UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL COMPOUNDS FROM Calophyllum buxifolium VESQUE, Calophyllum depressinervosum M.R. HEND. & WYATT-SM. AND Morinda citrifolia L. AND THEIR ANTI-PROLIFERATIVE ACTIVITY

NOR HISAM ZAMAKSHSHARI

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By

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

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PHYTOCHEMICAL COMPOUNDS FROM *Calophyllum buxifolium* VESQUE, *Calophyllum depressinervosum* M.R. HEND. & WYATT-SM. AND *Morinda citrifolia* L. AND THEIR ANTI-PROLIFERATIVE ACTIVITY

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October 2017

Chairman : Professor Gwendoline Ee Cheng Lian, PhD
Faculty : Science

Plants have been used in folk medicines for treatment of diseases. The stem bark of *Buxifolium, Calophyllum depressinervosum and Morinda citrifolia* were studied for their phytochemical and biological activities. Different chromatographic methods were used to isolate and purify pure compounds from these three plants species. The structural elucidations of these pure compounds were achieved through FT-IR, UV-Vis, GC-MS, 1D and 2D NMR. Extensive phytochemical studies on three different plants species which are *Buxifolium vesque, Calophyllum depressinervosum and Morinda citrifolia* have resulted in the isolation of 30 compounds of various classes such as xanthones, coumarins, kavalactones, flavonoids, terpenes and anthraquinone. *Morinda citrifolia* gave twelve anthraquinones. Meanwhile, *Calophyllum depressinervosum* and *Calophyllum buxifolium* gave 12 and 6 phenolic compounds, respectively. Surprisingly, desmethoxyyangonin (**88**) from the kavalactone class was found in *Calophyllum depressinervosum*. This is the first report on the existence of kavalactones in *Calophyllum* species.

This research reports the new anti-proliferative activities of the extracts and isolated compounds against three cancer cell lines which are SNU-1 (stomach cancer), LS174T (colon cancer) and K562 (leukemia). The hexane extract of *Calophyllum depressinervosum* showed strong inhibition activity against SNU-1 with an IC_{50} value of 9.50 µg/ml. Meanwhile, the hexane extract of *Calophyllum buxifolium* gave strong inhibition activities against LS174T and K562 cell lines. Caloxanthone B (**16**) gave the highest inhibition activity compared to the other compounds against SNU-1 and K562 with IC_{50} values of 1.47 µg/ml and 1.23 µg/ml, respectively. For LS174T, xanthochymone B (**86**) gave the highest inhibition activity with an IC_{50} value of 0.45 µg/ml.

Structural modifications were successfully carried out on two major compounds (ananixanthone (**83**) and thwaitesixanthone (**21**)) isolated from *Calophyllum depressinervosum* and *Calophyllum buxifolium* respectively and this resulted in 5 new synthesis compounds. The acetylation on ananixanthone (**83**) gave two new synthesised compounds which are ananixanthone monoacetate (**98**) and ananixanthone diacetate (**99**). Meanwhile, the acetylation on thwaitesixanthone (**21**) resulted in another new synthesised compound thwaitesixanthone monoacetate (**102**). Methoxyananixanthone (**100**) and O-benzylananixanthone (**101**) were successfully synthesised from ananixanthone (**83**) through methylation and benzylation reactions. These synthesised compounds were also screened for cytotoxic activity against the SNU-1, LS174T and K562 cell lines to check for any increase in inhibition activity after modification. Unfortunately, only methoxyananixanthone (**100**) had better inhibition towards LS174T cell line compared to ananixanthone (**83**) with an IC_{50} value of 5.76 µg/ml.

Antioxidant activity tests on the crude extracts were carried out using five different assays, DPPH, beta-carotene bleaching assays, and ferric reducing antioxidant power (FRAP) assay, nitric oxide scavenging activity and ferrous ion chelating. In the DPPH assay, the methanol extract of *Calophyllum depressinervosum* gave good antioxidant activity with an EC_{50} value of 16.02 µg/mL. Meanwhile, the extract with the highest percentage of β-carotene bleaching and comparable to the standard drug was the ethyl acetate extract of *Calophyllum depressinervosum* which is 72.89% at 100 µg/mL. The FRAP assay that showed the methanol extract from *Calophyllum buxifolium* possess the highest FRAP value which is 8.19 GAE compared to other extracts. Good nitric oxide scavenging activity was shown by the dichloromethane extract of *Morinda citrifolia.* Last but not least, the highest chelating effect was shown by the dichloromethane extract of *Calophyllum depressinervosum* with a percentage of 40.42% at 500 µg/ml.

Molecular docking study was attempted to elucidate the mechanisms by which the active compounds could induce anti-proliferative activities in SNU-1, K562 and LS174T. The molecular docking technique was used to model the binding interaction between active compounds and responsible protein chosen from each cancer cell. The responsible protein chosen for SNU-1, K562 and LS174T were HER2, Src and β-catenin respectively. The molecular docking studies showed that all the active compounds could bind well with the responsible protein and could act as anti-cancer inhibitors.

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

FITOKIMIA KOMPONEN DARI *Calophyllum Buxifolium, Calophyllum Depressinervosum Vesque, Calophyllum Depressinervosum* M.R. HEND. & WYATT-SM DAN *Morinda Citrifolia* L. DAN ANTI-PROLIFERATIF AKTIVITI

Oleh

NOR HISAM ZAMAKSHSHARI

Oktober 2017

Pengerusi : Profesor Gwendoline Ee Cheng Lian, PhD
Fakulti : Sains

Tumbuhan telah digunakan sebagai ubat-ubatan sejak zaman dahulu untuk mengubati pelbagai penyakit. Tiga jenis spesies dinamakan *Calophyllum depressinervosum, Calophyllum buxifolium* dan *Morinda citrifolia* yang banyak tumbuh di Malaysia telah dipilih bagi kajian fitokimia dan biologi. Teknik kromatografi yang berbeza telah digunakan untuk mengektrak sebatian tulen daripada ketiga-tiga jenis spesies yang telah dipilih. Semua sebatian tulen telah dikenalpasti dengan mengunakan analisis spektroskopi seperti FT-IR, UV-Vis, GC-MS, 1D dan 2D NMR. Kajian fitokimia secara terperinci terhadap tiga jenis tumbuhan iaitu *Calophyllum depressinervosum, Calophyllum buxifolium* dan *Citrifolia morinda* telah menghasilkan tiga puluh sebatian tulen terdiri daripada xanton, coumarin, flavonol kavalaktone, triterpenoid dan antrakuinon. *Citrifolia morinda* telah menghasilkan dua belas sebatian tulen jenis antrakuinon. Manakala, *Calophyllum depressinervosum* dan *Calophyllum buxifolium* masing-masing telah menghasilakan dua belas dan enam sebatian tulen. Sebatian desmethoxyyangonin (**88**) daripada kumpulan kavalaktone telah dijumpai didalam *Calophyllum depressinervosum*. Ini merupakan kajian pertama yang membuktikan kewujudan kumpulan kavalakton didalam Calophyllum species.

Kajian ini akan membentangkan tentang pertemuan baru mengenai anti-proliferatif aktiviti keatas semua ekstrak dan sebatian tulen terhadap tiga jenis sel kanser iaitu SNU-1 (kanser perut), LS174T (kanser kolon) dan K562 (kanser darah). Hasil daripada kajian ini, ekstrak heksana daripada *Calophyllum depressinervosum* telah menujukkkan aktiviti perencatan yang paling tinggi terhadap SNU-1dengan nilai bacaan IC_{50} pada 9.50 µg/ml. Manakala, ekstrak heksana daripada *Calophyllum buxifolium* telah memberikan aktiviti perencatan yang paling tinggi terhadap LS174T dan K562. Caloxanthone B (**16**) telah memberikan aktiviti perencatan yang tinggi terhadap SNU-1 dan K562 berbanding

sebatian tulen yang lain dengan nilai bacaan IC_{50} masing-masing pada 1.47 μg/ml dan 1.23 μg/ml. Bagi LS174T, xanthochymone B (**86**) telah menunjukkan aktiviti perencatan yang paling tinggi dengan nilai bacaan IC_{50} pada 0.45 μg/ml.

Modifikasi struktur telah berjaya dilaksanakan terhadap dua sebatian tulen (ananixantone (**83**) dan thwaitesixantone (**21**)) hasil pengekstrak daripada *Calophyllum depressinervosum* dan *Calophyllum buxifolium* dan menghasilkan 5 sebatian baru. Tindak balas pengasetilan terhadap ananixantone (**83**) telah menghasilkan dua komponen baru iaitu ananixantone monoasetil (**98**) dan ananixantone dwiasetil (**99**). Sementara itu, tindak balas asetilasi terhadap thwaitesixanthone (**21**) telah menghasilkan satu komponen baru iaitu thwaitesixantone monoasetil (**102**). Metoksiananixantone (**100**) dan benzilananixantone (**101**) telah berjaya dihasilkan daripada tindak balas metilasi dan benzoilasi. Kajian anti-proliferatif telah dijalankan keatas semua modifikasi komponen terhadap SNU-1, LS174T dan K562 bagi memeriksa sebarang peningkatan dalam aktiviti perencatan. Malangnya, hanya metoksiananixanthone (**100**) mempunyai peningkatan keatas aktiviti perencatan terhadap LS174T berbanding ananixantone (**83**) dengan nilai IC_{50} pada 5.76 μg/ml.

Keupayaan anti-pengoksidaan untuk semua ekstrak telah dijalankan dengan menggunakan lima jenis ujian yang berbeza iaitu DPPH, Pelunturan β-karotena, mengurangkan kuasa anti-pengoksidatan ferik (FRAP), aktiviti memerangkap oksida nitrik dan pengkhelatan ion besi. Extrak metanol daripada *Calophyllum depressinervosum* telah memberikan aktiviti yang tinggi terhadap perencatan radikal DPPH dengan bacaan EC_{50} pada 16.02 μg/ml. Sementara itu, ekstrak yang mempunyai peratusan β-karotena yang tinggi dan setanding dengan agen umum adalah ektrak etil asetat daripada *Calophyllum depressinervosum* dengan peratusan sebanyak 72.89% pada 100 μg/ml. Ekstrak metanol daripada *Calophyllum buxifolium* menunjukkan nilai FRAP yang tinggi berbanding ekstrak yang lain dengan bacaan 8.19 GAE. Didalam ujian aktiviti memerangkap oksida nitrik, diklorometana ekstrak daripada *Morinda citrifolia* telah menunjukkan nilai bacaan yang tinggi berbanding ekstrak lain. Akhir sekali, kesan pengkhelatan ion besi tertinggi telah ditunjukkan oleh ekstrak diklorometana daripada *Calophyllum depressinervosum* dengan peratusan sebanyak 40.42% pada 500 μg/ml.

Kajian terhadap melekular docking telah dijalankan untuk menjelaskan mekanisme anti-proliferatif yang mendorong sebatian tulen keatas aktiviti perencatan bagi SNU-1, LS174T dan K562. Teknik ini telah digunakan bagi menunjukkan model interaksi antara sebatian aktif dan protien yang bertangungjawab pada setiap jenis sel kanser. Protien bagi SNU-1 ialah HER2, manakala untuk LS174T ialah β-catenin dan K562 ialah Src. Hasil kajian molekular docking telah menunjukkan bahawa kesemua sebatian aktif boleh bertindak balas dengan protien yang bertanggungjawab dan bertindak sebagai perencat anti-kanser.

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I certify that a Thesis Examination Committee has met on 31 October 2017 to conduct the final examination of Nor Hisam binti Zamakshshari on her thesis entitled "Phytochemical Compounds from *Calophyllum buxifolium* Vesque, *Calophyllum depressinervosum* M.R. Hend. & Wyatt-Sm. and *Morinda citrifolia* L. and their Anti-Proliferative Activity" 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.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

s	singlet
d	doublet
dd	doublet of doublet
t	triplet
m	multiplet
NMR	Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass Spectra
EIMS	Electron Ionization Mass Spectrometer
UV	Ultra Violet
FTIR	Fourier Transform Infrared Spectrometer
COSY	Correlated Spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HMQC	Heteronuclear Multiple Quantum Correlation
TLC	Thin Layer Chromatography
ATCC	American Type Culture Collection
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Power
NO	Nitric Oxide
TPC	Total Phenolic Content
TFC	Total Flavonoids content
MTT	Micro Culture Tetrazolium Salt
RPMI	Roswell Memorial Park Institute

MEM	Eagle's Minimum Essential Media
FBS	Fetal Bovine Serum
PDB	Protein Data Bank
ATP	Adenosine Triphosphate
MMFF	Merck Molecular Force Field
DS	Discovery Studio

CHAPTER 1

INTRODUCTION

1.1 General Introduction

One of the important discussions in science is the study on natural products from plants. Plants are significantly important for human beings such as for respiration, food, medicine and many more. Plants have been used in medicine for a long time by human civilization in treatments to cure diseases. Previous scientific studies have shown that several therapeutic properties of plants that are usually used in old folk medicines have contributed to the development of drugs which are obtained from isolation from plants (Filho *et al.*, 2009).

Unfortunately, due to pressure from overexploitation and global climatic change, many tree species have become extinct. IUCN estimated that numerous species of plants are disappearing every day, at an estimated rate of 0.1%-0.01% per year, and another 17291 species is estimated to be critically endangered or at the verge of extinction (Tringali, 2014). This scenario has led to a decrease in earth biodiversity, hence reducing the availability of chemodiversity. For example, the genus *Papaver*(poppies) only has 50 species worldwide (Tringali, 2014). This species produces alkaloids of the morphine-type which has been used for thousands of years and is still one of the most valuable drugs known to mankind. If the destroying of natural habitats is still continued and plant species are allowed to go extinct before they could be explored, maybe some of the precious biologically active natural products will be missed.

Around half of the drugs in clinical use are from natural products (Peterson and Anderson, 2005). Synthetic drugs that have limited success due to adverse side effects make compounds from nature products with minimal toxicity gain much popularity as potential drug candidates (Manigandan *et al.*, 2015). Natural products such as secondary metabolites once considered unimportant have now become important as potential drug candidates (Culter and Culter, 2000). Secondary metabolites are compounds not involved in the normal growth, development, or reproduction of an organism but play an important role in plant defense against herbivory and other interspecies defenses.

Kurt (1926) reported that work on finding, separating, purifying and analyzing compounds from plants started in the eighteen centuries when modern science took place. Many techniques were introduced such as column chromatography, gas chromatography, thin layer chromatography and many more in order to determine the identities of compounds obtained from plants. Nowadays, most of these techniques have been upgraded and a lot of new techniques introduced in order for chemists to elucidate compound structures.

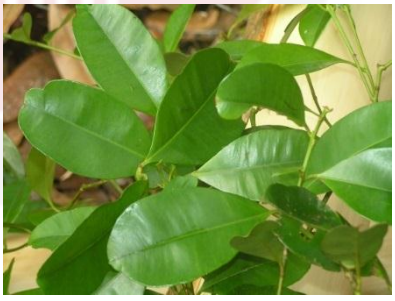
1.2 The Genus Calophyllum

The genus *Calopyllum* belongs to the Clusiaceae family. This *Calophyllum* genus comprises 180-200 tree species distributed in the tropical rain forests with some occurring in Malaysia (Filho *et al.*, 2009). In Malaysia, it consists of 80 species with 5 genera (Corner, 1952). This genus consists of many common trees of the lowland and mountain forests. They are one of the forest-trees easy to recognize; from the characteristic veining of the tough leaves through the leaves that vary considerably in size. The Malay name for this genus is *Bintagor* (Corner, 1952).

Calophyllum depressinervosum is one *Calophyllum* species that grows in Malaysia. It can be found abundantly in Pahang and Negeri Sembilan southward (Whitmore, 1973). It is locally known as bintagor lekok. It is a big tree reaching 35m tall and nearly 2m girth without buttresses. The bark color of this species is yellow-orange and grey-brown. Meanwhile, the inner bark surface is clear with golden-syrup color, slightly resinous exudate. The twigs are slender and usually appear as dark grey color. The stalk for the leaves is normally short about 8mm. It is slender and the blade is small. (Whitmore, 1973).

Calophyllum buxifolium is a higher plant mainly distributed in Philippines, Malaysia and Brunei. It can grow up to 20 to 25 meters tall. The bark is brown in color and consists of diamond-shape or boat shape (Whitmore, 1973). The inner bark of this species is orange brown and contents latex. The leave is elliptic. The flower is inflorescence consisting of 3 to 5 petal 7mm wide. Meanwhile, the fruit is green in color and 2.5 cm long and 1.8cm wide. (Stevens , 1980).

(**A**)



(**B**)

Figure 1.1 : A) The leaves of *Calophyllum depressinervosum, B)* The leaves of *Calophyllum buxifolium*

1.3 The genus *Morinda*

The genus *Morinda* belongs to the Rubiaceae family. This genus consists of about 80 species, mostly of Old World origin (Samoylenko *et al.,* 2006). In Malaysia, *Morinda* comprises nine species which include three species of trees and six species of climber. The tree species of *Morinda* are *Morinda citrifolia, Morinda elliptica* and *Morinda corneri.* Meanwhile the climber species are *Morinda lacunosa, Morinda rigida, Morinda calciphila, Morinda scortechinii, Morinda umbellata* and *Morinda ridleyi* (Wong, 1984).

Morinda citrifolia is known as *mengkudu* by locals in Malaysia. It is a small tree three to eight meters tall. The leaves are simple with an opposite arrangement along the branches that consist of 20 to 45 cm long and 7 to 25cm wide. Meanwhile, the flower is white in colour and can been seen in an ovoid to round flower head consisting of 70 to 90 flowers. The fruit of *Morinda citrifolia* is yellowish-white in colour and roughly cone like in shape that is 3 to 10 cm long and 2 to 3cm wide. The fruit it soft and have a strong smell. This species grow on wide range of soils and harsh environmental condition like brackish tide pools, lime stone soils or outcroppings on coral atolls (Selvan, 2007).

Figure 1.2 : The Fruits and Flowers of *Morinda* species

1.4 Molecular docking

Today, there are several methods used to assist in the drug discovery process which are in-vivo, in-vitro and in-silico. A combination of these three methods will speed up the drug discovery process hence increasing accuracy in finding the exact lead candidate. Molecular docking one of the in-silico methods that been uses for decades to study protein-ligand interaction (Leach *et al.,* 2006). By using pre-calculated search algorithm embedded in docking software, plausible ligand-target structures is generated, together with a scoring function that is represented in the form of binding energies or affinity

between ligand and target. Evaluation on the scoring function and relevant binding interactions allows identification of the most favourable protein-ligand complex structures (Eric *et al.,* 2012). There are several molecular docking software available such as AutoDock, DOCK, FlexX and GOLD (Chen, 2015). The difference between these software is the algorithm used when predicting ligand conformations and their scoring function. In this work, AutoDock Vina was used as a docking tool to position selected compound to determine the probable binding model. The scoring function in AutoDock Vina was based on the binding energy calculated from hydrophobic interaction (van der Waals), hydrogen bond and torsional penalty (Chang *et al.,* 2010). The search algorithm used in AutoDock Vina is a gradient-based local search (Trott and Olson, 2010). The AutoDock Vina was chosen in this work because of its speed and accuracy of docking (Trott and Olson, 2010). The resultant docked structures were manually inspected and evaluated in term of their protein-ligand interaction such as hydrogen bond and hydrophobic interaction between protein receptor and ligand. The hydrogen bond and hydrophobic interaction which are essential for binding peptide, secondary and tertiary protein structure (kubinyi, 2001). The binding of targeted ligand on a protein receptor was reported to alter the function or characteristic of protein receptor (Gohlke *et al.,* 2000).

1.5 Problem Statement

Nowadays, cancer has become a major public health problem worldwide. Many chemotherapeutic drugs have been developed based on organic or inorganic compounds. However, the commercial chemotherapeutic drugs available could lead to severe side-effects resulting in function loss of human organs. For instance, the use of cisplatin as a chemotherapeutic drug will lead to kidney and liver damage. This had led to the use of plants as a source in drug discovery due to their less cytotoxicity. Three species from two different genus were selected in this research due to their medicinal properties. *Calophyllum* genus had been used in old folk medicine to treat several diseases such as peptic ulcer, malaria, tumor, infection, blood pressure, pain and inflammation (Gomez verjan *et al.,* 2005). Similarly, for *Morinda* species which is used in traditional medicine for treatment of diabetics, hyper tension and cancer (Kamiya *et al.,* 2004). This is due to these two genus being an important source of active metabolites, mainly coumarins, xanthones, flavonoids, chromones, triterpenes, anthraquinone and so on. All the chemical constituents from *Calophyllum* and *Morinda* species were tested against three cancer cell lines which are stomach, colon and leukemia cancers with the hope of finding new potential candidates for chemotherapeutic drugs. Besides, this research will also determine the phytochemicals in the two-new species of *Calophyllum* which are *Calophyllum depressinervosum* and *Calophyllum buxifolium.*

1.6 Objective of Studies

Calophyllum and *Morinda* species are known for their anti-proliferative and anti-oxidant activities. These activities are contributed by the chemical constituents existing in the plant species. Two new *Calophyllum* species which were *Calophyllum depressinervosum* and *Calophyllum buxifolium* together with *Morinda citrifolia* were selected for phytochemical and biological studies. Besides isolation, structurally modified isolated compounds might also contribute to an increase the anti-proliferative activities. Molecular docking was used to understand the mechanism of drug inhibition in molecular level. Hence, the objective of this project was to research for lead compound which are drug candidates in cancer treatment. Below are the specific objectives needed to fulfil the study.

1. To isolate, identify and characterize compounds from *Calophyllum* and *Morinda* species using spectroscopic analyses.
2. To screen and evaluate the anti-proliferative activities of the crude extracts and pure compounds from *Calophyllum and Morinda* species against stomach, colon and leukemia cell lines.
3. To determine the correlations between the phytochemical analysis (TPC and TFC) and antioxidant and anti-proliferative activities of the plant extracts.
4. To carry out structural modifications of major compounds and determine their anti-proliferative activities toward stomach, colon and leukemia cell lines.
5. To simulate the binding interaction model between the active compounds and protein receptor using molecular docking.

LIST OF PUBLICATIONS

Zamakshshari, N. H., Ee, G. C. L., Teh, S. S., Daud, S., Karunakaran, T., and Ismail, I. S., (2016) Natural product compounds from *Calophyllum depressinervosum*. Pertanika Journal of Tropical Agricultural Science, 39:249-255.

Zamakshshari, N. H, Mah, S. H., Ee, G. C. L., Ibrahim, Z., Teh, S. S., and Daud Shaari. (2017). Cytotoxic Activities of Anthraquinones from *Morinda citrifolia* towards SNU-1, LS-174T and K562 Cell Lines. Archives of Natural and Medicinal Chemistry, volume 20017, Issue 02, 1-27.

Lee, K. W., Zamakshshari, N.H., Ee, G. C. L., Mah, S. H, and Mohd Nor, S. M., Isolation and Structural Modifications of Ananixanthone from *Calophyllum teysmannii* and their Cytotoxic Activities. Natural product research, 2017

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