PHYTOCHEMICALS AND BIOACTIVITIES OF *ARTOCARPUS ANISOPHYLLUS* MIQ.

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PHYTOCHEMICALS AND BIOACTIVITIES OF *ARTOCARPUS ANISOPHYLLUS* MIQ.

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

JANUARY 2013

For my beloved father, mother, brothers, sisters, brothers and sisters in law, and nephews.....

ACKNOWLEGDEMENTS

I would like to express my deepest sincere gratitude and appreciation to my research supervisor, Dr. Shajarahtunnur binti Jamil for her guidance, patience, motivations, encouragements and continuous support to me in order to complete this research and thesis writing. My special appreciation also goes to the lecturers at the Department of Chemistry, Faculty of Science especially to Assoc. Prof. Dr. Farediah Ahmad and Prof. Dr. Hasnah Mohd. Sirat for their kindness, encouragement and precious help.

I am grateful to eScience fund (Vote No. 79132) from the Ministry of Science, Technology and Innovation (MOSTI) for the financial support. My appreciation also goes to the Department of Chemistry for the access of using the UV, IR, GC, GC-MS, and NMR instruments. Sincerely thanks to all the lab assistants for their helped and assistance in carrying out this research.

I highly express my appreciation to my lab mates in the Natural Products/ Organic Chemistry Research Laboratory, Safina, Raihan, Nadya, Della, Saidah, Athirah, Nuzul, Tarmizi and Mira for their moral support and sharing to make the lab works meaningful. I also thank the seniors, Pak Em, Yau, Mala, Azlin, Syamil, Fariz, and Salihin for their helps and cooperation.

Last but not least, special thanks to all my family members and friends especially to my beloved father and mother for their love, spirits, and encouragement in every single thing I do. I am deeply grateful to everyone who has helped me in completing this research.

PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between December 2008 and December 2011 under the supervision of Dr. Shajarahtunnur binti Jamil. Parts of my work described in this thesis have been reported in the following publications:

1. Noraini binti Jema'on and Shajarahtunnur Jamil (2009). DPPH Radical Scavenging Effects on Crude Extracts from the Different Parts of Three *Artocarpus* Species. Poster presented at 2nd Junior Chemist Colloquium 2009 at Universiti Malaysia Sarawak (UNIMAS), Sarawak. 1 – 2 July 2009.

2. Noraini binti Jema'on and Shajarahtunnur Jamil (2011). Phytochemicals from Heartwood of *Artocarpus anisophyllus* Miq.(Moraceae). Paper presented at 3rd Junior Chemist Colloquium 2011 at Universiti Teknologi Malaysia, Johor Bahru. 18 – 19 January 2011.

ABSTRACT

Phytochemicals and bioactivities of the heartwood and stem bark of *Artocarpus anisophyllus* Miq. have been studied. Extraction of the air-dried and powdered form of both parts was carried out by cold extraction method using *n*-hexane, dichloromethane and ethyl acetate as the solvent system. Fractionation and purification of the crude extracts have resulted in the isolation of eight compounds including flavonoids, triterpenes and a plant sterol. A new compound was isolated from the heartwood of *A. anisophyllus* which was identified as 3′-hydroxycycloartocarpin together with chaplashin, cycloartocarpin, artocarpin, derivative of artoindonesianin Z-1, a mixture of stigmast-4-en-3-one and stigmasta-4,22-dien-3-one, and *β*-sitosterol. The last compound, 6-hydroxystigmast-4-en-3-one was isolated from the stem bark of *A. anisophyllus*, together with derivative of artoindonesianin Z-1 which previously isolated from the heartwood. Their structures were elucidated using spectroscopic methods including Nuclear Magnetic Resonance, Infrared, Ultraviolet spectroscopies and Mass spectrometry. Bioactivities were carried out on the crude extracts and pure compounds. The antibacterial test on the crude extracts and pure compounds were carried out against the Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. The most significant antibacterial activity was shown by the dichloromethane extract of the heartwood against *E. coli* with the MIC value of 125 μg/mL. Artocarpin showed moderate antibacterial activity against *B. subtilis* and *E. coli* with MIC value of 250 μg/mL. Derivative of artoindonesianin Z-1 showed significant antibacterial activity against *B. subtilis* with MIC value of 250 μg/mL. The antioxidant assay using DPPH radical showed that the dichloromethane extract of the stem bark of *A. anisophyllus* possessed the highest radical scavenging activity with IC_{50} value of 307.24 μg/mL. Among the pure compounds tested, artocarpin showed the strongest radical scavenging property with IC_{50} value of 69.63 μg/ml.

ABSTRAK

Fitokimia dan bioaktiviti daripada batang dan kulit batang *Artocarpus anisophyllus* Miq. telah dikaji. Pengekstrakan secara rendaman sejuk telah dijalankan ke atas kedua-dua sampel kering menggunakan *n*-heksana, diklorometana dan etil asetat sebagai system pelarut. Pemeringkatan dan penulenan ke atas ekstrak mentah telah berjaya menemukan lapan sebatian yang terdiri daripada flavonoid, triterpena dan sterol tumbuhan. Satu sebatian baru telah berjaya diasingkan daripada batang *A. anisophyllus* yang dikenalpasti sebagai 3′-hidroksisikloartokarpin bersama-sama dengan kaplasin, sikloartokarpin, artokarpin, terbitan artoindonesianin Z-1, campuran stigmast-4-en-3-on dan stigmasta-4,22-dien-3-on, dan *β*-sitosterol. Sebatian terakhir, 6-hidroksistigmast-4-en-3-on telah diasingkan daripada kulit batang *A. anisophyllus* bersama terbitan artoindonesianin Z-1 yang sebelum ini telah diasingkan daripada bahagian batang. Struktur sebatian telah dikenalpasti dengan menggunakan kaedah spektroskopi termasuk Spektroskopi Resonan Magnet Nukleus, Inframerah, Ultralembayung dan Spektrometri Jisim. Ujian bioaktiviti telah dijalankan ke atas ekstrak mentah dan sebatian tulen. Ujian antibakteria ke atas ekstrak mentah dan sebatian tulen telah dijalankan terhadap bakteria Gram-positif, *Bacillus subtilis* dan *Staphylococcus aureus* dan bakteria Gram-negatif, *Escherichia coli* dan *Pseudomonas aeruginosa*. Aktiviti antibakteria paling baik ditunjukkan oleh ekstrak dikrolometana bahagian batang terhadap *E. coli* dengan nilai MIC 125 μg/mL. Artokarpin menunjukkan aktiviti antibakteria yang sederhana terhadap *B. subtilis* dan *E. coli* dengan nilai MIC 250 μg/mL. Terbitan artoindonesianin Z-1 menunjukkan aktiviti antibakteria yang ketara terhadap *B. subtilis* dan *E. coli* dengan nilai MIC 250 μg/mL. Ujian antioksidan yang menggunakan radikal bebas DPPH menunjukkan bahawa ekstrak diklorometana kulit batang *A. anisophyllus* memiliki aktiviti perencatan radikal tertinggi dengan nilai IC_{50} 307.24 μg/mL. Antara sebatian tulen yang diuji, artokarpin didapati memiliki sifat perencatan radikal paling tinggi dengan nilai IC_{50} 69.63 μg/mL.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
br	Broad
^{13}C	Carbon-13
CC	Column Chromatography
COSY	Correlation Spectroscopy
$CDCl_3$	Deuterated chloroform
cm^{-1}	Per centimeter
δ	Chemical shift
d	Doublet
d*	Overlapping doublet
1D	1 Dimension
2D	2 Dimension
D_2O	Deuterium Oxide
DCM	Dichloromethane
dd	Doublet of doublets
dd*	Overlapping doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
DMAP	4-Dimethylaminopyridine
DMSO	Dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ε	Epsilon
E	Entgegen
ED_{50}	Effective Dose at 50%

EtOAc	Ethyl acetate
EIMS	Electron Impact Mass Spectrometry
FABMS	Fast Atom Bombardment Mass Spectrometry
FRAP	Ferric Reducing / Antioxidant Power
FT-IR	Fourier Transform Infrared
γ	Gamma
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMG-CoA	3-Hydroxy-3-methylglutaryl-Coenzyme A
HMQC	Heteronuclear Multiple Quantum Coherence
HREIMS	High Resolution Electron Impact Mass Spectrometry
HSV-1	*Herpes simplex* virus type-1
Hz	Hertz
IR	Infrared
IC_{50}	Inhibition Concentration at 50%
J	Coupling constant
λ	Lamda
L	Liter
LC_{50}	Lethal Concentration at 50%
LC-MS	Liquid Chromatography-Mass Spectrometry
lit.	Literature
M	Molar
M^{+}	Molecular ion
max	Maximum
MIC	Minimum Inhibition Concentration
min	Minimum
MBC	Minimum Bactericidal Concentration
mg	Milligram
mL	Milliliter
Mo	Molybdenum
MS	Mass Spectrometry
mM	Milimolar
m/z	Mass to charge ion
m.p.	Melting point

MHz	Megahertz
m	Multiplet
m*	Overlapping multiplet
NA	Nutrient Agar
NB	Nutrient Broth
$NaH_2PO_4.H_2O$	Sodium phosphate monobasic monohydrate
nm	Nanometer
NMR	Nuclear Magnetic Resonance
ORAC	Oxygen Radical Scavenging Capacity
p	Para
PE	Petroleum ether
PIV	*Parainfluenza*
ppm	Parts per million
prep-TLC	Preparative Thin Layer Chromatography
R_f	Retention factor
ROS	Reactive Oxygen Species
s	Singlet
SD	Standard Deviation
sh	Shoulder
S_N2	Bimolecular Nucleophilic Substitution
t	Triplet
t*	Overlapping triplet
TLC	Thin Layer Chromatography
TNF	Tumor Necrosis Factor
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography
Z	Zusammen
µg	Microgram

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Consumption of plant derived medicines is wide spread and increasing significantly in both traditional and modern medicine. The therapeutic use of plants certainly goes back to the Sumerian civilization and 400 years before the Common Era, it has been recorded that Hippocrates used approximately 400 different plant species for medicinal purpose. Natural products played a prominent role in ancient traditional medicine system, such as Chinese, Ayurveda, and Egyptian, which are still in common use today [1]. According to The World Health Organization, more than 80% of the world population in developing countries depends primarily on plant based medicines for basic healthcare needs [2, 3]. These plants have been used widely by the villagers to treat fever, diarrhea, cough, high blood pressure and many more [4].

Nature has been a source of therapeutic agents for thousands of years, and an impressive number of modern drugs have been derived from natural sources, many based on their use in traditional medicine. Over the last century, several drugs have been developed from medicinal plants. In recent years, studies on the medicinal plants have increased remarkably due to their rich sources of bioactive compounds. Several modern drugs have been developed from these plants [5]. However, new bioactive compounds and chemical modification, or lead compounds and more effective drugs with no side effect are urgently needed. Despite the magnificent success of chemotherapy, only 30 %

of about 2000 existing diseases can be cured at present. Therefore, it is important to continuously explore natural compounds from medicinal plants that can be developed into drug to treat severe diseases [6].

Based on the previous literatures, one of flowering plants that produce many compounds with their biological activity is Moraceae family which is commonly known as mulberry family. The family Moraceae consists mostly of trees, but also shrubs, herbs, and geophytes. It is a large family comprising 53 genera and nearly 1400 species. This family is widespread in tropical and subtropical regions, but it is less in temperate climates condition. In Malaysia, there are 9 genera and 137 species, mostly tropical from lowlands to mountain forests [7]. A few of these genera such as *Morus*, *Ficus* and *Artocarpus* are economic sources of food and widely used in traditional medicines, agriculture and industry [8]. The genera constitute valuable timbers for commercial purposes such as *Artocarpus* and *Ficus.* A few of *Ficus* species are also known to give edible fruits and are used for birth control, also for women after giving birth. The leaves are consumed as tea [9]. These genera also received great level of scientific interest as they contain medicinally important secondary metabolites possessing useful biological activities.

Artocarpus is one of the genus of the Moraceae family which is widely distributed in Sri Lanka, India, Burma, Thailand, Indo-China and Malaysia. A number of *Artocarpus* species are used as food and for traditional folk medicines especially in South-East Asia, Indonesia and India. *Artocarpus* plants offer advantages as a profitable multipurpose crop for producing fruits and timber. The exceptional medicinal value of *Artocarpus* has long been recognized and economically the genus is of appreciable importance as a source of edible aggregate fruit such as *Artocarpus heterophyllus* (jackfruit), *Artocarpus altilis* (breadfruit) and *Artocarpus champeden* (Chempedak) and yielding fairly good timber [10, 11].

Extracts of the aerial and underground plant parts have been applied in traditional medicine for the treatment of diarrhea, malarial fever, tapeworm infection diabetes, and other ailments [12]. The other uses include wound healing, anti-syphilis, expectorant properties and also use to treat anemia, asthma and dermatitis. Referring to which provides a botanical description of *Artocarpus* species and their phytochemical constituents in the aerial and underground parts. In addition, *in vitro* and *in vivo* pharmacological studies are reviewed and discussed, focusing on antibacterial, anti tubercular, antiviral, cytotoxic [13], anti diarrheal, antiarthritic, antimycobacterial [14], antihelmintic, antioxidative [15], anti-inflammatory [16], antiplatelet [17], tyrosinase, 5-lipoxygenase, 5-α reductase inhibitory activities and antifungal [18] of *Artocarpus* species. Most of the pharmacological effects can be explained by the phenolic compounds including flavonoids, stillbenoids, arylbenzofurans [19] present in all plant parts and Jacalin, a lectin [20] present in seeds of certain *Artocarpus* species.

Artocarpus anisophyllus Miq. is one of Malaysian rare *Artocarpus* species. *A. anisophyllus* Miq. is locally known as “keledang babi” or “mentawa”. It is a mid-canopy tree, stem with white sap and hairy. It has leaflet penni-veined and glabrous which is large and small in regular order. *A. anisophyllus* can be found in the lowland of Negeri Sembilan, Johor, Sumatera and Borneo. The barks of *A. anisophyllus* are locally used as rope for backpacks, whereas the leaves can be used against boils and itch by burning and mixed with coconut oil [6].

1.2 Problem Statement

The phytochemical investigation reported in the review is mostly carried out on *Artocarpus* species of South-East Asia including Indonesia that has medicinal purposes and isolation of phenolic compounds. However, only a few phytochemical studies had been carried out on Malaysian *Artocarpus* species. Furthermore, thorough literature search did not reveal any report on the chemical constituents or biological activity of *A.*

anisophyllus Miq. Therefore, this research will focus on the phytochemical and bioactivity studies of *A. anisophyllus* Miq. native of Malaysia.

1.3 Research Objectives

The objective of this research is to determine the chemical constituents of *A. anisophyllus* Miq. This research will focus on the two parts of *A. anisophyllus* Miq. which are the heartwoods and stem barks. This research will involve extraction of the samples, fractionation and purification to obtain pure compounds and to identify their structures. In addition, the next objective is to evaluate the bioactivities of the crude extracts as well as the pure compounds.

1.4 Scopes of the Study

This research will focus on the investigation of chemical constituents and biological activities of the two parts of *A. anisophyllus* Miq which are the heartwoods, and stem barks. The dried samples of each part will be used and extracted using cold extraction in different polarity of solvents. The crude extracts will be fractionated by using vacuum liquid chromatography (VLC). Purifications of the fractions will be carried out by multiple silica gel or sephadex LH-20 gravity column chromatography (CC), prep-TLC and recrystallization to obtain the pure compounds. Structure elucidation of the pure compounds will be carried out using several spectroscopic methods, including UV, IR, 1D NMR (^{1}H, ^{13}C, DEPT), 2D NMR (COSY, HMQC, HMBC), and MS.

The crude extracts and isolated compounds will be screened for antioxidant and antimicrobial activities. The antioxidant activity will be performed using DPPH radical scavenging method. The antimicrobial activity will be tested using disc diffusion method

with Gram-positive bacteria strain, *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacteria strain, *Pseudomonas aeruginosa* and *Escherichia coli*.

REFERENCES

1. Sarker, S. D., Latif, Z. and Gray, A. I. *Natural Product: History Perspective in Natural Product Isolation*. 2nd Ed. Humana Press, New Jersey, pp. 4-5. 2006.

2. Canter, P. H., Thomas, H. and Ernst, E. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. *Trends in Biotechnology*. 2005. 23: 180-185.

3. Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D. and Guo, Z. Medicinal plants in therapy. *Bull. WHO*. 1985. 63: 965-981.

4. Ahmad, F. and Raji, H. *"Kimia Hasilan Semulajadi dan Tumbuhan Ubatan"*. Dewan Bahasa dan Pustaka. Kuala Lumpur. 11-12. 1993.

5. Cragg, G. M., Newmann, D. J. and Snader, K. M. Natural products in drug discovery and development. *J. Nat. Prod.* 1997. 60: 52-60.

6. Yaniv, Z. and Bachrach, U. *Handbook of Medicinal Plants*. Food Product Press, Oxford. 10-12. 2005.

7. Phil, F. S. P., Ng, D. O. and F. L. S. *Tree Flora of* Malaysia. 3rd ed. Forest Department: Ministry of Primary Industries Malaysia. 1978

8. Andersen, J., Nilsson, C., Richelieu, T., Fridriksdottir, H., Gobilick, J., Mertz, O.and Gausset, Q. Local Use of Forest Products In Kuyongon, Sabah, Malaysia. *ASEAN Review of Biodiversity and Environmental Conservation (ARBEC)*.2003. 1-18.

9. Jagtap, U. B. and Baptap, V. A. *Artocarpus*: A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacology*, 2010. 129: 142-166.

10. Verheij, E.W.M. and Coronel, R.E. *Plant Resources of South-East Asia No. 2. Edible Fruits and Nut*. PROSEA, Bogor, Indonesia: pp. 79-96. 1992

11. Heyne, K. *The useful Indonesian Plants*. Research and Development Agency, The Ministry of Forestry, Jakarta: pp. 659-703. 1987

12. Hakim, E. H., A., Y., Aimi, N., Kitajima, M. and Takayama, H. Artoindonesianin P, a new prenylated flavones with cytotoxicity from *Artocarpus lanceifolius. Fitoterapia.* 2002. 73: 668-673.

13. Sato, M., Fujiwara, S., Tscuhiya, H., Fuji, T., Iimuna, M., Tosa, H. and Ohkawa, Y. Flavones with antibacterial activity against cariogenic bacteria. *J. Ethnopharmacol*. 1996. 54: 171-176.

14. Toshio, F., Kazue, S., Taro, N. and Hiroshi, S. Antinephritis and radical scavenging activity of prenylflavonoids. *Fitoterapia*. 2003. 74: 720-724.

15. Wei, B. L., Weng, J. R., Chiu, P. H., Hung, C. F., Wang, J. P. and Lin, C. N. Anti inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J. Agric. Food Chem.* 2005. 53: 3867-3871.

16. Weng, J. R., Chan, S. C., Lu, Y. H., Lin, H. C., Ko, H. H. and Lin, C. N. Antiplatelet prenylflavonoids from *Artocarpus communis*. *Phytochemistry*. 2006. 67: 824-829.

17. Jagadeesh, S. L., Reddy, B. S., Swamy, G. S. K., Gorbal, K., Hedge, L. and Raghavan, G. S. V. Chemical composition of jackfruit (*Artocarpus heterophyllus* Lam.) selections of Western Ghats of India. *Food Chem*. 2006. 102: 361-365.

18. Hakim, E. H., Achmad, S. A., Juliawaty, L. D., Makmur, I., Syah, Y. M., Aimi, N., Kitajima, M. and Ghisalberti, E. I. Prenylated flavonoids and

related compounds of the Indonesian *Artocarpus* (Moraceae). *J. Nat. Med.* 2006. 60: 161-184.

19. Kabir, S. Jacalin: a jackfruit (*Artocarpus heterophyllus*) seed derived lectin of versatile applications in immunological research. *J. Immunological Methods*. 1998. 212: 193-211.

20. Ventakaraman, K. Wood phenolics in the chemotaxonomy of the Moraceae. *Phytochemistry*, 1972. 11:1571-1586.

21. Patil, A. D., Freyer, A. J., Killmer, L., Offen, P., Taylor, P. B.m Votta, B. J. And Johnson, R. K. A New Dimeric Dihydrochalcone and a New Prenylated Flavone from the Bud covers *of Artocarpus altilis*: Potent Inhibitors of Cathepsin K. *J. Nat Prod*, 2002. 65: 624-627.

22. Perry, L. M. *Medicinal Plants of East and South East Asia Attributed Properties and Uses*. MIT Press, Cambridge: 1980. pp. 113.

23. Wang, Y., Deng, T., Lin, I., Pan, Y. and Zheng, X. Bioassay guided isolation of anti-atherosclerotic phytochemicals from *Artocarpus altilis*. *Phytotherapy Research*, 2006. 20: 1052-1055.

24. Kochummen, K. M. and Go, R. *Artocarpus* J. R. & G. Forster. In: Soepadmo, E. and Saw L. G. (Eds). *Tree Flora flora of Sabah and Sarawak*. Vol. 3. Sabah Forestry Department, Forest Research Institute Malaysia and Sarawak Forestry Department, Kuala Lumpur, Malaysia: pp. 187. 2000

25. Salguero, C. P. *A Thai Herbal Traditional Recipe for Health and Harmony*. Findhorn Press, Scotland: 2003. pp. 119.

26. Charoenlarp, P., Radomyos, P. and Harinasuta, T. Treatment of taeniasis with Puag-Haad: a crude extract of *Artocarpus lakoocha* wood. The Southeast Asian. *J. Tropic. Med. and Public Health*, 1981. 12: 568-570.

27. Jamil, S., Sirat, H. M., Jantan, I., Aimi, N. and Kitajima, M. A new prenylated dihydrochalcone from the leaves of *Artocarpus lowii*. *J. Nat. Med.*, 2008. 62: 321-324.

28. Nomura, T., Hano, Y. and Aida, M. Isoprenoid-substitued flavonoids from *Artocarpus* plants (Moraceae). *Heterocycles*, 1998. 47: 1179-1205.

29. Soekamto, N. H., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H. and Syah, Y. M. Artoindonesianins X and Y, two isoprenylated 2-arylbenzofurans from *Artocarpus fretessi* (Moraceae). *Phytochemistry*, 2003. 64: 831-834.

30. Kurniadewi, F. and Hakim, E. H. Three phenolic compounds from the tree bark of *Artocarpus gomezianus* (Moraceae). *Bull. Soc. Nat. Prod* (*Indonesia*), 2003. 3: 78-83.

31. Lin, C. N., Lu, C. M. and Huang, P. L. Flavonoids from *Artocarpus heterophyllus*. *Phytochemistry*, 1995. 39: 1447-1451.

32. Hakim, E. H., Aripin, A., Achmad, S. A., Aimi, N., Kitajima, M., Makmur, L., Mujahidin, D., Syah, Y. M. and Takayama, H. Artoindonesianin E, a new flavanone derivative from *Artocarpus champeden*. *Proc ITB* (*Indonesia*), 2001. 33: 69-73.

33. Lu, C. M. and Lin C. N. Two 2′,4′,6′-trioxygenated flavanones from *Artocarpus heterophyllus*. *Phytochemistry*, 1993. 33: 909-911.

34. Ersam, T., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H. and Tamin, R. An isoprenylated flavonoids from *Artocarpus bracteata* Hook. *J. Mat. Sci.* (*Indonesia*), 1999. 4: 172-177.

35. Nair, P. M., Rama, R. A. V., Ventakaraman, K. Cycloartocarpin. *Tetrahedron Letter*. 1964. 2: 125-128.

36. Wang, Y.H., Hou, A. J., Chen, L., Chen, D. F., Sun, H. D., Zhao, Q. S., Bastow, K. F., Nakanishi, Y., Wang, X. H. and Lee, K. H. New isoprenylated

flavones, artochamins, A-E and cytotoxic principles from *Artocarpus chama*. *J. Nat. Prod.*, 2004. 67:757-761.

37. Lin, C. N. and Lu, C. M. Flavonoids and 9-hydroxytridecyl docosanoate from *Artocarpus heterophyllus*. *Phytochemistry*, 1994. 35(3): 781-783.

38. Chung, M. I., Lu, C. M., Huang, P. L. and Lin, C. N. Prenylflavonoids of *Artocarpus heterophyllus*. *Phytochemistry*, 1995. 40: 1279-1282.

39. Arung, T. E., Shimizu, K., Tanaka, H. and Kondo, R. 3-Prenyl luteolin, a new prenylated flavones with melanin biosynthesis inhibitory activity from wood of *Artocarpus heterophyllus*. *Fitoterapia*, 2010. 81: 640-643.

40. Arung, T. E., Shimizu, K., Tanaka, H. and Kondo, R. Isoprenoid-subtituted flavonoids from wood of *Artocarpus heterophyllus* on B16 melanoma cells: Cytotoxicity and structural criteria. *Fitoterapia*, 2010. 81: 120-123.

41. Achmad, S. A., Hakim, E. H., Juliawaty, L. D., Makmur, L. and Suyatno. A new prenylated flavones from *Artocarpus champeden*. *J. Nat. Prod.*, 1996. 59: 878-879.

42. Syah, Y. M., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L. and Mujahidin, D. Artoindonesianins Q-T, four new isoprenylated flavones from *Artocarpus champeden* Spreng. (Moraceae). *Phytochemistry*, 2002. 61: 949-953.

43. Syah, Y. M., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H. and Mujahidin. Two new cytotoxic isoprenylated flavones, artoindonesianins U and V, from the heartwood of *Artocarpus champeden* Spreng. (Moraceae). *Fitoterapia*, 2004. 75: 134-140.

44. Nomura, T. Phenolic compounds of the mulberry tree and related plants. *Fortschr. Chem. Org. Naturst*., 1988. 53: 87-201.

45. Lin, C. N. and Shieh, W. L. Pyranoflavonoids from *Artocarpus communis*. *Phytochemistry*, 1992. 31: 2922-2924.

46. Rao, A. V. R., Varadan, M. and Ventakaraman, K. Coloring matters of the wood of *Artocarpus heterophyllus*. VI. Cycloheterophyllin, flavones linked to three isoprenoid groups. *Indian J. Chem*., 1971. 9: 7-13.

47. Ajizah, A. *Some flavonoids from the heartwood of Artocarpus champeden.* PhD Thesis. Department of Chemistry, Bandung Institute of Technology. 2001.

48. Syah, Y. M., Juliawaty, L. D., Achmad, S. A., Hakim, E. H. and Ghisalberti, E. L. Cytotoxic prenylated flavones from *Artocarpus champeden*. *J. Nat. Med.* 2006. 60: 308-312.

49. Hakim, E. H., Fahriyati, A., Kau, M. S., Achmad, S. A., Makmur, L., Ghisalberti, E. L. and Nomura, T. Artoindonesianins A and B, two prenylated flavones from the root bark of *Artocarpus champeden*. *J. Nat. Prod*. 1999. 62: 613-615.

50. Rao, A. V., Rama, Rathi, S. S. and Ventakaraman, K. Chaplashin, a flavone containing an oxepine ring from the heartwood of *Artocarpus chaplasha*. *Indian J. of Chem*. 1972. 10(9): 905-907

51. Erwin, Achmad, S. A., Syah, Y. M., Aimi, N., Hakim, E. H., Kitajima, M., Makmur, L., Mujahidin, D. and Takayama, H. Artoindonesianin B, a cytotoxic compound against P388 tumor cells from *Artocarpus altilis*. *Bull. Soc. Nat. Prod. Chem. (Indonesia)*. 2001. 1: 20-27.

52. Syah, Y. M., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L. and Mujahidin, D. Artoindonesianin M, a new prenylated flavones from *Artocarpus champeden*. *Bull. Soc. Nat. Prod. Chem. (Indonesia).* 2002. 2: 31-36.

53. Hakim, E. H., Fahriyati, A., Achmad, S. A., Makmur, L., Mujahidin, D., Nomura, T. and Syah, Y. M. Artonin A, a furanodihydrobenzoxanthone

derivative from *Artocarpus champeden*. *J. Kimia Andalas (Indonesia).* 2000. 6: 72-76.

54. Widyawaruyanti, A., S., Kalauni, A. K., Awale, S., Nindatu, M., Zaini, N. C., Syafrudin, D., Asih, P. B. S., Tezuka, Y. and Kadota, S. New prenylated flavones from *Artocarpus champeden,* and their antimalarial activity in vitro. *J. Nat. Prod.* 2007. 61: 410-413.

55. Hakim, E. H., Achmad, S. A., Juliawaty, L. D., Makmur, L., Syah, Y. M., Aimi, N., Kitajima, M., Takayama, K. and Ghisalberti, E. L. Prenylated flavonoids and related compounds of the Indonesian *Artocarpus* (Moraceae). *J. Nat.Med.,* 2007. 61: 229.

56. Syah, Y. M., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., Makmur, L. and Mujahidin, D. Artoindonesianin G-I, three new isoprenylated flavones from *Artocarpus lanceifolius*. *Fitoterapia*. 2001. 72: 765-773.

57. Hakim, E. H., A., Y., Aimi, N., Kitajima, M. and Takayama, H. Artoindonesianin P, a new prenylated flavones with cytotoxicity from *Artocarpus lanceifolius. Fitoterapia.* 2002. 73: 668-673.

58. Syah, Y. M., Achmad, S. A., Aimi, N., Hakim, E. H., Juliawaty, L. D. and Takayama, H. Two prenylated flavones from the tree bark of *Artocarpus lanceifolius*. *Z. Naturforsch.* 2006. 61b: 1134-1137.

59. Achmad, S. A., Hakim, E. H., Juliawaty, L. D., Makmur, L. and Syah, Y. M. New natural products from Indonesian Moraceae. *International Science Congress.* Kuala Lumpur, Malaysia. 2005.

60. Cao, S., Butler, M. S. and Buss, A. Flavonoids from *Artocarpus lanceifolius*. *Nat. Prod. Res*. 2002. 17: 79-81.

61. Sultanbawa, M. U. S. and Surendrakumar, S. Two pyranodihydrobenzoxanthones from *Artocarpus nobilis*. *Phytochemistry,* 1989. 28: 599-605.

62. Jayasinghe, L., Balasooriya, B. A. I. S., Padmini, W. C., Hara, N. and Fujimoto, Y. Geranyl chalcone derivatives with antifungal and radical scavenging properties from the leaves of *Artocarpus nobilis*. *Phytochemistry*, 2004. 65: 1287-1290.

63. Jayasinghe, L., Rupasinghe, G. K., Hara, N. and Fujimoto, Y. Geranylated phenolic constituents from the fruits of *Artocarpus nobilis*. *Phytochemistry*, 2006. 67: 1353-1358.

64. Jayasinghe, U. L. B., Samarakoon, T. B., Kumarihamy, B. M. M., Hara, N. and Fujimoto, Y. Four new prenylated flavonoids and xanthones from the root bark of *Artocarpus nobilis*. *Fitoterapia*, 2008. 79: 37-41.

65. Han, A. R., Kang, Y. J., Windono, T., Lee, S. K. and Seo, E. K. Prenylated flavonoids from the heartwood of *Artocarpus communis* with inhibitory activity on lipopolysaccharide-induced nitric oxide production. *J. Nat. Prod.* 2006. 69: 719-721.

66. Weng, J. R., Chan, S. C., Lu, Y. H., Lin, H. C., Ko, H. H. and Lin, C. N. Antiplatelet prenylflavonoids from *Artocarpus communis*. *Phytochemistry*. 2006. 67: 824-829.

67. Shamaun, S. S., Rahmani, M., Hashim, N. M., Ismail, H. B. M., Sukari, M. A., Lian, G. E. C. and Go, R. Prenylated flavones from *Artocarpus altilis. J. Nat. Med.* 2010. 64:478-481.

68. Chan, S. C., Ko, H. H. and Lin, C. N. New prenylflavonoids from *Artocarpus communis*. *J. Nat. Prod.* 2003. 66: 427-430.

69. Wei, B. L., Weng, J. R., Chiu, P. H., Hung, C. F., Wang, J. P. and Lin, C. N. Anti inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J. Agric. Food Chem.* 2005. 53: 3867-3871.

70. Lin, K. W., Liu, C. H., Tu, H. Y., Ko, H. H., Wei, B. L. Antioxidant prenylflavonoids from *Artocarpus communis* and *Artocarpus elastiscus*. *Food Chem*. 2009. 10: 1016-1020.

71. Wang, Y., Xu, K., Lin, L. Pan, Y. and Zheng, X. Geranyl flavonoids from the leaves of *Artocarpus altilis. Phytochemistry.* 2007. 68: 1300-1306.

72. McLean, S., Reynolds, W. F., Tinto, W. F., Chan, W. R. and Shepherd, V. Complete ^{13}C and ^{1}H spectral assignments of prenylated flavonoids and hydroxyl fatty acid from the leaves of Caribbean *Artocarpus communis*. *Magn. Reson. Chem.* 1996. 34: 719-722.

73. Hsu, C. L., Shyu, M. H., Lin, J. A., Yen, G. C. and Fang, S. C. Cytotoxic effect of geranyl flavanoid derivatives from the fruit of *Artocarpus communis* in SK-Hep-1 human hepatocellular carcinoma cells. *Food Chem.* 2011. 127: 127-134.

74. Amarasinghe, N. R., Jayasinghe, L., Hara, N. and Fujimoto, Y. Chemical constituents of the fruits of *Artocarpus altilis*. *Biochem. system. and ecol.* 2008. 36: 323-325.

75. Musthapa, Iqbal., Juliawaty, L. D., Syah, Y. M., Hakim, E. I., Latip, J. and Ghisalberti, E. L. An oxepinaflavone from *Artocarpus elastiscus* with cytotoxicity activity against P-388 cells. *Arch. Pharm. Res.* 2009. 32(2): 191-194.

76. Makmur, L., S., T., Achmad, S. A., Aimi, N., Hakim, E. H., Kitajima, M. and Takayama, H. Artoindonesianin C, a new xanthone derivative from *Artocarpus tesymanii*. *J. Nat. Prod.* 2000. 63: 243-244.

77. Makmur, L., Syamsurizal, Tukiran, Syamsu, Y., Achmad, S. A., Aimi, N., Hakim, E. H., Kitajima, M., Mujahidin, D. and Takayama, H. Artonol B and cycloartobiloxanthone from *Artocarpus tesymanii* Miq. *Proc ITB Indonesia*. 1999. 31: 63-68.

78. Tukiran, Achmad, S. A., Makmur, L., Hakim, E. H. and Juliawaty, L. D. Cycloartobiloxanthone and artonin J from the tree bark of *Artocarpus tesymanii* Miq. (Moraceae). *J. Mat. Sci (Indonesia)*. 1999. 4: 156-163.

79. Suharti, T., Achmad, S. A., Aimi, N., Hakim, E. H., Kitajima, M., Takayama, H. and Takeye, K. Artoindonesianin L, a new prenylated flavones with cytotoxic activity from *A. rotunda*. *Fitoterapia.* 2001. 72: 912-918.

80. Suharti, T., Achmad, S. A., Aimi, N. and Hakim, E. H. Artonin E, a flavonoids from *Artocarpus rotunda*. *J. Mat. Sci (Indonesia).* 1999. 4: 178-184.

81. Hakim, E. H., Afrida, Eliza, Achmad, S. A., Aimi, N., Kitajima, M., Makmur, L., Mujahidin, D., Syah, Y. M. and Takayama, H. Artoindonesianin D, a new bioactive pyranoflavone derivatve and chaplashin from *Artocarpus maingayii*. *Proc ITB (Indonesia).* 2000. 32: 13-19.

82. Radwan, M. M., Guzman, R. R., Manly, S. P., Jacob, M. and Ross, S. A. Sepicanin A- A new geranyl flavanone from *Artocarpus sepicanus* with activity against methilicin-resistant *Staphylococcus aureus* (MRSA). *Phytochem. Lett.* 2009. 84: 1-3.

83. 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., Constituents of the Bark and Twigs of *Artocarpus dadah* with Cyclooxygenase Inhibitory Activity, *J. Nat. Prod*. 2002. 65: 163-169.

84. Puntumchai, A., Kittakoop, P., Rajviroongit, S., Vimuttipong, S., Likhitwitayawuid, K., Thebtaranonth, Y. Lakoochins A and B, New Antimycobacterial Stilbene Derivatives from *Artocarpus lakoocha*. *J. Nat. Prod*. 2004.67: 485-486.

85. Boonlaksiri, C., OOnanat, W., Kongsaeree, P., Kittakoop, P., Tanticharoen, M. and Thebtaranonth, Y. An antimalarial stilbene from *Artocarpus integer*. *Phytochemistry*. 2000. 54: 415-417.

86. Hakim, E. H., Ulinnuha, U. Z., Syah, Y. M. and Ghisalberti, E. L. Artoindonesianin N and O, new prenylated stilbene and prenylated

arylbenzofuran derivatives from *Artocarpus gomezianus*. *Fitoterapia*. 2002. 73: 597-603.

87. Soekamto, N. H., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H. and Syah, Y. M. Artoindonesianin X and Y, two isoprenylated 2-arylbenzofurans from *Artocarpus fretessi* (Moraceae). *Phytochemistry*. 2003. 64: 831-834.

88. Hano, Y., Aida, M., and Nomura, T., Two New Natural Diels-Alder Type Adducts from The Root Bark of *Artocarpus heterophyllus*, *J. Nat. Prod*. 1990. 53: 391-395.

89. Shinomiya, K., Aida, M., Hano, Y., and Nomura, T., A Diels-Alder Type Adduct from *Artocarpus heterophyllus*, *Phytochemistry*, 1995. 40: 1317-1319.

90. Ko, F. N., Cheng, Z. J., Lin, C. N. and Teng, C. M. Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Rad. Bio. and Med.* 1998. 25: 160-168.

91. Cerqueira, F., Cidade, H., Van Ufford, I., Beukelman, C., Kijjoa, A. and Nascimento, M. S. The natural prenylated flavones artelastin is an inhibitor of ROS and NO production. *Inter*. *Immunopharm*. 208. 8: 597- 602.

92. Bakar, M. F. A., Mohamed, M., Rahmat, A. and Fry, J. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissmus*). *Food Chem*. 2009. 113: 479-483.

93. Khan, M. R., Omoloso, A. D. and Kihara, D. Antibacterial activity of *Artocarpus heterophyllus*. *Fitoterapia*. 2003. 74:501-505.

94. Arung, E. T., Wicaksono, B. D., Handoko, Y. A., Kusuma, I. W., Shimizu, K., Yulia, D. and Sandra, F. Cytotoxic effect of artocarpin on T47D cells. *J. Nat. Med.* 2010. 64: 423-429.

95. Mahato, S. B., Banerjee, S. K. and Chakvanarti, R. N. Triterpenes from stem bark of *Artocarpus chaplasha*. *Phytochemistry*. 1971. 10, pp: 1351-1354.

96. Markam, K. R. *Techniques of Flavonoid Identification*. London: Academic Press. 1982.

97. Wu, T. S., Wang, M. L., Shyur, H. J., Leu, Y. L., Chan, Y. Y., Teng, C. M. and Kuo, S. C. Chemical constituents and bioactive principles from the root bark of *Dictamnus dasycarpus*. *Chinese Pharm. Journal*. 1994. 46(5): 1015-1016.

98. Russel, A. D. and Chopra, I. Understanding Antibacterial Action and Resistance, Second Edition, Hertfordshire: Ellis Horwood Ltd, 1996.

99. Kunhan, J. The Flavonoids. A Class of Semi-Essential Food Components; Their Role in Human Nutrition. *World Review of Nutrition and Dietetics.* 1976. 24: 117-191.

100. Shahidi, F. and Zhong, Y. *Measurement of Antioxidant Activity in Food and Biological Systems*, In Antioxidant Measurement and Applications, ACS Symposium Series 956, Edited by F. Shahidi and C-T. Ho, United States of America: American Chemical Society, 2007.

101. Cook, N. C. and samman, S. Flavonoids – Chemistry, Metabolism, Cardioprotective Effects and Dietary Sources. *Nutritional Biochem*. 1996. 7: 66-76.

102. Ramamoorthy, P. K. and Bono, A. Antioxidant Activity, Total Phenolic and Flavonoid Content of *Morinda citrifolia* Fruit extracts from various Extraction Processes. *J. Eng. Sci. Tech*. 2007. 2(1): 70-80.

103. Mbaveng, A. T., Ngameni, B., Kuete, V., Simo, I. K., Ambassa, P., Roy, R., Bezabih, M., Etoa, F. X., Ngadjui, B. T., Abegaz, B. M., Meyer, J. J. M., Lall, N. and Beng, V. P. Antimicrobial Activity of the Crude Extract and Five

Flavonoids from the Twigs of *Dorstenia barteri* (Moraceae). *J. Ethnopharmacol*. 2008. 116: 483-489.

104. Tagashira, M. and Ohtake, Y. A. New Antioxidative 1,2-Benzodioxole from *Melissa officianalis*. *Planta Med*. 1998. 64: 555 – 558.