UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL AND BIOLOGICAL ACTIVITIES OF ALPINIA MUTICA, BOESENBERGIA ARMENIACA (ZINGIBERACEAE) AND AGLAIA VARIISQUAMA (MELIACEAE)

NOORUL ADAWIYAH BINTI MUSTAHIL

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By

NOORUL ADAWIYAH BINTI MUSTAHIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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

PHYTOCHEMICAL AND BIOLOGICAL ACTIVITIES OF *ALPINIA MUTICA*, *BOESENBERGIA ARMENIACA* (ZINGIBERACEAE) AND *AGLAIA VARIISQUAMA* (MELIACEAE)

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January 2014

Chairman : Professor Dr. Mohd. Aspollah Sukari, PhD
Faculty : Science

Phytochemical study on the rhizomes of *Alpinia mutica* afforded four compounds; a kavalactone, 5,6-dehydrokawain **(6)** as a major constituent and the flavonoids; flavokawin B **(12)**, pinostrobin **(33)** and pinocembrin **(9)** together with β-sitosterol **(14)** while, isolation work on the rhizome extracts of *Boesenbergia armeniaca* afforded two pyrone compounds; 6-aryl-4-methoxy-2-pyrone **(93)** and 6-*cis*-styryl-4-methoxy-2-pyrone **(94)**. Compounds **(93)** and **(94)** were new to the species and this is a first report on their structural elucidation using 2D NMR spectroscopy. Similar work on the leaves of *Aglaia variisquama* yielded two triterpene compounds; lupenone **(95)** and lupeol **(96)** together with β-sitosterol **(14)** and this is a first report on phytochemicals of the leaves of *A. variisquama*.

The crude hexane and chloroform extracts of the rhizomes of *A. mutica* showed significant cytotoxicity against HT-29 (human colon) cancer cells with IC_{50} values less than 15 µg/mL. The crude hexane extract also showed significant cytotoxic activity against MCF-7 (human breast) cancer cells with an IC_{50} value of 16.10 µg/mL. Flavokawin B **(12)** was the most cytotoxic constituent against HT-29 and MCF-7 cancer cells with an IC_{50} value of 4.68 µg/mL and 5.18 µg/mL, respectively. Besides, compounds **(6)**, **(33)** and **(9)** also showed significant cytotoxicity against HT-29 and MCF-7 cancer cells with an IC_{50} value ranging from 6 to 13 µg/mL, except for pinocembrin **(9)** which exhibited weak cytotoxicity against MCF-7 cancer cells. The cytotoxicity of the isolates of the rhizomes of *A. mutica* against human cancer cell lines using MTT assay is firstly reported here.

All the rhizome extracts of *B. armeniaca* demonstrated significant cytotoxicity against HeLa (human cervical) cancer cells with an IC_{50} value ranging from 16 to 18 µg/mL, except for the methanol extract which was inactive. The crude ethyl acetate extract was the most cytotoxic against MCF-7 cancer cells with an IC_{50} value of 1.10 µg/mL. Moreover, compounds **(93)** and **(94)** showed significant cytotoxicity against MCF-7 cancer cells with an IC_{50} value of 16.70 µg/mL and 14.10 µg/mL, respectively. All the crude extracts of the leaves of *A. variisquama* showed significant cytotoxicity against HeLa cancer cells with IC_{50} values less than 15 µg/mL while, its methanol extract exhibited weak cytotoxicity. Here, the cytotoxic activities of compounds **(93)** and **(94)** as well as the crude extracts of the leaves of *A. variisquama* against human cancer cells are firstly reported.

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

KAJIAN FITOKIMIA DAN AKTIVITI BIOLOGI BAGI *ALPINIA MUTICA*, *BOESENBERGIA ARMENIACA* (ZINGIBERACEAE) DAN *AGLAIA VARIISQUAMA* (MELIACEAE)

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Kerja pemencilan terhadap rizom *Alpinia mutica* telah menghasilkan empat sebatian iaitu 5,6-dehydrokawain **(6)**, flavokawin B **(12)**, pinostrobin **(33)** dan pinocembrin **(9)** bersama dengan β-sitosterol **(14)**. Manakala, kajian fitokimia terhadap rizom *Boesenbergia armeniaca* telah menghasilkan 6-aryl-4-methoxy-2-pyrone **(93)** dan 6-*cis*-styryl-4-methoxy-2-pyrone **(94)**. Pemencilan sebatian **(93)** dan **(94)** daripada rizom *B. armeniaca* serta penentuan struktur kimia sebatian tersebut menggunakan teknik NMR 2D adalah pertama kali dilaporkan di sini. Kerja pemencilan yang serupa terhadap daun *Aglaia variisquama* menghasilkan sebatian lupenone **(95)**, lupeol **(96)** dan β-sitosterol **(14)**, adalah pertama kali dilaporkan di sini.

Ekstrak heksana dan klorofom bahagian rizom *A. mutica* mempamerkan aktiviti sitotoksik yang signifikan terhadap sel kanser HT-29 dengan nilai IC_{50} kurang daripada 15 µg/mL. Ekstrak heksana juga menunjukkan aktiviti sitotoksik yang signifikan melawan sel kanser MCF-7 dengan nilai IC_{50} 16.10 µg/mL. Flavokawin B **(12)** adalah sebatian yang paling aktif melawan sel kanser HT-29 dan MCF-7 dengan nilai IC_{50} 4.68 µg/mL dan 5.18 µg/mL, masing-masing. Selain itu, sebatian **(6)**, **(33)** dan **(9)** turut menunjukkan aktiviti sitotoksik yang signifikan terhadap sel kanser HT-29 dan MCF-7 dengan nilai IC_{50} antara 6 hingga 13 µg/mL, manakala pinocembrin **(9)** mempamerkan aktiviti yang lemah melawan sel kanser MCF-7. Aktiviti sitotoksik oleh ekstrak rizom dan sebatian yang dipencilkan daripada *A. mutica* terhadap sel kanser manusia menggunakan kaedah MTT adalah pertama kali dilaporkan di sini.

Kesemua ekstrak bahagian rizom *B. armeniaca* telah menunjukkan aktiviti sitotoksik yang signifikan melawan sel kanser HeLa dengan nilai IC_{50} antara 16 hingga 18 µg/mL, kecuali ekstrak metanol yang tidak aktif. Bagi sel kanser MCF-7, ekstrak etil asetat adalah paling aktif dengan nilai IC_{50} 1.10 µg/mL. Di samping itu, sebatian **(93)** dan **(94)** telah mempamerkan aktiviti sitotoksik yang signifikan melawan sel kanser MCF-7 dengan nilai IC_{50} 16.70 µg/mL dan 14.10 µg/mL, masing-masing. Kesemua ekstrak bahagian daun *A. variisquama* pula mempamerkan ketoksikan yang signifikan terhadap sel kanser HeLa dengan nilai IC_{50} kurang daripada 15 µg/mL, kecuali ekstrak metanol yang lemah. Aktiviti sitotoksik untuk sebatian **(93)** dan **(94)** serta ekstrak daun *A. variisquama* melawan sel-sel kanser manusia adalah pertama kali dilaporkan di sini.

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I certify that a Thesis Examination Committee has met on 13 January 2014 to conduct the final examination of Noorul Adawiyah binti Mustahil on her thesis entitled "Phytochemical and Biological Activities of *Alpinia mutica, Boesenbergia armeniaca* (Zingiberaceae) and *Aglaia variisquama* (Meliaceae)" 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 Master of Science.

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

AU	Absorbance units
α	alpha
β	beta
δ	chemical shift in ppm
ppm	parts per million
^{1}H	proton
^{13}C	carbon-13
DEPT	Distortionless Enhancement by Polarization Transfer
COSY	Correlation Spectroscopy
HMQC	Heteronuclear Multiple Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
Hz	Hertz
GC-MS	Gas Chromatography-Mass Spectrometry
IR	Infrared
mm	milimetre
g	gram
kg	kilogram
J	coupling constant in Hertz
m/z	mass per charge
MeOD	deuterated methanol
$CDCl_3$	deuterated chloroform
MS	mass spectrometry
DMSO	dimethyl sulfoxide
m.p.	melting point
NMR	Nuclear Magnetic Resonance
s	singlet
d	doublet
m	multiplet
dd	doublet of doublet
TLC	Thin Layer Chromatography
µg	microgram
KBr	Potassium Bromide
µL	microlitre
mL	mililitre
M^{+}	Molecular ion
MHz	Mega Hertz
mg	miligram
CC	Column Chromatography
1D	one-dimensional
2D	two-dimensional
IC_{50}	concentration that yield 50 % inhibition
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide

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CHAPTER ONE

INTRODUCTION

1.1 Natural Products and Their Importance

Natural products can be defined as any products which being produced naturally by living organisms either by plants, animals or microorganisms. Natural products are categorized into two major groups namely primary metabolites (for example: amino acids, proteins, nucleic acids, lipids and carbohydrates) where they are used by all living organisms to control certain functions such as for their growth and development whereas, secondary metabolites can be classified into several major groups based on their chemical structures such as alkaloids, steroids, terpenes and phenolic compounds (Ahmad and Raji, 1993).

However, in most cases the term natural products refers to secondary metabolites which are small molecules with molecular weight less than 2000 atomic mass unit (amu) produced by an organism that are not strictly necessary for the survival of the organism (Sarker *et al.*, 2006). According to Sarker *et al*. (2006), natural products will certainly continue to be considered as one of the major sources of new drugs in the years to come because they offer incomparable structural diversity and have "drug-like" properties (as they can be absorbed and metabolized).

Numerous natural products possess therapeutic values and being used to promote health beyond basic nutrition. Sarker *et al*. (2006) also pointed out that natural products can contribute to the search for new drugs in several ways and one of them is being used directly in an unmodified state for example, vincristine isolated from *Catharanthus roseus* which is well known on its strong anticancer property. Besides, Ahmad and Raji (1993) summarized the importance of natural products research on medicinal field by a direct use of compounds which have been isolated from plants, as therapeutic agent. For examples, morphine from *Papaver somniferum*, atropine from *Atropa belladonna* and digitoxin from *Digitalis purpurea*. Plants products can also be used as starting material in the synthesis of many kinds of drugs. For instance, steroid hormone like adrenal cortex hormone is normally synthesized from sapogenin steroid. Natural products also play as an active pharmacology model in the synthesis of certain drugs. This may be due to the certain plant products with therapeutic values which cannot be used directly because of their side effects.

Many contemporary drugs have been originally discovered from natural sources through the traditional uses since ages ago by indigenous people for treating various diseases. An intensive study on plants used traditionally by folk people such as 'Tongkat Ali' (*Eurycoma longifolia*) has led to the isolation of compound names eurycomalactone and its derivatives as antimalarial agents; 'Seruntum/Putarwali' (*Tinospora crispa*) gave palmatine which can be used to treat hypertension and 'Letup-letup' (*Physalis minima*) gave physalin B, an antitumor agent (Ahmad and Raji, 1993).

There will always be a progress in the finding of new drugs equivalent to the increasing of population and diversity of ailments in addition of resistance of certain existing drugs to some diseases and microorganisms such as bacteria and fungi. Even though there are new sources and approaches in the drug discovery and development process, natural products still maintain their important role as one platform and basis in providing many diverse chemical entities with variety of medicinal values. These are true where natural products and natural product-derived compounds have been selected as drug candidates to undergo clinical trial and several phases of drug discovery processes.

Cragg *et al*. (1997) stated that among 92 anticancer drugs commercially available prior to 1983 in the United States and among worldwide approved anticancer drugs between 1983 and 1994, 60% are of natural origin. A review by Butler (2004), described that in the year 2000, natural products and natural product derivatives comprised 14 of the top 35 drugs (based on worldwide sales). Moreover, there has also been a steady introduction of new natural products and natural products-derived drugs in the United States, Europe and Japan from 2000 to 2003. A total of 15 drugs were launched (one in 2000, four in 2001, five in 2002 and five in 2003). Recent review by Mishra and Tiwari (2011) stated that a total of 19 natural products based drugs were approved for marketing worldwide in between the year 2005 to 2010, among which 7 are classified as natural products, 10 as semi-synthetic natural products and 2 as natural product-derived drugs.

1.2 Plant Species Introduction

1.2.1 Zingiberaceae

Zingiberaceae family or well known as ginger family consists of about 1,200 species of which 1,000 are found in tropical Asia (Larsen *et al*., 1999). The ginger species are basically rhizomatous, aromatic herbs ranging in size from as small as 15 cm to as tall as 5 m. Majority of the species grow in the wild and prefer shaded and moist habitats, for example *Alpinia katsumadai* grows wildly in Southern China mainly in Hainan, Guangxi and Guangdong provinces (Yuan *et al*., 2009). The Zingiberaceae family are widely used in traditional medicines with variety of therapeutic values, as flavouring agents and also as a source of certain dyes. Larsen *et al.* (1999) stated many studies have shown that at least more than ten cultivated species of Zingiberaceae have been used frequently in traditional medicine.

According to Habsah *et al*. (2003), the plant species from several genus of the ginger family such as *Alpinia*, *Zingiber*, *Curcuma* and *Kaempferia* are among the most often used as ingredients in traditionally prepared health supplements, tonics and ointments. Tushar *et al.* (2010) pointed out that Zingiberaceae can be established as a medicinal plant family by reporting that 41 % of the total 88 plant species from Zingiberaceae from the Northeast India have been found to possess medicinal properties.

Rhizomes of ginger plants have been widely used as spices or condiments (Larsen *et al*., 1999), eaten raw or cooked as vegetables and used for flavouring foods, and also taken as carminatives for relieving flatulence. In addition, they are also consumed by

women during ailment, illness and confinement (Chan *et al*., 2008). Powder of rhizomes of *Curcuma longa* or turmeric is commonly used as a dietary pigment and spice. It has also been used traditionally in Asian medicine for the treatment of inflammation, skin wounds and hepatic disorders, coughing and certain tumours (Sumiyoshi and Kimura, 2009).

Besides rhizomes, the leaves of ginger plants have been used for foods flavouring. For example, in Malaysia the leaves of *Curcuma longa* are used to wrap fish before steaming or baking (Larsen *et al*., 1999) and the combination of leaves of *Curcuma longa* and *Kaempferia galangal* are ingredients of curries (Chan *et al*., 2008). According to Ibrahim (1999), the leaves of *Kaempferia rotunda* and *Kaempferia galangal* are eaten fresh or cooked as vegetables, and used as cosmetic powder also as food flavouring agents. In Okinawa (Japan), leaves of *Alpinia zerumbet* are sold as herbal tea and commonly used to flavour noodles and to wrap rice cakes. Leaves of *Elettariopsis latiflora* have been used to relieve flatulence, to improve appetite and as an antidote to poisons while in Thailand while boiled leaves of *Hedychium coronarium* are applied to relieve stiff and sore joints (Chan *et al.*, 2008).

1.2.1.1 Genus *Alpinia*

Alpinia is a large, widespread and taxonomically complex genus in the Zingiberaceae family with 230 species occurring throughout tropical and subtropical Asia (Kress *et al*., 2005). The most remarkable trait of this genus is the beauty of its inflorescence, which explains its wide ornamental use through the commercialization of its seedlings and flowers (Victorio, 2011). Many species of the genus *Alpinia* provide a variety of medicinal properties. *Alpinia galanga* (galangal) which is the most common *Alpinia* species has been used as carminative, stomachic and as antibacterial agents in Thailand (Mayachiew and Devahastin, 2008). Mayachiew and Devahastin (2008) also pointed out that the rhizome extracts of galangal possessed high antioxidant activity and showed good activity against the growth of food poisoning bacteria, *Staphylococcus aureus*.

Besides galangal, *Alpinia zerumbet* and *Alpinia purpurata* were the *Alpinia* species that have been used in folk medicine to treat hypertension and as anti-inflammatory (Victorio, 2011). Lin *et al*. (2009) described that leaves of *Alpinia pricei* were used to make traditional zongzi (a glutinous rice dumpling) in Taiwan while its rhizomes were used as a folk medicine for dispelling abdominal distension and enhancing stomach secretion and peristalsis. Some other examples of *Alpinia* species are *A. officinarum, A. conchigera, A. ligulata* and *A. speciosa*.

1.2.1.2 Genus *Boesenbergia*

Boesenbergia genus comprises of approximately 80 species distributed throughout tropical Asia (Saensouk and Larsen, 2001). One-quarter of the total *Boesenbergia* species are indigenous to Borneo (21 species) and Thailand (20 species); therefore, these areas were proposed as the center of origin of *Boesenbergia* (Poulsen, 1993; Larsen and Larsen, 2006). *Boesenbergia* species is extremely rare if compared to other genus of Zingiberaceae family and most of them are found in very damp, shaded areas and usually close to streams or in boggy conditions (Jing *et al*., 2011).

Boesenbergia genus was regarded as closely related to *Kaempferia* and *Scaphochlamys* (Holttum, 1950) and they are often difficult to distinguish. They are classified in the tribe Hedychieae (Holttum, 1950) but Kress *et al*. (2002) suggested that they should be treated as subfamily Zingiberoideae, tribe Zingibereae. They are small herbaceous plants with short, fleshy or slender rhizomes, one to a few leaves, similar appearance in vegetative characters and occurring in similar habitats. However, *Boesenbergia* is believed to be closer to *Scaphochlamys* than to *Kaempferia* (Hussin *et al*., 2001).

Many variations in colour can occur in *Boesenbergia* species. For instance, *B. curtisii* can have black or white leaf-sheaths and *B. plicata* can be yellow or red flowers (Vanijajiva *et al*., 2003). Other examples of *Boesenbergia* species are *B. rotunda*, *B. oligosperma*, *B. pulchella*, *B. prainiana*, *B. stenophylla* and *B. cordata*. Among these, only *B. rotunda* is cultivated commercially and its rhizomes have been used for medicinal such as treatment of colic disorder and as an aphrodisiac in Thailand folk medicine (Jaipetch *et al*., 1982).

1.2.2 Meliaceae

Meliaceae family is well represented in Southern and Eastern Africa and Madagascar. The plants range in size from magnificent forest trees to small shrubs where certain species are important as timber trees (Mulholland *et al*., 2000). Some common examples genera from this family are *Trichilia*, *Dysoxylum*, *Aglaia* and *Azadirachta*. According to Ting *et al*. (2011), *Dysoxylum* species have long been used to treat inflammatory conditions and fever throughout Asia. *Trichilia hirta* is a tree traditionally used in the folk medicine of Cuba to treat asthma, cancer and ulcers (Sosa *et al*., 2011), while *Trichilia emetica* is a plant native to Africa has been used traditionally as a remedy for abdominal pains, dermatitis, haemorrhoids, jaundice and chest pain (Komane *et al*., 2011).

A lot of study has been carried out on Meliaceae species and most of them have been proved to possessed significant insecticidal activity against varieties of insects. A study by Xie *et al*. (1994) indicated that most of extracts of *Trichilia* species collected from Costa Rica region can significantly deter larval growth of polyphagous lepidopterans *Peridroma saucia*. Nathan *et al.* (2006) reported that the Indian neem tree *Azadirachta indica* is a promising source of botanical insecticides with several limonoid compounds isolated from this plant species demonstrated high mortality effects and proved to be the most potent growth inhibitor on *Cnaphalocrocis medinalis*, a major insect pest of rice (*Oryza sativa*).

1.2.2.1 Genus *Aglaia*

Aglaia is the largest genus of the tropical and subtropical plant family, Meliaceae (Kim *et al*., 2006), comprises of more than 100 woody species ranging from small to large trees up to 40 m high (Joycharat *et al*., 2008) and represents an important component of the tropical rainforests of the Indomalaysian region (Pannell, 1992). Proksch *et al*. (2005) pointing out that crude extracts from leaves and flowers of various *Aglaia* species are used in traditional medicine in several countries of

Southeast Asia. For example, in Vietnam they are being used for the treatment of inflammatory skin diseases and allergic inflammatory disorders such as asthma.

Instead of being used medicinally, other uses of *Aglaia* species can be seen by the utilization of flowers of *Aglaia odorata* in China and Java, where this species has been used for flavouring tea and scenting clothes, respectively (Pannell, 1992). Some other examples of *Aglaia* species are *A. oligophylla*, *A. leucophylla*, *A. argentea* and *A. elliptifolia*. Besides their traditional usage, the plants species from Meliaceae family are well recognized and acknowledged as sources of insecticidal and anticancer agents.

1.3 Problems Statement

Cancer continues to be a major disease that caused many people to die and suffer. Cancer can be considered as one of the main killers besides heart attack and it is a disease that many people worry about and try to avoid. In the mean time, there are hundreds thousand high plants, shrubs and herbs existing on our earth but only a small portion has been studied for their phytochemicals and biological activities. Whereas, naturally occurring entities mainly, plant products have played an important role in the treatment of cancer. They continue to provide promising bioactive compounds for the development of new 'leads' to combat cancer diseases.

Alpinia mutica or locally known as 'chengkenam' has been cultivated mostly as ornamentals. The rhizomes of this plant has been reported to possess significant cytotoxicity towards several cancer cells and showed potential antioxidant activity. While, *Boesenbergia armeniaca* is a rather rare ginger species from *Boesenbergia* genus and has been reported to shows significant cytotoxicity towards breast cancer cells (MCF-7). Due to these properties, both plant species were selected for further research on its phytochemicals and biological activities.

Aglaia species have been reported to possess some interesting biological activities mainly its strong *in vitro* anticancer activity against several of human cancer cells. As for *Aglaia variisquama*, there is a need to study extensively on its phytochemicals and biological activities since no previous study has been reported. Besides, previous investigation carried out by our group on *Boesenbergia* and *Aglaia* species afforded flavonoids and triterpenes, some of which demonstrated significant cytotoxic activity.

Figure 1.1: Pictures of various parts of *Alpinia mutica*, A: Fruits & Leaves; B: Rhizomes; C: Flowers

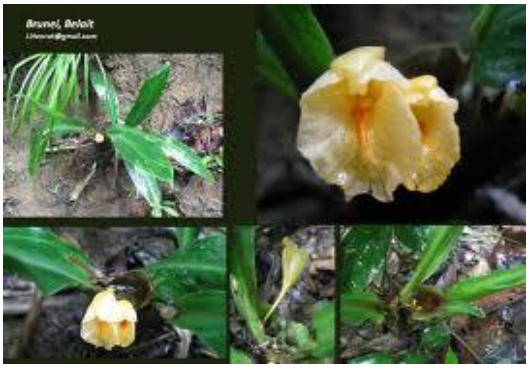

Figure 1.2: *Boesenbergia armeniaca* Adapted from: http://sites.google.com/site/florimages

Figure 1.3: Leaves of *Aglaia variisquama*

1.4 Objectives of the Study

The objectives of the study are:

1) To extract and isolate pure compounds from the rhizomes of *Alpinia mutica* and *Boesenbergia armeniaca* and the leaves of *Aglaia variisquama* using various chromatographic methods.
2) To elucidate the chemical structures of isolated compounds using spectroscopic techniques including Infrared (IR), Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR 1D and 2D) spectroscopy.
3) To evaluate the biological activities (cytotoxic, antioxidant and antimicrobial) of the crude extracts and isolated compounds from the plant species.

REFERENCES

Abdelwahab, S. I., Mohan, S., Abdulla, M. A., Sukari, M. A., Abdul, A. B., Taha, M. M. E., Syam, S., Ahmad, S. and Lee, Ka- H. (2011). The methanolic extract of *Boesenbergia rotunda* (L.) Mansf. And its major compound pinostrobin induces anti-ulcerogenic property in vivo: Possible involvement of indirect antioxidant action. *J. Ethnopharmacol.,* **137:** 963-970.

Ahmad, F. and Raji, H. (1993). *Kimia Hasilan Semulajadi dan Tumbuhan Ubatan*. Kuala Lumpur: Dewan Bahasa dan Pustaka, pp. 1-5.

Ahmad, F., Ali, M. and Alam, P. (2010). New phytoconstituents from the stem bark of *Tinospora cordifolia* Miers. *Nat. Prod. Res.*, **24(10):** 926-934.

Andrade da Mata Rezende, C. M., Von Bulow, M. V., Gottlieb, O. R., Lamego Vieira Pinho, S. and Da Rocha, A. I. (1971). The 2-Pyrones of *Aniba* Species. *Phytochem.,* **10:** 3167-3172.

Awale, S., Miyamoto, T., Linn, T. Z., Li, F., Win, N. N., Tezuka, Y., Esumi, H. and Kadota, S. (2009). Cytotoxic Constituents of *Soymida febrifuga* from Myanmar. *J. Nat. Prod.,* **72:** 1631-1636.

Benosman, A., Richomme, P., Sevenet, T., Hadi, A. H. A. and Bruneton, J. (1994). Secotirucallane Triterpenes from the Stembark of *Aglaia leucophylla. Phytochem.,* **37(4):** 1143-1145.

Benosman, A., Richomme, P., Sevenet, T., Perromat, G., Hadi, A. H. A. and Bruneton, J. (1995). Tirucallane Triterpenes fron the Stem bark of *Aglaia leucophylla. Phytochem.,* **40(5):** 1485-1487.

Butler, M. S. (2004). The Role of Natural Product Chemistry in Drug Discovery. *J. Nat. Prod.,* **67(12):** 2141-2153.

Chan, E. W. C., Lim, Y. Y., Wong, L. F., Lianto, F. S., Wong, S. K., Lim, K. K., Joe, C. E. and Lim, T. Y. (2008). Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chem.,* **109:** 477-483.

Cheah, S. C., Appleton, D. R., Lee, S. T., Lam, M. L., Hadi, A. H. A. and Mustafa, M. R. (2011). Panduratin A Inhibits the Growth of A549 Cells through Induction of Apoptosis and Inhibition of NF-KappaB Translocation. *Molecules,* **16:** 2583-2598.

Chou, T. H., Chen, J. J., Lee, S. J., Chiang, M. Y., Yang, C. W. and Chen, I. S. (2010). Cytotoxic Flavonoids from the Leaves of *Cryptocarya chinensis*. *J. Nat. Prod.,* **73:** 1470-1475.

Cowley, J. (2000). Three new gingers from Borneo. *Kew Bull.,* **55(3):** 669-678.

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

Cui, B., Chai, H., Santisuk, T., Reutrakul, V., Farnsworth, N. R., Cordell, G. A., Pezzuto, J. M. and Kinghorn, A. D. (1997). Novel Cytotoxic 1*H*-Cyclopenta [*b*]benzofuran Lignans from *Aglaia elliptica. Tetrahedron,* **53(52):** 17625-17632.

Dharmaratne, H. R. W., Nanayakkara, N. P. D. and Khan, I. A. (2002). Kavalactones from *Piper methysticum*, and their ^{13}C NMR spectroscopic analysis. *Phytochem.,* **59:** 429-433.

Dhavale, D. D., Aidhen, I. S. and Mohammed, S. (1989). Acyl Anion Equivalents in the Synthesis of *2H*-Pyran-2-ones: An Efficient Synthesis of Anibine. *J. Org. Chem.,* **54(16):** 3985-3987.

Elzaawely, A. A., Xuan, T. D., Koyama, H. and Tawata, S. (2007). Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds *of Alpinia zerumbet* (Pers.) B. L. Burtt. & R. M. Sm. *Food Chem.,* **104:** 1648-1653.

Ficker, C. E., Smith, M. L., Susiarti, S., Leaman, D. J., Irawati, C. and Arnason, J. T. (2003). Inhibition of human pathogenic fungi by members of Zingiberaceae used by the Kenyah (Indonesian Borneo). *J. Ethnopharmacol.,* **85:** 289-293.

Fujita, T., Nishimura, H., Kaburagi, K. and Mizutani, J. (1994). Plant Growth Inhibiting α-Pyrones from *Alpinia speciosa. Phytochem.,* **36(1):** 23-27.

Gupta, R., Sharma, A. K., Sharma, M. C., Dobhal, M. P. and Gupta, R. S. (2012). Evaluation of antidiabetic and antioxidant potential of lupeol in experimental hyperglycaemia. *Nat. Prod. Res.,* **26(12):** 1125-1129.

Habsah, M., Amran, M., Mackeen, M. M., Lajis, N. H., Kikuzaki, H., Nakatani, N., Rahman, A. A. and Ali, A. M. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *J. Ethnopharmacol.,* **72:** 403-410.

Habsah, M., Lajis, N. H., Ali, A. M., Sukari, M. A., Hin, Y. Y., Kikuzaki, H. and Nakatani, N. (2003). The Antioxidative Components from *Alpinia nutans*. *Pharm. Biol.,* **41(1):** 7-9.

Habsah, M., Faridah, A., Dharma, P., Lajis, N. H., Ali, A. M., Sukari, M. A., Hin, Y. Y., Kikuzaki, H. and Nakatani, N. (2004). DPPH Free Radical Scavenger Components from the Fruits of *Alpinia rafflesiana* Wall. ex. Bak. (Zingiberaceae). *Z. Naturforsch,* **59c:** 811-815.

Harneti, D., Tjokronegoro, R., Safari, A., Supratman, U., Loong, X. M., Mukhtar, M. R., Mohamad, K., Awang, K. and Hayashi, H. (2012). Cytotoxic triterpenoids from the bark of *Aglaia Smithii* (Meliaceae). *Phytochem. Lett.,* **5:** 496-499.

Hayashi, N., Lee, K. H., Hall, I. H., Mcphail, A. T. and Huang, H. C. (1982). Structure and Stereochemistry of (-)-Odorinol, An Antileukemic Diamide from *Aglaia odorata. Phytochem.,* **21(9):** 2371-2373.

Hema, P. S. and Nair, M. S. (2009). Flavonoids and other constituents from the rhizomes of *Alpinia calcarata*. *Biochem. Systematics Ecol.,* **37:** 52-54.

Holttum, R. E. (1950). The Zingiberaceae of the Malay peninsular. *Gard. Bull. Sing.,* **13:** 1–282.

Hui, W. H. and Li, M. M. (1976). Lupene Triterpenoids from *Glochidion eriocarpum*. *Phytochem.,* **15:** 561-562.

Hussin, K. H., Ibrahim, H. and Ali, D. A. H. A. (2001). Anatomical variations in leaves of *Boesenbergia* O. Kuntze and *Kaempferia* L. species (Zingiberaceae). *J. Trop. Subtrop. Bot.,* **9:** 49–54.

Ibrahim, H. (1999). *Kaempferia*. In L. S. de Padua, N. Bunyapraphatsara and R. H. M. J. Lemmens (Eds.). *Plant resources of South-east Asia, Vol. 12*. 331–335. Netherlands: Backhuys Publisher.

Inada, A., Nishino, H., Kuchide, M., Takayasu, J., Mukainaka, T., Nobukuni, Y., Okuda, M. and Tokuda, H. (2001). Cancer Chemopreventive Activity of Odorine and Odorinol from *Aglaia odorata*. *Biol. Pharm. Bull.,* **24(11):** 1282-1285.

Itokawa, H., Morita, M. and Mihashi, S. (1981). Phenolic compounds from the rhizomes of *Alpinia speciosa. Phytochem.,* **20(11):** 2503-2506.

Jaipetch, T., Kanghae, S., Pancharoen, O., Patrick, V. A., Reutrakul, V., Tuntiwachwuttikul, P. and White, A. H. (1982). Constituents of

Boesenbergia pandurata (syn *Kaempferia pandurata*): isolation, crystal structure and synthesis of (±)-boesenbergin A. *Australian J. Chem.,* **35:** 351–361.

Jamal, A. K., Yaacob, W. A. and Laily, D. (2009). A Chemical Study on *Phyllanthus Columnaris. European J. Sci. Res.,* **28(1):** 76-81.

Janaki, S., Vijayasekaran, V., Viswanathan, S. and Balakrishna, K. (1999). Anti-inflammatory activity of *Aglaia roxburghiana* var. *beddomei* extract and triterpenes roxburghiadiol A and B. *J. Ethnopharmacol.,* **67:** 45-51.

Jantan, I., Pisar, M., Sirat, H. M., Basar, N., Jamil, S., Ali, R. M. and Jalil, J. (2004). Inhibitory Effects of Compounds from Zingiberaceae Species on Platelet Activating Factor Receptor Binding. *Phytotherap. Res.,* **18:** 1005-1007.

Jantan, I., Raweh, S. M., Sirat, H. M., Jamil, S., Mohd Yasin, Y. H., Jalil, J. and Jamal, J. A. (2008). Inhibitory effect of compounds from Zingiberaceae species on human platelet aggregation. *Phytomed.,* **15:** 306-309.

Jhoo, J. W., Freeman, J. P., Heinze, T. M., Moody, J. D., Schnackenberg, L. K., Beger, R. D., Dragull, K., Tang, C. S. and Ang, C. Y. W. (2006). In Vitro Cytotoxicity of Nonpolar Constituents from Different Parts of Kava Plant (*Piper methysticum*). *J. Agric. Food Chem.,* **54:** 3157-3162.

Jing, L. J., Mohamed, M., Rahmat, A. and Abu Bakar, M. F. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*). *J. Med. Plant. Res.,* **4(1):** 27-32.

Jing, L. J., Abu Bakar, M. F., Mohamed, M. and Rahmat, A. (2011). Effects of Selected *Boesenbergia* Species on the Proliferation of Several Cancer Cell Lines. *J. Pharmacol. Toxicol.,* **6(3):** 272-282.

Joycharat, N., Greger, H., Hofer, O. and Saifah, E. (2008). Flavaglines and triterpenes as chemical markers of *Aglaia oligophylla*. *Biochem. Systematics Ecol.,* **36:** 584-587.

Kikuzaki, H., Tesaki, S., Yonemori, S. and Nakatani, N. (2001). Phenylbutanoid dimers from the leaves of *Alpinia flabellata*. *Phytochem.,* **56:** 109-114.

Kim, S., Salim, A. A., Swanson, S. M. and Kinghorn, A. D. (2006). Potential of Cyclopenta[*b*]benzofurans from *Aglaia* Species in Cancer Chemotheraphy. *Anti-Cancer Agents in Med. Chem.,* **6:** 319-345.

Kirana, C., Jones, G. P., Record, I. R. and McIntosh, G. H. (2007). Anticancer properties of panduratin A isolated from *Boesenbergia pandurata* (Zingiberaceae). *J. Nat. Med.,* **61:** 131-137.

Komane, B. M., Olivier, E. I. and Viljoen, A. M. (2011). *Trichilia emetica* (Meliaceae). A review of traditional uses, biological activities and phytochemistry. *Phytochem. Lett.,* **4:** 1-9.

Kress, W. J., Prince, L. M. and Williams, K. J. (2002). The Phylogeny and a New Classification of the Gingers (Zingiberaceae): Evidence from Molecular Data. *Am. J. Bot.,* **89:** 1682-1696.

Kress, W. J., Liu, A. Z., Newman, M. and Li, Q. J. (2005). The molecular phylogeny of *Alpinia* (Zingiberaceae): a complex and polyphyletic genus of gingers. *Am. J. Bot.,* **92(1):** 167-178.

Kurniadewi, F., Juliawaty, L. D., Syah, Y. M., Achmad, S. A., Hakim, E. H., Koyama, K., Kinoshita, K. and Takahashi, K. (2010). Phenolic compounds from *Cryptocarya konishii*: their cytotoxic and tyrosine kinase inhibitory properties. *J. Nat. Med.*, **64:** 121-125.

Larsen, K., Ibrahim, H., Khaw, S. H. and Saw, L. G. (1999). *Gingers of Peninsular Malaysia and Singapore*. Kota Kinabalu: Natural History Publications, pp. 87-89.

Larsen, K. and Larsen, S. S. (2006). *Gingers of Thailand*. Bangkok: Academic Press. 67-73.

Li, F., Awale, S., Tezuka, Y., Esumi, H. and Kadota, S. (2010). Study on the Constituents of Mexican Propolis and Their Cytotoxic Activity against PANC-1 Human Pancreatic Cancer Cells. *J. Nat. Prod.*, **73:** 623-627.

Li, Y. X., Qiao, W. T. and Yuan, K. (2011). Isolation and structural elucidation of chemical constituents of *Mussaenda hainanensis* Merr. *J. Med. Plants Res.*, **5(8):** 1459-1465.

Lin, C. T., Kumar, K. J. S., Tseng, Y. H., Wang, Z. J., Pan, M. Y., Xiao, J. H., Chien, S. C. and Wang, S. Y. (2009). Anti-inflammatory Activity of Flavokawain B from *Alpinia pricei* Hayata. *J. Agric. Food Chem.*, **57:** 6060-6065.

Mackeen, M. M., Ali, A. M., El-Sharkawy, S. H., Manap, M. Y., Salleh, K. M., Lajis, N. H. and Kawazu, K. (1997). Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables (ulam). *Int. J. Pharmacog.*, **35:** 174-178.

Mahidol, C., Tunticachwuttikul, P., Reutrakul, V. and Taylor, W. C. (1984). Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata*). III Isolation and synthesis of (±)-boesenbergin B. *Australian J. Chem.*, **37:** 1739–1745.

Mai, H. D. T., Minh, H. N. T., Pham, V. C., Bui, K. N., Nguyen, V. H. and Chau, V. M. (2011). Lignans and other constituents from the roots of the Vietnamese medicinal plant *Pseuderanthemum palatiferum. Planta Medica*, **77(9):** 951-954.

Malek, S. N. A., Phang, C. W., Ibrahim, H., Wahab, N. A. and Kae, S. S. (2011). Phytochemical and Cytotoxic Investigations of *Alpinia mutica* Rhizomes. *Molecules*, **16:** 583-589.

Mayachiew, P. and Devahastin, S. (2008). Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. *LWT*, **41:** 1153-1159.

Mishra, B. B. and Tiwari, V. K. (2011). Natural products: An evolving role in future drug discovery. *European J. Med. Chem.*, **46:** 4769-4807.

Mulholland, D.A., Parel, B. and Coombes, P. H. (2000). The Chemistry of the Meliaceae and Ptaeroxylaceae of Southern and Eastern Africa and Madagascar. *Curr. Org. Chem.*, **4(10):** 1011-1054.

Mohamad, K., Martin, M. T., Leroy, E., Tempete, C., Sevenet, T., Awang, K. and Pais, M. (1997). Argenteanones C-E and Argenteanols B-E, Cytotoxic Cycloartanes from *Aglaia argentea*. *J. Nat. Prod.*, **60(2):** 81-85.

Molleyres, L. P., Rindlisbacher, A., Winkler, T. and Kumar, V. (1999). Insecticidal natural products: new rocaglamide derivatives from *Aglaia roxburghiana*. *Pestic. Sci.*, **55:** 486-503.

Moriarity, D. M., Huang, J., Yancey, C. A., Zhang, P., Setzer, W. N., Lawton, R. O., Bates, R. B. and Caldera, S. (1998). Lupeol is the cytotoxic principle in the leaf extract of *Dendropanax cf. querceti. Planta Medica,* **64(4):** 370-372.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods,* **65:** 55-63.

Nathan, S. S., Kalaivani, K., Sehoon, K. and Murugan, K. (2006). The toxicity and behavioural effects of neem limonoids on *Cnaphalocrocis medinalis,* the rice leaffolder. *Chemosphere,* **62:** 1381-1387.

Nugroho, B. W., Gussregen, B., Wray, V., Witte, L., Bringmann, G. and Proksch, P. (1997). Insecticidal Rocaglamide Derivatives from *Aglaia elliptica* and *A. harmsiana*. *Phytochem.,* **45(8):** 1579-1585.

Nuntawong, N. and Suksamrarn, A. (2008). Chemical constituents of the rhizomes of *Alpinia malaccensis. Biochem. Systematics Ecol.,* **36:** 661-664.

Omobuwajo, O. R., Martin, M. T., Perromat, G., Sevenet, T., Awang, K. and Pais, M. (1996). Cytotoxic Cycloartanes from *Aglaia argentea*. *Phytochem.,* **41(5):** 1325-1328.

Pan, Li., Acuna, U. M., Li, J., Jena, N., Ninh, T. N., Pannell, C. M., Chai, H., Fuchs, J. R., Carcache de Blanco, E. J., Soejarto, D. D. and Kinghorn, A. D. (2013). Bioactive Flavaglines and Other Constituents Isolated from *Aglaia perviridis*. *J. Nat. Prod.,* **76:** 394-404.

Pandji, C., Grimm, C., Wray, V., Witte, L., Proksch, P. (1993). Insecticidal constituents from four species of the Zingiberaceae. *Phytochem.,* **34:** 415-419.

Pannell, C. M. (1992). *A Taxonomic Monograph of the Genus Aglaia Lour. (Meliaceae).* London: HMSO, pp. 1-379.

Phang, C. W., Malek, S. N. A., Ibrahim, H. and Wahab, N. A. (2011). Antioxidant properties of crude and fractionated extracts of *Alpinia mutica* rhizomes and their total phenolic content. *Afr. J. Pharm. Pharmacol.,* **5(7):** 842-852.

Pouget, C., Lauthier, F., Simon, A., Fagnere, C., Basly, J. P., Delage, C. and Chulia, A. J. (2001). Flavonoids: Structural Requirements for Antiproliferative Activity on Breast Cancer Cells. *Bioorg. Med. Chem. Lett.,* **11:** 3095-3097.

Poulsen, A. D. (1993). Two new species of *Boesenbergia* (Zingiberaceae) from Borneo. *Northen J. Bot.,* **13:** 289-294.

Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S. and Prachayasittikul, V. (2010). New Bioactive Triterpenoids and Antimalarial Activity of *Diospyros rubra* Lec. *EXCLI Journal,* **9:** 1-10.

Prasad, S., Kalra, N. and Shukla, Y. (2007). Hepatoprotective effects of lupeol and mango pulp extract of carcinogen induced alteration in Swiss albino mice. *Molecular Nutri. Food Res.,* **51(3):** 352-359.

Prasad, S., Kalra, N. and Shukla, Y. (2008). Induction of apoptosis by lupeol and mango extract in mouse prostate and LNCaP cells. *Nutrit. Cancer,* **60(1):** 120-130.

Proksch, P., Giaisi, M., Treiber, M. K., Palfi, K., Merling, A., Spring, H., Krammer, P. H. and Li-Weber, M. (2005). Rocaglamide Derivatives are Immunosuppressive Phytochemicals that Target NF-AT Activity in T Cells. *J. Immunol.,* **174:** 7075-7084.

Saensouk, S. and Larsen, K. (2001). *Boesenbergia baimaii*, a new species of Zingiberaceae from Thailand. *Northern J. Bot.,* **21:** 595-597.

Saha, K., Lajis, N. H., Israf, D. A., Hamzah, A. S., Khozirah, S., Khamis, S. and Syahida, A. (2004). Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *J. Ethnopharmacol.,* **92:** 263-267.

Saleem, M., Maddodi, N., Abu Zaid, M., Khan, N., Hafeez, B., Asim, M., Suh, Y., Yun, J. M., Setaluri, V. and Mukhtar, H. (2008). Lupeol Inhibits Growth of Highly Aggressive Human Metastatic Melanoma Cells *In vitro* and *In vivo* by Inducing Apoptosis. *Clin. Cancer Res.,* **14(7):** 2119-2127.

Sarker, S. D., Latif, Z. and Gray, A. I. (2006). *Natural Product Isolation*. In S. D. Sarker, Z. Latif and A. I. Gray (Eds.). *Natural Products Isolation*. 1-24. New Jersey: Humana Press.

Sawamura, R., Sun, Y., Yasukawa, K., Shimizu, T., Watanabe, W. and Kurokawa, M. (2010). Antiviral activities of diarylheptanoids against influenza virus *in vitro*. *J. Nat. Med.,* **64:** 117-120.

Shaik, A. A., Hermanson, D. L. and Xing, C. (2009). Identification of methysticin as a potent and non-toxic NF-kB inhibitor from kava, potentially responsible for kava's chemopreventive activity. *Bioorg. Med. Chem Lett.,* **19:** 5732-5736.

Shindo, K., Kato, M., Kinoshita, A., Kobayashi, A. and Yukiko, Koike. (2006). Analysis of Antioxidant Activities Contained in the *Boesenbergia pandurata* Schult. Rhizome. *Biosci. Biotechnol. Biochem.,* **70(9):** 2281-2284.

Sirat, H. M., Masri, D. and Rahman, A. A. (1994). The Distribution of Labdane Diterpenes in the Zingiberaceae of Malaysia. *Phytochem.,* **36(3):** 699-701.

Sirat, H. M., Rahman, A. A., Itokawa, H. and Morita, H. (1996). Constituents of two *Alpinia* species. *Planta Medica,* **62:** 188-189.

Sohn, J. H., Han, K. L., Lee, S. H. and Hwang, J. K. (2005). Protective Effects of Panduratin A against Oxidative Damage of *tert*-Butylhydroperoxide in Human HepG2 Cells. *Biol. Pharm. Bull.,* **28(6):** 1083-1086.

Sosa, E. H., Castejon, Y. M., Duharte, A. B., Portuondo, D., Tamayo, V., Quevedo, H. J. M. and Manrique, C. E. M. (2011). Leukocyte-Stimulating Effect and Phytochemical Screening of *Trichilia hirta* Extracts. *J. Med. Food,* **14(9):** 1057-1059.

Sumiyoshi, M. and Kimura, Y. (2009). Effects of a turmeric extract (*Curcuma longa*) on chronic ultraviolet B irradiation-induced skin damage in melanin-possessing hairless mice. *Phytomed.,* **16:** 1137-1143.

Tewtrakul, S., Subhadhirasakul, S. and Kummee, S. (2003). HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt. *Songklanakarin J. Sci. Technol.,* **25:** 505-507.

Thanakijcharoenpath, W. and Theanphong, O. (2007). Triterpenoids from the Stem of *Diospyros glandulosa. Thai. J. Pharm. Sci.*, **31:** 1-8.

Ting, K. N., Oyhman, M., Telford, G., Clarke, G., Bradshaw, T. D., Khoo, T. J., Loh, H. S., Wiart, C., Pritchard, D. and Fry, J. R. (2011). Antioxidant, cytoprotective, growth inhibitory and immunomodulatory activities of extracts of *Dysoxylum cauliflorum* Hiern. A Malaysian Meliaceae. *J. Med. Plants Res.,* **5(24):** 5867-5872.

Trakoontivakorn, G., Nakahara, K., Shinmoto, H., Takenaka, M., Onishi, K. M., Ono, H., Yoshida, M., Nagata, T. and Tsushida, T. (2001). Structural analysis of a novel anti-mutagenic compound, 4-hydroxypanduratin A, and the anti-mutagenic activity of flavonoids in a Thai spice, Fingerroot (*Boesenbergia*

pandurata Schult.) against mutagenic heterocyclic amines. *J. Agric. Food Chem.,* **49:** 3046-3050.

Tuchinda, P., Reutrakul, V., Claeson, P., Pongprayoon, U., Sematong, T., Santisuk, T. and Taylor, W. C. (2002). Anti-inflammatory cyclohexenyl chalcone derivatives in *Boesenbergia pandurata*. *Phytochem.,* **59:** 169-173.

Tushar, S. B., Gajen, C. S. and Latha, R. (2010). Ethnomedical uses of Zingiberaceous plants of Northeast India. *J. Ethnopharmacol.,* **132:** 286-296.

Umehara, K., Nemoto, K., Matsushita, A., Terada, E., Monthakantirat, O., De-Eknamkul, W., Miyase, T., Warashina, T., Degawa, M. and Noguchi, H. (2009). Flavonoids from the Heartwood of the Thai Medicinal Plant *Dalbergia parviflora* and Their Effects on Estrogenic-Responsive Human Breast Cancer Cells. *J. Nat. Prod.,* **72:** 2163-2168.

Usia, T., Banskota, A. H., Tezuka, Y., Midorikawa, K., Matsushige, K. and Kadota, S. (2002). Constituents of Chinese Propolis and Their Antiproliferative Activities. *J. Nat. Prod.,* **65:** 673-676.

Vanijajiva, O., Suvachittanont, W. and Sirirugsa, P. (2003). Isozyme Analysis of Relationships Among *Boesenbergia* (Zingiberaceae) and Related Genera in Southern Thailand. *Biochem. Systematics Ecol.,* **31:** 499-511.

Victorio, C. P. (2011). Therapeutic value of the genus *Alpinia,* Zingiberaceae. *Brazilian J. Pharmacog.*, **21(1):** 194-201.

Win, N. N., Awale, S., Esumi, H., Tezuka, Y. and Kadota, S. (2007). Bioactive Secondary Metabolites from *Boesenbergia pandurata* of Myanmar and Their Preferential Cytotoxicity against Human Pancreatic Cancer PANC-1 Cell Line in Nutrient-Deprived Medium. *J. Nat. Prod.,* **70:** 1582-1587.

Xie, Y. S., Isman, M. B., Gunning, P., Mackinnon, S., Arnason, J. T., Taylor, D. R., Sanchez, P., Hasbun, C. and Towers, G. H. N. (1994). Biological Activity of Extracts of *Trichilia* Species and the Limonoid Hirtin Against Lepidopteran Larvae. *Biochem. Systematics Ecol.,* **22(2):** 129-136.

Yap, A. L. C., Tang, S. W., Sukari, M. A., Ee, G. C. L., Rahmani, M. and Khalid, K. (2007). Characterization of Flavonoid Derivative from *Boesenbergia Rotunda* (L.). *The Malaysian J. Anal. Sci.,* **11(1):** 154-159.

Yasukawa, K., Sun, Y. and Kitanaka, S. (2008). Inhibitory effect of the rhizomes of *Alpinia officinarum* on TPA-induced inflammation and tumor promotion in two-stage carcinogenesis in mouse skin. *J. Nat. Med.,* **62:** 374-378.

Yuan, L. Y., Xin, C. G. and Tao, W. Z. (2009). Chemical Constituents in *n*-butanol Extract from the Seeds of *Alpinia katsumadai. Chin. J. Nat. Med.,* **7(6):** 417-420.

Yun, J. M., Kwon, H. J. and Hwang, J. K. (2003). *In vitro* anti-inflammatory activity of panduratin A isolated from *Kaempferia pandurata* in RAW 264.7 cells. *Planta Medica*, **69:** 1102–1108.

Yun, J. M., Kwon, H., Mukhtar, H. and Hwang, J. K. (2005). Induction of apoptosis by panduratin A isolated from *Kaempferia pandurata* in human colon cancer HT-29 cells. *Planta Medica*, **71:** 501–507.

Yun, J. M., Kweon, M. H., Kwon, H., Hwang, J. K. and Mukhtar, H. (2006). Induction of apoptosis and cell cycle arrest by a chalcone panduratin A isolated from *Kaempferia pandurata* in androgen-independent human prostate cancer cells PC3 and DU145. *Carcinogenesis*, **27(7):** 1454-1464.