# PHYTOCHEMICAL AND BIOACTIVITIES OF MALAYSIAN *ARTOCARPUS LOWII* KING, *A. SCORTECHINII* KING AND *A. TEYSMANII* MIQ.

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UNIVERSITI TEKNOLOGI MALAYSIA

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
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*Dedicated to...*

*My parents*

*My beloved husband, Mr Kamaruddin bin Mohammed*

*My sons, Uzair bin Kamaruddin, Zaid bin Kamaruddin and*

*Adam bin Kamaruddin*

## ACKNOWLEDGEMENTS

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My greatest debt is to my husband, Mr Kamaruddin Mohammed whose patient love enabled me to complete this work. My deepest appreciation goes to my parents who never fail to pray for my success.

# ABSTRACT

Phytochemical studies of *Artocarpus lowii* King, *A. scortechinii* King and *A. teysmanii* Miq. have resulted in the isolation of four new compounds and eight known compounds. Three new compounds have been successfully isolated from *A. lowii* King, i.e 2′,4′-dihydroxy-4-methoxy-3′-prenyldihydrochalcone, 2′,3,4′,4-tetrahydroxy-3′-prenylchalcone and 2-hydroxyparatocarpin C. Three known compounds were identified as cycloheterophyllin, 2′,4′,4-trihydroxy-3′-prenylchalcone and 4-hydroxylonchocarpin. Methylation of 2′,4′-dihydroxy-4-methoxy-3′-prenyldihydrochalcone and 2′,4′,4-trihydroxy-3′-prenylchalcone gave 2′,4′,4-trimethoxy-3′-prenyldihydrochalcone and 2′-hydroxy-4′,4-dimethoxy-3′-prenylchalcone, respectively while methylation of cycloheterophyllin gave mixtures of dimethoxy and trimethoxy derivatives. Acetylation of cycloheterophyllin afforded cycloheterophyllin diacetate. A new compound was isolated from *A. scortechinii* King and was identified as 2′,4′,5′,5-tetrahydroxy-3-geranyl-7,8-(2,2-dimethyl-6*H*-pyrano)-6-prenylflavone together with three known compounds, i.e artonin E, artobiloxanthone and lupeol 3-acetate. Methylation of artonin E gave artonin E trimethyl ether while acetylation of artonin E afforded artonin E tetraacetate. Four known compounds were isolated from *A. teysmanii* Miq., which were identified as artonin E, artobiloxanthone, artonol B and cycloartobiloxanthone. The structures of all compounds were established based on spectral studies using nuclear magnetic resonance spectroscopy, mass spectrometry, infrared spectroscopy and ultraviolet spectroscopy. The biological studies on the crude extracts and pure compounds of these three species showed that several pure compounds have significant biological activity especially in the antioxidant, platelet aggregation and cytotoxicity assays. Cycloheterophyllin and artonin E showed high ability to act as free radical scavengers with scavenging concentration values of 51.6 μg/mL and 48.3 μg/mL, respectively. Cycloheterophyllin, artonin E, isobavachalcone and 2′,4′-dihydroxy-4-methoxy-3′-prenyldihydrochalcone totally inhibited adenosine diphosphate-induced platelet aggregation compared to standard aspirin which suppressed only 31.6% of the platelet aggregation. Cyloheterophyllin and artonin E were found to be active against breast cancer cell line, MCF7 comparable to the standard tamoxifen citrate. Finally, 2′-hydroxy-4,4′,6′-trimethoxy-3′-prenylchalcone was synthesized through a three step synthesis. The steps involved Friedel-Crafts prenylation followed by methylation and Claisen-Schmidt condensation. Oxidative cyclization of this chalcone yielded an aldehyde-type chalcone derivative. 2′-Hydroxy-4-methoxy-4′-*O*-prenylchalcone was synthesized through a two steps synthesis involving Friedel-Crafts prenylation followed by Claisen-Schmidt condensation. Attempted Claisen rearrangement produced 4′-hydroxy-4-methoxy-2′-*O*-prenylchalcone as the major product.

# ABSTRAK

Kajian fitokimia ke atas *Artocarpus lowii* King, *A. scortechinii* King dan *A. teysmanii* Miq. berjaya menemukan empat sebatian baru dan lapan sebatian yang diketahui. Tiga sebatian baru ditemui daripada *A. lowii* King dikenalpasti sebagai 2′,4′-dihidroksi-4-metoksi-3′-prenildihidrokalkon, 2′,3,4′,4-tetrahidroksi-3′-prenil-kalkon dan 2-hidroksiparatokarpin C. Tiga sebatian lain dikenalpasti sebagai sikloheterofilin, 2′,4′,4-trihidroksi-3′-prenilkalkon dan 4-hidroksilonchokarpin. Pemetilan 2′,4′-dihidroksi-4-metoksi-3′-prenildihidrokalkon dan 2′,4′,4-trihidroksi-3′-prenilkalkon masing-masing berjaya menghasilkan 2′,4′,4-trimetoksi-3′-prenildihidrokalkon dan 2′-hidroksi-4′,4-dimetoksi-3-prenilkalkon, sementara pemetilan sikloheterofilin menghasilkan campuran terbitan dimetoksi dan trimetoksi. Pengasetilan terhadap sikloheterofilin berjaya menghasilkan sikloheterofilin diasetat. Satu sebatian baru telah berjaya diasingkan daripada *A. scortechinii* King dan dikenalpasti sebagai 2′,4′,5′,5-tetrahidroksi-3-geranil-7,8-(2,2-dimetil-6*H*-pirano)-6-prenilflavon bersama-sama tiga sebatian diketahui iaitu artonin E, artobiloxanton dan lupeol 3-asetat. Pemetilan artonin E berjaya menghasilkan artonin E trimetil eter, sementara pengasetilan artonin E berjaya menghasilkan artonin E tetraasetat. Empat sebatian berjaya diasingkan daripada *A. teysmanii* Miq. dan dikenalpasti sebagai artonin E, artobiloxanton, artonol B dan sikloartobiloxanton. Struktur kesemua sebatian dikenalpasti berdasarkan kepada kajian spektrum dengan menggunakan spektroskopi resonan magnet nukleus, spektrometri jisim, spektroskopi inframerah dan spektroskopi ultralembayung. Kajian aktiviti biologi ke atas ekstrak mentah dan sebatian tulen daripada ketiga-tiga spesies *Artocarpus* ini mendapati beberapa sebatian menunjukkan aktiviti biologi yang signifikan terutamanya di dalam cerakin antioksidan, aggregasi platelet dan sitoketoksikan. Sikloheterofilin dan artonin E menunjukkan keupayaan yang tinggi sebagai perencat radikal bebas dengan nilai perencatan masing-masing 51.6 μg/mL dan 48.3 μg/mL. Sikloheterofilin, artonin E, isobavakalkon dan 2′,4′-dihidroksi-4-metoksi-3′-prenildihidrokalkon merencat 100% aggregasi platelet yang dirangsang oleh adenosin difosfat, berbanding dengan aspirin yang hanya mampu merencat sebanyak 31.6% sahaja. Sikloheterofilin dan artonin E juga didapati aktif terhadap MCF7 iaitu sel kanser payu dara, setanding dengan tamoksifen sitrat iaitu dadah anti kanser piawai. 2′-Hidroksi-4,4′,6′-trimetoksi-3′-prenilkalkon telah berjaya disintesis melalui tiga langkah tindak balas. Tindak balas yang terlibat ialah pemprenilan Friedel-Crafts, tindak balas pemetilan dan kondensasi Claisen-Schmidt. Pensiklikan oksidatif terhadap kalkon ini menghasilkan satu terbitan kalkon jenis aldehid. 2′-Hidroksi-4-metoksi-4′-*O*-prenilkalkon pula telah berjaya disintesis melalui dua langkah tindak balas iaitu tindak balas pemprenilan Friedel-Crafts, diikuti dengan kondensasi Claisen-Schmidt. Percubaan melakukan penyusunan semula Claisen menghasilkan 4′-hidroksi-4-metoksi-2′-*O*-prenilkalkon sebagai hasil utama.

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| | |
|---|---|
| AA | arachidonic acid |
| $Ac_2O$ | acetic anhydride |
| ADP | Adenosine diphosphate |
| $AlCl_3$ | Aluminium trichloride |
| b.p | boiling point |
| br | Broad |
| $^{13}C$ | Carbon-13 |
| $cm^{-1}$ | Per centimetre |
| cm | Centimetre |
| $CDCl_3$ | deuterated chloroform |
| $CD_3OD$ | deuterated methanol |
| $CHCl_3$ | Chloroform |
| $CH_2Cl_2$ | Dichloromethane |
| COSY | Correlation Spectroscopy |
| $CO_2$ | Carbon dioxide |
| d | Doublet |
| dd | doublet of doublets |
| DEPT | Distortionless Enhancement of Polarisation Transfer |
| DMSO | dimethyl sulphoxide |
| DMAP | 4-(*N*,*N*-dimethylamino)pyridine |
| DMEM | Dulbecco's Modified Eagle Medium |
| *E* | *Entgegen* |
| $Et_2O$ | Diethyl ether |
| EtOH | Ethanol |
| EIMS | Electron Impact Mass Spectrometry |

| | |
|---|---|
| ESR | Electron Spin Resonance |
| GHz | Gigahertz |
| $^{1}H$ | Proton |
| $^{3}H$-PAF | Radiolabelled platelet activating factor |
| HEPES | N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid] |
| HMBC | Heteronuclear Multiple Bond Correlation |
| HMQC | Heteronuclear Multiple Quantum Coherence |
| HRMS | High Resolution Mass Spectroscopy |
| HCl | Hydrochloric Acid |
| Hz | Hertz |
| IC | inhibition concentration |
| IR | Infrared |
| *J* | coupling constant |
| $K_2HPO_4$ | Potassium hydrogen phosphate |
| lit. | Literature |
| m | Multiplet |
| M | Molar |
| mg | Milligram |
| mM | Millimolar |
| MBC | Minimum bactericidal concentration |
| MeOH | Methanol |
| MHz | Megahertz |
| MIC | minimum inhibition concentration |
| m.p | melting point |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| *m/z* | mass-to-charge ratio |
| nM | Nanomolar |
| nm | Nanometer |
| NMR | Nuclear Magnetic Resonance |
| pet. ether | Petroleum ether |
| ppm | Part per million |
| $R_f$ | retention factor |

| | |
|---|---|
| rt | room temperature |
| s | Singlet |
| t | Triplet |
| TLC | Thin-layer chromatography |
| VLC | vacuum liquid chromatography |
| δ | chemical shift |
| UV | Ultraviolet |
| μM | Micromolar |
| γ | Gamma |
| λ | Lamda |

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# CHAPTER 1

# INTRODUCTION

## 1.1 General Introduction

Malaysia is blessed with an abundance of untapped variety of medicinal plants as she is among the world's 12 mega biodiversity-rich countries. Our diverse ethnic communities meant that Malaysia has inherited a unique confluence of deep-rooted traditional medicine systems, including those practiced by our indigenous people. These elements have put Malaysia in an exceptional position to tap into the herbal medicine potential. Researchers from all over the world are racing against time to find cures for diseases and ailments such as HIV, cancer, Alzheimer's, Parkinsons and meningitis. Phytochemical research has provided stimulation in organic synthesis of potentially superior agents and in providing more compounds to be used as tools to understand the biochemical mechanisms involved in the occurrence of certain diseases. We believe the elixir is locked in the secrets of the rainforest plants. Due to this reason, initiation is taken to further explore higher plants for biologically active compounds.

Malaysia has about 12,000 species of flowering plants of which about 1300 are said to be medicinal [1]. The Taman Negara Reserve Rainforest in Pahang is considered the grandmother to the rest of the world's rainforests. It is considered to be one of the richest natural environments on earth with 10,000 species of plants, 350 species of birds,

100 types of snakes, 1000 varieties of butterflies, 150,000 kinds of insects and 140 types of animals. The huge diversity of Malaysian flora means that we can expect well diverse chemical structures from their secondary metabolites. However, there is still more effort to be made locally to establish and to develop the available plants into useful and valuable pharmaceutical products [2].

Natural products isolated from higher plants have been providing novel, clinically active drugs. The development of medicinal plants into therapeutic drugs takes several years and a substantial amount of money is needed. The process is very capital-intensive, high risk and the success rate is not very good. Despite all these, natural products drug discovery programmes in Malaysia are still existing, mainly because the potential of high chemical diversity from natural products is largely unknown and the large number of our terrestrial species have yet to be investigated. It is believed that there is still a lot more waiting for discovery as what have been studied to date is just a small fraction [3]. Bioassay-guided research and multidisciplinary concepts were introduced so that the research carried out can be more meaningful. The Government of Malaysia has taken steps to increase the scientific knowledge of our rich source of medicinal plants by making available funds for research beginning in 1985 with the Intensification of Research in Priority Areas (IRPA) programme. One of the areas that have been identified as a priority is the commercialization of biotechnology, which also takes into account the development and production of biopharmaceuticals from plant genetic resources.

## 1.2 Family Moraceae

Moraceae is a large family comprising of about 60 genera and approximately 1400 species that form a significant element in the flora of the tropical region of Southeast Asia [4]. The most important genera are *Morus*, *Ficus* and *Artocarpus*. Only nine genera and 137 species could be found in Malaysia, distributed from lowlands to mountain forests. *Ficus* and *Artocarpus* plants are quite abundant in Malaysian forests. Several members of this family produce valuable timbers and edible fruits. Another important economic plant is the mulberry tree or *Morus*.

*Morus* is a small genus found primarily in temperate and subtropical regions of the Northern Hemisphere and has been widely cultivated in China and Japan for its leaves, which are fed to silkworms. *Morus nigra* (black mulberry) can grow up to 35 feet high and forms a compact crown. The heart-shaped leaves are usually whole, except when younger, they often separate into several lobes. The berries are dark red when ripe and can be eaten fresh or used to make jams. *M. rubra* (red mulberry) is the largest of all the Mulberries and can grow from 60 to 70 feet high. The leaves are oval or oblong heart-shaped with a pointed tip and serrated edges. The edible berries are dark red while the wood can be used for light carpentry. *M. alba* (white mulberry) can grow up to 50 feet high and produces extremely sweet, pinkish, white, or purplish berries.

*Ficus* is a large genus with about 600 species that is commonly found in the tropical regions. *Ficus* in Malaysia is a big and ubiquitous genus, consists of 101 species. The trees grow easily in all types of forest where the seeds are spread by small mammals and birds. Some of the species are deciduous while others like *Ficus benjamina* and *F. microcarpa* are evergreen. *Ficus* is popular as a landscape tree because of its deep green leaves and long red stipules. The trees are suitable for 'bonsai' as it can grow fast, tolerant to most soil and light condition. Only several species of *Ficus* produce edible fruits [4].

### 1.3 Genus *Artocarpus*

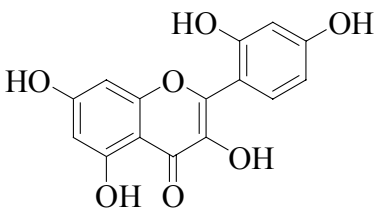
*Artocarpus* is the most commonly encountered genus, representatives of the Moraceae in the lowland forest of the tropical Southeast Asia, apart from *Ficus*. There are 47 species of *Artocarpus* in which only 20 species including the cultivated plants could be found in Malaysia. This genus is known world wide for its edible fruits like the jackfruit, *A. heterophyllus* locally known as 'nangka', bread fruit, *A. communis* ('sukun') and 'cempedak', *A. integer*. These species are widely cultivated in Malaysia as villagers and traders commercially sell their fruits in local market. The lightwood known locally as 'terap' and the medium hardwood known as 'keledang' constitute valuable timber resources [4-5]. Some of Malaysian *Artocarpus* species are rare. Most of these species

have never been chemically investigated including *A. anisophyllus*, *A. bracteata*, *A. fulvicortex*. *A. hispidus*, *A. kemando*, *A. lowii*, *A. nitidus*, and *A. odoratissima* [4].

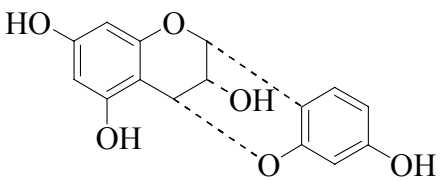
## 1.4 A Review of Phytochemicals and Biological Properties of *Artocarpus* Species

*Artocarpus* species have been studied quite thoroughly, chemically or biologically by few groups of researchers from Indonesia, Japan, and Taiwan. Most of the plants studied were collected from the rain forest of Indonesia. Some studies cover *Artocarpus* species of Taiwan, Carribean and Thailand. Several new and interesting compounds have been isolated, characterized and evaluated for their biological activities. *Artocarpus* species are noted as an abundant source of phenolic constituents. These constituents can be classified into isoprenylflavonoids, stilbenoid and 2-arylbenzofuran derivatives, phenolic compounds with oxepine ring and natural Diels-Alder type adducts.

The earlier work on the phytochemical investigation of *Artocarpus* species started long ago in 1895 where morin **(1)** and cyanomaclurin **(2)** were isolated from *A. heterophyllus*. It was only in 1963 that a study of the NMR spectrum of the acetate of cyanomaclurin trimethyl ether led to the structure of cyanomaclurin **(2)** [6]. Two more prenylflavonoids were isolated in very minute quantities from the same species and identified as cycloheterophyllin **(3)** and heterophyllin **(4)** [7]. Since then, many types of new isoprenoid-substituted phenolic compounds were isolated from *Artocarpus* species.




**(1)**

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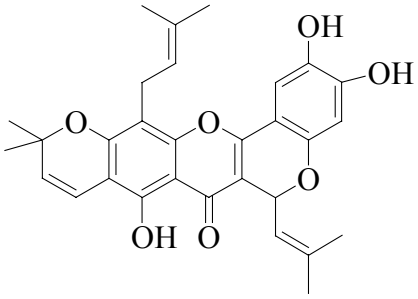


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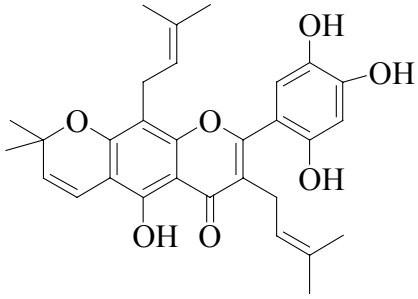
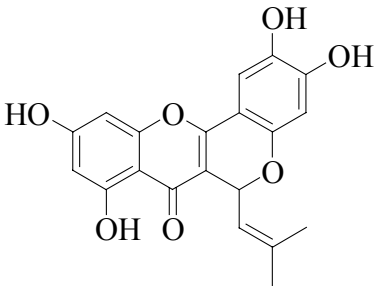



**(4)**

### 1.4.1 Isoprenylflavonoids

Most of the isolated isoprenylflavonoids have common features of hydroxyl groups in the 5,7,2′,4′- positions and C-γ, γ-dimethylallyl or isoprenyl substituents in the 6-, 3,6- or 3,6,8-positions of the flavone skeleton [6]. These remarkable structural features of the flavonoids correlate with their biological activities very significantly.

Nine new isoprenylflavonoids were isolated from the tree bark, root and heartwood of *Artocarpus champeden*, Spreng. This species is an endemic species found throughout the Indonesian archipelago. The fruits, locally known as ‘cempedak’ are eaten as staple food and its wood is used commercially as timber. In Malaysia, this species is known as *A. integer* [4]. The compounds isolated were characterized spectroscopically as cyclochampedol **(5)**, artoindonesianins A-B **(6-7)**, artoindonesianins Q-T **(8-11)**, and artoindonesianins U-V **(12-13)** [8-11]. Cyclochampedol **(5)** was shown to be toxic to brine shrimps (*Artemia salina*) [8], whereas artoindonesianin A-B **(6-7)** and artoindonesianins U-V **(12-13)** exhibited cytotoxic effects against murine P388 leukemia cells [9, 11].



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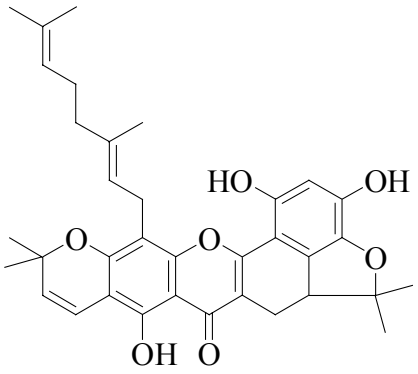

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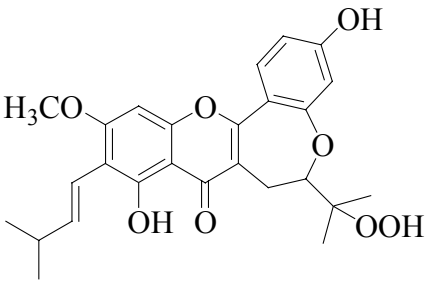

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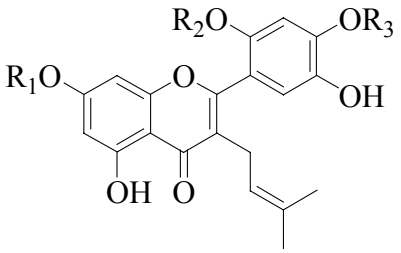
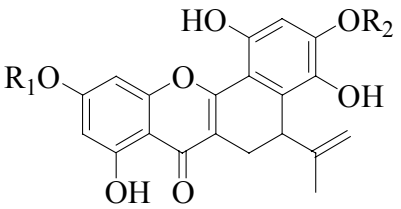
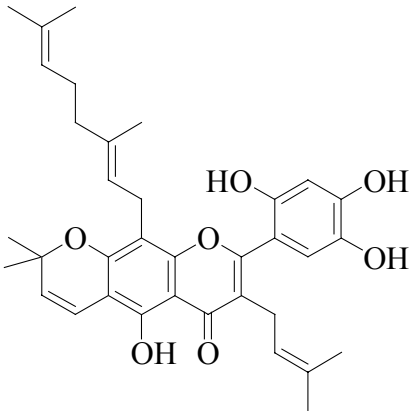


**(8)** $R_1 = R_3 = CH_3$, $R_2 = H$
**(9)** $R_1 = H$, $R_2 = R_3 = CH_3$



**(10)** $R_1 = R_2 = CH_3$
**(11)** $R_1 = H$, $R_2 = CH_3$



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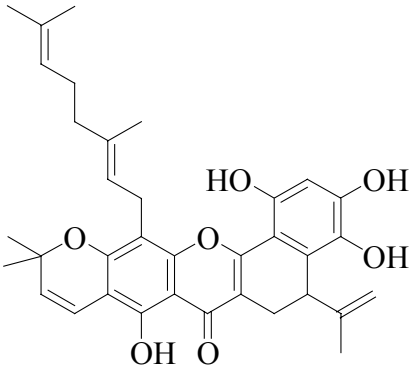


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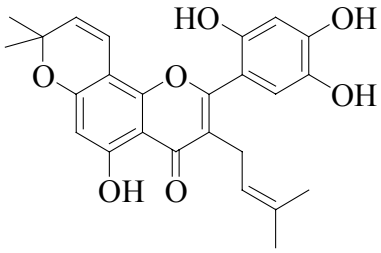
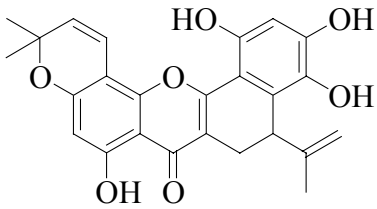



**(14)**

The dried bark of *Artocarpus communis* Forst. (synonym *A. altilis*) contained isoprenylflavonoids identified as artonin E **(14)**, artobiloxanthone **(15)**, artonin F **(16)**, cycloartobiloxanthone **(17)**, and artonols A-E **(18-22)** [12-14]. This species is widely distributed throughout the tropical area in Southeast Asia, especially in Malaysia and Indonesia. In Malaysia, this species is known as 'sukun' while in Indonesia, there are two varieties; namely 'kulur' (breadfruit tree) and the other which produce edible fruits called 'sukun' (seedless breadfruit tree) [12]. The leaves are used for hepatomegalis and febris, while the flowers are used against parulis and adontalgia. Furthermore, the dried flower of the plant has been used as a mosquito repellent [13].

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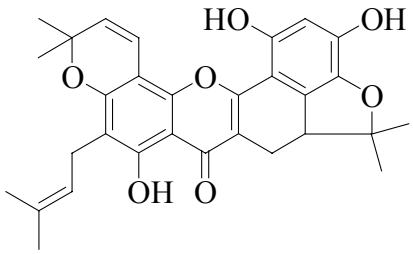


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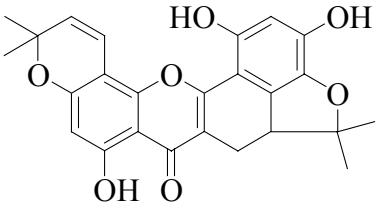

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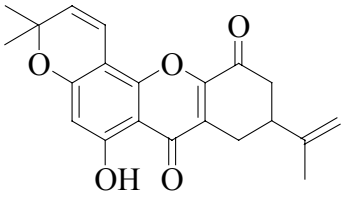

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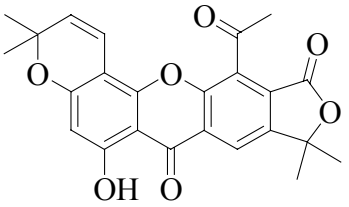
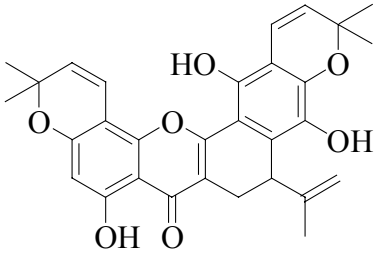


**(19)**



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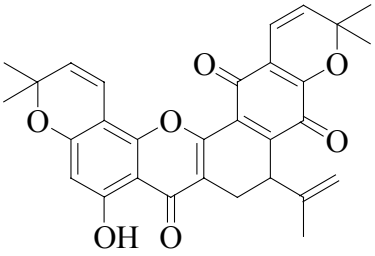

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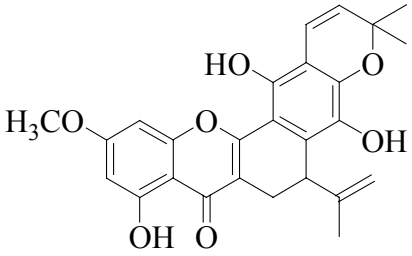
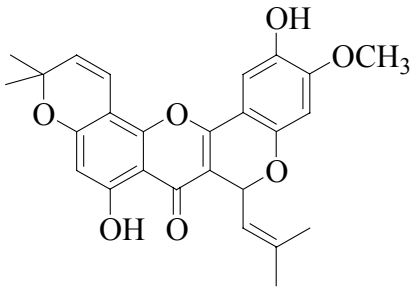


**(22)**

Nine new isoprenylflavonoids were successfully isolated from the root bark of *Artocarpus communis* of Taiwan [15-18]. The compounds were characterized as cycloartomunin **(23)**, dihydrocycloartomunin **(24)**, cycloartomunoxanthone **(25)**, artomunoxanthone **(26)**, artomunoxanthentrione **(27)**, artomunoxanthotrione epoxide **(28)**, cyclocommunol **(29)**, cyclocommunin **(30)** and dihydroisocycloartomunin **(31)** [15-18]. Another five prenylflavonoids were isolated from the cortex of the roots of *A. communis* of Taiwan. These compounds were characterized as artocommunols CA **(32)**, CB **(33)**, CC **(34)**, CD **(35)** and CE **(36)** [19]. However, these studies did not include any work on their biological activities.



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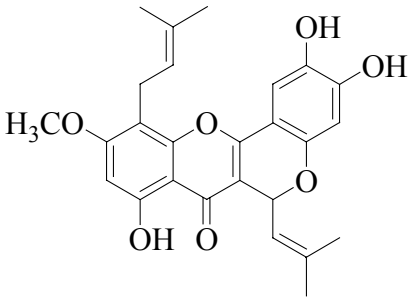

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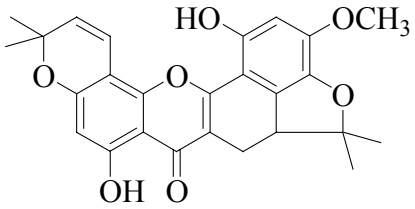

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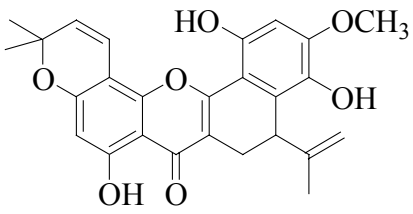

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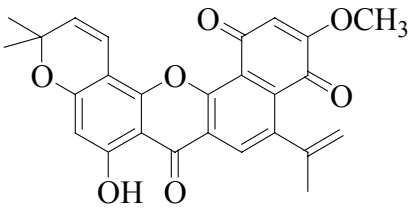

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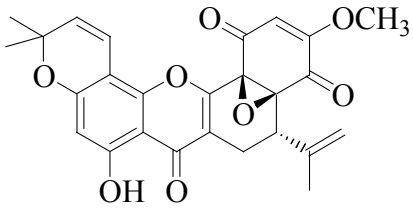

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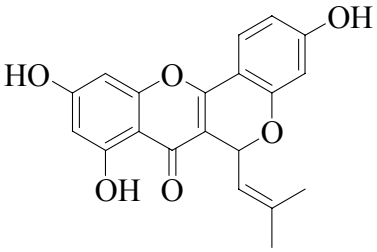

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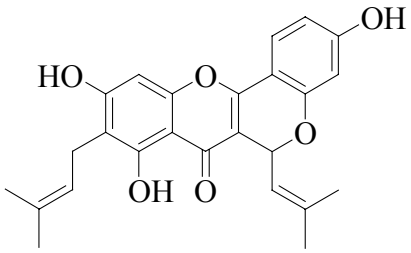

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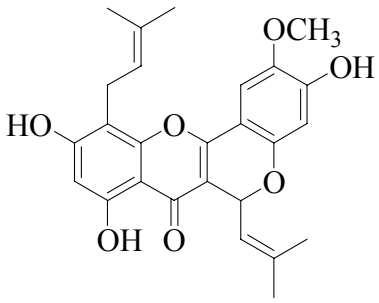
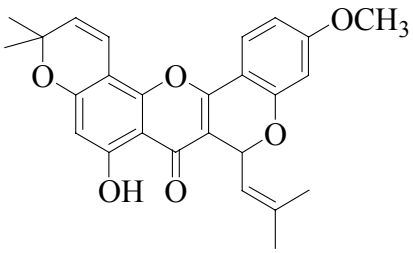



**(31)**

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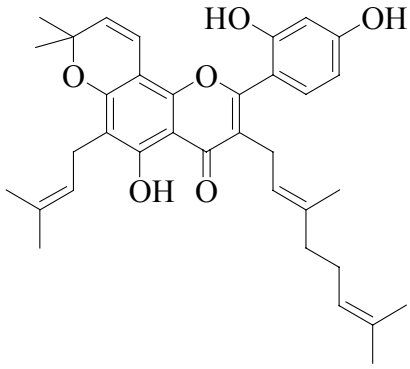


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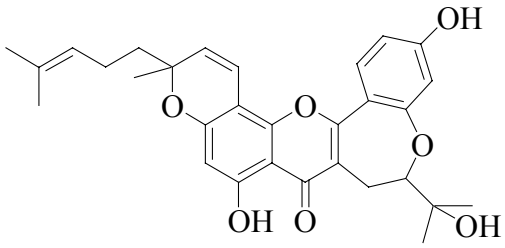
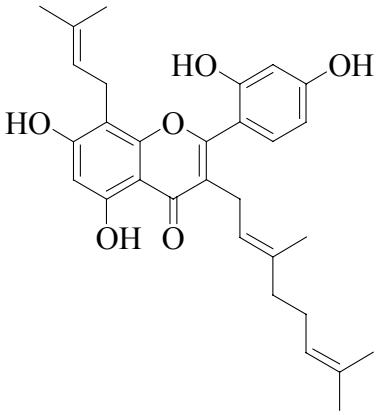



**(34)**

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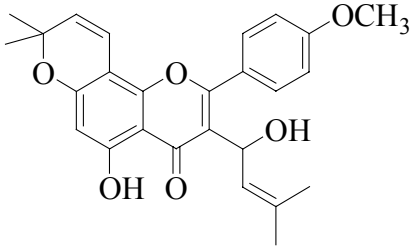
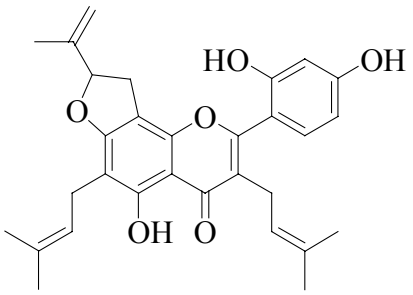


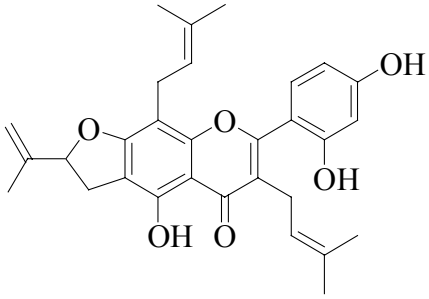

**(36)**

*Artocarpus lanceifolius*, Roxb. is a rare species endemic to lowlands and hill forests in Malaysia and the province of West Sumatra, Indonesia. It is locally known as ‘keledang’. The wood is used for making coffins and for heavy construction [4].

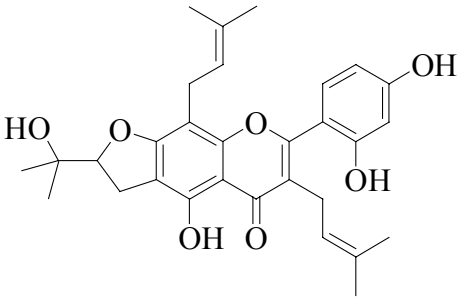
Phytochemical and biological studies have been carried out on the heartwood and the tree bark of *A. lanceifolius* of Indonesia. Five new prenylated flavones were isolated. These flavones were identified as artoindonesianins G-I **(37-39)**, artoindonesianin P **(40)** and 14-hydroartonin E **(41)** [20-23]. Biological evaluation of artoindonesianins G-I **(37-39)** and artoindonesianin P **(40)** showed that these compounds exhibited cytotoxicity effect against murine P388 leukemia cells [21-22].



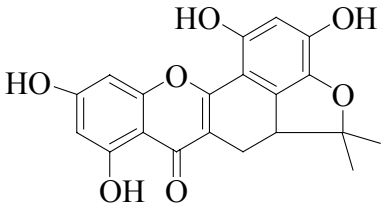

**(37)**




**(38)**




**(39)**

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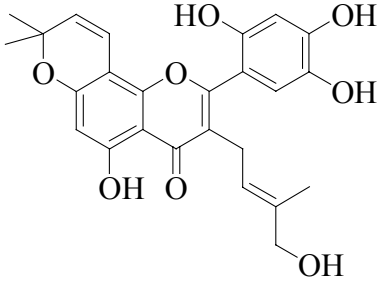


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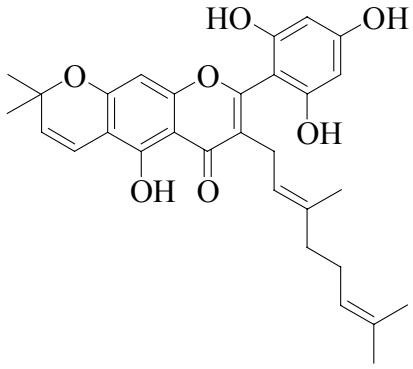
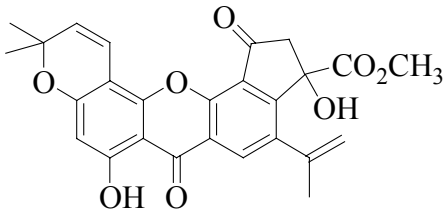



**(42)**

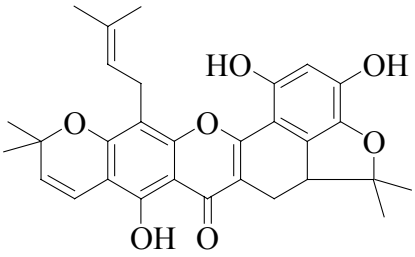
*Artocarpus rotunda* (Hout) Panzer is a wild tree growing in Southern Sumatera, Indonesia. Studies on the root bark of this species have resulted in the isolation of a new prenylated flavone, named artoindonesianin L **(42)**. This flavone showed significant cytotoxicity against murine P388 leukemia cells [24].

*Artocarpus teysmanii* Miq. is a rare species found in swampy areas of West Coast of Peninsular Malaysia, Sumatera, South Sulawesi and Western New Guinea [4]. A new xanthone derivative, artoindonesianin C **(43)** together with known cycloartobiloxanthone **(17)** and artonol B **(19)** were isolated from the root bark of this species. The two known compounds were found to be active in the *Artemia salina* bioassay, while artoindonesianin C **(43)** was shown to be inactive [25-26].

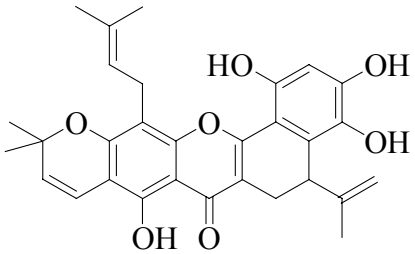



**(43)**

*Artocarpus heterophyllus* Lamk. or jackfruit is a laticiferous fruit tree probably native to Southern India and cultivated throughout the tropical world including Malaysia. *A. rigida* Bl. or *A. rigidus* Bl. is locally known as 'temponek'. There are four varieties of *A. rigidus* Bl. in Malaysia viz. "hispidus", "asperulus", "tomentosa", and "glabra". These species can be found throughout the lowland and hill forests of Malaysia, Thailand and Indonesia [4]. These two species had been intensively investigated and contained a substantial amount of prenylflavonoids. Among the new prenylflavonoids isolated were artonins A-B **(44-45)**, artonins J-L **(46-48)**, artonins Q-U **(49-53)** from the root bark and tree bark of *A. heterophyllus* Lamk. [27-29] and artonins G-H **(54-55)**, artonins M-P **(56-59)** from the tree bark of *A. rigida* Bl. [30-31].



**(44)**



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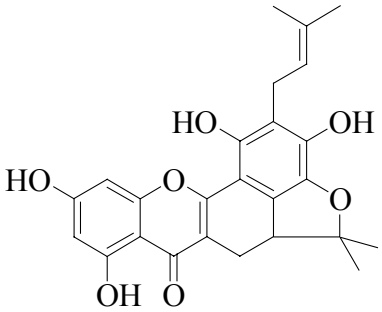
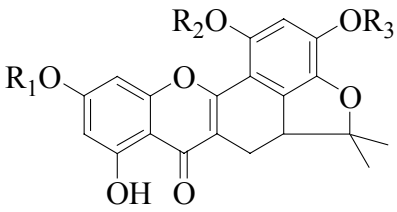
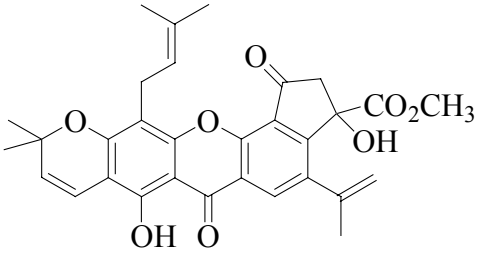


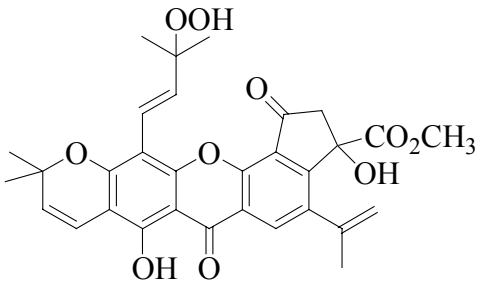
**(46)**



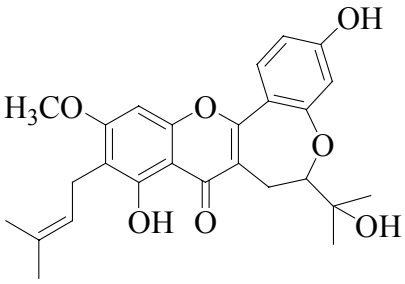
**(47)** $R_1 = CH_3$, $R_2 = R_3 = H$
**(48)** $R_1 = R_2 = CH_3$, $R_3 = H$



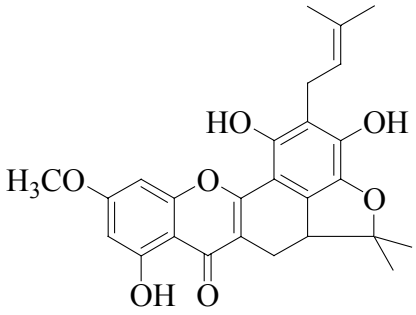
**(49)**



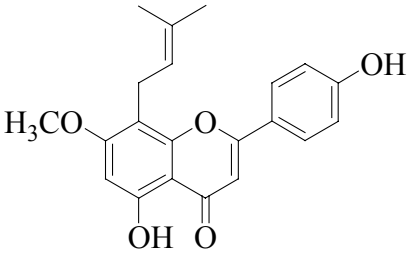
**(50)**


OH
H3CO
O
O
OH
O
OH

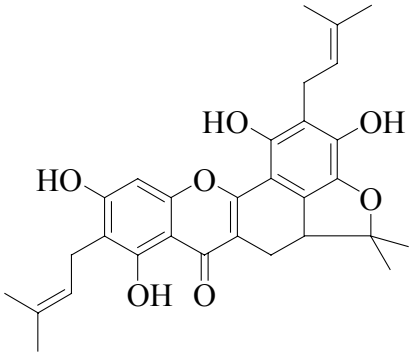
**(51)**


HO
OH
H3CO
O
O
OH
O

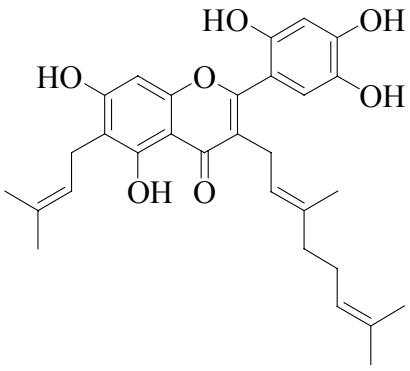
**(52)**


OH
H3CO
O
OH
O

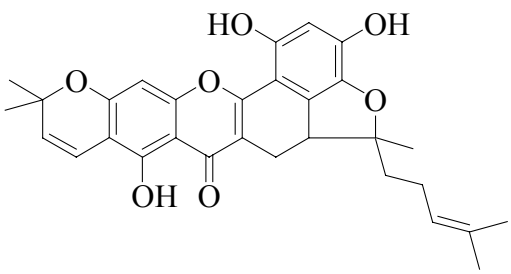
**(53)**


HO
OH
HO
O
O
OH
O

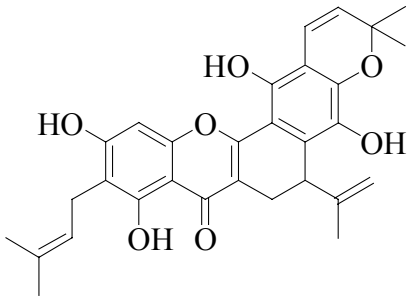
**(54)**


HO
OH
HO
O
OH
OH
O

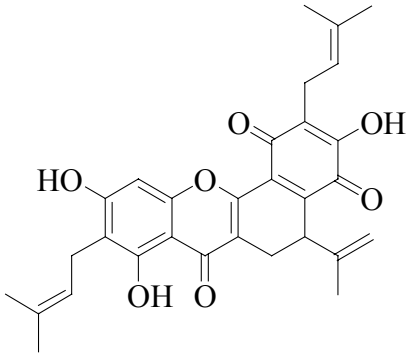
**(55)**



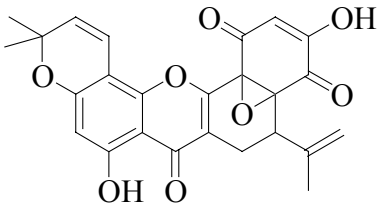
**(56)**



**(57)**

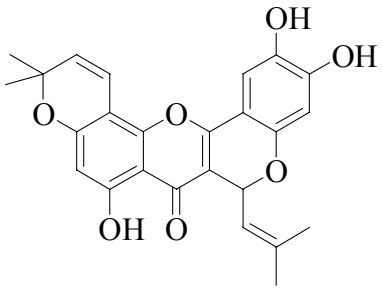


**(58)**

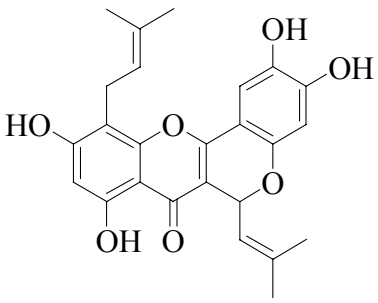


**(59)**

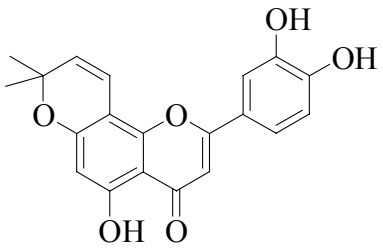
*Artocarpus chama* Buch.-Ham can be found growing in Yunnan, China. Investigation on the roots of this species has led to a report of five new isoprenylated flavones. These flavones were elucidated spectroscopically and identified as artochamins A-E **(60-64)**. Artochamin C **(62)** was found to be potent against human lung carcinoma (A549) and breast adenocarcinoma (MCF7) [32].



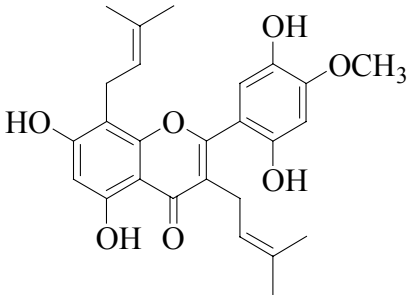
**(60)**



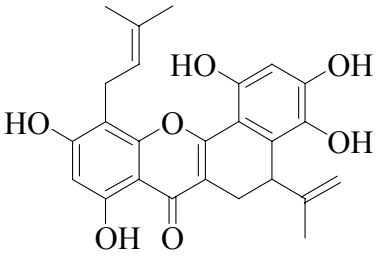
**(61)**



**(62)**

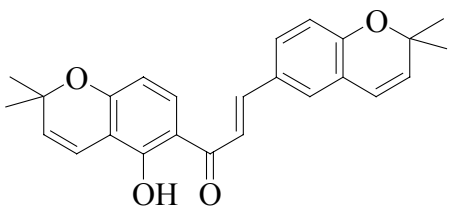


**(63)**

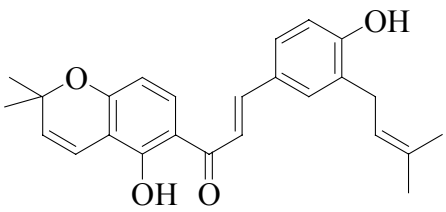


**(64)**

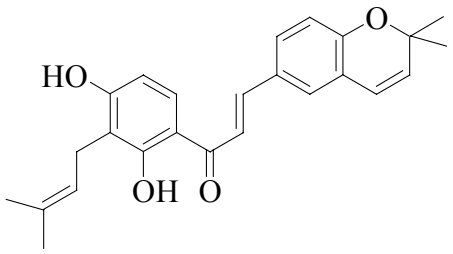
Besides isoprenylflavones, there are a few reports on isoprenylchalcones, dihydrochalcones and isoprenylflavanones from *Artocarpus* species. Among the species that contained these constituents are *Paratocarpus venenosa* Zoll., *A. altilis*, and *A. nobilis* Thw.. Seven new isoprenoid-substituted chalcones and five isoprenoid-substituted flavanones were isolated from the tree bark of *Paratocarpus venenosa* Zoll. collected in Bogor, Indonesia. These compounds were characterized as paratocarpins A-G **(65-71)** and paratocarpins H-L **(72-76)** [33-34].



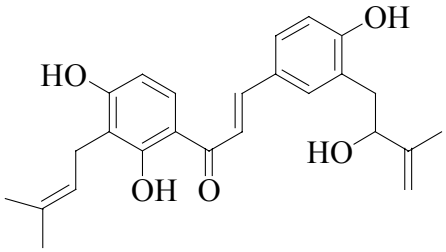
**(65)**



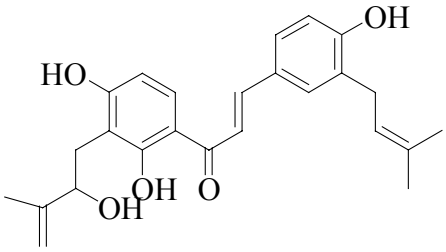
**(66)**



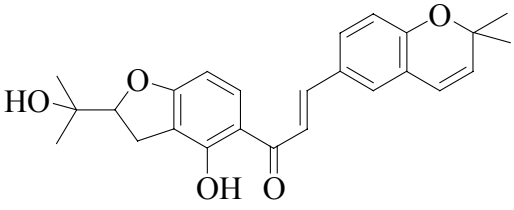

**(67)**




**(68)**




**(69)**

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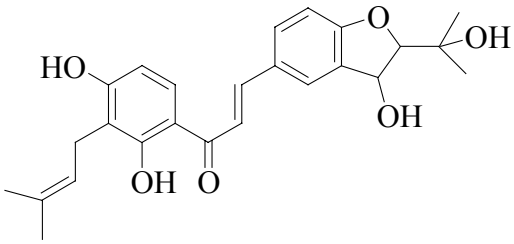


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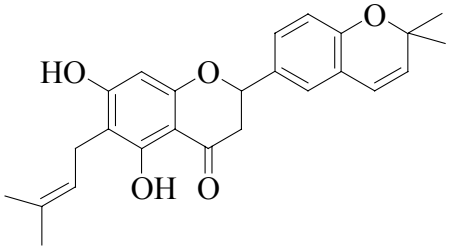


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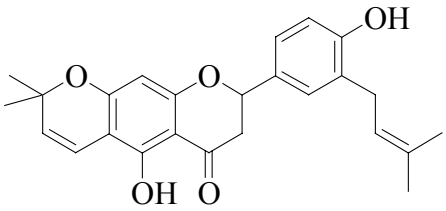


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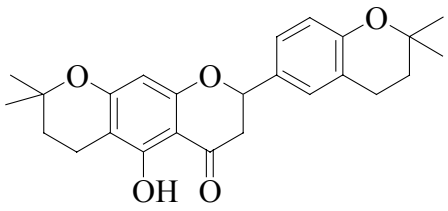


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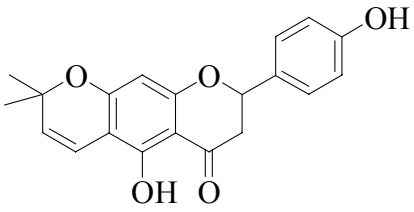


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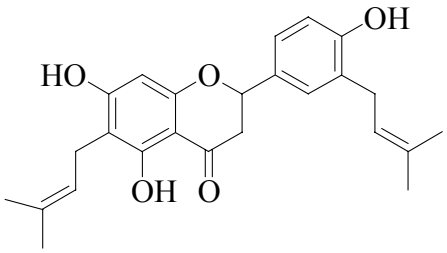
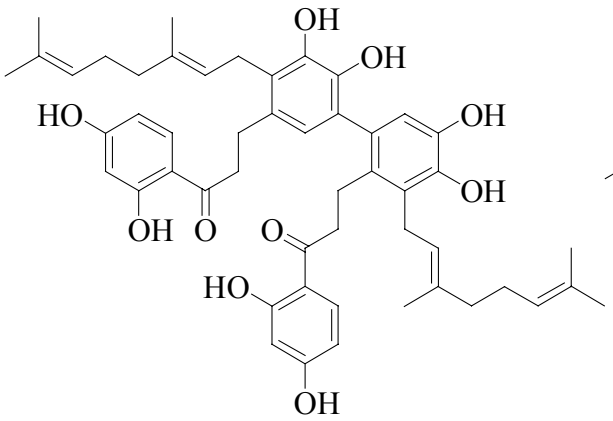



**(76)**

The buds of *Artocarpus altilis* have been used traditionally in Taiwan for the treatment of liver cirrhosis and hypertension and have been reported to possess anti-inflammatory and detoxifying effects. Investigation on the bud covers of *A. altilis* has led to the isolation of a new dimeric dihydrochalcone, cycloaltisin 6 **(77)** and an isoprenylflavanone, cycloaltisin 7 **(78)**. Both compounds showed activity in a cathepsin K inhibition assay with $IC_{50}$ values of 98 and 840 nM, respectively. Cathepsin K is a novel cysteine protease that has been implicated in osteoporosis. It has been established that cysteine protease inhibitors are very effective in preventing bone resorption [35].

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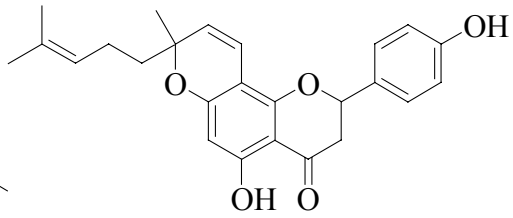
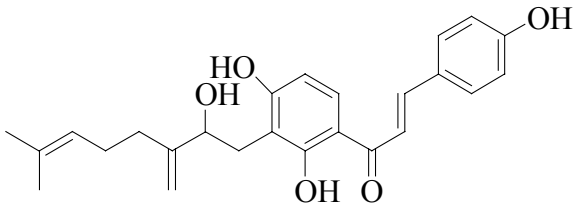



**(78)**

*Artocarpus nobilis* is a tree of moderate size and the only endemic species of the genus *Artocarpus* found in Sri Lanka. Two new chalcones were isolated from the leaves of this species and identified as 2′, 4′, 4-trihydroxy-3′-(2-hydroxy-7-methyl-3-methylene-6-octaenyl)chalcone **(79)** and 2′, 3, 4, 4′-tetrahydroxy-3′-(6-hydroxy-3,7-dimethyl-2*E*,7-octadienyl)chalcone **(80)**. These compounds showed significant fungicidal activity against *Cladosporium cladosporioides* and high radical scavenging activity towards DPPH radical in TLC bio-autography method [36].

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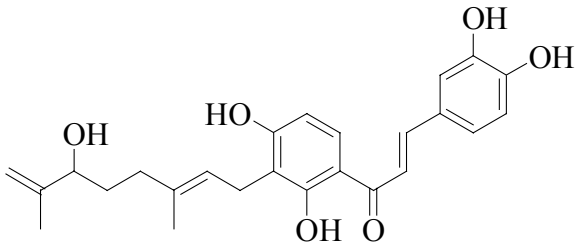
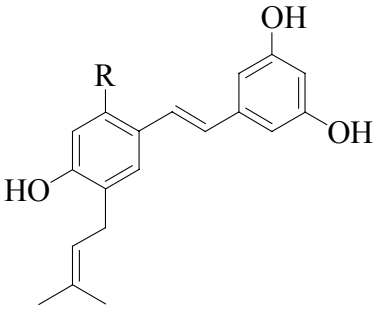



**(80)**

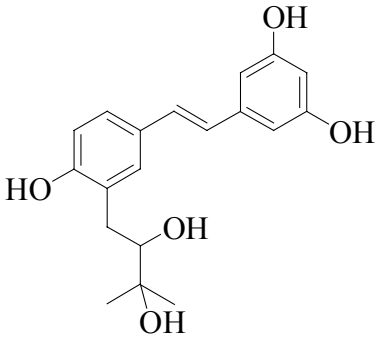
### 1.4.2 Stilbenoid and 2-Arylbenzofuran Derivatives

*Artocarpus* species also contain the biosynthetically related stilbene and 2-arylbenzofuran derivatives although their distributions are more limited. Stilbenoids are bibenzyl compounds produced *via* the mixed phenylpropanoid or polyketide biosynthetic pathway. Among the species reported to have these types of compounds are *A. dadah*, *A. fretessi*, *A. gomezianus*, *A. heterophyllus*, *A. integer*, *A. incisus*, and *A. tonkinensis* [37-43].

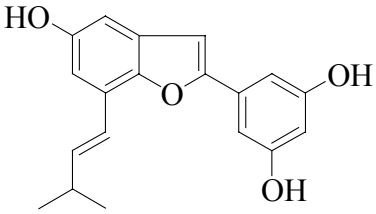
*A. dadah* Miq. is known as ‘tampang’ in Malaysia and Kalimantan, Indonesia and its bark has been used as an ingredient in the betel nut chewing mixture. Three new prenylated stilbenoid derivatives, 3-(3, 3-dimethylallyl)resveratrol **(81)**, 5-(3, 3-dimethylallyl)oxyresveratrol **(82)** and 3-(2,3-dihydroxy-3-methylbutyl)resveratrol **(83)**, and a new benzofuran derivatives, 3-(3, 3-dimethylpropenyl)morusin M **(84)** were isolated from the bark and twigs of *A. dadah* Miq. [37].




**(81)** R = H
**(82)** R = OH

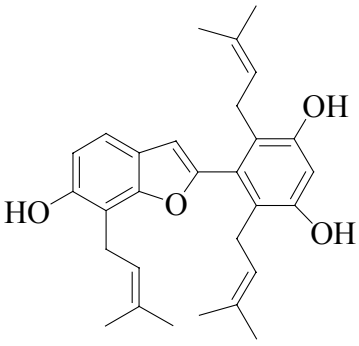



**(83)**




**(84)**

Two new isoprenylated arylbenzofurans, namely artoindonesianins X **(85)** and Y **(86)** were isolated from the root bark of *Artocarpus fretessi* Hassk. These compounds showed moderate activity against the brine shrimp, *Artemia salina* [38].

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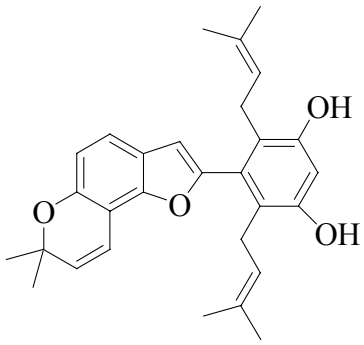
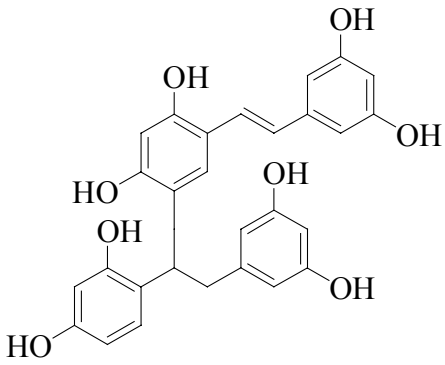
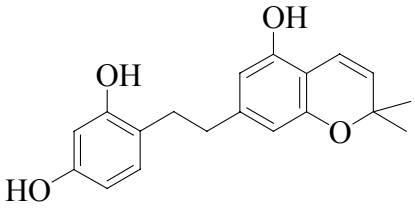



**(86)**

Study on the roots of *Artocarpus gomezianus*, Wall ex Tre'c revealed the presence of a new dimeric stilbene, artogomezianus **(87)** which displayed potent tyrosinase inhibitory activity [39]. Investigation of tyrosinase inhibitors may provide important clues for developing new insects control agents [40]. In plants, this enzyme is responsible for the browning of some fruits and vegetables; therefore its inhibitors may have potential uses as food preservatives. In man, potent tyrosinase inhibitors, such as kojic acid have been used as whitening agents in cosmetic products, due to their ability to suppress dermal melanin production [39-40].

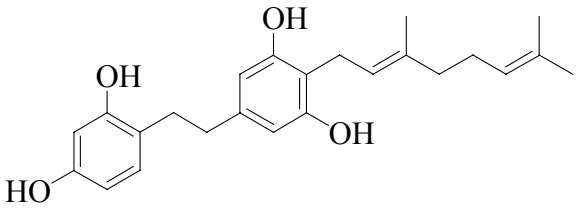



**(87)**




**(88)**

Investigation on the heartwood of *Artocarpus incisus* also revealed the presence of stilbenoid derivatives with tyrosinase inhibitory properties. These compounds were identified as artocarbene **(88)**, chlorophorin **(89)** and 4-prenyloxyresveratrol **(90)** [41-42].

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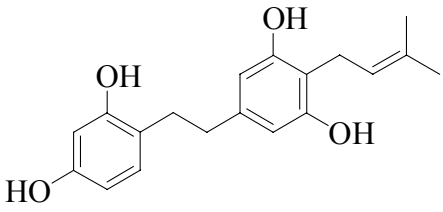
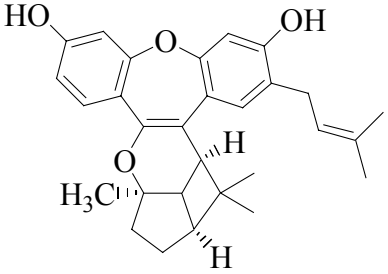


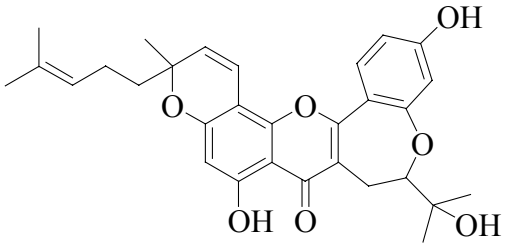

**(90)**

### 1.4.3 Phenolic Compounds with Oxepine Ring

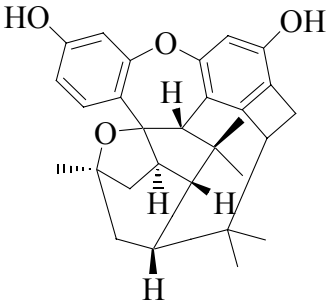
The root barks of *Artocarpus rigida* of Taiwan were found to contain some novel compounds with an oxepine ring. These compounds were named as artocarpols A-F **(91-96)**. Artocarpol A **(91)** strongly inhibited superoxide formation in phorbol 12-myristate 13-acetate (PMA) stimulated rat neutophils in a concentration-dependent manner with an $IC_{50}$ value of 13.7 ± 0.7 μM and also showed a significant inhibitory effect on tumour necrosis factor-α (TNF-α) formation in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Artocarpols A **(91)**, C **(93)**, D **(94)** and E **(95)** are the first natural products containing an oxepine ring with a novel skeleton [43-45].



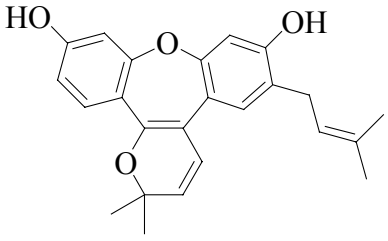

**(91)**



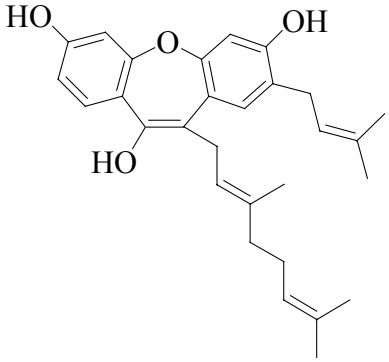

**(92)**



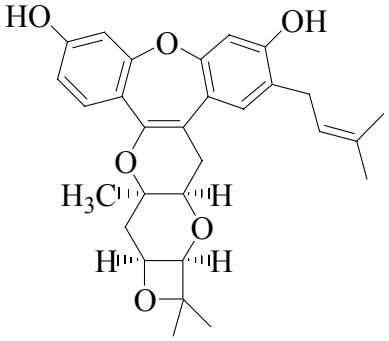

**(93)**




**(94)**



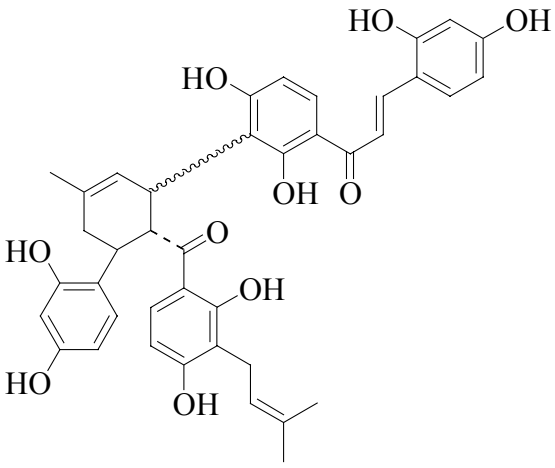
**(95)**



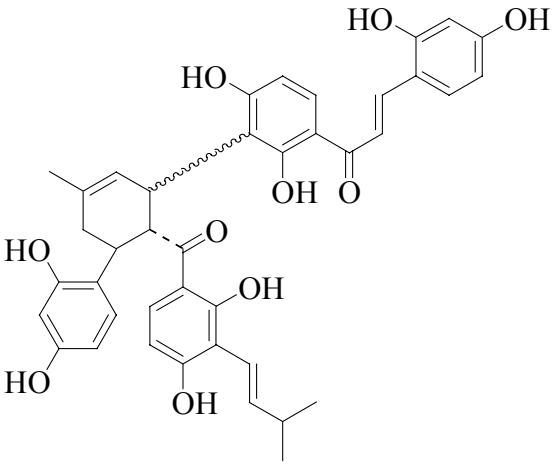
**(96)**

### 1.4.4 Diels-Alder Type Adducts

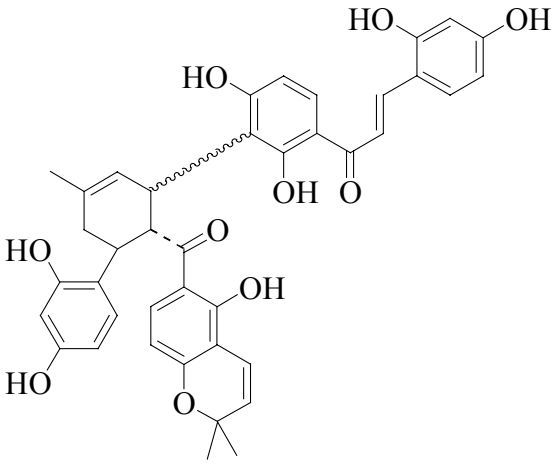
Diels-Alder type adducts are regarded as an intermolecular [4+2] cycloaddition products from the isoprenyl portion of a dehydroprenylphenols, as a diene, and the α,β-unsaturated carbon double bond of a chalcone skeleton, as a dienophile. Artonins C-D **(97-98)** and artonin X **(99)** are examples of this type of compounds, isolated from the root bark of *Artocarpus heterophyllus* [46-47].



**(97)**



**(98)**




**(99)**

## 1.5 Biosynthesis of Flavonoids

Over the past 30 years, there have been increasing reports of isoprenylated compounds belonging to different classes of flavonoids especially the isoprenylated flavones, flavanones and chalcones. In spite of the rich variety and structural diversity of isoprenylated flavonoids, these compounds have been isolated from a rather limited number of unrelated plant families especially the Leguminosae, Moraceae and Asteraceae. Prenylated flavonoids are most frequently found in roots, barks and heartwoods, but also occur in the aerial parts, buds and seeds [48].

All flavonoids derive their carbon skeletons from two basic compounds, malonyl CoA that is synthesized from the glycolysis intermediate acetyl-CoA and carbon dioxide, and the CoA ester of a hydrocinnamic acid **(Figure 1.1)**. The aromatic ring B and its adjacent 3-carbon side chain are derived from L-phenylalanine *via* the shikimate pathway, whereas ring A is formed by the head-to-tail condensation of three acetate units *via* the polyketide pathway leading to the formation of the $C_{15}$ chalcone intermediate [48]. Flavonoids, aurones and other diphenylpropanoids are derived from the $C_{15}$ chalcone intermediate and the first flavonoid, flavanone is formed by stereospecific

action of chalcone isomerase on this compound. Oxidative rearrangement of this flavanone yields an isoflavone. Introduction of a double bond between C-2 and C-3 of the flavanone leads to the abundant class of flavone. Dihydroflavonol is formed by direct hydroxylation of flavanone at C-3, which is catalysed by flavanone 3-hydroxylase. Dihydroflavonol is biosynthetic intermediate in the formation of flavonol, catechin, proanthocyanidin and anthocyanidin. The large class of flavonol is formed by introduction of a double bond between C-2 and C-3 of the dihydroflavonol [48-49]. They are summarized in **Figure 1.1**.

Generally, most flavonoids are *C*-prenylated, whereas *O*-prenylation is quite rare. *C*-prenylation takes place more frequently on ring A at C-6 or C-8, as well as C-3′ or C-5′ especially in flavanones and flavones. From the biosynthetic point of view, it is agreed that the basic skeleton of the different flavonoid classes, including isoflavonoids, is constructed before any isoprenoid substituents are added. A structural analysis of prenylated flavonoids from Leguminosae suggests that all modifications of ring A occur at the chalcone stage including isoprenylation, *β*-hydroxylation and elimination of the C-6′ hydroxyl group or 2′-*O*-methylation. In addition, cyclization of the prenyl substituent is determined by the *O*-methylation pattern of C-2′ and/or C-6′ hydroxyl groups. *C*-prenylation usually occurs *ortho* to a phenolic hydroxyl group e.g. at positions C-6, C-8, C-3′, C-4′ or C-5′, except for C-3 prenylation [49].

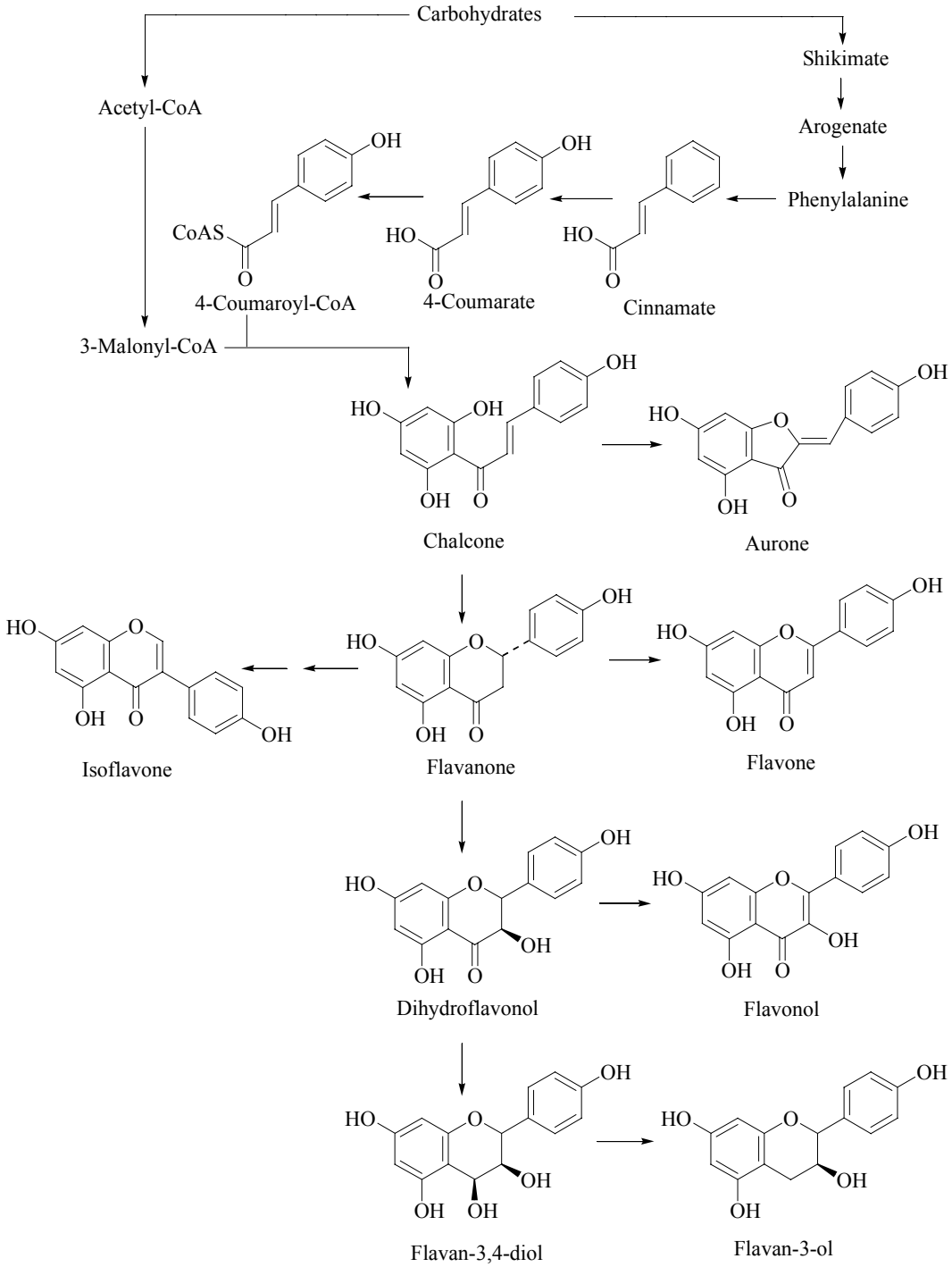
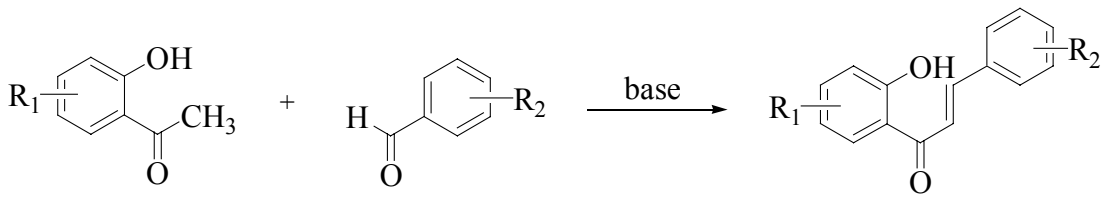



**Figure 1.1:** Biosynthetic Pathway of Flavonoids [48-49]

## 1.6 Synthesis of Flavonoids

The flavonoids are a very well known family of natural products found almost exclusively in the plant kingdom, most of them are highly coloured and, as a consequence, play a vital role in the ecology of plants by making flowers and fruits attractive to bees and birds. Many naturally occurring flavonoids are known to have significant biological activities. They were reported to be useful as antioxidants, anti-inflammatories, pulmonary carcinogenesis inhibitors, antimalarials, and antileishmanials [50]. With their vast biological potential, synthesis of flavonoids and flavonoid precursors have become great interests to many researchers nowadays. The classical flavonoid synthetic routes are known by the name of their developers, viz., Claisen-Schmidt, Baker-Venkataraman, Allan-Robinson, and Algar-Flynn-Oyamada [51].

The Claisen-Schmidt reaction **(Figure 1.2)** is the most frequently used means of establishing the $C_6$-$C_3$-$C_6$ flavonoid nucleus owing to the availability of starting materials and comparative ease with which the reaction can be run. This reaction involves condensation of a $C_6$-$C_2$ unit, substituted 2-hydroxyacetophenone **(100)** and a $C_6$-$C_1$ unit, benzaldehyde derivatives **(101)** to obtain a 2′-hydroxychalcone **(102)** or the isomeric flavanone, bearing A-ring substituents provided by the acetophenone (indicated as $R_1$) and B-ring substituents provided by the benzaldehyde (indicated as $R_2$). The classical Claisen-Schmidt reaction is routinely run using aqueous sodium or potassium hydroxide or ethanolic sodium ethoxide at about 50°C over a period of several hours. The benzaldehyde is often used in slightly more than equivalent amounts. Besides the Claisen-Schmidt reaction, chalcones can also be synthesized by the direct Friedel-Crafts acylation of a phenol. In this approach the phenol becomes the A-ring while the acylating agent provides both the B-ring carbons and the three-carbon bridge to form the $C_6$-$C_3$-$C_6$ unit [51]. Chalcones became the key precursors in the synthesis of various flavonoids as they can be transformed easily to other classes of flavonoids by using different reagents and conditions **(Figure 1.3)** [51].




**(100)** **(101)** **(102)**

**Figure 1.2:** The Claisen-Schmidt Reaction

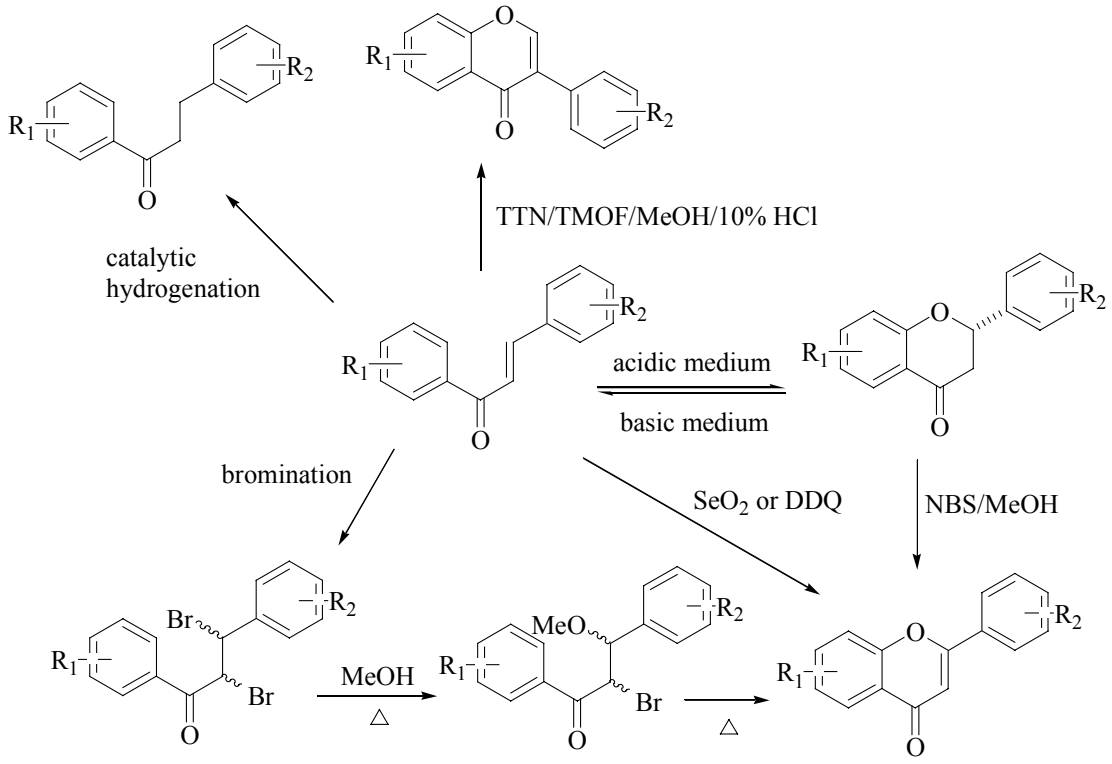
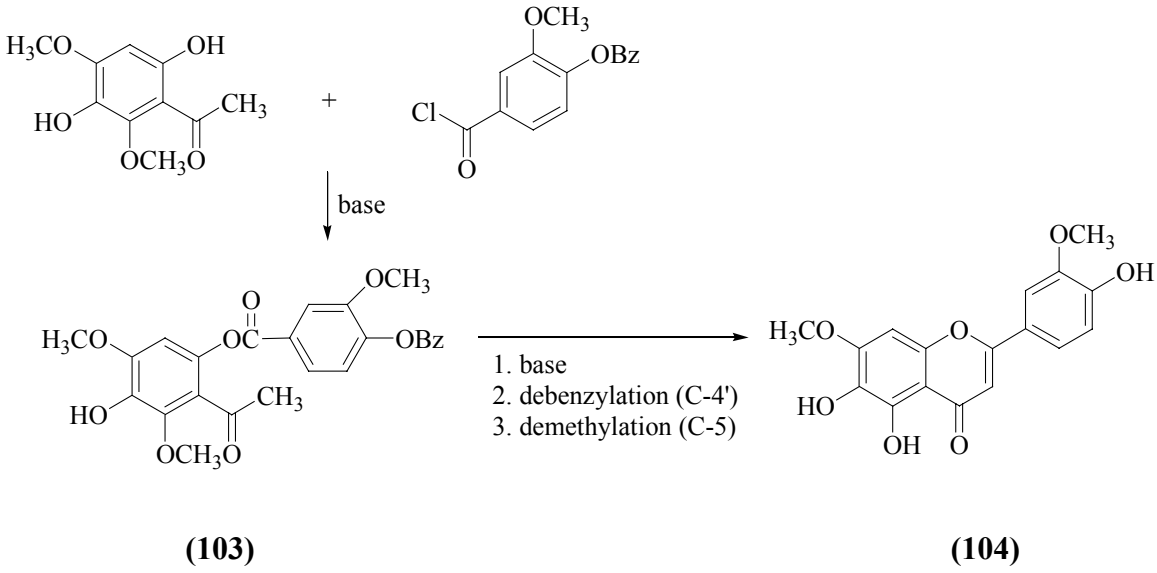



**Figure 1.3:** Conversion of Chalcone to Various Types of Flavonoids

The Baker-Venkataraman rearrangement involves acylation of a 2′-hydroxy-acetophenone with an aromatic acid chloride at oil bath temperature in the presence of a base such as potassium carbonate or pyridine. The resulting esters are converted into β-diketone with a strong base, potassium hydroxide in pyridine or with sodium hydride. Treatment of the β-diketone with ethanol-sulphuric acid, in glacial acetic acid and

anhydrous sodium acetate, results in recyclisation to the hemiketal followed by elimination of water to form the flavone. A typical example of this method involves the conversion of the 3-methoxy-4-benzyloxybenzoyl ester of 2,5-dihydroxy-4,6-dimethoxyacetophenone **(103)** to 4′,5,6-trihydroxy-3′,7-dimethoxyflavone **(104)**. Removal of the benzyl group from the 4′- position and the methyl group from the 5-position afforded the desired compound **(Figure 1.4)** [51-52].




**(103)** **(104)**

**Figure 1.4:** Baker-Venkataraman Rearrangement

In 1993, Ares and co-workers described a simple and convenient large-scale synthesis of 5-methoxyflavone **(108)**, which employs potassium *tert*-butoxide (*t*-BuOK) in a modified Baker-Venkataraman process **(Figure 1.5)**. The starting material, 2-hydroxy-6-methoxyacetophenone **(106)** was readily prepared from commercially available 2,6-dihydroxyacetophenone **(105)** using methyl iodide and potassium carbonate in acetone. Transformation of **(106)** into its potassium phenoxide anion with 1.1 equivalent of *t*-BuOK was followed by treatment with benzoyl chloride to form the benzoyl ester. A second 1.1 equivalent of *t*-BuOK was directly added to this reaction mixture, which after refluxing overnight, provided crystalline diketone **(107)** in 64-68% isolated yield. Treatment of **(107)** with sulphuric acid in refluxing acetic acid afforded 5-methoxyflavone **(108)** in 70-75% yield [53].

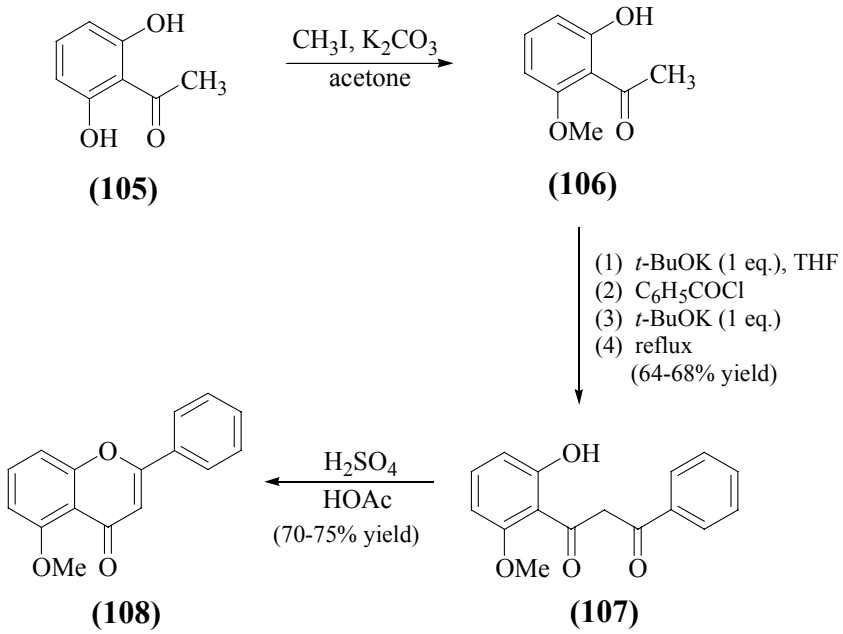



**Figure 1.5:** Modified Baker-Venkataraman Rearrangement

The Allan-Robinson synthesis is a variation of the Baker-Venkataraman route in which a 2′-hydroxyacetophenone derivative is heated at oil bath temperature with the anhydride of an aromatic acid and the sodium salt of that acid, or in the presence of pyridine or triethylamine as catalyst. This method has been used for the preparation of corymbosin **(109)** isolated from *Webera corymbosa* Willd. **(Figure 1.6)** [52].

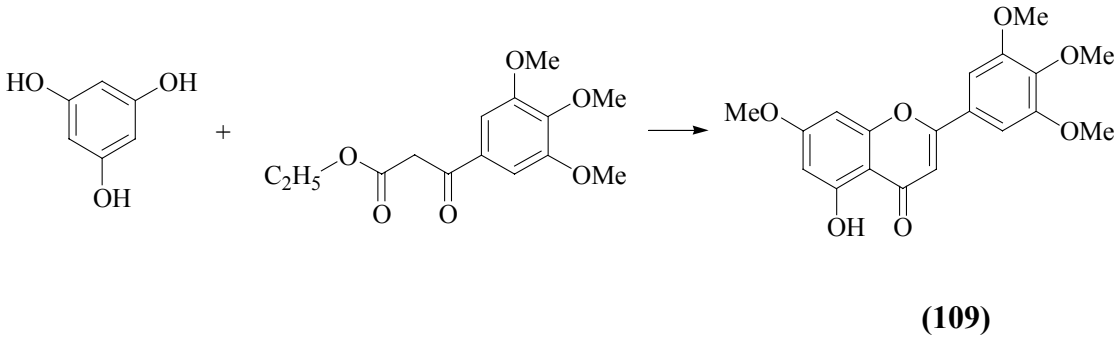



**Figure 1.6:** Allan-Robinson Synthesis

Algar-Flynn-Oyamada described a method for converting chalcones to flavonols or flavonol 3-methyl ethers in a single step. The method which came to be known as the AFO reaction involves oxidizing a chalcone with hydrogen peroxide in an alkaline medium **(Figure 1.7)**. Epoxide **(110)** was the key intermediate in these reactions, which proceeded by the intramolecular displacement of the oxirane oxygen by the phenoxide at the β-position to give dihydroflavonol **(111)**, and subsequently flavonol **(112)**, or at the α-position to give aurone **(113)** [54].

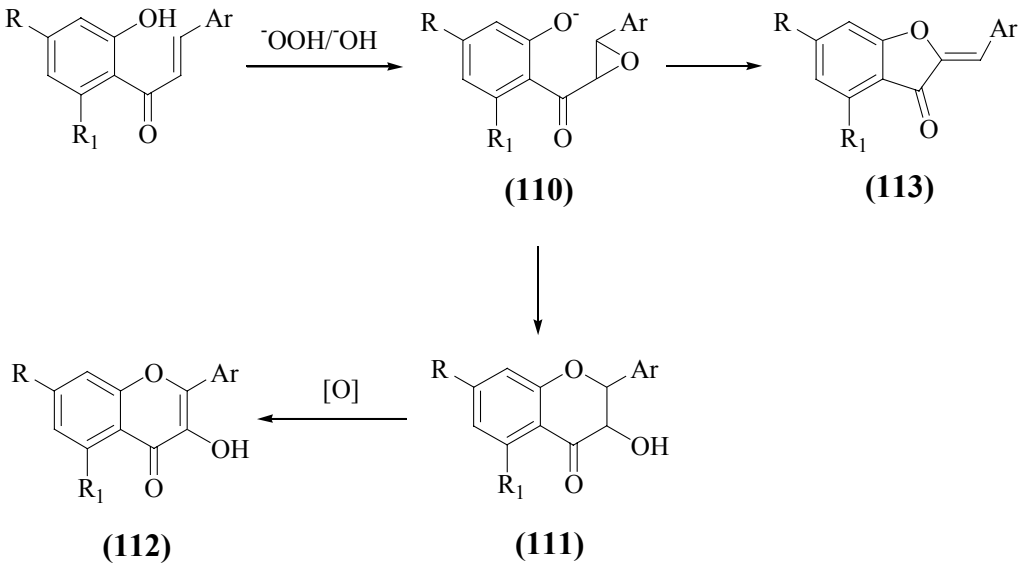



**Figure 1.7:** Algar-Flynn-Oyamada (AFO) Reaction

## 1.7 Research Objectives

The phytochemical investigations reported in the review are studies mostly on the *Artocarpus* species of Indonesia and Taiwan. A thorough literature search did not reveal any report on the chemical constituents or biological activity of Malaysian *Artocarpus* except for a report on the volatile flavour constituents of *A. polyphema* Pers. and *A. heterophyllus* Lamk. [55]. Therefore, this research will focus on the phytochemical and biological activity studies of three Malaysian *Artocarpus* i.e. *A. lowii* King ('miku'), *A. teysmanii* Miq. and *A. scortechinii* King ('terap hitam').

The objectives of this research are to extract the plants samples using different polarity of organic solvents at room temperature. The crude extracts obtained after removal of solvents will be fractionated into several fractions based on polarity using vacuum column chromatographic technique. The natural compounds of each fraction will be analyzed using thin layer chromatography and then, will be isolated by using various chromatographic techniques on silica gel or Sephadex LH20. The structures of the isolated pure compounds will be spectroscopically identified by using high field NMR, 2D NMR (COSY, HMQC, HMBC), high resolution MS, FTIR and UV. Synthesis of prenylated chalcone and flavone will also be attempted by using 2,4,6-trihydroxyacetophenone and 2,4-dihydroxyacetophenone as starting materials. Finally, evaluation on the biological activities of the crude extracts and pure compounds will be carried out by using several bioassays including antioxidant (FTC method and free radical scavenging on 2,2-diphenyl-1-picrylhidrazine (DPPH) method), antibacterial, platelet activating factor (PAF) receptor binding, platelet aggregation and cytotoxic assays.

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