

**PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF  
*MELASTOMA MALABATHRICUM* L. AND *MELASTOMA IMBRICATUM* WALL.**

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PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF  
*MELASTOMA MALABATHRICUM* L. AND *MELASTOMA IMBRICATUM* WALL.

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*In the memory of my dearest father, Darnis*

*for being an endless sources of spirit*

*To my mother, Enimar*

*for her love, support and .....especially her patience*

*To my sister and brother, Devy and Dody*

*for their support and always beside me*

*To my dearest husband, Muhammad Taher*

*for his deepest of love, support, inspiration and understanding which have been  
essential to the success in the completion of this work*

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## ABSTRACT

Phytochemical and bioactivity studies of *Melastoma malabathricum* and *M. imbricatum* have been investigated. Isolation of the compounds was carried out using several chromatographic techniques. Chemical structures of isolated compounds were identified by spectroscopic methods including UV, IR, NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT, COSY, HMQC and HMBC) and MS. The *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH) extracts of the leaves of *M. malabathricum* yielded three new compounds, namely 2,5,6-trihydroxynaphtoic carbonic acid, methyl-2,5,6-trihydroxynaphtalene carbonate and flavonol glycoside derivative together with the known of auranamide, patriscabratine,  $\alpha$ -amyrin, quercetin, quercitrin and kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)-glucoside. The *n*-hexane extract of the roots gave betulinic acid, serrat-14-en-16-one and 2-(2'-hydroxyvinyl)-1-methyl-4-propoxyphthalate. The EtOAc extract of the flowers yielded three compounds, kaempferol-3-*O*- $\beta$ -D-glucoside, kaempferol and naringenin. The MeOH extract of the flowers gave kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)-glucoside and kaempferol-3-*O*- $\beta$ -D-glucoside. The EtOAc extract of the fruits afforded betulinic acid, while the *n*-hexane extract of the stems gave  $\alpha$ -amyrin. Phytochemical studies of the EtOAc extract of the leaves of *M. imbricatum* afforded quercitrin and the MeOH extract gave hyperin and kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)-glucoside. The EtOAc extract of the roots and fruits yielded betulinic acid. The *n*-hexane of the stems gave  $\alpha$ -amyrin. The EtOAc extract of the flowers yielded kaempferol, kaempferol-3-*O*- $\beta$ -D-glucoside and quercitrin. Methylation and acetylation of isolated compounds gave the methyl ether and acetyl derivatives, respectively. The pure compounds and crude extracts were subjected to antimicrobial, antioxidant, anti-inflammatory and cytotoxic assays. The MeOH extract of the fruits of *M. malabathricum* exhibited the strongest inhibition against bacteria, *Bacillus subtilis*, *Streptococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* with MIC values of 62.5, 62.5, 125.0 and 62.5  $\mu\text{g/mL}$ , respectively, in the antimicrobial assay. The antioxidant assay was carried using FTC and DPPH (UV and ESR spectroscopic) methods. Kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)glucoside, kaempferol-3-*O*- $\beta$ -D-glucose, kaempferol, hyperin, quercetin and quercitrin showed strong activities with inhibition more than 90% in the FTC method. Quercetin was found to be the most active as radical scavenger in DPPH-UV and ESR method with  $\text{IC}_{50}$  of 0.69 and 0.65  $\mu\text{M}$ , respectively.  $\alpha$ -Amyrin and kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)glucoside demonstrated the strongest activities in the anti-inflammatory assay of TPA mouse ear oedema with  $\text{IC}_{50}$  of 0.11 and 0.34  $\text{mM/ear}$ , respectively. The EtOAc extract of the leaves of *M. malabathricum* displayed high activity with the inhibition of 94.3%. Kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)glucoside gave an  $\text{IC}_{50}$  of 5.6  $\mu\text{M}$  in the PAF anti-inflammatory assay, while the MeOH extract of the leaves of *M. imbricatum* showed moderate activity with the inhibition of 78.0%. The cytotoxicity study was carried out using MTT assay on MCF7 cell line showed that kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-coumaroyl)glucoside and naringenin were found to be active in inhibiting cell proliferation of MCF7 with  $\text{IC}_{50}$  of 0.28 and 1.3  $\mu\text{M}$ , respectively.

## ABSTRAK

Kajian kimia dan bioaktiviti telah dijalankan ke atas *Melastoma malabathricum* dan *M. imbricatum*. Pengasingan sebatian dijalankan dengan pelbagai kaedah kromatografi. Struktur sebatian tulen dikenal pasti dengan menggunakan teknik spektroskopi termasuk UL, IM, RMN ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT, COSY, HMQC dan HMBC) serta SJ. Tiga sebatian baru telah diasingkan daripada ekstrak *n*-heksana, etil asetat (EtOAc) dan metanol (MeOH) bagi daun *M. Malabathricum*, iaitu asid karbonik 2,5,6-trihidroksinaftoat, metil-2,5,6-trihidroksinaftalen karbonat, terbitan flavonol glukosida dan sebatian yang sudah diketahui iaitu auranamida, patriskabratin,  $\alpha$ -amirin, kuersetin, kuersitrin dan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida. Ekstrak *n*-heksana akar menghasilkan asid betulinik, serat-14-en-16-on dan asid 2-(2'-hidroksivinil)-1-metil-4-propoksifalat. Ekstrak EtOAc bunga menghasilkan tiga sebatian, iaitu kaempferol-3-*O*- $\beta$ -D-glukosida, kaempferol dan naringenin. Ekstrak MeOH pula menghasilkan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida dan kaempferol-3-*O*- $\beta$ -D-glukosida. Ekstrak EtOAc bagibuah menghasilkan asid betulinik, manakala ekstrak *n*-heksana batang menghasilkan  $\alpha$ -amirin. Kajian fitokimia ke atas ekstrak EtOAc daun *M. imbricatum* menghasilkan kuersitrin dan ekstrak MeOH menghasilkan hiperin dan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida. Ekstrak EtOAc akar dan buah menghasilkan asid betulinik. Ekstrak *n*-heksana batang menghasilkan  $\alpha$ -amirin. Ekstrak EtOAc bunga menghasilkan kaempferol, kaempferol-3-*O*- $\beta$ -D-glukosida dan kuersitrin. Pemetilan dan pengasetilan sebatian tulen menghasilkan terbitan masing-masing eter metil dan ester asetil. Kajian keaktifan biologi ke atas sebatian tulen dan ekstrak mentah dilakukan menggunakan ujian antimikrob, antioksidan, antibengkak dan sitotoksik. Ekstrak bagi MeOH buah *M. malabathricum* menunjukkan perencatan yang kuat terhadap bakteria *Bacillus subtilis*, *Streptococcus aureus*, *Pseudomonas aeruginosa* dan *Escherichia coli* dengan nilai MIC 62.5, 62.5, 125.0 dan 62.5  $\mu\text{g/mL}$  dalam ujian antimikrob. Kajian antioksidan dengan kaedah FTC dan DPPH (spektroskopi UV dan ESR) memperlihatkan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida, kaempferol-3-*O*- $\beta$ -D-glukosida, kaempferol, hiperin, kuersetin dan kuersitrin memberikan aktiviti yang kuat dengan peratus perencatan lebih daripada 90% dengan kaedah FTC. Kuersetin didapati paling aktif sebagai perangkap radikal dalam kaedah DPPH-UV dan ESR dengan nilai  $\text{IC}_{50}$  0.69 dan 0.65  $\mu\text{M}$ . Ujian antibengkak menggunakan kaedah TPA memperlihatkan  $\alpha$ -amirin dan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida memberikan  $\text{IC}_{50}$  0.11 dan 0.34 mM/telinga. Ekstrak EtOAc daripada daun *M. malabathricum* memperlihatkan aktiviti yang tinggi dengan peratus perencatan 94.3%. Kajian antibengkak dengan kaedah PAF memperlihatkan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida mempunyai  $\text{IC}_{50}$  5.6  $\mu\text{M}$ , manakala ekstrak metanol daripada daun *M. imbricatum* memperlihatkan peratus perencatan 78.0%. Kajian sitotoksik dengan kaedah MTT pada sel MCF7 memperlihatkan kaempferol-3-*O*-(2'',6''-di-*O-p-trans*-kumaril)glukosida dan naringenin merencat pembiakan sel MCF7 dengan  $\text{IC}_{50}$  0.28 dan 1.3  $\mu\text{M}$ .

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**LIST OF SYMBOLS**

|                    |   |                              |
|--------------------|---|------------------------------|
| $[\alpha]_D^{24}$  | - | Specific rotation            |
| $\delta$           | - | Chemical shift               |
| $\epsilon$         | - | Molar extinction coefficient |
| $^{13}\text{C}$    | - | Carbon-13                    |
| $^{\circ}\text{C}$ | - | degree of Celsius            |
| $^1\text{H}$       | - | Proton                       |
| $J$                | - | Coupling constant in Hertz   |
| $\lambda$          | - | Wavelength                   |
| $\nu$              | - | Wavenumber                   |

**LIST OF ABBREVIATIONS**

|                             |   |  |
|-----------------------------|---|--|
| Abs.                        | - | absorbance                                       |
| Ac <sub>2</sub> O           | - | acetic acid anhydride                            |
| AlCl <sub>3</sub>           | - | aluminium chloride                               |
| BHT                         | - | Butylated Hydroxy Toluene                        |
| br                          | - | broad  |
| CC                          | - | Column Chromatography                            |
| CDCl <sub>3</sub>           | - | deuterated chloroform                            |
| CD <sub>3</sub> OD          | - | deuterated methanol                              |
| CHCl <sub>3</sub>           | - | chloroform                                       |
| CIMS                        | - | Chemical Ionization Mass Spectrometry            |
| COSY                        | - | Correlation Spectroscopy                         |
| cm <sup>-1</sup>            | - | reciprocal centimeter (wavenumber)               |
| <sup>13</sup> C NMR         | - | carbon-13 Nuclear Magnetic Resonance             |
| d                           | - | doublet  |
| dd                          | - | double doublet                                   |
| dt                          | - | double triplet                                   |
| dec.                        | - | decomposed                                       |
| DEPT                        | - | Distortionless Enhancement Polarization Transfer |
| DMEM                        | - | Dulbecco's Modified Eagle Medium                 |
| DMSO- <i>d</i> <sub>6</sub> | - | deuterated dimethyl sulphoxide                   |
| DNA                         | - | deoxyribonucleic acid                            |
| DPPH                        | - | 2,2-diphenyl-1-picrylhydrazyl                    |
| EIMS                        | - | Electron Impact Mass Spectrometry                |
| EPRT                        | - | estrogens/progesterone replacement therapy       |

|                                |   |  |
|--------------------------------|---|--|
| EtOAc                          | - | ethyl acetate  |
| ESR                            | - | Electron Spin Resonance  |
| EPR                            | - | Electronic Paramagnetic Resonance  |
| FABMS                          | - | Fast Atom Bombardment Mass Spectrometry                                      |
| FTC                            | - | Ferric Thiocyanate   |
| g                              | - | gram   |
| GC                             | - | Gas Chromatography   |
| GSH                            | - | Glutathione peroxide   |
| H <sub>3</sub> BO <sub>3</sub> | - | boric acid   |
| HCl                            | - | hydrochloric acid  |
| HEPES                          | - | <i>N</i> -[2-Hydroxyethyl]piperazine- <i>N'</i> -[2-ethanesulfonic acid]     |
| HHDP                           | - | hexahydroxydiphenoyl   |
| <sup>1</sup> H NMR             | - | Proton Nuclear Magnetic Resonance  |
| HMBC                           | - | Heteronuclear Multiple-Bond Connectivity                                     |
| HMQC                           | - | Heteronuclear Multiple Quantum Coherence                                     |
| HRT                            | - | estrogens replacement therapy  |
| Hz                             | - | Hertz  |
| IC <sub>50</sub>               | - | concentration that inhibits a response by 50% relative to a negative control |
| IgE                            | - | immunoglobulin E   |
| IR                             | - | Infrared   |
| <i>J</i>                       | - | coupling constant  |
| KBr                            | - | potassium bromide  |
| L                              | - | liter  |
| m                              | - | multiplet  |
| M                              | - | molar concentration  |
| M <sup>+</sup>                 | - | molecular ion  |
| MAO                            | - | monoamine oxidase  |
| max                            | - | maximum  |
| MBC                            | - | Minimum Bactericidal Concentration   |
| MCF7                           | - | Human Breast Cancer cell line  |

|                   |   |  |
|-------------------|---|--|
| MeOH              | - | methanol   |
| MFC               | - | Minimum Fungicidal Concentration                             |
| MgSO <sub>4</sub> | - | magnesium sulphate   |
| μg                | - | microgram  |
| MHz               | - | megahertz  |
| MIC               | - | Minimum Inhibition Concentration                             |
| mL                | - | milliliter   |
| mM                | - | millimolar   |
| μM                | - | micromolar   |
| mp.               | - | melting point  |
| MS                | - | Mass Spectra   |
| MTT               | - | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| <i>m/z</i>        | - | mass to charge ratio   |
| NA                | - | Nutrient Agar  |
| NB                | - | Nutrient Broth   |
| NaOAc             | - | sodium acetate   |
| NaOMe             | - | sodium methoxide   |
| nm                | - | nanometer  |
| PAF               | - | platelet activating factor                                   |
| PBS               | - | phosphate buffer saline                                      |
| PGI1              | - | prostaglandin I1   |
| PGE2              | - | prostaglandin E2   |
| PGF2              | - | prostaglandin F2   |
| PGH               | - | prostaglandin H  |
| PHGPX             | - | phospholipids hydroperoxide glutathione peroxidase           |
| PKC               | - | protein kinase C   |
| %                 | - | percent  |
| ppm               | - | parts per million  |
| PTLC              | - | Preparative Thin Layer Chromatography                        |
| q                 | - | quartet  |
| rel. int.         | - | relative intensity   |

|       |   |   |
|-------|---|---|
| $R_f$ | - | retention factor                              |
| s     | - | singlet                                       |
| SD    | - | standard deviation                            |
| sept  | - | septet  |
| SOD   | - | superoxide dismutase                          |
| t     | - | triplet                                       |
| TLC   | - | Thin Layer Chromatography                     |
| TPA   | - | 12- <i>O</i> -Tetradecanoylphorbol-13-acetate |
| UV    | - | Ultraviolet                                   |
| VLC   | - | Vacuum Liquid Chromatography                  |

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## **CHAPTER I**

### **INTRODUCTION**

#### **1.1 General Introduction**

Bioactive natural products have an enormous economic importance as specialty chemicals. They can be used as drugs, lead compounds, biological or pharmacological tools, feed stock products (raw materials for the production of drugs) and nutraceuticals. They are found in herbs, dietary supplements, spices and foods. Some of them are important flavours, fragrances, dyes and cosmetic, and others are used as insecticides, antifeedants, pesticides and antirodentocides [1].

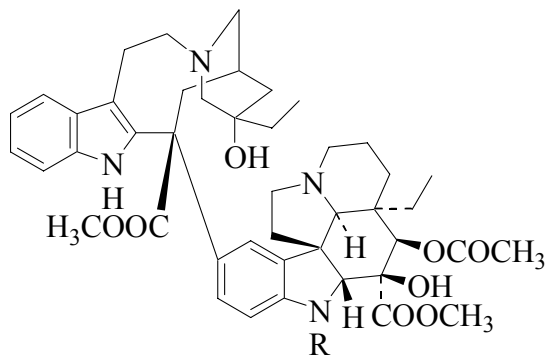
Plants have been the source of medicinal agents for thousands of years, and impressive numbers of modern drugs have been isolated from natural sources, many based on their uses in traditional medicines. In fact, natural products once served as the source of all drugs. Since more than 80% of the world's population use plants as their primary source of medicinal agents, it is not surprising to find that in many countries of the world there is a well-established system of traditional medicine, whose remedies are still being compiled. In some instances, the Chinese and the Ayurvedic systems have documented the remedies of their traditional medicines and these documents are commercially available [2, 3].

## 1.2 Drug Discovery

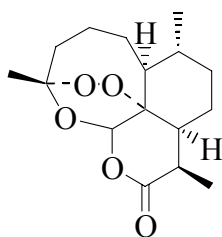
A few drug discovery programmes based on plants has restarted in the late 1980's when pharmacologists, physicians and chemists started reinvestigating the active ingredients in plants used for medicinal purposes. The more recent advances in chromatographic and spectroscopic technical equipments have decreased the time taken to identify and determine the molecular structures of the active compounds in these plants. This has resulted in an increase in the number of natural products being isolated and identified every year, although very few have been developed as drugs. However, due to the novel structures of some of these active compounds, they might serve as templates or lead compounds for drugs [4].

Different approaches to drug discovery using higher plants can be distinguished: random selection followed by chemical screening, random selection followed by one or more biological assays, follow up of biological activity reports, follow up of ethnomedical (traditional medicines) uses of plants. The latter approach includes plants used in organized traditional medical system; herbalism and folklore; the use of database. The objective is the targeted isolation of new bioactive plant product, i. e. lead substances with novel structure and novel mechanisms of action [1].

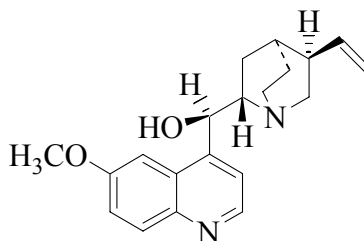
Among the 45 drugs of known structures derived from the tropical rain forest species, including those that are of major important used in therapy, none is currently produced through synthesis. For example, the anticancer vinblastine (**1**) and vincristine (**2**) from *Catharanthus roseus* L. G. Don (Apocynaceae) in which (**1**) has been used as part of chemotherapy combination for the treatment of acute lymphoblastic leukemia and testicular teratoma since 1979's. The antiplasmodial artemisinin (**3**) from *Artemisia annua* L. (Compositae) as well as the anti malarial quinine (**4**) isolated from the bark of *Cinchona* spp (Rubiaceae). Ginkgolide A (**5**) was isolated from the root barks and leaves of *Ginkgo biloba* (Ginkgoaceae) have been used from the natural sources. There is evidence that ginkgolides are able to competitively inhibit the specific platelet aggregating factors to cell membranes. This type of activity could explain the use of these plants in the treatment of inflammation and for respiratory and panic disorder [4, 5].



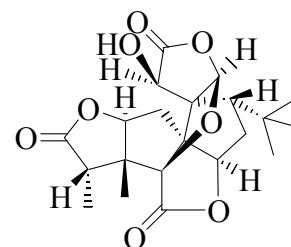
- (1) R = CH<sub>3</sub>  
 (2) R = CHO



(3)

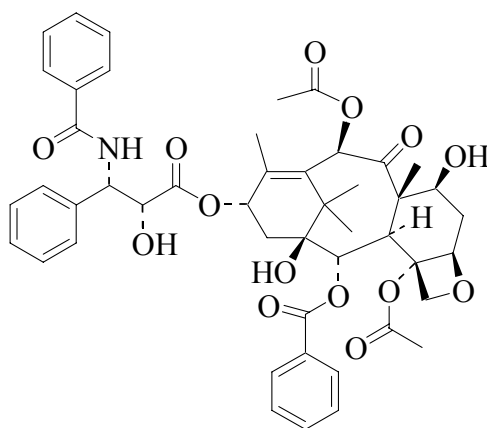


(4)



(5)

The anti cancer drug, taxol (6) was isolated from the bark of yew trees as a result of NCI's systematic effort in collecting and screening plants for antitumor activity [4, 6]. Taxol has now been approved to be used for the treatment of ovarian and breast cancers, as well as small cell and non small cell lung cancer which include neck and head cancers [4, 7].



(6)

The commercial value of drug derived from higher plants should not be underestimated. For example, in 1980 American consumers paid about USD 8 billion for prescription of drugs derived solely from higher plants. From 1959 to 1980, drugs derived from higher plants represented a constant 25% of all new and refilled prescriptions dispensed from community pharmacies in the United State [8].

Phytochemical investigation will provide data on the chemical constituents of the plant concerned. However, many new natural compounds were isolated, characterised and published without any biological testing and their useful biological activities then remain unknown for a long time. The significance of the phytochemical work is greatly increased if the fine chemicals isolated from the plant possess certain biological activities, particularly if the activity is in line with the development of medicinal agents [9].

### **1.3 Botany and Distribution of Melastomataceae**

The Melastomataceae is an exclusively tropical plant family with about 4500 species; 3000 species in South America, 250 species in tropical west and east African harbour, and 250 species in Madagascar. The remaining 1000 species occur in Asia Oceania and Northern Australia with a concentration in Central Malaysia (Borneo). This family has 25 genera and 180 species in Malaysia and usually occurs in lowlands and mountains. Melastomataceae comprises a wide range of plants, such as terrestrial herbs, epiphytic shrubs, trees and woody climbers, ranging in height from 10 cm to 30-50 cm. Some of the trees are among the few plants in Malaysia that have blue flowers [11].

The botanical characters of this family are the opposite leaves, simple, generally with three prominent longitudinal veins. Flowers are small to large, clustered, regular or bilaterally symmetrical; four or five sepals, or apparently absent; four or five petals, separate, pink, purple or blue, rarely white; stamens



twice as many as the petals, eight or ten, with rather thick, pink or blue stalks (rarely white) and large yellow, pink or blue anthers; ovary inferior. The fruit is a berry with many small seeds or with one large seed; in other cases capsular and opening, with dry or pulpy contents. The family is close to the Myrtales (Myrtaceae) and differs chiefly in the absence of oil-glands, so that the tissues are not aromatic [11].

The family of Melastomaceae comprises of several genera, for example *Melastoma*, *Huberia*, *Lavoisiera*, *Microlicia*, *Trembleya*, *Memycelon*, *Dissostis*, *Tibouchina*, *Heterocentron* and *Osbeckia*.

#### 1.4 Botany and Distribution of Genus *Melastoma*

Botanic classification of *Melastoma* [10]:

Sub-division : Angiospermae  
 Class : Dicotyledoneae  
 Sub-class : Archichlamideae  
 Order : Myrtiflorae  
 Family : Melastomataceae  
 Genus : *Melastoma*

The genus of *Melastoma* is categorized as shrubs and rarely small trees, found from the Mascarene Island to Pacific. Most of the species of this genus are known as ‘senduduk’ and some of them can be distinguished, e.g. *M. decemfidum*, Roxb. and *M. parkense*, Ridl. as ‘senduduk gajah’ and *M. imbricatum*, Wall as ‘senduduk rimba’ [12]

Plants of *Melastoma* if repeatedly burnt or cut, and left undisturbed, will grow as small trees 12-13 ft. high, occasionally even up to 20 ft., and they may be found in the forest at the edge of the stream, on landslip or in old clearing, and they are evergreen and flowering throughout the year.

The flower lasts only one day, opening about 8 a.m. and closing in the late afternoon, the petals fall a few days later. The fruits with purple pulp in *M. malabathricum* are sweet, often eaten by the children, and thus stain the mouth like bilberries [11].

The characteristics of these genus are leaves with 3-5 longitudinal veins, tapered to each end; large flower, in terminal clusters, bilaterally symmetrical through the arrangement of the stamens; 5 sepals, usually with an epicalyx; 5 petals, pinkish, large; 10 stamens, with two kinds, 5 short stamens with yellow stalk and anthers, 5 long stamens with a straight basal part to the stalk; style pink with green stigma. Fruits as berry-like capsules, opening irregularly and disclosing a yellow, red or commonly purple pulpy mass with the tiny seeds embedded on it [11].

This genus consists of about 40 species in Madagascar, Australia and 5 species in Malaysia [11].

#### **1.4.1 *Melastoma malabathricum* L**

*M. malabathricum* L. (senduduk) is a very common herb or shrub found throughout the tropic in the moist part mostly from India, Thailand and Malaysia. The plants have been used in traditional Malay medicine for the treatment of diarrhoea, puerperal infection, dysentery, leucorrhoea, wound healing, post-partum treatment and haemorrhoids [12].

This species has at least three varieties, i.e. large, medium and small size flower with dark purple-magenta petals, light pink-magenta petals and the rare variety with white petals [11].

The characteristic of these species are leaves 0.25-2 inches wide, with stalk 0.25-0.5 inches long; flower 1-3 inches wide; calyx closely set with short chaffy,

silky or silvery scale. This species spread in Madagascar, India to Australia and very common throughout Malaysia in the lowland and mountain forests, chiefly in open places and ever flowering [11].



**Figure 1.1 *Melastoma malabathricum* L**

#### **1.4.2 *Melastoma imbricatum* Wall**

The characteristics of this species are the leaves 2-4 inches wide with stalk 0.25-2 inches long, flower 1-2 inches wide, hardly stalked, set in dense cluster; calyx set with tiny scales. Occasionally spread in the mountain and scarce in the lowlands [11].



**Figure 1.2** *Melastoma imbricatum* Wall

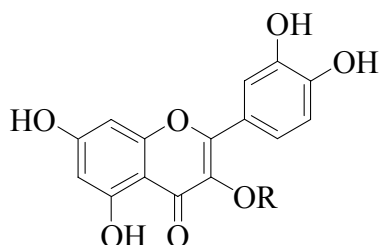
### 1.5 Chemical Investigation of Melastomataceae

The chemistry of Melastomaceae is poorly known. The family is characterized by tannin (very common) and alkaloids (rare). Acylated anthocyanins have been found in fruits and flowers. Mimura *et al.* (2004) had carried out the distribution of foliar alkenes with the chemotaxonomic approaches on the genus of *Huberia* [13].

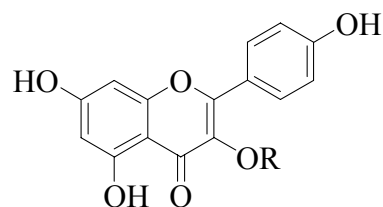
The reports on the distribution of foliar flavonoids of approximately 33% of the known species of *Lavoisiera* and *Trembleya*, and 15% of *Microlicia*, with the purpose of establishing affinity relationship and adding evidence towards solving delimitation problems between the genus [14].

A diverse of flavonoid structures has been found in this family, with the predominant of flavonol glycoside, mainly glucoside of quercetin (**7**) and kaempferol (**8**). The most frequent sugars are glucose and galactose, e.g. quercetin-3-*O*-glucoside (**9**), quercetin-3-*O*-galactoside (hyperin) (**10**) and kaempferol-3-*O*-glucoside (**11**). Glycoside of isorhamnetin (**12**), rhamnetin (**13**) and myricetin (**14**) were also found, although less frequently, e.g. isorhamnetin-3-*O*-galactoside (**15**), rhamnetin-3-*O*-

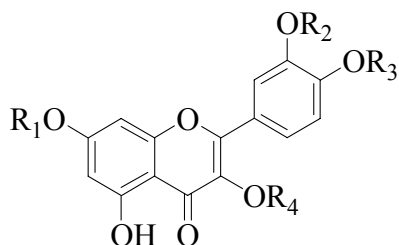
glucoside (**16**) and myricetin-3-*O*-galactose (**17**). Derivatives of apigenin (**18**), luteolin (**19**) and chrysoeriol (**20**) were among the flavones found in the Melastomaceae family e.g. apigenin-7-*O*-glucoside (**21**), luteolin-7-*O*-glucoside (**22**) and chrysoeriol-7-*O*-glucoside (**23**) [14].



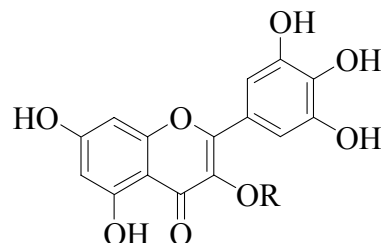
- (7) R = H  
 (9) R = Glc  
 (10) R = Gal



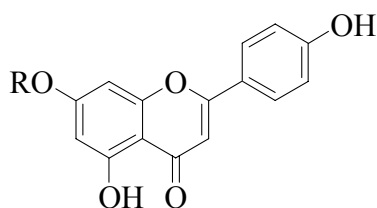
- (8) R = H  
 (11) R = Glc



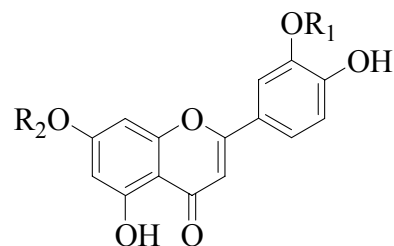
- (12) R<sub>1</sub>, R<sub>3</sub> = H, R<sub>2</sub> = CH<sub>3</sub>, R<sub>4</sub> = H  
 (13) R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub>, R<sub>3</sub> = H, R<sub>4</sub> = H  
 (15) R<sub>1</sub>, R<sub>3</sub> = H, R<sub>2</sub> = CH<sub>3</sub>, R<sub>4</sub> = Gal  
 (16) R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub>, R<sub>3</sub> = H, R<sub>4</sub> = Glc



- (14) R = H  
 (17) R = Gal

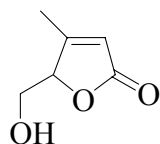


- (18) R = H  
 (21) R = Glc



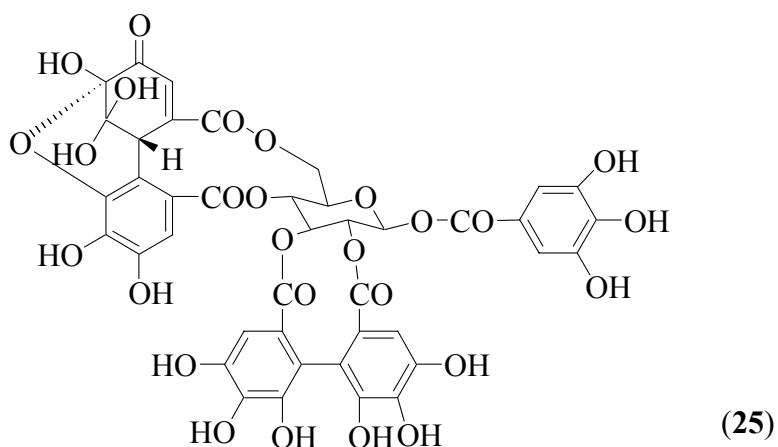
- (19) R<sub>1</sub> = H, R<sub>2</sub> = H  
 (20) R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = H  
 (22) R<sub>1</sub> = H, R<sub>2</sub> = Glc  
 (23) R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = Glc

The chemical investigation on the aerial parts of *Memycelon umbelatum* yielded a new compound named as umbelactone (**24**) [15].

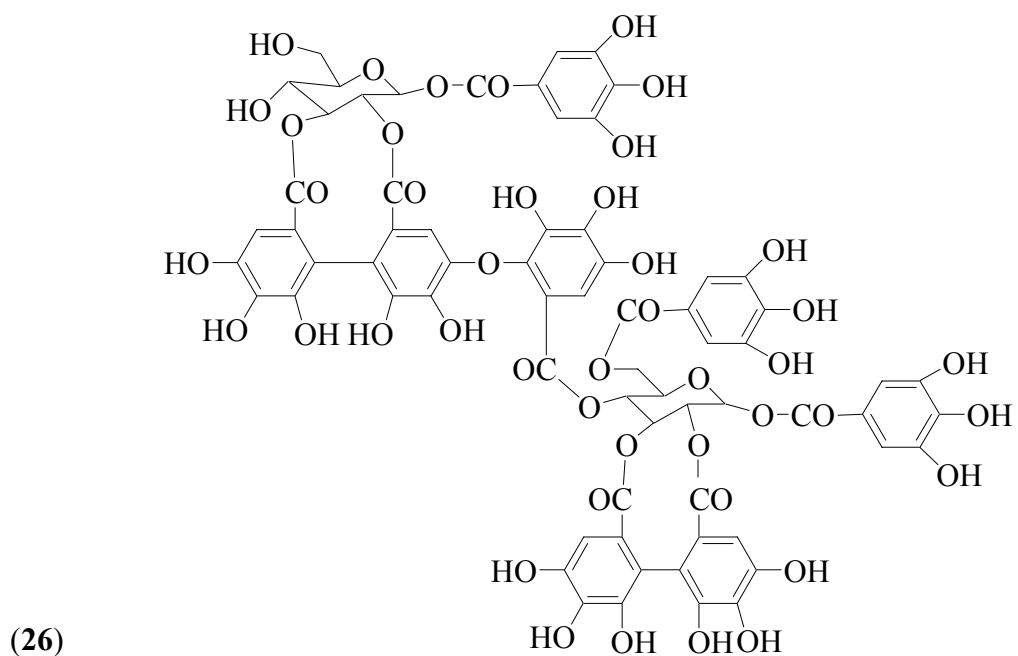


(24)

In continuing study on this family, Yoshida *et al.* (1994) [16] isolated two new polyphenols from *Bredia tuberculata* named brediatins A (**25**) and brediatins B (**26**).

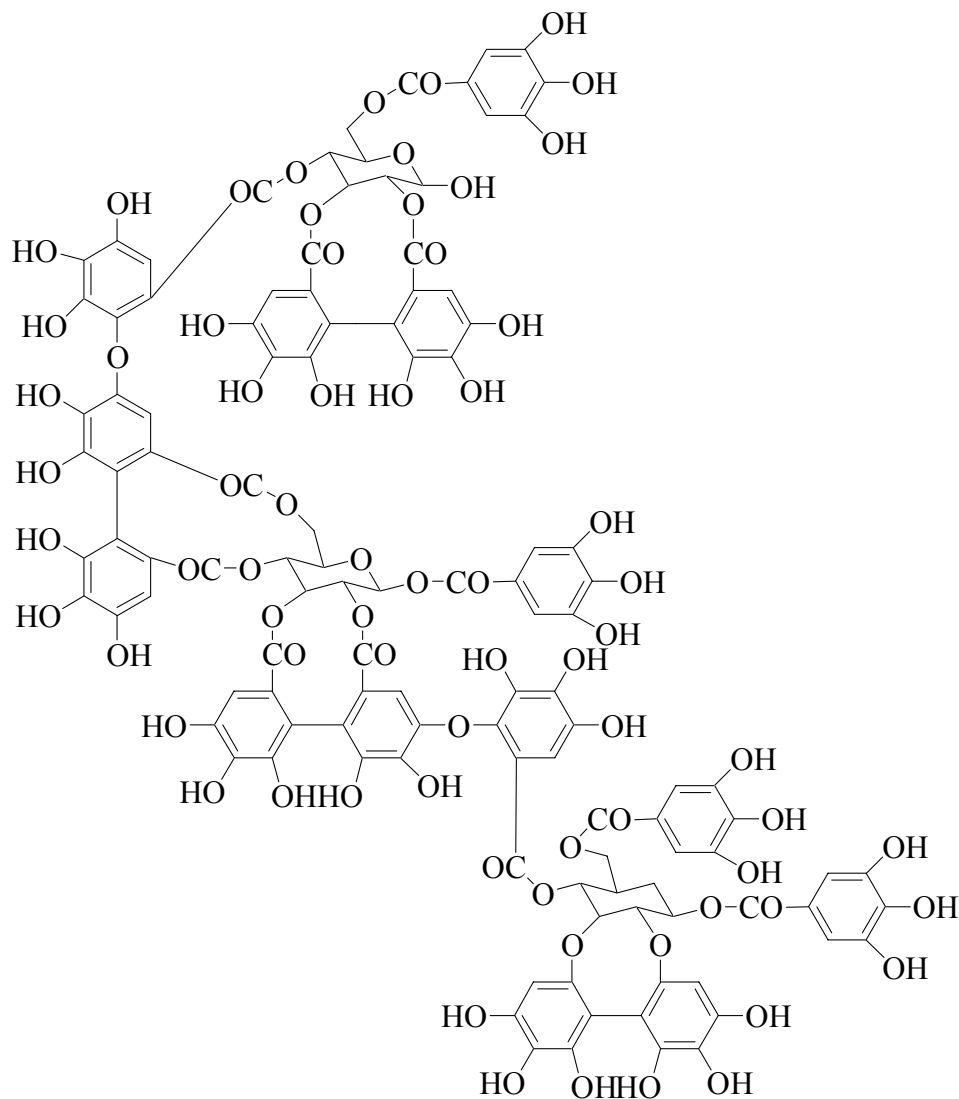


(25)

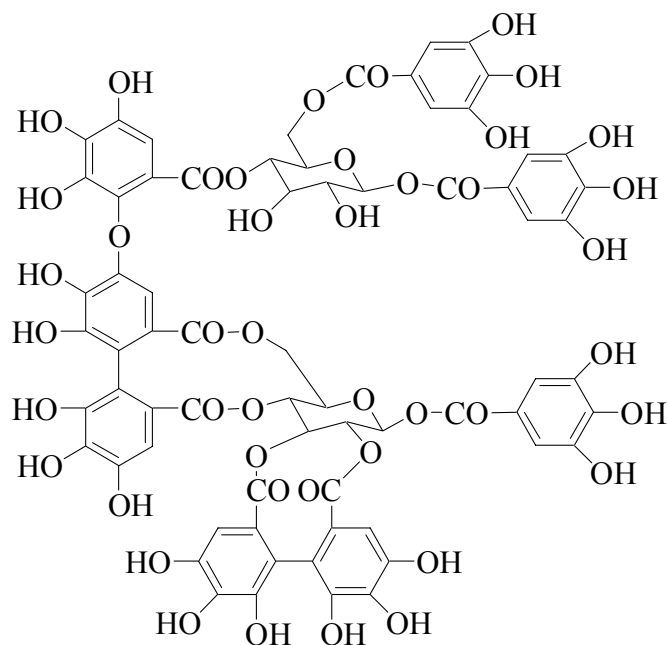


(26)

The chromatographic survey of the tannins in this family [17] revealed that *Tibouchina multiflora* is rich in tannins, particularly in oligomeric hydrolysable tannins. Two new oligomeric hydrolysable tannins named nobotanins O (**27**) and nobotanins P (**28**) were isolated from the leaf extract of *T. multiflora*.

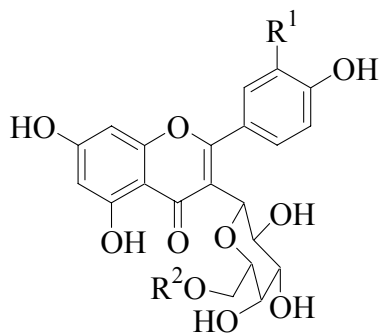


(27)

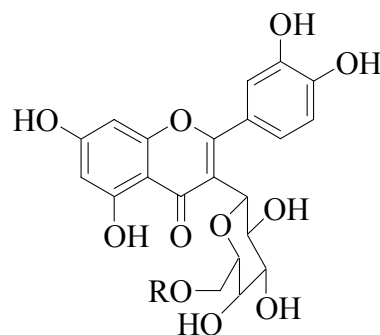


(28)

The chemical investigation on *Monochaetum multiflorum* yielded trifolin (29), hyperin (10), quercetin 3-(6'-*O*-caffeoyl)- $\beta$ -D-galactoside (30), isoquercitrin (31), quercetin 3-(6'-*O*-caffeoyl)- $\beta$ -D-glucopyranoside (32), 4-*O*- $\beta$ -D-glucopyranosyl-2-*O*-methylphloroacetophenone (33), 4-*O*-(6'-*O*-galloyl- $\beta$ -glucopyranosyl)-*cis-p*-coumaric acid (34), 6'-*O*-galloylprunasin (35), benzyl 6'-*O*-galloyl- $\beta$ -gluco-piranoside (36) and a novel diester of tetrahydroxy- $\mu$ -truxinic acid with 2 moles of hyperin (monochaetin) (37) [18].

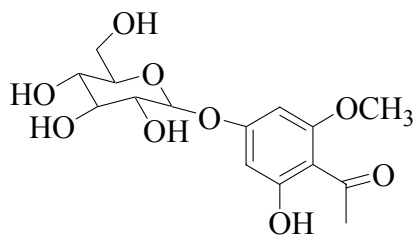


(29)  $R^1 = R^2 = H$   
 (30)  $R^1 = OH, R^2 = trans\text{-caffeoyl}$

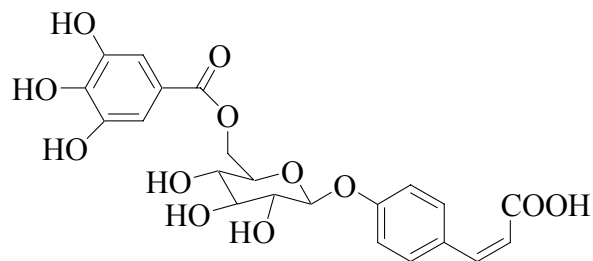


(31)  $R = H$   
 (32)  $R = trans\text{-caffeoyl}$

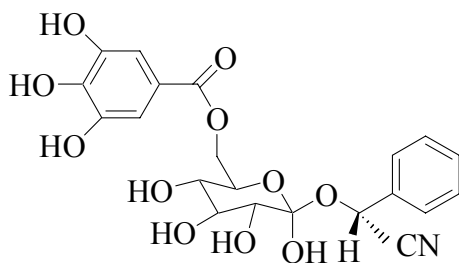




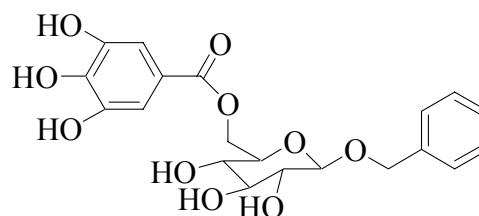
(33)



(34)

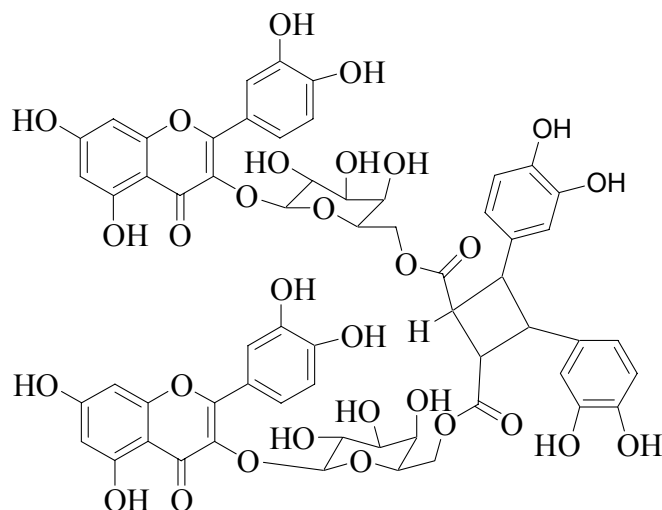


(35)

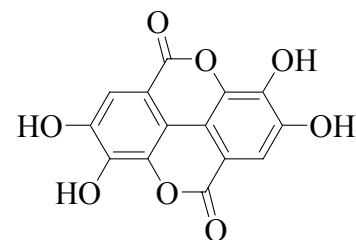
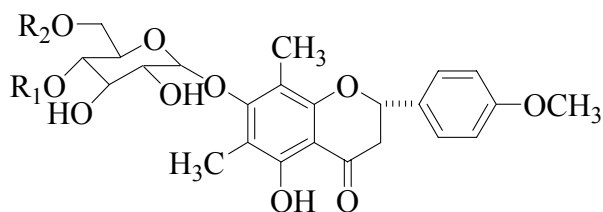


(36)

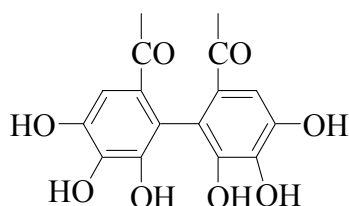
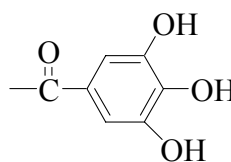
Bioassay-guided fractionation on secreted aspartic protease (SAP) on *Candida albicans* of the ethanol extract of twigs and leaves of *Miconia myriantha* yielded mattucinol-7-*O*-[4'',6''-*O*-(*S*)-hexahydroxydiphenoyl]- $\beta$ -D-glucopyranoside (38), mattucinol-7-*O*-[4'',6''-di-*O*-galloyl]- $\beta$ -D-glucopyranoside (39), mattucinol-7-*O*- $\beta$ -D-glucopyranoside (40) and ellagic acid (41) [19].



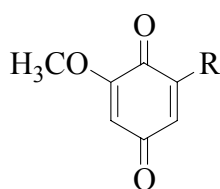
(37)



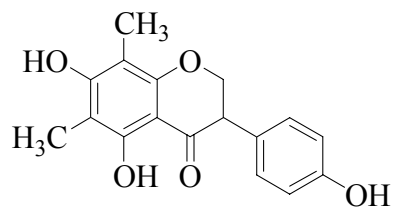
(41)

(38)  $R_1, R_2 = \text{HHDP} =$ (39)  $R_1, R_2 = \text{Galloyl} =$ (40)  $R_1, R_2 = \text{H}$ 

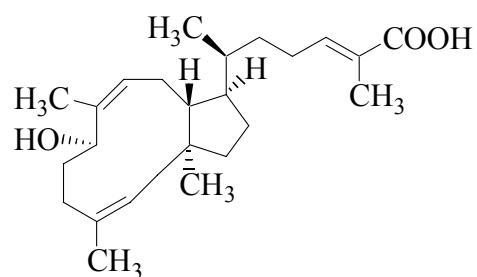
Bioactivity-directed fractionation of EtOAc extract from the leaves of *Miconia lepidota*, afforded two benzoquinones, namely 2-methoxy-6-heptyl-1,4-benzoquinone (42) and 2-methoxy-6-pentyl-1,4-benzoquinone (43) [20].

(42)  $R = \text{C}_7\text{H}_{15}$ (43)  $R = \text{C}_5\text{H}_{11}$ 

The chemical investigation of *Henriettella fascicularis* afforded 4',5,7-trihydroxy-6,8-dimethylisoflavone (44) and sesterterpenoic acid (45) [21].

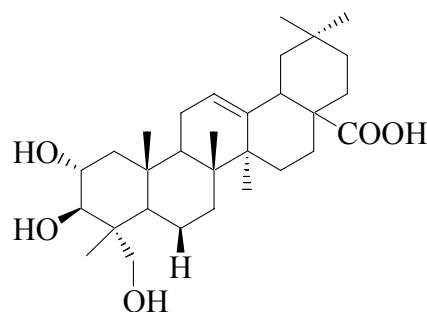


(44)



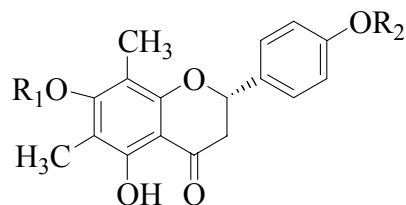
(45)

The ethanol extract of *Miconia pilgeriana* yielded a triterpene compound, which was characterized as arjunolic acid (**46**) [22].

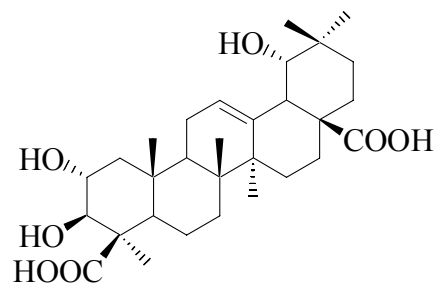


(46)

Bioactivity-guided fractionation of the ethanol extract of *Miconia trailii*, yielded miconioside A (**47**), miconioside B (**48**), matteucinol (**49**), bartogenic acid (**50**), arjunolic acid (**46**) and myrianthic acid (**51**) [23].

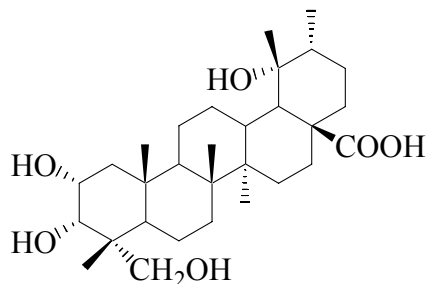


- (47)  $R_1 = \alpha\text{-L-Ara}(1 \rightarrow 6)\text{-}\beta\text{-D-Glc}$ ,  $R_2 = \text{CH}_3$   
 (48)  $R_1 = \beta\text{-D-Api}(1 \rightarrow 6)\text{-}\beta\text{-D-Glc}$ ,  $R_2 = \text{H}$   
 (49)  $R_1 = \text{H}$ ,  $R_2 = \text{H}$

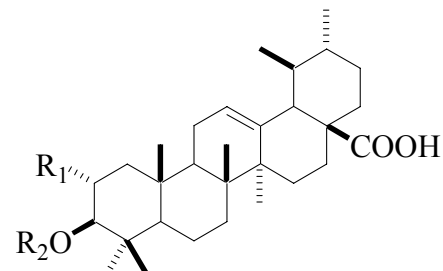


(50)

The chemical investigation of *Monochaetum vulcanicum* resulted in the isolation of 3 $\beta$ -acetoxy-2 $\alpha$ -hydroxyurs-12-en-28-oic acid (**52**), ursolic acid (**53**), 2 $\alpha$ -hydroxy-ursolic acid (**54**) and 3-(*p*-coumaroyl)ursolic acid (**55**) [24].

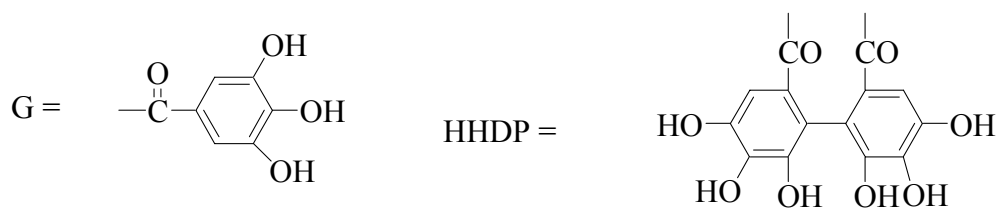
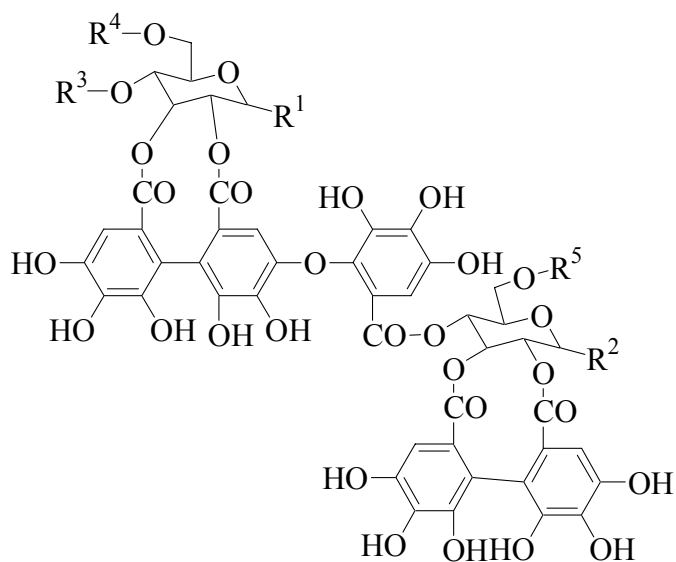


(51)

(52)  $R_1 = \text{OH}$ ,  $R_2 = \text{Ac}$ (53)  $R_1 = R_2 = \text{H}$ (54)  $R_1 = \text{OH}$ ,  $R_2 = \text{H}$ (55)  $R_1 = \text{H}$ ,  $R_2 = p\text{-coumaroyl}$ 

## 1.6 Chemical Investigation of *Melastoma*

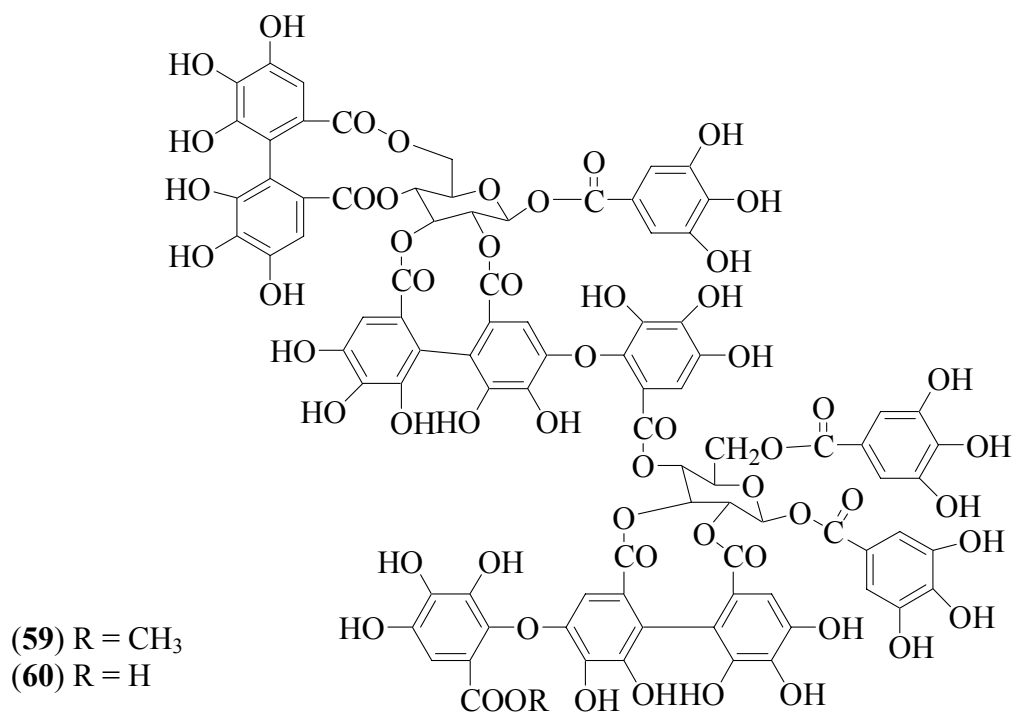
Several tannins have been isolated from the dry leaves of *M. malabathricum*. The main tannin was hydrolysable tannin oligomers named nobotanin B (**56**), which was recently found to exhibit potent *in vitro* antiviral activity against human immunodeficiency virus. The other tannins were hydrolysable tannin dimmers named malabathrins B (**57**), malabathrins C (**58**) and malabathrins D (**59**), hydrolysable tannin oligomers nobotanin G (**60**), hydrolysable tannin monomers named 1,4,6-tri-*O*-galloyl- $\beta$ -D-glucoside (**61**), 1,2,4,6-tetra-*O*-galloyl- $\beta$ -D-glucoside (**62**), strictinin (**63**), casuarictin (**64**), pedunculagin (**65**), nobotanin D (**66**), pterocarinin (**67**), nobotanin H (**68**) and nobotanin J (**69**) [25].

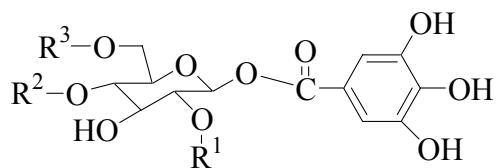


(56)  $R^1, R^2 = (\beta)\text{-OG}$ ,  $R^3, R^4 = (S)\text{-HHDP}$ ,  $R^5 = G$

(57)  $R^1 = (\beta)\text{-OG}$ ,  $R^2 = \text{OH}$ ,  $R^3, R^4 = (S)\text{-HHDP}$ ,  $R^5 = G$

(58)  $R^1 = \text{OH}$ ,  $R^2 = (\beta)\text{-OG}$ ,  $R^3, R^4 = (S)\text{-HHDP}$ ,  $R^5 = G$

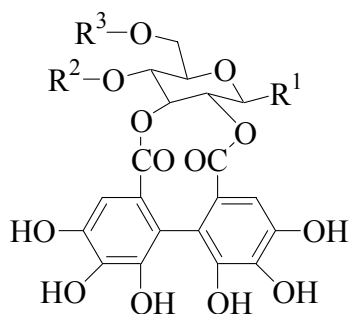




(61)  $R^1 = H, R^2, R^3 = \text{Glc}$

(62)  $R^1, R^2, R^3 = \text{Glc}$

(63)  $R^1 = H, R^2, R^3 = (S)\text{-HHDP}$

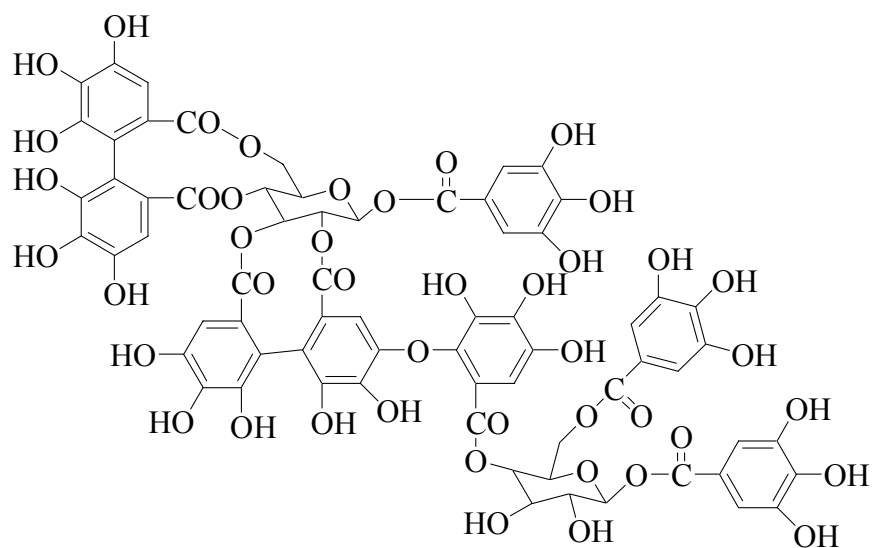


(64)  $R^1 = (\beta)\text{-OG}, R^2, R^3 = (S)\text{-HHDP}$

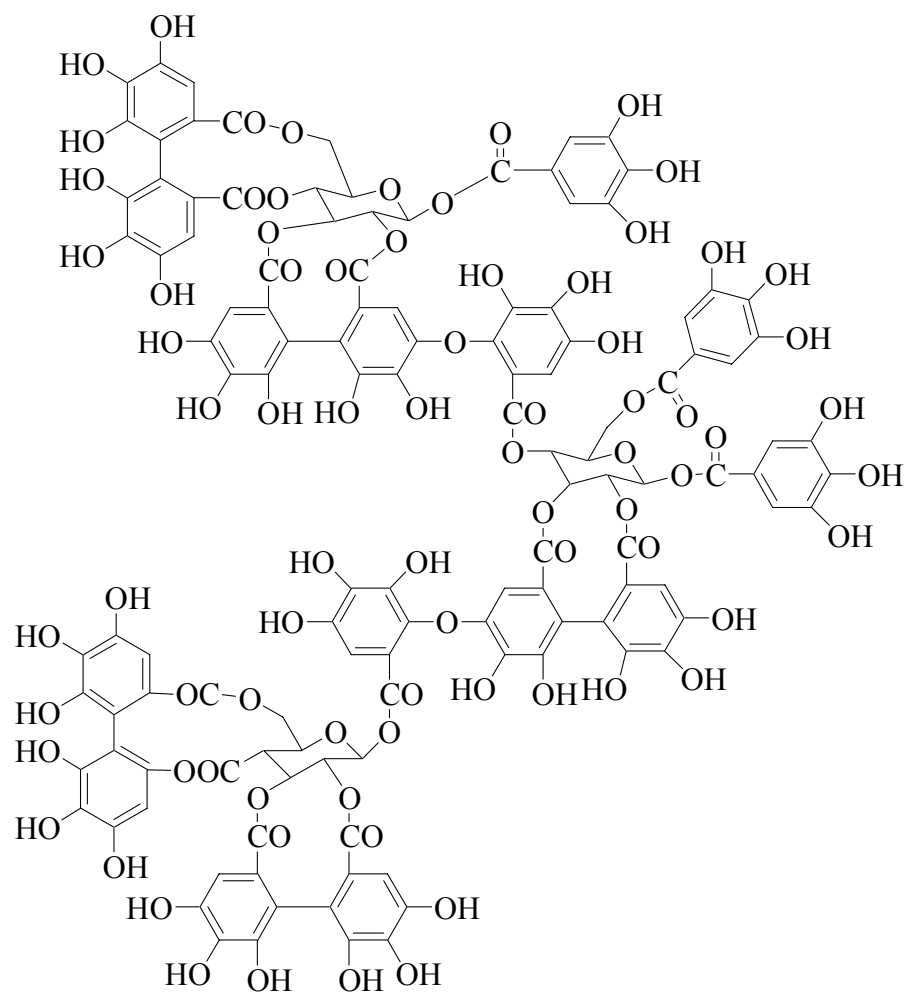
(65)  $R^1 = \text{OH}, R^2, R^3 = (S)\text{-HHDP}$

(66)  $R^1 = (\beta)\text{-OG}, R^2 = H, R^3 = G$

(67)  $R^1 = (\beta)\text{-OG}, R^2, R^3 = G$

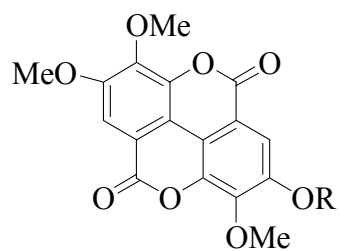


(68)



(69)

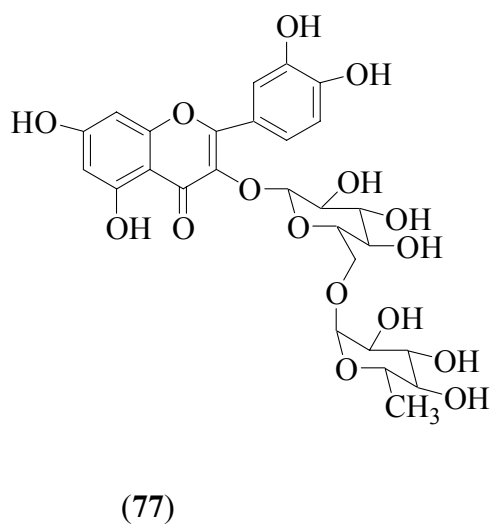
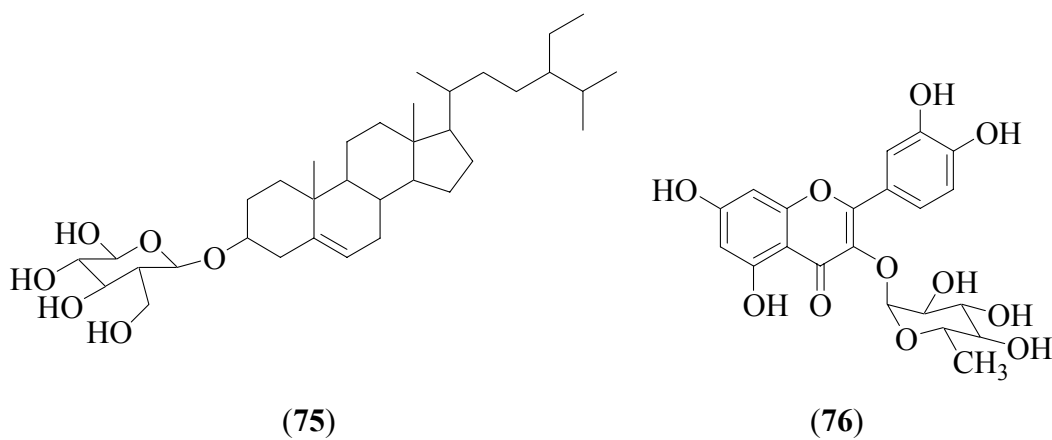
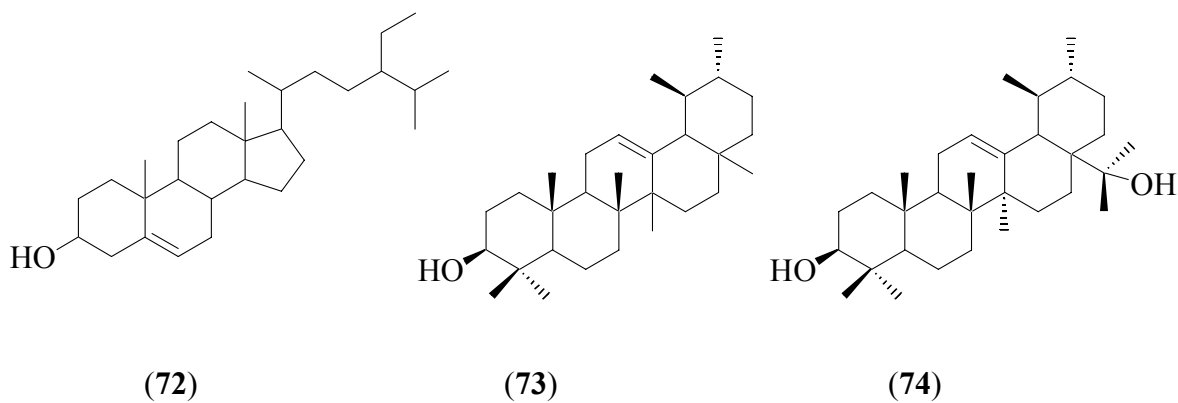
The chemical investigation of *M. polyanthum* yielded tri-*O*-methyl ellagic acid (70) and tri-*O*-methyl ellagic acid glucoside (71) [26].



(70) R = H

(71) R = Glc

The polyphenols named strictinin (**62**), casuarictin (**63**) and nobotanin B (**56**) have been reported from *M. normale* [16]. While, *M. malabathricum* with dark purple-magenta petals contains  $\beta$ -sitosterol (**72**),  $\alpha$ -amyrin (**73**), uvaol (**74**), sitosterol 3-*O*- $\beta$ -D-glucopyranoside (**75**), quercetin (**7**), quercitrin (**76**) and rutin (**77**) [27].





## 1.7 Bioactivity Investigation on Melastomataceae

Monoamine oxidase type B (MAO-B) activity and free radical scavenger are elevated in certain neurological disease. Four natural flavonoids, quercitrin (**76**), rutin (**77**), quercetin (**7**) and isoquercitrin (quercetin-3-*O*-glucoside) (**31**), isolated for the first time from the leaves of *M. candidum*, were found to inhibit the MAO-B. These four potent compounds, also exhibited hydroxyl radical scavenging activity. These important properties may be use for preventing some neurodegenerative disease [28].

The methanol extract of *M. malabathricum* L. exhibited attractive antiviral and cytotoxic activities on murine cell lines. The biological activities of *M. malabathricum* could be attributed to the hydrolysable tannin [29].

*Dissotis brazae* Cogn. was tested for *in vitro* antiplasmodium activity against chloroquin-resistant (ENT36). The IC<sub>50</sub> was found to be ≤ 10µg/mL [30].

The methanol extract of various Sumatran plants were tested *in vivo* for antinematodal activity against *Bursaphelenchus xylophilus*. In this screening, the root extract of *M. malabathricum* showed strong activity [31].

Mattucinol-7-*O*-[4'',6''-*O*-(*S*)- hexahydroxydiphenoyl]-β-D-glucopyranoside (**38**) which was isolated from *Miconia myriantha* exhibited inhibitory effect against SAP with IC<sub>50</sub> of 8.4 µM [22].

Bioactivity-directed fractionation of the leaves of *Miconia lepidota* in the *in vitro* antitumor cytotoxicity assay with Madison Lung Carcinoma (M109) murine cell line, showed that 2-methoxy-6-heptyl-1,4-benzoquinone (**42**) and 2-methoxy-6-pentyl-1,4-benzoquinone (**43**) were potential anticancer agent [20].

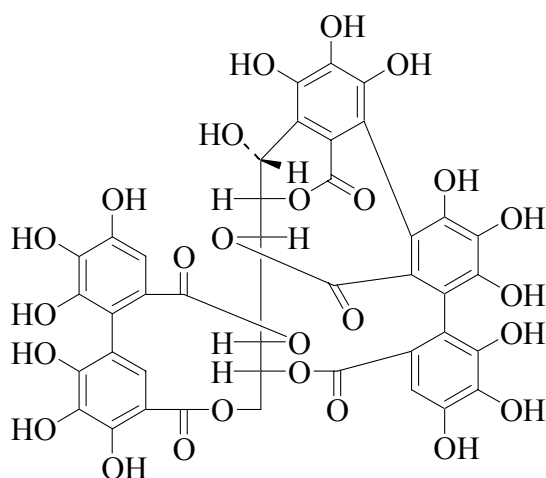
The esterogen receptor (ER) competitive binding experiments revealed higher affinity of 4',5,7-trihydroxy-6,8-dimethylisoflavone (**44**) for ERβ than for

ER $\alpha$ , isolated from *Henriettella fascicularis*. In Ishikawa cells, when alkaline phosphatase was induced by treatment with estradiol, 4',5,7-trihydroxy-6,8-dimethylisoflavone (**44**) mediated a decrease in activity, suggestive of an antiestrogenic effect [21].

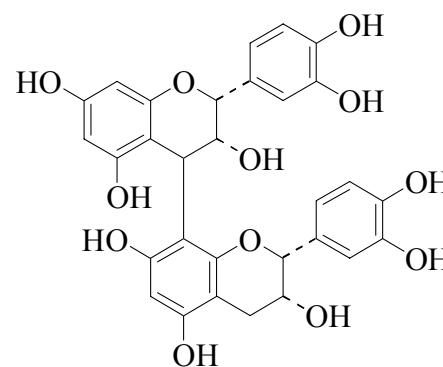
Fatty Acid Synthase (FAS) has been identified as a potential antifungal target. Bioactivity-guided fractionation on *Miconia pilgeriana* showed that arjunolic acid (**46**) isolated from this plant gave moderate activity against FAS (IC<sub>50</sub> 27.5 mg/mL) [22].

The antinociceptive effect of the ethanolic extract of *M. malabathricum* using acetic acid-induced abdominal writhing test and hot-plate test in mice has been done by Sulaiman *et al.* It was demonstrated that the extract (30-300 mg/kg, i.p.) strongly and dose-dependently inhibited the acetic acid-induced writhing test with an ED<sub>50</sub> of 100 mg/kg i.p., suggesting that, the ethanolic extract of *M. malabathricum* is a potentially antinociceptive agent that acts at both peripheral and central levels of nerves [32].

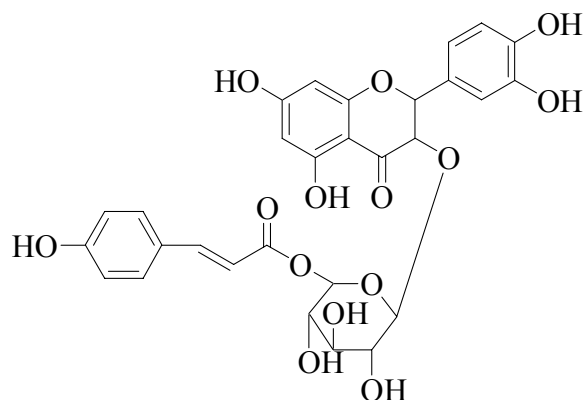
Three active compounds, castalagin (**78**), procyanidin B-2 (**79**) and helichryoside (**80**), which were isolated from the leaves of *M. candidum* possess the ability to lower blood pressure through a decrease of sympathetic tone as well as due to direct vasodilatation in SHR (spontaneously hypertensive rat) [33].



(78)



(79)



(80)

The analgesic effects of the hexane, methylene chloride and ethanol extracts of *Miconia rubiginosa* were evaluated in mice and rats using the acetic acid-induced writhing and hot plate tests. The extracts (100, 200 and 300 mg/kg body wt.) and indomethacin (5 mg/kg body wt.) produced a significant ( $p < 0.05$  and  $p < 0.01$ ) inhibition of acetic acid-induced abdominal writhing [34].

## 1.8 Background of the Research

The reviews on several *Melastoma* species did not mention the work carried out on *Melastoma imbricatum* and *Melastoma malabathricum* with white petals. In fact a thorough literature search on these species did not reveal any report on the chemical constituents or their biological activities. It is believed that both plants have never been investigated before. These plants are chosen in this research because they are used prominently in Malaysian society as traditional medicine, for the treatment of diarrhea, puerperal infection, dysentery, leucorrhoea, wound healing, post-partum treatment and hemorrhoids especially for woman after child birth [12]. The *M. imbricatum* is also endemic to Malaysian forest, while *M. malabathricum* with white petals is known to grow mainly in southern part of Malaysia especially in Johor.

## 1.9 Objectives of the Study

The objectives of this study are to investigate the chemical constituent of two Malaysian traditional medicinal plants of Melastomataceae i.e. *M. malabathricum* L. with white petals and *M. imbricatum* and to screen the biological activities (antimicrobial, antioxidant, anti-inflammatory and cytotoxicity) of the crude extracts and the pure isolated compounds.

## 1.10 Scopes of the Study

In natural products research, there are two main approaches mostly conducted by many researchers including chemical investigation and bioactivity testing. Thus, these two major approaches were also carried out in investigating the Melastomaceae plants.

The first approach was the extraction, isolation and characterization of the chemical components from the whole parts of the plants. The extraction was carried out by successive soxhlet extraction using organic solvents. Isolation procedure was done by chromatographic technique such as vacuum liquid chromatography and gravity column chromatography on silica gel as well as sephadex LH-20. Characterizations of the isolated compounds were carried out by means of physical and chemical properties such as melting point, optical rotation and chemical reaction. The structures were elucidated using spectroscopic methods including ultraviolet, infrared, nuclear magnetic resonance spectroscopies and mass spectrometry.

The second approach was bioactivity screening on the extracts and pure compounds. The bioactivity assays conducted were antibacterial, antifungal, anti-inflammatory, antioxidant and cytotoxicity. Antibacterial activity was tested using disc diffusion methods with four strains of bacteria i.e. *Staphylococcus aureus*,

*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antifungal activity was tested against *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Microsporum gypseum*, *Trichophyton mentagophytes*, *Trichophyton rubrum*, *Cryptococcus neoformans*, and *Candida albicans*. Anti-inflammatory activity was carried out using 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced inflammation and platelet activating factor receptor binding antagonist on mouse ear oedema and rabbit platelet, respectively. Antioxidant assay was carried out using lipid peroxidation and radical scavenging analyzed with ultraviolet and electron spin resonance, respectively. Cytotoxic activity was conducted using cell culture of human breast cancer cells (MCF7).

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