UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS, ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF Aglaia oligophylla MIQ. AND Kaempferia angustifolia ROSC.

YUNIE YEAP SOON YU

FS 2018 38

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YUNIE YEAP SOON YU

DOCTOR OF PHILOSOPHY

UNIVERSITI PUTRA MALAYSIA

2017

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By

YUNIE YEAP SOON YU

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

November 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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November 2017

Chairman : Nur Kartinee Binti Kassim, PhD
Faculty : Science

Natural products are chemical substances produced by plants, microbial sources and other living organisms. Some natural products are used for medicinal purpose due to their distinctive pharmacological active properties. *Aglaia oligophylla* Miq. belong to the genus *Aglaia* under the Meliaceae family whilst *Kaempferia angustifolia* Rosc. is a species from the genus of *Kaempferia* under the Zingiberaceae family. The genera are recognized for their versatile medicinal properties including antioxidant, anti-malarial, antiviral, antimicrobial and anti-tumor. However, there are limited studies on cytotoxicities and antioxidant properties of *A. oligophylla* and *K. angustifolia*. Therefore, the objective of the studies is to investigate the phytochemistry, antioxidant and cytotoxic properties of *A. oligophylla* and *K. angustifolia*.

The elucidation and assignation of structure of the pure compound were carried out using spectroscopic methods such as UV (Ultra-violet), IR (Infrared Spectroscopy), NMR (Nuclear Magnetic Resonance), and MS (Mass Spectrometry) and as well as comparison with published data. Column chromatographic separation and purification of trunks and stem bark of *A. oligophylla* furnished three new compounds and three know compounds. The trunks of *A. oligophylla* afforded two new compounds identified as 20*S*,24*R*-epoxy-3-oxo-A-dinor-5α-dammaran-31-al (oligophyllin) (**101**) and 5-hydroxy-7-methoxy-2-[(5'*S*,6'*S*,8'*S*,11'*R*)-12'-(5',6',8',9'-tetramethyl-11'-hydroxycyclonon-9'-enyl)-phenyl]-4*H*-1-benzopyran-4-one (oligolin A) (**102**) whilst the stem of *A. oligophylla* obtained 20*S*,24*S*-epoxy-25-hydroxy-4-propyl-2-secodammarane-2-oic acid (oligophyllic acid) (**105**) as new compound. Meanwhile, three known compounds namely cabraleone (**104**), β-sitosterol (**98**) and stigmasterol (**103**) were isolated from the trunks and stem bark of *A. oligophylla*. Phytochemical investigaton on rhizomes of *K. angustifolia* afforded six known compounds which were boesenboxide (**89**), crotepoxide (**94**), flavokawain A (**92**), kaempfolienol (**96**), β-sitosterol (**98**) and zeylenol (**95**). Various chromatography techniques such as thin layer, gravity column and centrifugal thin layer have been used to isolate these compounds.

The extracts and chemical constituents of the plants were evaluated for antioxidant potential using *in vitro* assays such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP) and β-carotene-linoleate bleaching assays. The comparison of the antioxidant properties of the extracts from *A. oligophylla* in DPPH assay revealed that the methanol extract from the stem bark showed the strongest antioxidant property. The ethyl acetate extract of the trunks of *A. oligophylla* exhibited the strongest antioxidant property among the extracts in ABTS and CUPRAC assays with the values of 96.97 and 1696.48 mg TE/g respectively whilst the methanol extract from the trunks showed the strongest antioxidant activity among the extracts in FRAP assay with the value of 1312.03 mg TE/g. The comparison of the antioxidant properties of the extracts and chemical constituents from *A. oligophylla* in β-carotene-linoleate bleaching assay indicated ethyl acetate extract from the trunks of the plant showed the strongest antioxidant property with the value of 88.77 %. The comparison of the antioxidant properties of the extracts and chemical constituents from *K. angustifolia* revealed that the methanol extract presented the strongest antioxidant in DPPH assay with the value of 607.29 mg TE/g whilst flavokawain A (**92**) showed the strongest antioxidant property in ABTS, CUPRAC and FRAP assays with the values of 42.01, 1470.19 and 780.77 mg TE/g respectively. Meanhwile, boesenboxide (**89**) showed the strongest antioxidant property in β-carotene-linoleate bleaching assay with the value of 98.31 %.

The extracts and phytochemicals of both plants were also subjected to cytotoxicity screening against hormone dependent human breast adenocarcinoma (MCF-7), hormone independent human breast adenocarcinoma (MDA-MB-231) and Swiss mouse embryo fibroblast (3T3-L1) cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Characterisation of cellular morphology was done using inverted light microscope. The chloroform extract from the trunks of *A. oligophylla* showed the strongest cytotoxicity against MCF-7 cell line with the IC_{50} value of 35.45 µg/mL whilst oligolin A (**102**) from *A. oligophylla* displayed the strongest cytotoxic effect against MDA-MB-231 cell line with the IC_{50} value of 14.23 ± 0.48 µg/mL. The oligolin A (**102**)-treated MDA-MB-231 cell underwent morphological change such as cell shrinkage, nuclear compaction and membrane blebbing which further support the cytotoxicity result of the compound. Oligolin A (**102**) showed insignificant cytotoxicity against 3T3-L1 cell line. The flavokawain A (**92**) from *K. angustifolia* showed the strongest cytotoxicity against MCF-7 and MDA-MB-231 cell lines with IC_{50} values of 53.98 and 22.48 µg/mL respectively. The results pinpointed the potential compounds isolated from *A. oligophylla* and *K. angustifolia* as the possible candidates for the breast cancer treatment and therapy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SEBATIAN KIMIA, SIFAT ANTIOKSIDAN DAN SITOTOKSIK DARIPADA *Aglaia oligophylla* Miq. DAN *Kaempferia angustifolia* Rosc.

Oleh

YUNIE YEAP SOON YU

November 2017

Pengerusi : Nur Kartinee Binti Kassim, PhD
Fakulti : Sains

Produk semula jadi ialah bahan kimia yang dihasilkan oleh tumbuh-tumbuhan, sumber mikroorganisma dan organisma hidupan yang lain. Sesetengah produk semula jadi digunakan untuk tujuan perubatan disebabkan ciri-ciri aktif farmakologi mereka yang tersendiri. *Aglala oligophylla* Miq. tergolong dalam genus *Aglaia* dalam famili Meliaceae manakala *Kaempferia angustifolia* Rosc. merupakan salah satu spesies dari genus *Kaempferia* di bawah famili Zingiberaceae. Genera tersebut dikenali dengan ciri-ciri perubatan yang luas termasuk antioksidan, anti-malaria, anti-virus, anti-mikrobia dan anti-tumor. Walaubagaimanapun, kajian tentang kesitotoksikan dan antioksidan dalam *A. oligophylla* dan *K. angustifolia* adalah terhad. Oleh itu, objektif dalam kajian ini adalah untuk menyiasat fitokimia, sifat antioksidan dan sitotoksik *A. oligophylla* dan *K. angustifolia*.

Struktur sebatian tersebut ditentukan melalui kaedah spektroskopi seperti UV (Ultralembayung), IR (Inframerah Spektroskopi), NMR (Resonans Magnet Nukleus) dan MS (Spektrometri jisim) dan juga perbandingan dengan data terbitan. Kerja pemisahan dan penulenan pada batang dan pangkal *A. oligophylla* memperolehi tiga sebatian baru dan tiga sebatian yang telah dikenali. Batang *A. oligophylla* memberi dua sebatian baru yang dikenali sebagai 20*S*,24*R*-epoksi-3-okso-A-dinor-5α-dammaran-31-al (oligofilin) (**101**) dan 5-hidroksi-7-metoksi-2-[(5'*S*,6'*S*,8'*S*,11'*R*)-12'-(5',6',8',9'-tetrametil-11'-hidroksisiklonon-9'-enil)-phenil]-4*H*-1-benzopiran-4-on (oligolin A) (**102**) manakala pangkal *A. oligophylla* memberi 20*S*,24*S*-epoksi-25-hidroksi-4-propil-2-sekodammaran-2-oik asid (oligofilic asid) (**105**) sebagai sebatian baru. Sementara itu, tiga sebatian yang telah dikenali iaitu cabraleon (**104**), β-sitosterol (**98**) dan stigmasterol (**103**) telah didapati daripada batang dan pangkal *A. oligophylla*. Penyiasatan fitokimia pada rizom *K. angustifolia* memberi enam sebatian yang telah dikenali iaitu boesenboksida (**89**), krotepoksida (**94**), flavokawain A (**92**), kaempfolienol (**96**), β-sitosterol (**98**) and zeylenol (**95**). Pelbagai teknik kromatografi seperti kromatografi lapisan nipis, kromatografi kolum gravity dan kromatografi emparan lapisan nipis telad digunakan untuk mengasingkan sebatian tersebut.

Antioksidan ekstrak dan sebatian tersebut dinilaikan dengan ujian *in vitro* seperti asai 1,1-difenil-2-pikrilhidrazil (DPPH), azinobis (3-etil-benzothiazoline-6-sulfonik asid) (ABTS) , kuprik penurunan kapasiti antioksidan (CUPRAC), ferik penurunan kuasa antioksidan (FRAP) dan β-karotena-linoleat pelunturan asai. Perbandingan sifat antioksidan antara ekstrak dari *A. oligophylla* dalam DPPH asai mendedahkan ekstrak metanol dari pangkal *A. oligophylla* menunjukkan sifat antioksidan yang paling kuat. Ekstrak etil asetat batang *A. oligophylla* menunjukkan sifat antioksidan yang paling kuat di kalangan ekstrak yang lain dalam ABTS dan CUPRAC asai dengan nilai 96.97 dan 1696.48 mg TE/g masing-masing manakala ekstrak metanol dari batang menunjukkan aktiviti antioksidan yang paling kuat di kalangan ekstrak yang lain dalam FRAP asai dengan nilai 1312.03 mg TE/g. Perbandingan sifat antioksidan antara ekstrak dan sebatian dari *A. oligophylla* dalam β-karotena-linoleat pelunturan asai menunjukkan ekstrak etil asetat dari batang tumbuh-tumbuhan menunjukkan sifat antioksidan yang paling kuat dengan nilai 88.77 %. Perbandingan sifat antioksidan antara ekstrak dan sebatian dari *K. angustifolia* mendedahkan ekstrak metanol sebagai antioksidan yang paling kuat dalam DPPH asai dengan nilai 607.29 mg TE/g manakala flavokawain A (**92**) menunjukkan antioksidan yang terkuat dalam ABTS, CUPRAC, FRAP dan β-karotena-linoleat pelunturan asai dengan nilai 42.01, 1470.19 and 780.77 mg TE/g masing-masing. Sementara itu, boesenboksida (**89**) menunjukkan sifat antioksidan yang terkuat dalam β-karotena-linoleat pelunturan asai dengan nilai 98.31 %.

Kajian kesitotoksikan terhadap dua jenis sel kanser payudara manusia (MDA-MB-231 dan MCF-7) dan sel normal tikus Swiss (3T3-L1) juga dijalankan pada ekstrak dan sebatian yang didapati daripada species tumbuh-tumbuhan tersebut dengan menggunakan 3-(4,5-dimetilthiazol-2-il)-2,5-dipheniltetrazolium bromid (MTT) asai. Pencirian dan pemerhatian morfologi sel telah dijalankan dengan menggunakan mikroskop. Ekstrak kloroform daripada batang *A. oligophylla* menunjukkan sitotoksik akitiviti terkuat terhadap sel MCF-7 dengan nilai IC_{50} 35.45 µg/mL manakala oligolin A (**102**) daripada *A. oligophylla* menunjukkan kesan sitotoksik yang paling kuat terhadap sel MDA-MB-231 dengan nilai IC_{50} 14.23 ± 0.48 µg/mL. Sel MDA-MB-231 yang dirawat dengan oligolin A (**102**) mengalami perubahan morfologi seperti pengecutan sel, pemadatan nuclear dan pelepuhan membran menyokong data hasil kesitotoksikan tersebut. Oligolin A (**102**) menunjukkan kesitotoksikan yang rendah terhadap sel 3T3-L1. Flavokawain A (**92**) daripada *K. angustifolia* menunjukkan kesitotoksikan terhadap sel-sel MCF-7 dan MDA-MB-231 dengan nilai IC_{50} 53.98 and 22.48 µg/mL masing-masing. Data hasil kajian tersebut menunjukkan sebatian yang didapati daripada *A. oligophylla* dan *K. angustifolia* berpotensi untuk dikembang dan digunakan dalam rawatan terapi kanser payudara.

ACKNOWLEDGEMENTS

Apart from the efforts of me, the success of any research depends largely on the encouragement and guidelines of many others. I take this opportunity to express my profound gratitude and deep regards to my guide (Dr Nur Kartinee Binti Kassim) for her guidance, constant encouragement and monitoring throughout the whole course of this thesis research. Her willingness to encourage me contributed tremendously to the research. The blessing and help that given by her from time to time shall carry me a long way in the journey of life on which I am about to embark. I would like to express my gratitude and appreciation to my co-supervisors, Prof. Gwendoline Ee Cheng Lian and Assoc. Prof. Dr. Latifah Saiful Yazan for their precious advices during the research study as well as Prof Dr. Mohd Aspollah Bin Hj. Sukari for providing the plant sample and guidance throughout the research.

I would like to express my appreciation to the staff and postgraduates from Cell Signaling Laboratory for providing facilities and useful advices in cytotoxicity screening and testing. I would like to thanks to Science Officers Mr. Johadi Iskandar, Ms. Sharina, Mr. Fazley, Madam Rusnani Amirudin and Mr. Zainal Kassim.

I would also like to take this opportunity to express a deep sense of gratitude to my mentors, Noorul Adawiyah Mustahil, Halimatul Saadiah Mohd Noor, Sadikah Ahmad and Faiqah Ramli for their cordial support, valuable information for laboratory skills and technique of running spectroscopic analysis, guidance and experience which helped me in completing the project throughout the various stages.

Last but not least, I thank my parents for their financial support; my beloved, my sisters and friends for their constant encouragement without which this research study would not be possible.

I certify that a Thesis Examination Committee has met on 14 November 2017 to conduct the final examination of Yunie Yeap Soon Yu on her thesis entitled "Chemical Constituents, Antioxidant and Cytotoxic Properties of *Aglaia oligophylla* Miq. and *Kaempferia angustifolia* Rosc." in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Khozirah binti Shaari, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Emilia binti Abd Malek, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Muhajir bin Hamid, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Ikhlas Khan, PhD
Professor
University of Mississippi
United States
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 February 2018

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

Nur Kartinee Binti Kassim, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Gwendoline Ee Cheng Lian, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Latifah Saiful Yazan, PhD
Associate Professor
Faculty of Medicine
Universiti Putra Malaysia
(Member)

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A	Absorbance
Ace	Acetone
α	Alpha
LNCaP	Androgen-Dependent Human Prostate Adenocarcinoma Cell Line
DU145	Androgen-Independent Human Prostate Adenocarcinoma Cell Line
PC3	Androgen-Independent Human Prostate Adenocarcinoma Cell Line
AA	Antioxidant Activity
ABTS	Azinobis (3-Ethyl-Benzothiazoline-6-Sulfonic Acid)
β	Beta
br	Broad
BHT	Butylated Hydroxytoluene
C	Carbon
^{13}C	Carbon-13
CO_2	Carbon Dioxide
δ	Chemical Shift
$CHCl_3$	Chloroform
R^2	Coefficient of Determination
c	Concentration
COSY	Correlation Spectroscopy
J	Coupling Constant
$CuCl_2$	Cupric Chloride
Cu^{2+}	Cupric Ion
CUPRAC	Cupric Reducing Antioxidant Capacity
Cu^{+}	Cuprous Ion

DR	Degradation Rate
°C	Degree in Celcius
DNA	Deoxyribonucleic Acid
$CDCI_3$	Deuterated Chloroform
CD_3OD	Deuterated Methanol
D	Dextrorotatory
Et_2O	Diethyl Ether
DMSO	Dimethyl Sulfoxide
DPPH	1,1-Diphenyl-2-Picrylhydrazyl
DIP	Direct-Induction Probe
d	Doublet
dd	Doublet of Doublets
Et_2O	Diethyl Ether
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
DEPT	Distortionless Enhancement by Polarization Transfer
EtOH	Ethanol
EI-MS	Electron Impact – Mass Spectrometry
eV	Electronvolt
MCF-7	Estrogen-Dependent Human Breast Adenocarcinoma Cell Line
MDA-MB-231	Estrogen-Independent Human Breast Adenocarcinoma Cell Line
EtOAc	Ethyl Acetate
EDTA	Ethylenediaminetetraacetic Acid
$FeCl_3$-$6H_2O$	Ferric Chloride Hexahydrate
Fe^{3+}	Ferric Ion
FRAP	Ferric Reducing Antioxidant Power

Fe(III)-TPTZ	Ferric-Tripyridyltriazine
Fe^{2+}	Ferrous Ion
GCMS	Gas Chromatography Mass Spectrometry
$^{2}J_{CH}$	Geminal Carbon-Proton Coupling Constant
$^{2}J_{HH}$	Geminal Proton-Proton Coupling Constant
g	Gram
GCC	Gravity Column Chromatography
IC_{50}	Half Maximal Inhibitory Concentration
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteonuclear Multiple Quantum Correlation
Hz	Hertz
Hex	Hexane
HR-ESI-MS	High Resolution-Electrospray Ionization-Mass Spectrometry
HeLa	Human Cervical Adenocarcinoma Cell Line
HT-29	Human Colorectal Adenocarcinoma Cell Line
SGC-7901	Human Gastric Adenocarcinoma Cell Line
HepG2	Human Hepatocellular Liver Carcinoma Cell Line
SMMC-7721	Human Hepatocellular Carcinoma Cell Line
HIV	Human Immunodeficiency Virus
Lu1	Human Lung Adenocarcinoma Cell Line
A-549	Human Lung Carcinoma Epithelial Cell Line
HL-60	Human Promyelocytic Leukemia Cell Line
HUVEC	Human Umbilical Vein Endothelial Cell Line
HCl	Hydrochloric Acid
Trolox	6-Hydroxy-2,5,7,8-Tetramethylchroman-2-Carboxylic Acid

IR	Infrared
kg	Kilogram
LC-MS	Liquid Chromatography-Mass Spectrometry
L	Liter
Lit.	Literature
Log	Logarithm
$^4J_{CH}$	Long-Range Carbon-Proton Coupling Constant
$^4J_{HH}$	Long-Range Proton-Proton Coupling Constant
$^4J_{HH}$(*meta*)	Long-Range Proton-Proton (*Meta*) Coupling Constant
m/z	Mass over Charge Ratio
MS	Mass Spectrometry
λ_{max}	Maximum Wavelength
ν_{max}	Maximum Wavenumber
MHz	Mega Hertz
m.p.	Melting Point
P388	Menogaril-Resistant Mouse Leukemia Cell Line
MeOH	Methanol
µg	Microgram
µL	Microliter
µM	Micromolar
mg	Milligram
mL	Milliliter
mM	Millimolar
min	Minute
ε	Molar Absorptivity

M	Molar Mass
M^+	Molecular Ion
m	Multiplet
nm	Nanometer
In	Natural Log
Nc	Neocuproine
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
1-D	One-Dimensional
$[\alpha]_D^{20}$	Optical Rotation in the Sodium D Line Region at 20℃
$[\alpha]_D^{25}$	Optical Rotation in the Sodium D Line Region at 25℃
ppm	Part Per Million
%	Percent
PCV	Percentage of Cell Viability
PET	Petroleum Ether
PBS	Phosphate Buffered Saline
Π	Pi
KBr	Potassium Bromide
$K_2S_2O_8$	Potassium Persulfate
pH	Potential of Hydrogen
p	Probability Value
H	Proton
^1H	Proton-1
q	Quartet
RNS	Reactive Nitrogen Species

ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
s	Singlet
Na	Sodium
NaCI	Sodium Chloride
SD	Standard Deviation
H_2SO_4	Sulfuric Acid
3T3-L1	Swiss Mouse Embryo Fibroblast Cell Line
TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
t	Triplet
TPTZ	2,4,6-Tris(2-Pyridyl)-s-Triazine
TE	Trolox Equivalent
2-D	Two-Dimensional
UATR	Universal Attenuated Total Reflection
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
$^3J_{CH}$	Vicinal Carbon-Proton Coupling Constant
$^3J_{HH}$(*cis*)	Vicinal Proton-Proton (*Cis*) Coupling Constant
$^3J_{HH}$	Vicinal Proton-Proton Coupling Constant
$^3J_{HH}$(*ortho*)	Vicinal Proton-Proton (*Ortho*) Coupling Constant
$^3J_{HH}$(*trans*)	Vicinal Proton-Proton (*Trans*) Coupling Constant
v/v	Volume/Volume
λ	Wavelength
w/v	Weight/Volume

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CHAPTER 1

INTRODUCTION

Natural products are naturally occurring chemical substances produced mostly by terrestrial type of plants and animals, microbial sources and other living organisms. Some of the natural products exert distinctive pharmacological activities with wide applicability and minimum side effects (McMurry, 2014). Therefore, the study of chemistry and bioactivity in natural product has been the major driving force in the development of organic and medicinal chemistry which lead to the drug discovery (Rahman, 2012).

Species of *Aglaia* such as *Aglaia odorata* Lour., *Aglaia silvestris* (M. Roemer) Merr. and *Aglaia foveolata* Pannell have showed promising bioactivities such as antioxidant, cytotoxic, tumoricidal, insecticidal and bactericidal activities over the past decade. Several species have been used as heart stimulants and treatments of coughing, injuries and diarrhoea by locals (Proksch et al., 2001). Some of the species have drawn attention of researchers due to their prominent insecticidal activities, which were suggested to be linked to the structure of the rocaglamide derivative, the characteristic phytochemical of the species such as *Aglaia oligophylla* Miq. Rocaglamide derivatives showed considerable interest as they may afford novel lead structures with interesting biological activities (Dreyer et al., 2001; Proksch et al., 2001).

Kaempferia species are commonly used as spice and traditional medicine by locals. Previous studies of *Kaempferia* species showed interesting bioactivities such as anti-malarial, antiviral, antimicrobial, anti-tumour, anti-cancer and antioxidant activities. Cyclohexane oxide derivatives are the chemical constituents that commonly found in *Kaempferia* species. For examples, *Kaempferia angustifolia* Rosc. and *Kaempferia rotunda* Linn. are the species with abundance of cyclohexane oxide derivatives which showed interesting bioactivities (Stevenson et al., 2007).

Based on literature review of *A. oligophylla*, a few phytochemical studies have been done on the twigs and leaves of the plant but no phytochemical study was reported on the stem bark and trunks of *A. oligophylla*. Triterpenoids, flavaglines and bisamides were obtained from the twigs and leaves parts of *A. oligophylla* with some of the compounds exhibited insecticidal properties (Joycharat et al., 2008; Bringmann et al., 2003; Dreyer et al., 2001). Zingiberaceae are well-known with their medicinal properties. The species of the family have been used widely for medicinal purpose by locals such as *Kaempferia galanga* Linn. and *K. angustifolia*. Previous reports showed triterpenoids, flavonoids and cyclohexane oxide derivatives were isolated from *K. angustifolia* with prominent cytotoxicities against several cancer cell lines (Tang et al., 2014).

As mentioned above, limited studies have been done on cytotoxic activities and antioxidant properties of *A. oligophylla* and *K. angustifolia*. Meanwhile, previous studies on *Aglaia* and *Kaempferia* species discovered numerous of natural products as potential antioxidant and anticancer agents such as *A. odorata*, *A. silvetris*, *A. foveolata*, *Aglaia forbesii* King, *Kaempferia ethelae* J. M. Wood, *K. galanga* and *K. rotunda* (Brown, 2002; Wang et al., 2001; Greger, 2000). Meanhwile, the application of antioxidants in cancer treatments has been well practiced for a period of time. Cancer treatments such as chemotherapy, immunotherapy and radiotherapy produce free radicals which induce fragmentation of DNA and biological molecules that lead to adverse effects (Vanessa, 2013). The correlation between antioxidant property and anticancer effect has led to the application of antioxidants as prescription medicines in some of the cancer treatments (Simone et al., 2007). Hence, antioxidants are encouraged in cancer treatments to reduce the side effects. Therefore, further investigation on phytochemistry and biological activities of *A. oligophylla* and *K. angustifolia* were planned with the aim of obtaining potential antioxidant and cytotoxic compounds from the plant species.

Breast cancer is the second most common cancer in the world. It is also the most prevalent cancer in woman population (Basile et al., 2016). The progressive improvements in breast cancer treatments still failed to resolve the potential toxicities and adverse effects from the drug consumption in the treatments. Drugs with enhanced efficacy and reduced side effects are the primary goal of pharmaceutical industry to overcome breast cancer disease (Ozcelik et al., 2010; Chatterjee et al., 2009; Zachariae, 1990). Previous studies on natural products of plant origin had revealed the potency of natural products as anti-breast cancer agents with minimum side effects (Roleira et al., 2015). Therefore, the study of cytotoxicity of the *A. oligophylla* and *K. angustifolia* were focus on breast cancer cell lines.

In our attempt to isolate phytochemicals from plant extracts, various column chromatographic techniques were incorporated into the isolation procedure. The structures of phytochemicals were elucidated by various spectroscopic methods (UV, IR, NMR and MS) and comparison with literature reviews. The antioxidant properties of phytochemicals and plant extracts were evaluated using DPPH, ABTS, CUPRAC, FRAP and β-carotene-linoleate bleaching assays whilst the cytotoxicities of the phytochemicals and plant extracts were determined using MTT assay.

The objectives of the study were to:

i. Isolate and purify the pure compounds from *A. oligophylla* and *K. angustifolia*.
ii. Elucidate the chemical structure structures of pure compounds.
iii. Evaluate the antioxidant activities of the extracts and pure compounds isolated from *A. oligophylla* and *K. angustifolia*.
iv. Evaluate the cytotoxic activities of the extracts and pure compounds isolated from *A. oligophylla* and *K. angustifolia* against breast cancer cell lines

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