., Khammee, T., Kuno, M., and Suksamrarn, S. (2018). 2-Arylbenzofurans from *Artocarpus lakoocha* and methyl ether analogs with potent cholinesterase inhibitory activity. *Eur. J. Med. Chem.* 143.1301-1311.

46. Zhang, P. Z., Gua, J., and Zhang, G. L. (2015). Novel stilbenes from *Artocarpus nanchuanensis. J Asian Nat Prod Res.* 17(3), 217-223.

47. Roberts, E., Magis, A., Ortiz, J. O., Baumeister, W., and Luthey-Schulten, Z. (2011). Noise Contributions in an Inducible Genetic Switch: A Whole-Cell Simulation *PLoS Comput Biol.* 7(3).

48. Tunev, S. S., Hastey, C. J., Hodzic, E., Feng, S., Barthold, S. W., and Baumgarth, N. (2011). Lymphoadenopathy during Lyme Borreliosis is caused by Spirochete Migration- Induced Specific B Cell Activation. *PLoS Pathog.* 7(5).

49. Kinnings, S. L., Liu, N., Buchmeier, N., Tonge, P. J., Xie, L., and Bourne, P. E. (2009). Drug Discovery Using Chemical Systems Biology: Repositioning the Safe Medicine Comtan to Treat Multi-Drug and Extensively Drug Resistant Tuberculosis. *PLoS Comput Biol.* 5(7).

50. Efferth, T., and Koch, E. (2011). Complex Interactions between Phytochemicals. The Multi-Target Therapeutic Concept of Phytotherapy Home. C*urr Drug Targets*. 12(1), 122-132.

51. Pereira, V. J., and Kaplan, M. A. C. (2013). The High Bioactivity of Artocarpus - An Exotic Genus. *Floresta e Ambiente*. 20(1), 1-15.

52. Rayburn, E. R., Ezell, S. J., and Zhang, R. (2009). Anti-Inflammatory Agents for Cancer Therapy. *Mol Cell Pharmacol.* 1(1), 29-43.

53. Chung, M. I., Ko, H. H., Yen, M. H., Lin, C. N., Yang, S. Z., Tsao, L. T., and Wang, J. P. (2000). Artocarpol A, a Novel Constituent with Potent Anti-Inflammatory Effect, Isolated from *Artocarpus rigida. Helv Chim Acta.* 83, 1200-1204.

54. Kuan, Y. H., Lin, R. H., Tsao, L. T., Lin, C. N., and Wang, J. P. (2005). Artocarpol A stimulation of superoxide anion generation in neutrophils involved the activation of PLC, PKC and p38 mitogen-activated PK signaling pathways. *Br. J. Pharmacol.*145, 460-468.

55. Kuan, Y. H., Lin, R. H., Lin, H. Y., Huang, L. J., Tsai, C. R., Tsao, L. T., Lin, C. N., Chang, L. C., and Wang, J. P. (2006). Activation of phosphoinositide 3-kinase

and Src family kinase is required for respiratory burst in rat neutrophils stimulated with artocarpol A. *Biochem Pharmacol.* 71, 1735-1746.

56. Su, B. N., Cuendet, M., Hawthorne, M. E., Kardono, L. B. S., Riswan, S., Fong, H. H. S., Mehta, R. G., Pezzuto, J. M., and Kinghorn, A. D. (2002). Constituents of the Bark and Twigs of *Artocarpus dadah* with Cyclooxygenase Inhibitory Activity. *J Nat Prod.* 65, 163-169.
57. Jantan, I., Yasin, Y. H. M., Jamil, S., Sirat, H., Basar, N. (2010). Effect of prenylated flavonoids and chalcones isolated from *Artocarpus* species on platelet aggregation in human whole blood. *J Nat Med.* 64, 365–369.
58. Sritularak, B., Tantituvanont, A., Chanvorachote, P., Meksawan, K., Miyamoto, T., Kohno, Y., and Likhitwitayawuid, K. (2010). Flavonoids with free radical scavenging activity and nitric oxide inhibitory effect from the stem bark of *Artocarpus gomezianus. J Med Plants Res.* 4(5), 387-392.
59. Han, A. R., Kang, Y. J., Windono, T., Lee, S. K., and Seo, S. K. (2006). Prenylated Flavonoids from the Heartwood of *Artocarpus communis* with Inhibitory Activity on Lipopolysaccharide-Induced Nitric Oxide Production. *J Nat Prod.* 69, 719-721.
60. Wei, B. L., Weng, J. R., Chiu, P.H., Hung, C. F., Wang, J. P., and Lin, C. N. (2005). Anti-inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis. J Agric Food Chem.* 53(10), 3867-3871.
61. Perez, G. (2001). Anti-Inflammatory Activity of Compounds Isolated from Plants. *Sci World J.* 1, 713-784.
62. Smith, H. S. (2006). Arachidonic Acid Pathways in Nociception. *J Support Oncol.* 4(6), 277-287.
63. Klein, D. R. (2012). *Organic Chemistry*. United States of America: John Wiley & Sons, Inc.
64. Rainsford, K. D. (2007). *Anti-Inflammatory Drugs in the 21st Century*. R. E. Harris (Ed.). *Inflammation in the Pathogenesis of Chronic Diseases* (pp 3-27). United Kingdom: Springer.
65. Ricciotti E., and FitzGerald G. A. (2011). Prostaglandins and Inflammation. *Arterioscler Thromb Vasc Biol.* 31(5), 986-1000.
66. Jalil, J., Sabandar, C. W., Ahmat, N., Jamal, J. A., Jantan, I., Aladdin, N., Muhammad, K., Buang, F., Mohamad, H. F., and Sahidin, I. (2015). Inhibitory Effects on Triterpenoids from *Dillenia serrata* (Dilleniaceae) on prostaglandin E_2

production and quantitative HPLC analysis of its koetjapic acid and betulinic acid contents. *Molecules*. 20, 3206-3220.

67. Mashima, R., and Okuyama, T. (2015). The role of lipoxygenases in pathophysiology; new insights and future perspectives. *Redox Biol*. 6. 297-310.
68. Nassar, G.M., Morrow, J.D., Roberts, L.J., II, Lakkis, F. G., and Badr, K. F. Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes. (1994). *J Biol Chem*. 269, 27631-27634.
69. Suardiaz, R., Jambrina, P. G., Masgrau, L., González-Lafont, A.,Rosta, E., and Lluch, J.M. (2016). Understanding the Mechanism of the Hydrogen Abstraction from Arachidonic Acid Catalyzed by the Human Enzyme 15-Lipoxygenase-2. A Quantum Mechanics/Molecular Mechanics Free Energy Simulation. *J. Chem. Theory Comput.* 12 (4). 2079-2090.
70. Bishnoi, M., Patil, C. S., Kumar, A., and Kulkarni, S. K. (2009). Relative Role of Cyclooxygenase-2 (COX-2) Inhibitors and Lipoxygenase (LOX) Inhibitors in Aging Induced Dementia and Oxidative Damage. *Indo J. Chem*. 9, 328-331.
71. Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C. B., and Rahu, N. (2016). Oxidative Stress and Inflammation: What Polyphenols Can Do for Us?. *Oxidative Medicine and Cellular Longevity*.
72. Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., and Buga, A. M. (2013). ROS and Brain Diseases: The Good, the Bad, and the Ugly. *Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity.*
73. Susidarti, R. A., Rahmani, M., Ali, A. M., Sukari, M. A., and Ismail, H. B. M. (2007). *Friedelin from Kelat Merah (Eugenia chlorantha Duthie)*. Majalah Obat Traditional. (Volume 11).
74. Sousa, G. F., Duarte, L. P., Alcântara, A. F. C., Silva, G. D. F., Vieira-Filho, S. A., Silva, R. R., Oliveira, D. M., and Takahashi, J. A. (2012). New Triterpenes from *Maytenus robusta*: Structural Elucidation Based on NMR Experimental Data and Theoretical Calculations. *Molecules*. 17, 13439-13456.
75. Queiroga, C. L., Silva, G. F., Dias, P. C., Possenti, A., and de Carvalho, J. E. (2000). Evaluation of the antiulcerogenic activity of friedelan-3β-ol and friedelin isolated from *Maytenus ilicifolia* (Celastraceae). *J Ethnopharmacol*. 72, 465-468.
76. Picker, K., Ritchie, E., and Taylor, W. C. (1976). The Chemical Constituents of Australian *Flindersia* Species. XXI* an Examination of the Bark and the Leaves of *F. laevicarpa*. *Aust. J. Chem*. 29. 2023-2036.

77. Ali, M. I., Ahmed, Z., Waffo, A. F., and Ali, M. S. (2010). Flavonoids from *Erythrina vogelii* (Fabaceae) of Cameroon. *Nat Prod Commun*. 5(6), 889-892.

78. Ersam, T., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L., and Syah, Y. M. (2002). A New Isoprenylated Chalcone, Artoindonesianin J, from root and tree bark of *Artocarpus bracteata*. *J Chem Res*. 4, 186-187.

79. Zheng, Z. P., Cheng, K. W., To, J. T. K., Li, H., and Wang, M. (2008). Isolation of tyrosinase inhibitors from *Artocarpus heterophyllus* and use of its extract as antibrowning agent. *Mol. Nutr. Food Res*. 52, 1530-1538.

80. Soekamto, N. H., Nafie, N. L., Mandey, F. W., and Garson, M. (2009). Norartocarpetin, Flavone Derivative from Leaves of *Artocarpus fretessi. Indo. J. Chem*. 9 (2), 328-331.

81. Jamal, A. K., Yaacob, W. A., and Din, L. (2008). A Chemical Study on *Phyllanthus reticulatus*. *J Phys Sci*. 19(2), 45-50.

82. Chaturvedula, V. S. P., and Prakash, I. (2012). Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus*. *Int Curr Pharm J*. 1(9), 239-242.

83. Pierre, L. L., and Moses, M. N. (2015). Isolation and Characterisation of Stigmasterol and β-Sitosterol from *Odontonema Strictum* (Acanthaceae). *J Innov Pharm Biol Sci*. 2(1), 88-95.

84. Povichit, N., Phrutivorapongkul, A., Suttajit, M., and Leelapornpisid, P. (2010). Antiglycation and antioxidant activities of oxyresveratrol extracted from the heartwood of *Artocarpus lakoocha* Roxb. *Maejo Int. J. Sci. Technol*. 4(03), 454-461.

85. He, S. H., Seung, H. J., and Yong, S. K. (2007). Cytotoxic constituents from the stem of *Rhododendron mucronulatum*. *Kor. J. Pharmacogn*. 38(3), 227-233.

86. Atomssa, T., and Gholap, A. V. (2015). Characterization and determination of catechins in green tea leaves using UV-visible spectrometer. *J. Eng. Technol. Res*. 7(1), 22-31.

87. Chai, M. L., and Chun, N. L. (1993). Two 2',4',6'-Trioxygenated Flavanones From *Artocarpus heterophyllus*. *Phytochemistry*. 33(4), 909-911.

88. Hakim, E. H., Aripin, A., Achmad, S. A., Aimi, N., Kitajima, M., Makmur, L., Mujahidin, D., Syah, Y. M., and Takayama, H. (2001). Artoindonesianin-E suatu senyawa baru turunan flavanon dari tumbuhan *Artocarpus champeden*. *Proc. ITB*. 33(3), 69-73.

89. Kweon, M. H., Kwak, J. H., Ra, K. S., Sung, H. C., and Yang, H. C. (1996). Structural Characterization of a Flavanoid Compound Scavenging Superoxide Anion Radical Isolated from *Capsella bursa-pastoris*. *J. Biochem. Mol. Biol.* 29(5), 423-428.

90. Hano, Y., Yamagami, Y., Kobayashi, M., Isohata, R., and Nomura, T. (1990). Artonins E and F, Two New Prenylflavones from the Bark of *Artocarpus communis* Forst. *Heterocycles*. 31(5). 879- 882

91. Chun, N. L., and Wen, L. S. (1992). Pyranoflavonoids from *Artocarpus communis*. *Phytochemistry*. 31(8). 2922-2924.

92. Hano, Y., Matsumoto, Y., Shinohara, K., Sun, J. Y., and Nomura, T. (1990). Cudraflavones C and D, Two New Prenylflavones from the Root Bark of *Cudrania Tricuspidata* (Carr.) Bur. *Heterocycles*. 31(7), 1339-1344.

93. Wang, L., Wang, H. Q., and Chen, R. Y. (2007). Studies on chemical constituents from bark of *Morus nigra*. *Zhongguo Zhong Yao Za Zhi*. 32(23), 2497-2499

94. Jayasinghe, U. L. B., Samarakoon, T. B., Kumarihamy, B. M. M., Hara, N., and Fujimoto, Y. (2008). Four New Prenylated Flavonoids and Xanthones from the Root Bark of *Artocarpus nobilis*. *Fitoterapia*. 79, 37–41.

95. Suhartati, T., Achmad, S. A., Aimi, N., Hakim, E. H., Kitajima, M., Takayama, H., and Takeya, K. (2001). Artoindonesianin L, A New Prenylated Flavone with Cytotoxic Activity from *Artocarpus rotunda. Fitoterapia.* 72. 912-918.

96. Darwish, I. A. (2006). Immunoassay Methods and their Applications in Pharmaceutical Analysis: Basic Methodology and Recent Advances. *Int J Biomed Sci*. 2(3), 217-235.

97. Fowler, E., and Cheng, N. (1983). Comparison of Radioimmunoassay and Elisa Methods for Detection of Antibodies to Chromatin Components. *Journal of Immunological Methods*. 62, 297-303.

98. Grange, R.D., Thompson J. P., and Lambert, D. G. (2014) Radioimmunoassay, enzyme and non-enzyme-based Immunoassays. *Br J Anaesth.* 112(2), 213-216.

99. Middleton, E., J., Kandaswami, C., and Theoharides, T. C. (2000). The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease and Cancer. *Pharmacol. Rev.* 52(4), 673-751.

100. Lago, J. H. G., Toledo-Arruda, A. C., Mernak, M., Barrosa, K. H., Martins, M. A., Tibério, I. F. L. C., and Prado, C. M. (2014). Structure-Activity Association of Flavonoids in Lung Diseases. *Molecules*. 19, 3570-3595.

101. Rosenkranz, H. S., and Thampatty, B. P. (2012). SAR: Flavonoids and COX-2 Inhibition. *Oncol Res*. 13(12), 529-535.

102. Saadawi. S., Jalil, J., Jasamai, M., and Jantan, I. (2012). Inhibitory Effects on Acetylmelodorinol, Chrysin and Polycarpol from *Mitrella kentia* on Prostaglandin E_2 and Thromboxane B_2 Production and Platelet Activating Factor Receptor Binding. *Molecules*. 17. 4824-4835.

103. Blanco, F. J., Guitian, R., Moreno, J., de Toro, F. J., and Galdo, F. (1999). Effect of anti-inflammatory drugs on COX-1 and COX-2 activity in human articular chondrocytes. *J Rheumatol*. 26. 1366-1373.

104. Sautebin, L., Ialenti, A., Ianaro, A., and Di Rosa, M. (1998). Relationship between Nitric Oxide and Prostaglandins in Carrageenan Pleurisy. *Biochem Pharmacol*. 55(7), 1113-1117.

105. Dobrian, A. D., Lieb, D. C., Coleb, B. K., Taylor-Fishwick, D. A., Chakrabarti, S. K., and Nadler, J. L. (2011). Functional and pathological roles of the 12- and 15-Lipoxygenases. *Prog Lipid Res*. 50(1), 115–131.

106. Xu, Z. G., Li, S. L., Lanting, L., Kim, Y. S., Shanmugam, N., Reddy, M. A., and Natarajan, R. (2006). Relationship between 12/15-lipoxygenase and COX-2 in mesangial cells: potential role in diabetic nephropathy. *Kidney Int*. 69, 512-519.

107. Sadik, C. D., Sies, H., and Schewe, T. (2003). Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action. *Biochem Pharmacol*. 65, 773-781.

108. Noguchi, N., Yamashita, H., Hamahara, J., Nakamura, A., Kühn, H., and Niki, E. (2002).The Specificity of Lipoxygenase-Catalyzed Lipid Peroxidation and the Effects of Radical-Scavenging Antioxidants. *Biol Chem*. 383, 619-626.

109. Ellman, G. L., Courtney, K. D., Andres, V. Jr., and Featherstone, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem Pharmacol*. 7, 88-95.

110. Das, S., Laskar, M. A., Sarker, S. D., Choudhury, M. D., Choudhury, P. R., Mitra, A., Jamil, S., Lathiff, S. M. A., Abdullah, S. A., Basar, N., Nahar, L., and Talukdar, A. D. (2017) Prediction of anti-Alzheimer's activity of flavonoids targeting acetylcholinesterase in silico. *Phytochem Anal*. 28 (4). 324-331.

111. Rahman, A., Parveen, S., Khalid, A., Farooq, A., and Choudhary, M. I. (2001). Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa*. *Phytochemistry*. 58(6), 963-968.

112. Orhan, I., Kartal, M., Tosun, F., and Sener, B. (2007). Screening of Various Phenolic Acids and Flavonoid Derivatives for their Anticholinesterase Potential. *Z. Naturforsch*. 62, 829-832.

113. Rhee, I. K., Rijn, R. M., and Verpoorte, R. (2003). Qualitative Determination of False-positive Effects in the Acetylcholinesterase Assay using Thin Layer Chromatography. *Phytochem. Anal.* 14, 127-131.

114. Chagas-Paula, D. A. 1, Zhang, T., Costa, F. B. D., and Edrada-Ebel, R. (2015). A Metabolomic Approach to Target Compounds from the Asteraceae Family for Dual COX and LOX Inhibition. *Metabolites*. 5, 404-430.