PHYTOCHEMICAL STUDY ON *SESBANIA GRANDIFLORA* ROOTS AND SYNTHESIS OF SELECTED FLAVONOID PRECURSORS (CHALCONES DERIVATIVES)

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by

NOVIANY

Thesis submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy

FEBRUARY 2014

Dedicated to my husband Riswan Harnata Jusran

my kids, Syafiq Ubaid Alwandanii Harnata

Zaky Taufiqurrahman Harnata,

Balqis Alifah Dzatill Izzah Harnata and

Muhammad Al Fatih Harnata

Also to my parents, Muhammad Hasan and Aisyah

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Bismillahirrohmanirrohiim

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February 2014

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LIST OF ABBREVIATIONS

Solvents

EtOH	Ethanol
MeOH	Methanol
EtOAc	Ethyl acetate
$CHCl_3$	Chloroform
DMF	Dimethyl Formamide
DMSO-d_6	Deuterated Dimethyl sulfoxide
$CDCl_3$	Deuterated chloroform
Ac-d_6	Deuterated Acetone

Chemicals

NH_4OH	Ammonium Hydroxide
NA	Nutrient Agar
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
MTT	(3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-Tetrazolium Bromide

Instruments and Techniques

HRESIMS	High resolution electrospray mass spectrometry
EI	Electron Ionization
TOF-MS	Time Of Flight Mass Spectrometry
UV	Ultraviolet
IR	Infrared
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
COSY	Correlated Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMQC	Heteronuclear Multiple Quantum Coherence

HMBC	Heteronuclear Multiple Bond Connectivity
NOESY	Nuclear Overhauser Enhancement Spectroscopy
NOE	Nuclear Overhauser Effect
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
PCTLC	Preparative Centrifugal Thin Layer Chromatography
GCMS	Gas Chromatography-Mass Spectrometry
R_t	Retention Time

Microbes

M. tuberculosis	*Mycobacterium tuberculosis*

LIST OF APPENDICES

KAJIAN FITOKIMIA TERHADAP

AKAR *SESBANIA GRANDIFLORA* DAN SINTESIS

PEMULA BIOSINTETIK FLAVONOID TERPILIH (TERBITAN KALKON)

ABSTRAK

Sesbania grandiflora, sejenis pokok setinggi 15 m, adalah tumbuhan ubatan tradisional yang telah dikutip dari daerah Lampung Selatan, Indonesia. Beberapa sebatian baru dan telah dikenal pasti disisihkan dari akar pokok ini. Semua sebatian tersebut dikenal pasti sebagai satu sebatian jenis terpenoid, asid betulinik (**81**), sepuluh flavonoid, malindonesianin A-C (**82**, **86**, **88**), xenognosin B (**83**), likuiritigenin (**84**), 7,2',4'-trihidroksiisoflavon (**85**), demetilvestitol (**87**), vestitol (**89**), medikarpin (**90**), sativan (**91**), dan satu sebatian jenis biaril, l,l'-binaftalen-2,2'-diol (**92**). Tiga sebatian (**82**, **86**, **88**) dikenal pasti sebagai dimer isoflavonoid baru, manakala satu sebatian (**92**) dikenali sebagai sebatian semulajadi biaril baru.

Kalkon merupakan pemula biosintetik penting disebabkan kepelbagaian aktiviti farmakologinya. Kajian ini bertujuan untuk mensintesis 28 terbitan baru kalkon, melalui penggabungan *p*-alkoksi asetofenon dengan benzaldehid, menggunakan alkilasi dan kondensasi Claisen-Schmidt dengan bes sebagai mangkin. Semua sebatian terbitan dicirikan dengan menggunakan IR, 1D NMR (^{1}H dan ^{13}C NMR), 2D NMR (COSY, HMQC, HMBC) dan spektrometri jisim.

Semua sebatian semulajadi dan terbitan baru telah disaring secara *in vitro* bagi mengesan aktiviti antituberkulosis terhadap strain *Mycobacterium tuberculosis*. Di antara sebatian semulajadi, aktiviti antituberkulosis yang ketara terhadap *Mycobacterium tuberculosis* telah ditunjukkan oleh sebatian **92** dengan nilai MIC yang terendah iaitu 312.5 x 10^{-2} µg/mL, manakala antara sebatian terbitan tersebut, sebatian **5a**, **5b**, dan **5d** menunjukkan aktiviti antimikrob yang amat baik dengan nilai MIC terendah iaitu 12.5 µg/mL.

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ABSTRACT

Sesbania grandiflora, a small tree up to 15 m tall, is a traditional medicinal plant collected from South Lampung, Indonesia. Some new and known compounds were isolated from the roots of this plant. They were identified as one terpenoid, betulinic acid (**81**), ten flavonoids, malindonesianin A-C (**82**, **86**, **88**), xenognosin B (**83**), liquiritigenin (**84**), 7,2',4'-trihydroxyisoflavone (**85**), demethylvestitol (**87**), vestitol (**89**), medicarpin (**90**) and sativan (**91**), together with one biaryl compound, l,l'-binaphthalene-2,2'-diol (**92**). Among them, three compounds (**82**, **86**, **88**) were established as novel isoflavonoid dimers, while one compound (**92**) was found as new biaryl natural product.

Chalcone is an important biosynthetic precursor, due to the diverse pharmacological activities. The aim of this current study was to synthesize 28 new chalcone derivatives compounds by incorporating *p*-alkoxyacetophenones with substituted benzaldehydes. Four new series of chalcone derivatives have been synthesized using the alkylation and the base catalysed Claisen-Schmidt condensation. All the synthesized compounds were fully characterized by IR, 1D NMR (^{1}H and ^{13}C NMR) and 2D NMR (COSY, HMQC, HMBC) as well as mass spectrometry analysis.

All the isolated and the synthesized compounds were assayed *in vitro* for their antituberculosis activities against *Mycobacterium tuberculosis* strain. Among isolated compounds, significant activity against *Mycobacterium tuberculosis* was exhibited by compound **92** with the lowest MIC value of 312.5 x 10^{-2} µg/mL, while among the synthesized compounds, compounds **5a**, **5b**, and **5d** showed good activities with the lowest MIC value of 12.5 µg/mL.

CHAPTER ONE

INTRODUCTION

1.1 General

The term "natural products' is generally used to describe a broad collection of the chemical compounds or substances produced by a living organism found in nature, including plants, animals, marine organisms and microorganism (Bart, 2011). Natural products have been exploited by humans as medicine, flavour, poison, food, dye and many other uses (Verpoorte, 2007). In recent years, there has been growing interest in the therapeutic use of natural products, particularly those derived from plants (Chin *et al.*, 2006; Newman & Cragg, 2012).

Plants produce a wide variety of so called secondary metabolites that play an important role in producing the medicinal properties and in the survival of the plant in its ecosystem. Each plant species has its own specific set of secondary metabolites that is usually unique in its structural features (Verpoorte *et al.*, 1999). The well known classes secondary metabolites which displayed medicinal properties are terpenoids, steroids, alkaloids, and phenolics (Hasan, 2007).

In the past years, there has been a rapidly increasing interest in plant secondary metabolites. About more than 100,000 plant secondary metabolites are already known, however, only a small percentage of all plants species have been studied for their phytopharmacological properties (Verpoorte *et al.*, 2000). Fabaceae plants, particularly species in the Papilionoideae subfamily, have long been extensively investigated for their phytochemical and pharmacological potentials. Several types of

secondary metabolites have been found in this family, including alkaloids, non-protein amino acids, flavonoids, isoflavonoids, coumarins, phenylpropanoids, antraquinones, terpenoids and cyanogenic glycosides (Wink & Mohamed, 2003). Among them, isoflavonoids are found predominantly in the Papilionoideae subfamily of the Fabaceae. A recent checklist of isoflavonoids reported in the Fabaceae, indicates that more than 420 isoflavonoids are new compounds (Veitch, 2007). In nature, isoflavonoids provide a wide range of functions, for instance, as antimicrobial, anti-insect and allelopathic agents (Dixon & Sumner, 2003), as signal molecules for the induction of nod genes (Ferrer *et al.*, 2008; Novák *et al.*, 2004) and as pathogen attack inhibitor (Graham & Graham, 2000). Moreover, these compounds are well known as common constituents of the human diet (Du *et al.*, 2010). Clinical studies on these constituents have suggested a positive effect of isoflavonoids in human health and nutrition, such as in preventing heart disease, hormonally dependent cancers, menopausal symptoms and osteoporosis (Cogolludo *et al.*, 2007; Cornwell *et al.*, 2004; Di *et al.*, 2008; Joung *et al.*, 2003; Kottra & Daniel, 2007; Sarkar & Li, 2003).

Due to their significant role in plant defense and health-related benefits, the various studies of Fabaceae plant have been conducted, including the isolation and screening of biological activity of purified compounds. The biologically active compounds can be lead compounds, allowing the design and rational planning of new drugs, the synthesis development and the discovery of new therapeutic properties.

1.2 Fabaceae

The Fabaceae is the third largest and one of the most economically important families of flowering plants. This family comprises of approximately 19325 species in 727 genera and it distributes into three sub-families, including the Caesalpinioideae (2250 species), the Mimosoideae (3270 species) and the Papilionoideae (13800 species).

Fabaceae is a family of the pea and/or bean plants. They are used as crops, forages, and green manures. Their wide variety of natural products lead to the production of flavours, drugs, poison, and dyes (Lewis *et al.*, 2005). This large family is also known for its variety of compounds, either primary metabolites (lectins, chitinases, numerous proteases and α-amylase inhibitors) or secondary metabolites (alkaloids, terpenoids, tannins and phenolics compounds) (Carlinia & Grossi-de-Sá, 2002; Sotheeswaran & Pasupathy, 1993; Wink & Mohamed, 2003). Among the three subfamilies, Papilionoideae is a remarkable chemistry feature of the Fabaceace due to the restricted occurrence of isoflavonoids (Veitch, 2009).

Most of the Fabaceae family are frequently used as traditional herbal medicine in the treatment of disorders such as diabetes, cough, urinary diseases, eye diseases, lung disease, toothache, fever, dysentery and different kinds of infections as well as inflammation of skin and mucous membrane (Neto *et al.*, 2008; Roosita *et al.*, 2008; Vitor *et al.*, 2004; Watjen *et al.*, 2007).

1.3 *Sesbania grandiflora* (L.) Pers

Taxonomy Classification

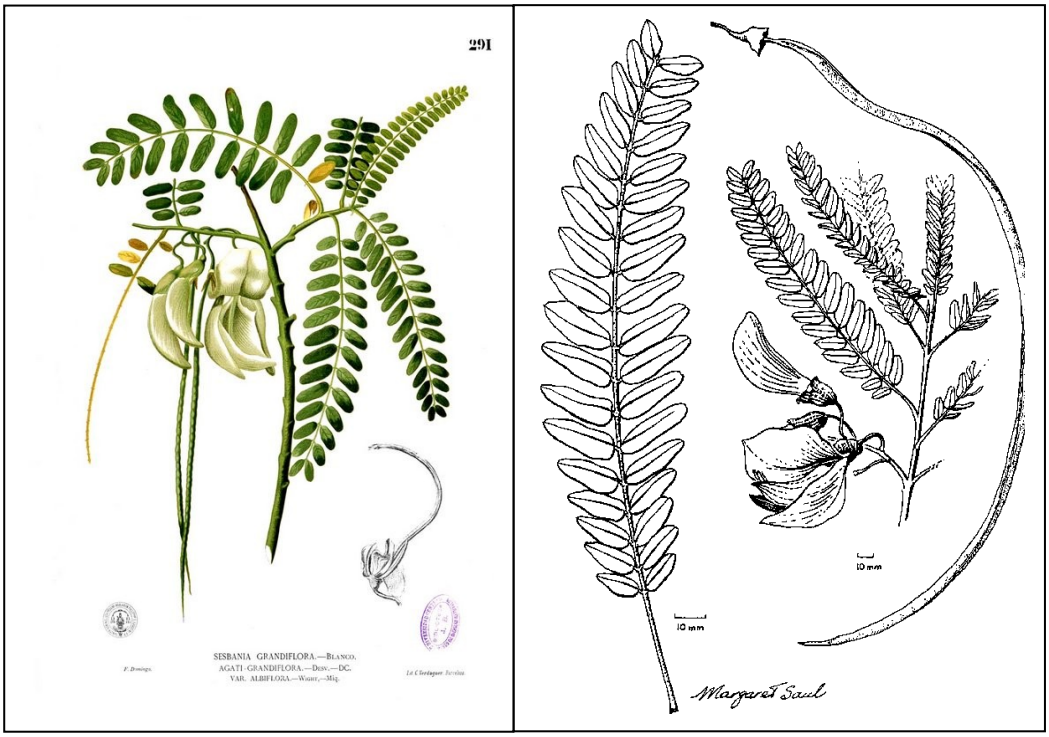
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Sesbania*
Scientific name	*Sesbania grandiflora*
Species authority	(L.) Pers
Common names	agati, white dragon tree, gauai-gauai, katuday, katurai, pan, colbri vegetal, agasti, turi, petai belalang, sesban, sesban getih, baculo, agathi, ton kae

Sesbania grandiflora (Plates 1.1) is a small erect, quick-growing, and sparsely branched tree that reaches up to 10-15 m in height. *S. grandiflora* is a member of the Fabaceae, subfamily Papilionoideae and tribe Robinieae. This plant is native to tropical Asia including India, Malaysia, Indonesia, Myanmar and Philippines and is found in tropical dry and moist forest from sea level up to 800 m (Heering & Gutteridge, 1992).

The tree of *S. grandiflora* can develop floating roots and aerenchyma tissue. The roots are heavily modulated. The leaves (Plates 1.2) are up to 30 cm long including a petiole 7-15 mm long and leaflets 20-50, in pairs opposite to alternate on the same leaf, oblong to elliptical, 12-44 mm x 5-15 mm, rounded to obtuse to slightly emarginated at the apex, glabrous or sparsely pubescent on both surfaces. Stipels filiform found in a 0.75 to 1 mm long, pubescent, persistent, and stipules broadly lanceolate. The raceme axillary has 2-4 flowers with rachis up to 65 mm long and peduncle 15-35 mm long. The flowers (Plates 1.3a) are white, yellowish, rose-pink or red with calyx 15-22 mm long. They are closed in young buds, splitting or

breaking at anthesis. The basal part persistent in the fruits (Plates 1.3b) are up to 10.5 x 8 cm and curved for most of its length, ovary and style glabrous. The pods are linear to slightly falcate, 20-60 x 6-9 mm with broad sutures, 15-50 seeded, septa 7.5-10 mm apart, glabrous, hanging vertically and indehiscent (Plates 1.3b). The seeds are dark brown and subreniform, 6.5 x 5 mm x 2.5-3 mm. The seed weight is 17,000-30,000 seeds/kg (Heering & Gutteridge, 1992).

Plates 1.1 *Sesbania grandiflora* tree (Heering & Gutteridge, 1992)



Plates 1.2 The leaves of *S. grandiflora* (Gutteridge, 1994)

(**a**) (**b**)

Plates 1.3 The flowers (**a**), the fruits (**b**) and the seeds (**c**) of *S. grandiflora* (Gutteridge, 1994)

1.3.1 Medicinal Uses

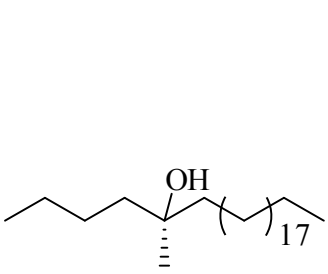
All parts of *S. grandiflora* are traditionally used as medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers, and fruits. Generally, the root is applied as a poultice to relieve from inflammation and fever. Ground root of *S. grandiflora* var. *coccinea* mixed with water is applied externally as a poultice to treat rheumatic swellings. The bark is used as astringent to cure smallpox. In Philippines, the decoction from the crushed bark is used for the treatment of ulcers in the mouth and alimentary canal. In Java, the local healers use the crushed bark for the treatment of thrush and infantile disorders of the stomach, and in Cambodia, the pounded bark is applied to treat the scabies. The leaves juice is used to treat worms, biliousness, fever, gout, itchiness and leprosy. In Malaysia, the crushed leaves are applied to cure sprains and bruises. While in Ayurveda, the leaves are employed for the treatment of epileptic fits and the fruits are used for the treatment of anaemia, bronchitis, fever and tumors. The leaves and flowers juice is a popular remedy for nasal catarrh and headache, head congestion or stuffy nose. The flowers juice is dropped into the eyes to correct dim visions (Wagh *et al.*, 2009).

Several studies have been conducted on the extracts obtained from *S. grandiflora* tree for providing a scientific validation of their acclaimed traditional utilisation. A study by Fojas *et al.* (1982) showed that the crude extracts of *S. grandiflora* possessed a hypotensive, oedema and diuretic properties. A study done by Tamboli *et al.* (2000) on three different extracts of *S. grandiflora* flowers, demonstrated that the ethyl acetate extract showed better analgesic and antipyretic activities compared with petroleum ether and ethanol extracts. The ethanolic extract of *S. grandiflora* barks displayed antiulcer and anti-inflammatory activities (Serti *et al.*, 2001). Another

study done by Kasture *et al.* (2002) demonstrated that the fraction of *S. grandiflora* leaves exhibited a wide spectrum of anticonvulsant profile and anxiolytic activities. Further studies revealed that *S. grandiflora* leaves could also establish a remarkable protective shield against erythromycin estolate-induced hepatotoxicity (Pari & Uma, 2003). The dichloromethane and methanol extracts of the aerial parts of *S. grandiflora* have also shown to have some antifungal activities (Goun *et al.*, 2003). Recently, *S. grandiflora* leaves supplements have shown to prevent oxidative damage in lung, liver and kidney and reverse cigarette smoke-induced oxidative damage in rats *via* its antioxidant potential (Ramesh & Begum, 2006; Ramesh *et al.*, 2007; Ramesh *et al.*, 2010). A subsequent research by Doddola *et al.* (2008) revealed that the leaf juice of *S. grandiflora* exhibits significant antiurolithiatic activity against calcium oxalate-type stones as well as antioxidant properties. Besides that, Laladhas *et al.* (2010) had evaluated the flowers of *S. grandiflora in vivo* and *in vitro* using different cancer cell lines, disclosed that the flowers may serve as a potential anticancer drug candidate. Moreover, the *n*-hexane extract of *S. grandiflora* seeds which was investigated by Shareef *et al.* (2012), showed that it possessed antioxidant, anti-inflammatory, analgesic and antipyretic activities.

1.3.2 Previous Phytochemical Studies on *Sesbania grandiflora*

The earliest phytochemical investigation on *S. grandiflora* started in the 1960s. The first simple alcoholic compound, α-5-methyl-5-pentacosanol or grandiflorol **1**, was isolated from the leaves of *S. grandiflora* (Tiwari & Bajpai, 1964). Later, Srivastava *et al.* (1968) and Pollard *et al.* (2011) reported a galactomannan **2** obtained from the seeds of *S. grandiflora*.



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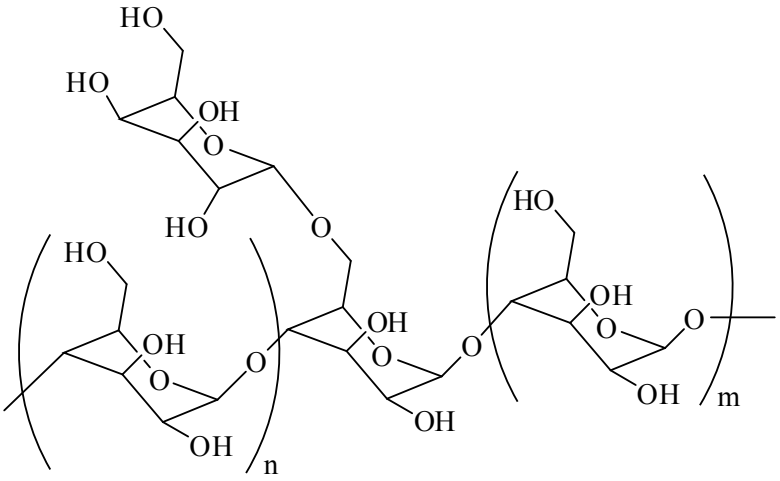

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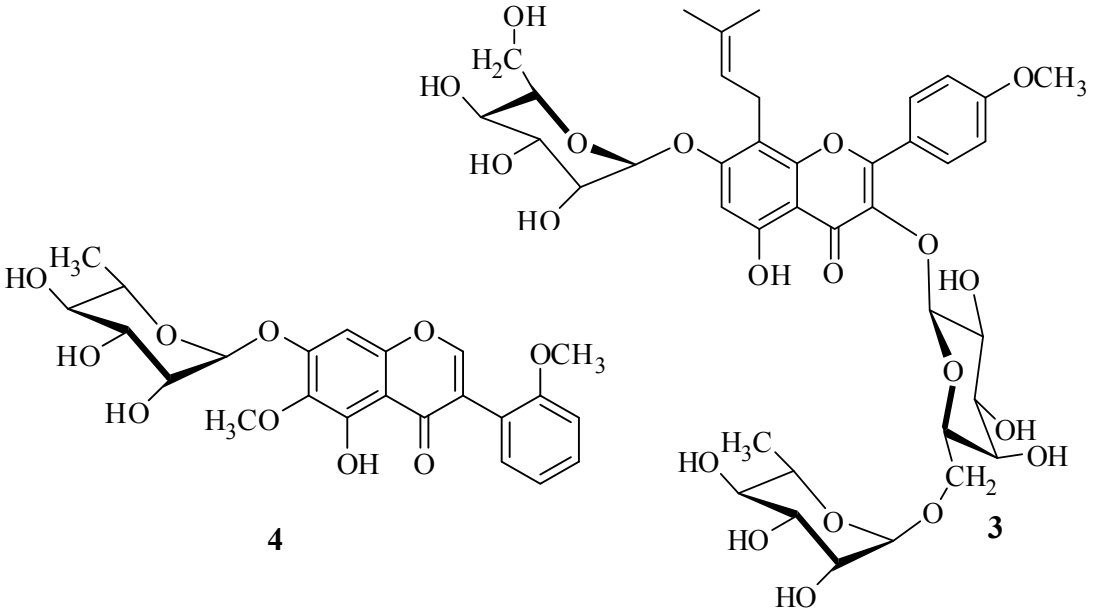
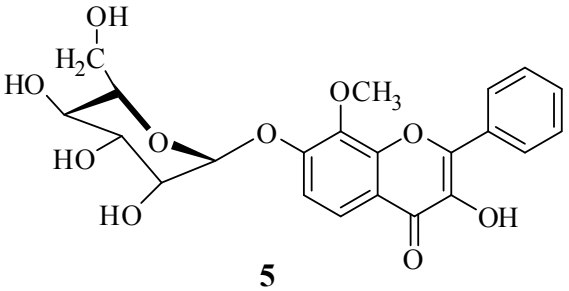


4 **3**

Das and Tripathi (1998) successfully isolated the first flavonol glycoside from *S. grandiflora* bark, identified as 4'-*O*-methyl-8-prenylkaempferol-3-*O*-(α-L-rhamnosyl-(1→6)-β-D-galactopyranoside)-7-*O*-β-D-galactopyranoside **3**. Further study done on the roots of *S. grandiflora* by Saxena and Mishra (1999a), furnished isoflavone glycoside-type flavonoid, 5,7-dihydroxy-6,2'-dimethoxyisoflavone-7-*O*-α-L-rhamnopyranoside **4**. In addition, another flavone glycoside, 3,7-dihydroxy-8-methoxyflavone-7-*O*-β-D-galactoside **5** has been isolated from the stems of

S. grandiflora (Saxena & Mishra, 1999b). Furthermore, a triterpenoid, 3-β-hydroxy-28-*p*-hydroxyphenoxyolean-12-ene **6** has been isolated from the bark fraction of *S. grandiflora* (Das & Tripathi, 1999).



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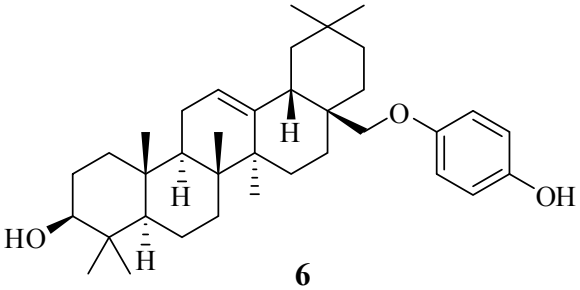


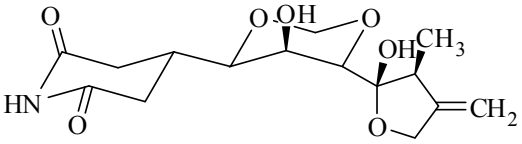
6

Additionally, an analytical study on the gum exudates of *S. grandiflora* by Anderson and Weiping (1990) afforded arabinogalactans with dextrorotatory, acidic and low viscosity properties. Recently, Shareef *et al.* (2012) had studied the sterols profile of *S. grandiflora* oil seeds collected in Pakistan and the results showed the presence of significant amount (74.06 %) of β-sitosterol.

1.3.3 Studies on Other *Sesbania* Species

The other four species from the genus *Sesbania* have also been investigated phytochemically. From the seeds of *S. drummondii*, three unusual glutarimide compounds, sesbanimide A-C **7-9**, were isolated by Powell *et al.* (1984). These three compounds showed inhibitory activity in PS leukimia and KB cell cultures. Later, Powell *et al.* (1990) had re-studied the occurrence of sesbanimide A and its isomers in seeds of *S. drummondii*, *S. punicea*, *S. vesicaria* and *S. exaltala* species, by using a tandem mass spectrometric procedure. Wagh *et al.* (2009) reported that the active ingredients of *Sesbania* were leucocyanidin **10** and cyanidin **11** obtained from the seeds, while oleanolic acid **12**, its methyl ester **13** and kaempferol-3-rutinoside **14**

found in the flowers. Recently, three new and six known oleanane saponins **15**-**23** have been successfully isolated from the seeds of *S. vesicaria* (Yuan *et al.*, 2013).



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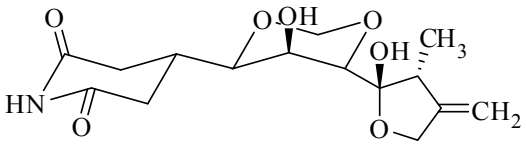

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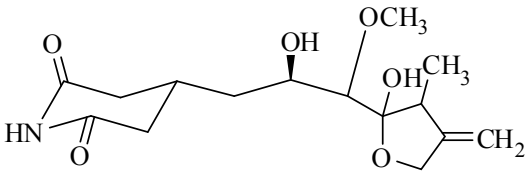

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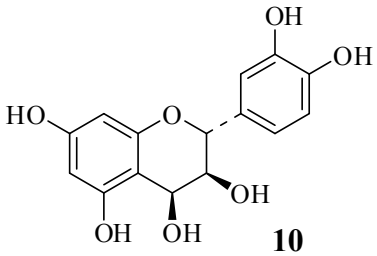

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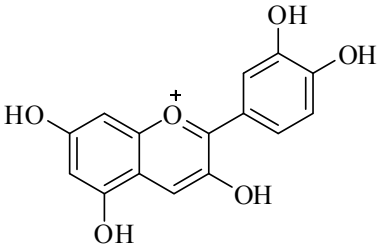

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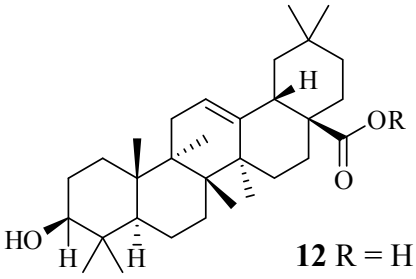
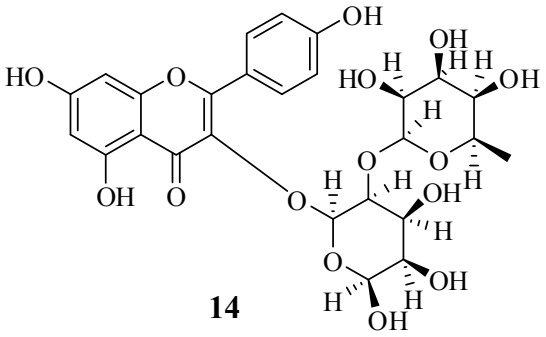


12 R = H
13 R = CH_3



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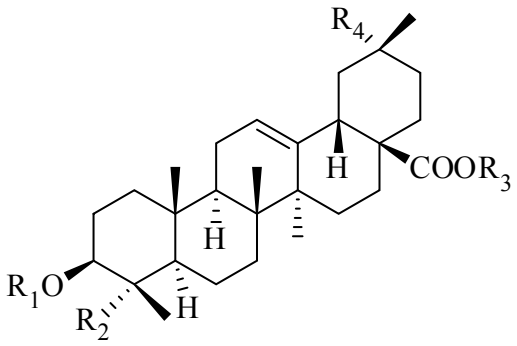
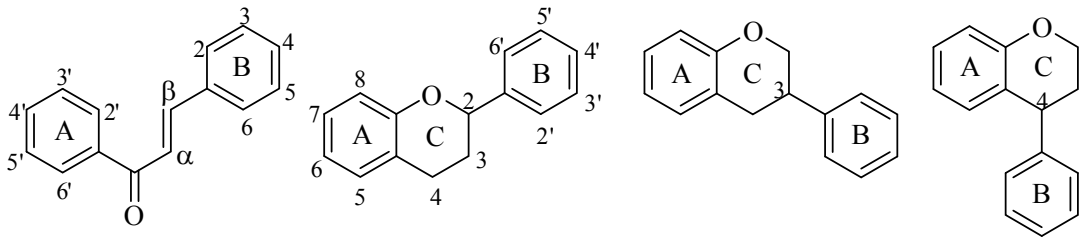


15 R_1 = α-L-Rha-(1→3)-β-D-GlcA, R_2 = CH_3, R_3 = H, R_4 = CH_2OH
16 R_1 = α-L-Rha, R_2 = CHO, R_3 = β-D-Glc, R_4 = CH_3
17 R_1 = α-L-Rha, R_2 = CH_2OH, R_3 = β-D-Glc, R_4 = CH_3
18 R_1 = β-D-GlcA, R_2 = COOH, R_3 = β-D-Glc, R_4 = CH_3
19 R_1 = β-D-GlcA, R_2 = CH_3, R_3 = β-D-Glc, R_4 = CH_3
20 R_1 = α-L-Rha-(1→3)-β-D-GlcA, R_2 = CH_3, R_3 = β-D-Glc, R_4 = CH_3
21 R_1 = α-L-Rha-(1→3)-6-OMe-β-D-GlcA, R_2 = CH_3, R_3 = β-D-Glc, R_4 = CH_3
22 R_1 = β-D-GlcA-(1→2)-[α-L-Rha-(1→3)]-β-D-GlcA, R_2 = CH_3, R_3 = β-D-Glc, R_4 = CH_3
23 R_1 = β-D-Xyl-(1→2)-[α-L-Rha-(1→3)]-β-D-GlcA, R_2 = CH_3, R_3 = β-D-Glc, R_4 = CH_3

1.4 Flavonoids

Flavonoids are the most ubiquitous polyphenolics in natural products, which are derived upon fifteen-carbon skeleton arranged in C_6-C_3-C_6 fashion (Grotewold, 2006). The two C_6 units form aromatic nuclei and the C_3 unit links them either to form an open chain connection, namely chalcone **24** or to fuse with (ring A), giving rise to extra heterocyclic ring (ring C) in flavonoid **25**, the so-called phenylbenzopyran core (Hasan, 2007). Depending on the position of the linkage between the aromatic ring and the benzopyrano (chromano) moiety, flavonoids can be divided into three sub-categories, including the flavonoids (2-phenylbenzopyrans) **25**, isoflavonoids (3-benzopyrans) **26**, and the neoflavonoids (4-benzopyrans) **27**. These three groups usually share a common chalcone **24** entity, thus they are biogenetically and structurally related (Grotewold, 2006).



24 **25** **26** **27**

Structural diversification in many types of flavonoids principally emanates from the variation in the degree of oxidation and saturation present in the heterocyclic C-ring (Grotewold, 2006). In the known type of flavonoids, the range of oxidation level extends from highly reduced catechin to highly oxidised flavonol (Fig.1.1).

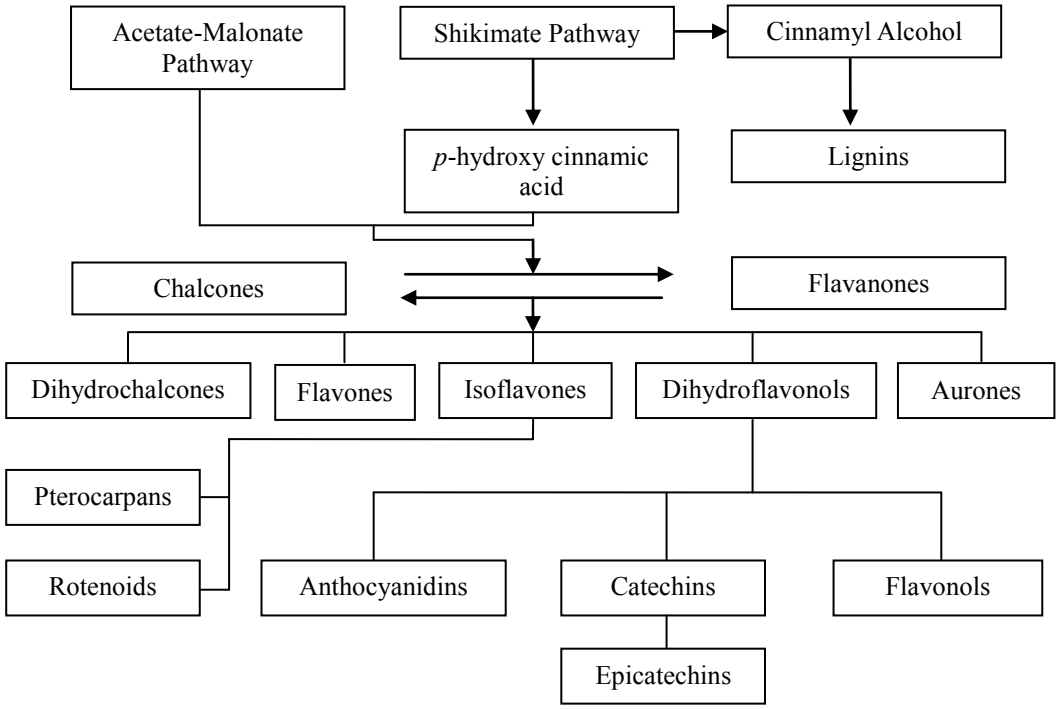


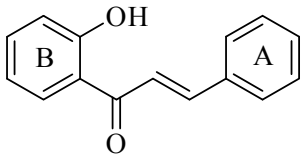
Figure 1.1 Biosynthetic origin of flavonoids (Hasan, 2007)

Combination of the shikimate pathway and the acetate-malonate pathway are responsible for the biosynthesis of flavonoids. Chalcone is the first flavonoid which being formed immediately following the confluence of the two pathways and all

other variants of flavonoids are derived from this way by a variety of routes (Hasan, 2007). Due to a wide variety of flavonoid types, this study is mainly focused on chalcones, isoflavonoids and dimer flavonoids which are related with the phytochemical and pharmacological investigation on Fabaceae plant.

1.4.1 Chalcones

Chalcone **24** or 1,3-diaryl-2-propen-1-one is the simplest compound of an open chain flavonoids incorporating two aromatic rings through the three-carbon α,β-unsaturated carbonyl system. Chalcones are considered to be the minor flavonoids and act as precursor for a vast range of flavonoid derivatives found throughout the plant kingdom (Grotewold, 2006). These groups of compounds include the 2′-hydroxychalcones **28**, 2′-OH-dihydrochalcones **29**, 2′-OH-*retro*-chalcone **30**, aurones (2-benzylidenecoumaranone) **31**, and auronols **32**. It is interesting to note that chalcones play an important role in initiating flavonoid biosynthesis as illustrated in Fig. 1.2 (Austin & Noel, 2003). In recent years, diverse biological activities associated with the chalcone analogues have drawn considerable attention of synthetic chemists. The growing number of publications on synthesis and biological evaluation of chalcones, demonstrate the growing interest in these compounds and their potential use in medicinal chemistry.



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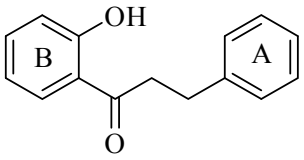

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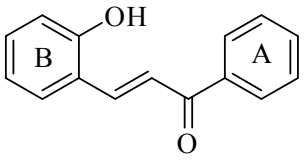

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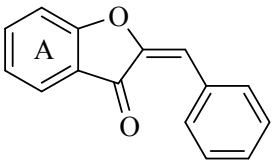

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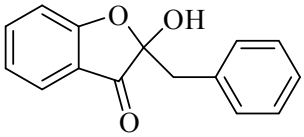

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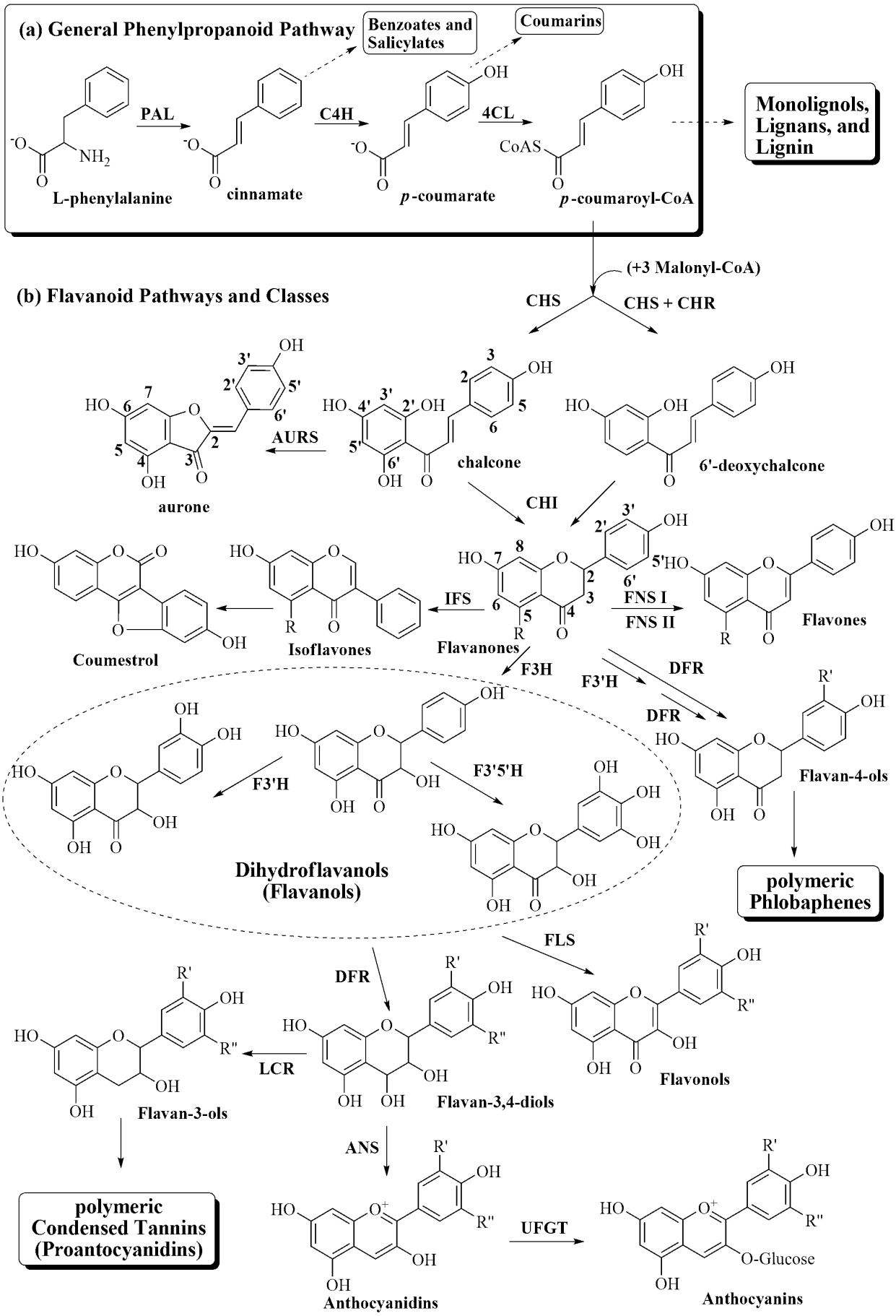
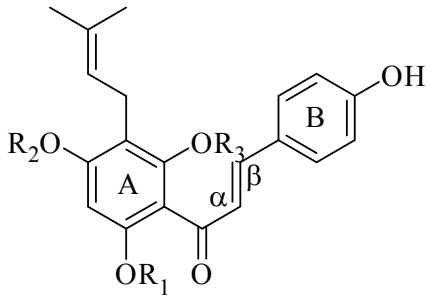


Figure 1.2 Plant pathways relevant to chalcone synthase (CHS) and resulting classes of natural products. (**a**) The general phenylpropanoid pathway. (**b**) CHS and its importance in flavonoid biosynthesis (Austin & Noel, 2003).

1.4.1.1 Biological Activities of Chalcones

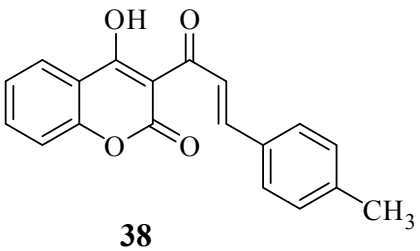
The natural and synthetic chalcone derivatives display a variety of biological activities against bacteria, fungi, viruses and tumours. The physiological, bacteriostatic and anti-tumour activities of chalcones lead to modification and screening of their analogues in search for novel therapeutic agents.

Xanthohumol **33** is the most naturally abundant prenylated chalcone. This compound has been isolated from hop cones of *Humulus lupulus* (Stevens & Page, 2004) and showed an interesting spectrum of pharmacological activities, such as apoptotic activity against different cell lines, anti-HIV agent, and inhibitor of prostate cancer (Colgate *et al.*, 2007; Drenzek *et al.*, 2011; Szliszka *et al.*, 2010; Wang *et al.*, 2004). Xanthohumol and its derivatives **34**-**37** were also successfully synthesised by Vogel *et al.* (2008) and were evaluated the cytotoxicity and anti-oxidative activities. All the tested derivatives showed remarkable cytotoxicity and antioxidant properties. Additionally, the results indicated that the variation of the hydroxyl group of ring A (concerning number and position) in xanthohumol derivatives effected the strength of the cytotoxicity in comparison to **33**.



33 $R_1 = CH_3$, $R_2 = H$; $R_3 = H$
34 $R_1 = H$, $R_2 = H$; $R_3 = H$
35 $R_1 = H$, $R_2 = CH_3$; $R_3 = H$
36 $R_1 = CH_3$, $R_2 = CH_3$; $R_3 = H$
37 $R_1 = H$, $R_2 = CH_3$; $R_3 = CH_3$

Hamdi *et al.* (2010), has synthesised a series of chalcone associated with the coumarin moiety and screened them against *Staphylococcus aureus* bacteria. The coumarin-chalcone **38** was the most active derivative with IC_{50} value of 2.07 μM.



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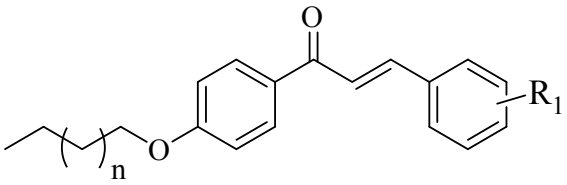


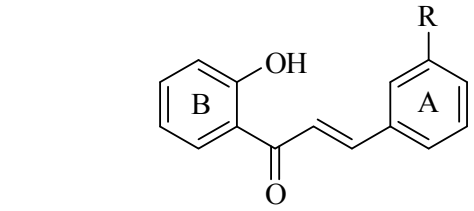
39

The effect of natural or synthetic alkyl chains in these type of compounds has also been investigated thoroughly by many researchers (Birnie *et al.*, 2000; Williams *et al.*, 2007), due to the ability of lipophilic alkyl chains to disrupt microorganism cell wall (Park *et al.*, 2004; Sheu & Freese, 1973).

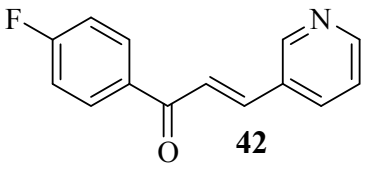
Recently, the synthesis of hydroxylated chalcone derivatives **39** with variable chain length (C_6, C_{10}, C_{12} and C_{14}) by treating benzaldehyde with 4-hydroxyacetophenone in the presence of potassium carbonate was investigated by Ngaini *et al.* (2012). All these derivatives were screened for antibacterial activity against *Escherichia coli* and were found to inhibit the growth of this strain in relevant concentrations. The results also indicated that the presence of hydroxyl groups at *meta* position with C_6 alkyl chains has considerable effect on antimicrobial activities observed.

Mycobacterium tuberculosis is one of the most pathogenic bacterial strains responsible for most of the tuberculosis (TB) cases. Among the infectious diseases, tuberculosis is the world's leading cause of death among women of reproductive age (Zealand, 1991). Although the improved prevention, detection, diagnosis and treatment methods have greatly reduced the affected individuals but the resistance of *M. tuberculosis* against the drug lines, have amplified the incidence of TB (Mitchison, 1984). Therefore, there is an urgent need to develop new molecules

against this deadly pathogen. The compounds having chalcone moiety and its analogues as well as some flavones were evaluated for their activity against *M. tuberculosis* H37Rv (Lin *et al.*, 2002). Among the series of natural chalcones and chalcone-like compounds consisting 47 and 21 compounds respectively, two chalcones **40**, **41** and four chalcone-like compounds **42**–**45** demonstrated >90% inhibitory activity. Furthermore, the results indicated that the substitution of a halogen group on the A-ring of 2'-hydroxychalcone improved anti-TB activity, whereby halogen-substitution on the 3-position displayed higher anti-TB activity than those substituent on 2- or 4-position. Besides, chalcone-like compounds demonstrated the most significant anti-TB activity among all the compounds evaluated. It was also mentioned that the active compounds in this series might require a lipophilic group on one side and a hydrophilic group on the other side of 2-propen-1-one.



40 R = Cl
41 R = I



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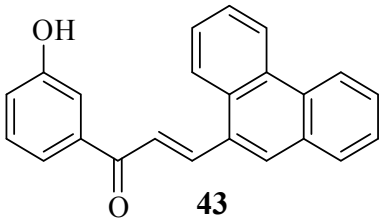

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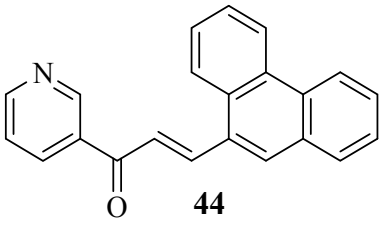

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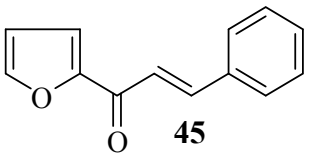

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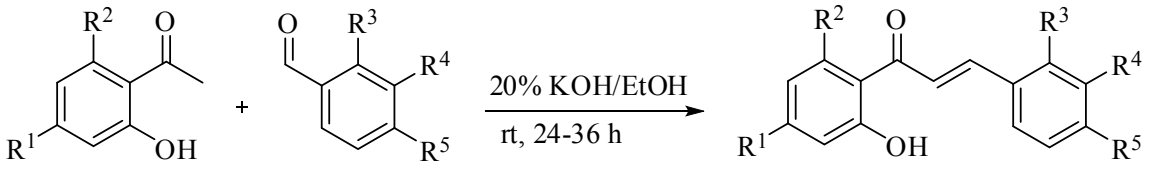
1.4.1.2 Methods for The Synthesis of Chalcones

The synthesis of chalcone derivatives can be carried out *via* various synthetic procedures, including Claisen-Schmidt reaction, Baker-Venkataraman rearrangement method, Algar-Flynn-Oyamada and Suzuki coupling reaction. The Claisen–Schmidt condensation is a general method to synthesize chalcone derivatives and their

analogues, which was used to synthesize novel chalcone derivatives in this study. It is a simple, straight-forward and fast procedure to prepare chalcones derivatives, *via* the condensation aromatic aldehydes with acetophenone derivatives in the presence of aqueous alkali media (Augustyn *et al.*, 1990). The base-catalyzed Claisen-Schmidt condensation have also been attempted by many synthetic chemists to synthesize chalcones. Detsi *et al.* (2009) have successfully synthesised chalcone and aurone derivatives with a Claisen-Schmidt reaction between appropriately substituted 2'-hydroxy-acetophenones and benzaldehydes in basic condition to give 41-88% yield of the final product.



Scheme 1.1 Synthesis of chalcones derivatives by Claisen–Schmidt condensation

1.4.2. Isoflavonoids

The isoflavonoids are a distinctive subclass of the flavonoids comprising 3-phenyl-4*H*-1-benzopyran-4-one (3-phenylchromone) as parent ring system (Grotewold, 2006). Biogenetically, isoflavonoids are derived from 2-phenylchroman as precursor by 1,2-arylmigration to the adjacent carbon of the heterocyclic ring as illustrated in Fig.1.3. This arrangement requires NADPH and O_2 cofactors to transforms the flavanones, liquiritigenin **85** or naringenin **45** into the isoflavones, daidzein **46** or genistein **47** *via* hydroxyisoflavanones intermediate **48** by radical mechanism as proposed in Fig. 1.3 (Dewick, 2005). Despite their limited distribution in the plants, isoflavonoids are remarkably diverse as far as structural variations are concerned.

Their structural diversity arises not only from the number and complexity of substituents on the basic 3-phenylchroman system, but also from the different oxidation levels and presence of additional heterocyclic rings as well as the hydroxylation and alkylation reactions (Grotewold, 2006).

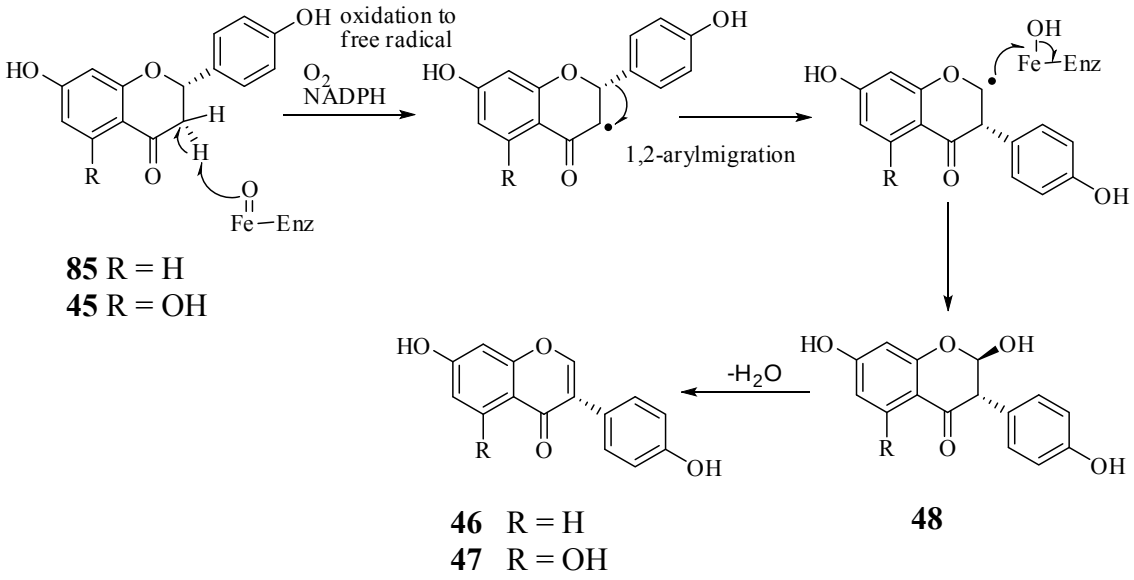
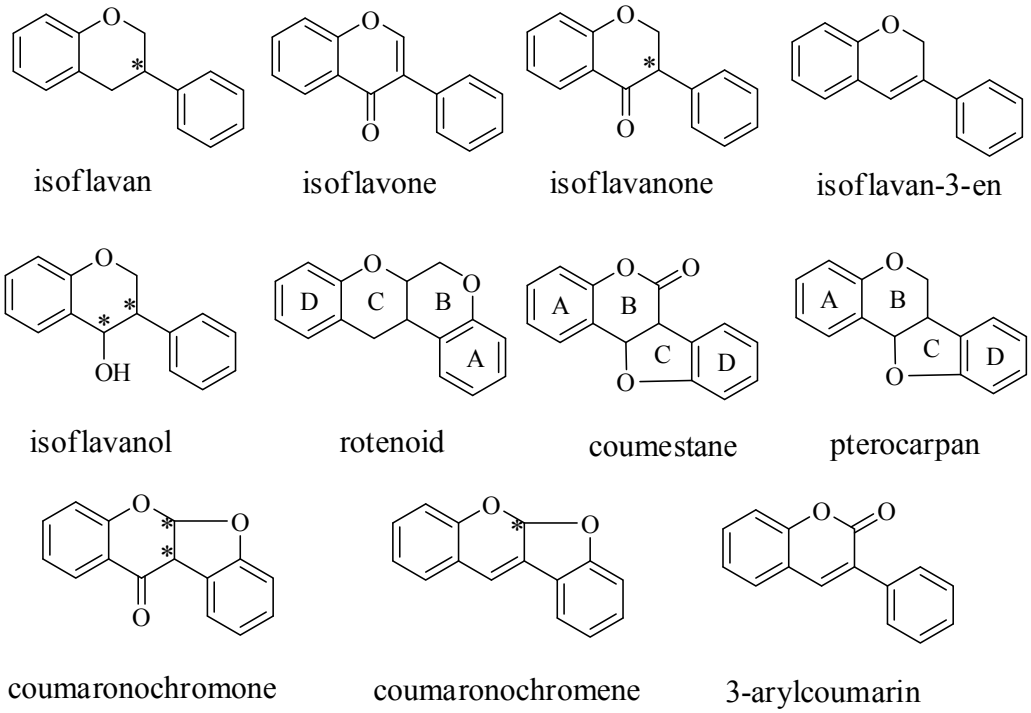


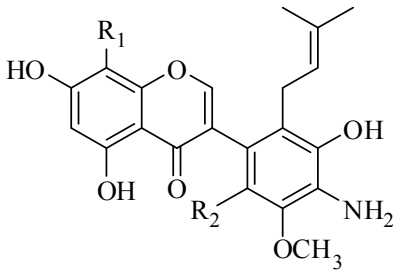
Figure 1.3 Proposed biogenetic routes of isoflavonoids (Dewick, 2005)

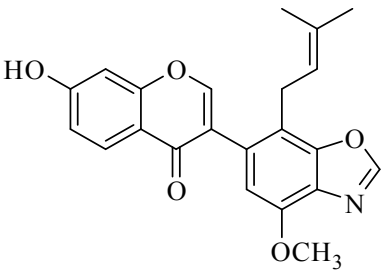
Isoflavonoids are subdivided into the following groups as described below:

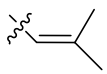


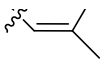
1.4.2.1 Naturally Occurring Isoflavonoids

The discovery of isoflavonoids as natural products has started in the mid-nineteenth century. To date, a large number of new and known isoflavonoids has been characterized from plants in which the majority of these compounds were isolated from the Fabaceae (Dewick, 2005; Veitch, 2007). An elaborated study done by Tahara and Ibrahim (1995) on isoflavonoid containing constituents in the root bark of *Piscidia erythrina,* showed unusual isoflavones with a highly substituted B-ring. Their exotic structures were established as 4'-amino-5,7,3'-trihydroxy-5'-methoxy-2',6'-di-(3,3-dimethylallyl)isoflavone **49**, 4'-amino-5,7,3'-trihydroxy-5'-methoxy-8,2'-di-(3,3-dimethylallyl)isoflavone **50**, and 7-hydroxy-5'-methoxy-2'-(3,3-dimethylallyl)-oxazolo-[4''',5''':4',3']isoflavone **51**. It was also reported that these compounds were the first naturally occurring flavonoids, directly nitrogenated on the C_6-C_3-C_6 skeleton. Later, five highly-prenylated isoflavonoids **52-56** have been obtained from the roots of *Erythrina zeyhery* (Tanaka *et al.*, 2003).





49 R_1 = H; R_2 = 

50 R_2 = H; R_1= 

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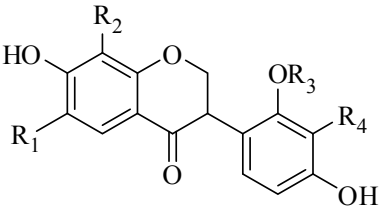
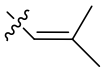
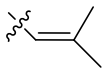
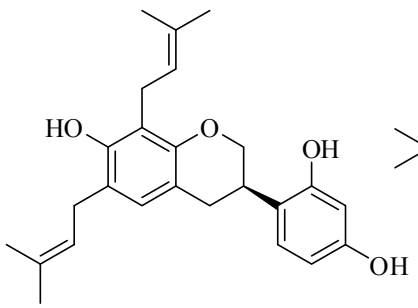


52 R_1 = R_3= H; R_2 = R_4 = 

53 R_3 = CH_3; R_4 = H; R_1 = R_2 



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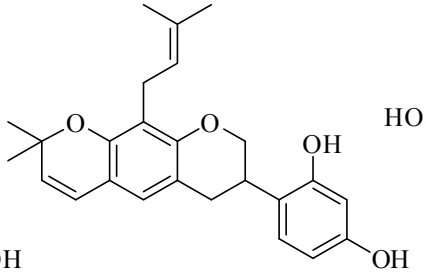

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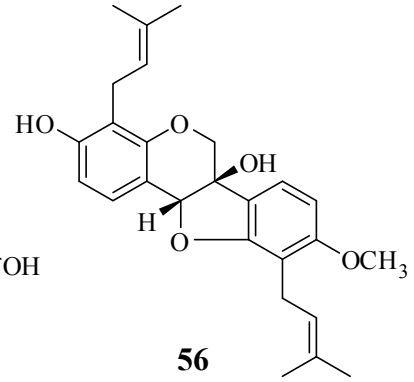
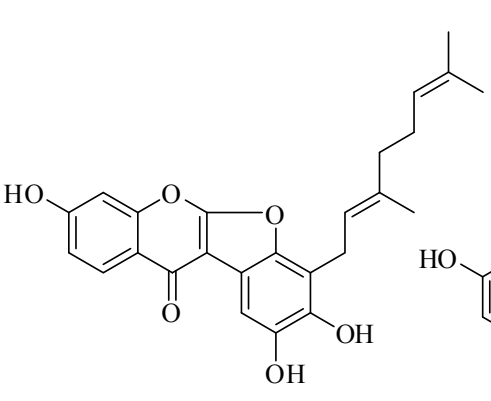


56

Another coumaronochromone-type of isoflavonoids, lespedezol C **57** has been successfully isolated for the first time from the stems of *Lespedeza homoloba*, including two geranyl-substituted isoflavones, lespedezol E_1 **58** and E_2 **59** (Miyase *et al.*, 1999a; Miyase *et al.*, 1999b). Veitch (2007) in his review paper on naturally occurring isoflavonoids in Fabaceae plant, reported that **59** is a potential precursor of compound **57**. Recently, Zappia *et al.* (2009) have isolated the first isoflavan featuring a *p*-quinol nucleus in the A-ring of isoflavonoid moiety, desmodian D **60**, together with two new isoflavanones, desmodianone F **61** and desmodianone G **62** from the roots of *Desmodium canum.*



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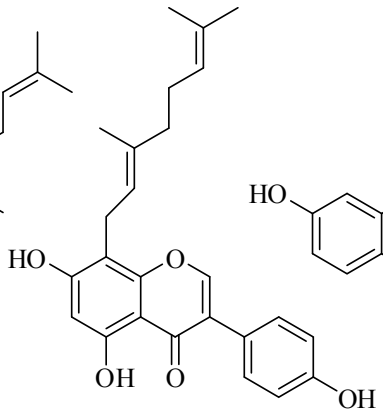

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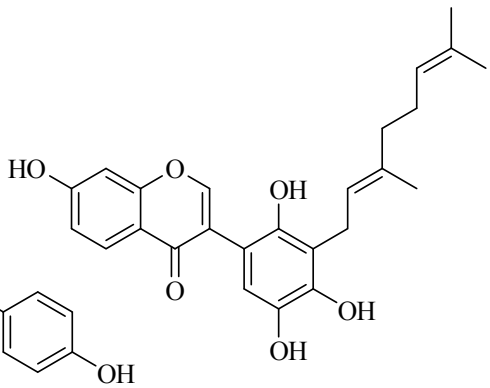

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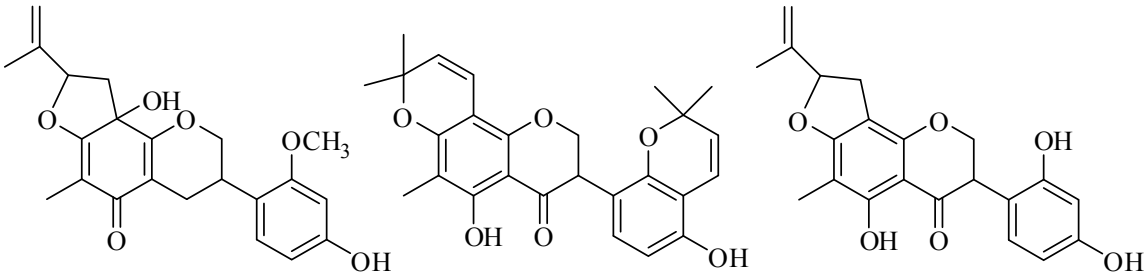
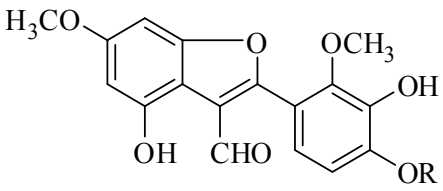


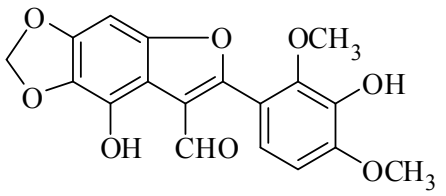
60 **61** **62**

1.4.2.2 Biological Activities of Isoflavonoids

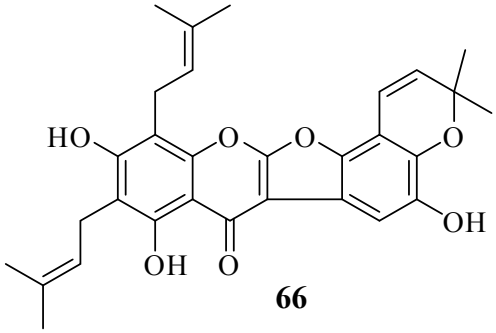
Isoflavonoids with their structural diversity, are reported to possess wide range of biological activities against different strains of bacteria, fungi, viruses, plasmodium and various cancer cell-lines. Kraft and co-workers (2001) have isolated three novel 2-arylbenzofuran-3-carbaldehydes, andinermals A-C **63-65** by using bioassay-guided fractionation of *Andira inermis* leaves. They found that among the tested compounds, compounds **63** and **65** are largely responsible for the antiplasmodial activity shown by leaves extract of *Andira inermis*. A subsequent study (Lo *et al.*, 2002) represented some new naturally coumaronochromones-type of isoflavonoids from the roots of *Euchresta formosana* that further evaluated against eight human cancer cells-lines. The results indicated that euchretins A **66**, J **67** and M **68** showed moderate cytotoxicity in a human hepatoma cell line (59T), while compound **67** was found to be active against stomach adencarcinoma cell (SCM-1). Moreover, another new isoflavone **69** has been obtained from the stems of *Derris indica* by a group of researchers (Koysomboon *et al.*, 2006) and they found that, **69** exhibited the activity against *Mycobacterium tuberculosis* H37Rv.



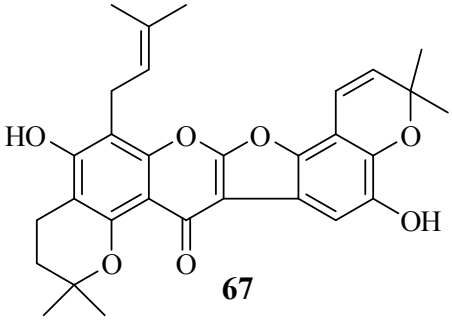
63 R = CH_3
65 R = H



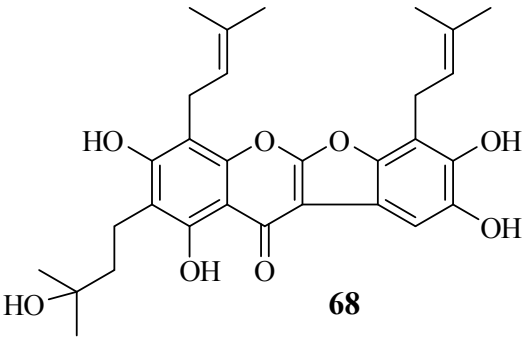
64



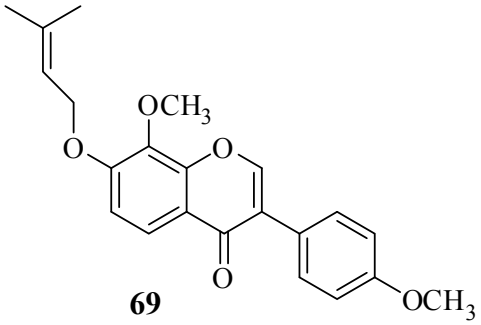
66



67



68



69

1.4.3 Flavonoid Dimer

A literature survey revealed that the main source of flavonoid dimers, heterodimers and conjugates are from the genus *Dalbergia* of Fabaceae plant following by other legume genera. A few compounds of this type have also been reported from non-legumes (Mackova *et al.*, 2006). Basically, dimeric flavonoids can be formed by incorporating two monomers of flavonoids with different linkages.

1.4.3.1 Naturally Occurring of Flavonoid Dimers

To date, the number of new naturally occurring flavonoid dimers is relatively small comparing to their monomers and structural diversity of this flavonoids type is