ISOLATION AND CHARACTERIZATION OF LIMONOIDS FROM *SWIETENIA MACROPHYLLA* AND THEIR ANDTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

by

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LIST OF ABBREVIATIONS

Solvents

CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
$CDCl_3$	Chloroform-*d*
MeOH	Methanol
EtOAc	Ethyl acetate
DMSO	Dimethyl sulfoxide

Chemicals

ZnSe	Zinc selenide
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
PG	Propyl gallate
Na_2CO_3	Sodium carbonate
Al_2Cl_3	Aluminium chloride
$BaCl_2$	Barium chloride
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
TMS	Tetramethylsilane

Chromatography

TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CC	Column Chromatography

Instrumental and Experimental

IR	Infra Red
UV	Ultra Violet
HRESIMS	High Resolution Electro Spray Ionization Mass Spectrometry
NMR	Nuclear Magnetic Resonance
HPLC	High Pressure Liquid Chromatography
DEPT	Distortionless Enhancement by Polarization Transfer
COSY	Correlated Spectroscopy
HMQC	Heteronuclear Multiple Quantum Coherence
HMBC	Heteronuclear Multiple Bond Connectivity
NOESY	Nuclear Overhauser Effect Spectroscopy

Symbols

$[\alpha]_D$	specific optical rotation
m/z	mass/charge
v/v	volume/volume
w/v	weight/volume
R_f	retention index
c	concentration
m. p.	melting point
nm	nano meter
SD	standard deviation
MHz	mega hertz
J	coupling constant
s	singlet
br s	broad singlet
d	doublet
br d	broad doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
t	triplet
dt	doublet of triplets
q	quartet
dq	doublet of quartets
qq	quartet of quartets
m	multiplet

Others

$DPPH^{\cdot}$	1,1-diphenyl-2-picrylhydrazyl radical
$O_2^{\cdot -}$	superoxide radical
$^{\cdot}OH$	hydroxyl radical
$ROO^{\cdot}$	peroxyl radical
ROS	reactive oxygen species
CAE	catechin equivalent
QE	quercetin equivalent
CFU	colony formation unit
ID	internal diameter
1D	one dimensional
2D	two dimensional
3D	three dimensional

PEMENCILAN DAN PENCIRIAN LIMONOID DARIPADA *SWIETENIA MACROPHYLLA* DAN AKTIVITI ANTIOKSIDAN DAN ANTIMIKROB

ABSTRAK

Swietenia macrophylla King. adalah sejenis pokok setinggi 30-35 m. Ia digunakan secara tradisional oleh masyarakat tempatan untuk mengubati penyakit kencing manis dan tekanan darah tinggi. Daun pokok ini telah dikutip di dalam kampus Universiti Sains Malaysia, dikeringkan di dalam udara, dijadikan serbuk and diekstrak dengan *n*-heksana, diklorometana dan metanol. Ekstrak diklorometana dan *n*-heksana telah dipisahkan dengan kromatografi turus and kromatografi lapisan nipis persediaan dengan menggunakan gel silika untuk memberikan tujuh sebatian, di mana empat daripadanya adalah sebatian limonoid yang baru, dinamakan sebagai swietefrakmin H-J [***1***-***3***] dan swietemakrofin [***4***]. Struktur sebatian baru tersebut telah ditentukan dengan menggunakan kaedah spektroskopi seperti NMR 1D dan 2D, HRESIMS, UV dan IR. Mereka telah dikenalpasti sebagai limonoid jenis frakmalin. Berdasarkan data spektroskopi dan juga melalui perbandingan dengan data literatur yang berkaitan, sebatian yang diketahui yang dipisahkan telah dikenalpasti sebagai swietenin [***5***], swietenolid [***6***] dan 3-*O*-tigloil-swietenolid [***7***], iaitu sebatian limonoid jenis meksikanolid.

Kandungan keseluruhan fenolik, tanin dan flavonoid dalam ekstrak daun *Swietenia macrophylla* telah ditentukan yang mana tertib kandungan-kandungan tersebut secara menurun adalah: ekstrak metanol > ekstrak diklorometana > *n*-heksana. Aktiviti antioksidan untuk ekstrak metanol, diklorometana and *n*-heksana, dan juga swietefrakmin H [***1***], swietefrakmin I [***2***] dan swietenolid [***6***] turut dinilai dengan menggunakan kaedah pemerangkapan radikal DPPH$^{\cdot}$. Keputusan

menunjukkan bahawa kesemua ekstrak yang diuji aktif sebagai pemerangkap radikal, dengan ekstrak metanol, diklorometana dan *n*-heksana menunjukkan IC_{50} pada 7.67 ± 0.29, 15.00 ± 0.50 dan 26.00 ± 1.73 μg/mL masing-masing. Untuk sebatian tulen yang diuji, swietenolid [***6***] menunjukkan IC_{50} pada 121.50 ± 0.87 μg/mL manakala swietefrakmin H [***1***] and swietefrakmin I [***2***] menunjukkan aktiviti yang rendah, yang mana nilai IC_{50} untuk kedua-dua sebatian ini tidak dapat ditentukan.

Aktiviti antimikrob untuk ektrak dan sebatian [***1***]-[***7***] yang dipisahkan dari daun *S. macrophylla* juga dinilai terhadap empat spesis bakteria dan satu spesis fungi. Ekstrak metanol dan diklorometana didapati aktif terhadap bakteria Gram-positif yang diuji, *Staphylococcus aureus* and *Bacillus subtilis*, dan sederhana atau tidak aktif terhadap bakteria Gram-negatif yang diuji, *Escherichia coli* and *Pseudomonas aeruginosa*. Ekstrak metanol juga menunjukkan sifat antifungi. Sementara itu, ekstrak *n*-heksana dan sebatian [***1***]-[***7***] didapati tidak aktif terhadap kesemua mikroorganisma yang diuji.

ISOLATION AND CHARACTERIZATION OF LIMONOIDS FROM *SWIETENIA MACROPHYLLA* AND THEIR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

ABSTRACT

Swietenia macrophylla King, a tree up to 30-35 m tall, is used traditionally by local folks to cure diabetes and high-blood pressure. The leaves of this tree, collected from the campus of Universiti Sains Malaysia, were air-dried, powdered and extracted with *n*-hexane, dichloromethane and methanol. The dichloromethane and *n*-hexane extracts were separated by column chromatography and preparative thin layer chromatography on silica gel to give seven limonoids, among which four are new limonoids, namely swietephragmins H-J [***1-3***] and swietemacrophine [***4***]. The structures of the new compounds were elucidated by spectroscopic methods using 1D and 2D NMR, HRESIMS, UV and IR, and were identified as phragmalin-type limonoids. Meanwhile, the known compounds were identified as swietenine [***5***], swietenolide [***6***] and 3-*O*-tigloyl-swietenolide [***7***], which are mexicanolide-type limonoids. These known compounds were identified based on their spectroscopic data and also by comparison with related literature.

The total phenolic, total tannin and total flavonoid contents of *Swietenia macrophylla* leaf extracts were examined and the contents were decreased in the following order: methanol extract > dichloromethane extract > *n*-hexane extract. The methanol, dichloromethane and *n*-hexane extracts, together with swietephragmin H [***1***], swietephragmin I [***2***] and swietenolide [***6***] were further evaluated for their antioxidant activity using $DPPH^{\cdot}$ radical scavenging assay. The

results showed that all of the extracts tested were active as radical scavengers, with methanol extract, dichloromethane extract and *n*-hexane extract showing IC_{50} at 7.67 ± 0.29, 15.00 ± 0.50 and 26.00 ± 1.73 μg/mL, respectively. For the pure compounds tested, swietenolide [***6***] showed IC_{50} at 121.50 ± 0.87 μg/mL, while the IC_{50} value for swietephramin H [***1***] and swietephragmin I [***2***], which showed low activity, cannot be determined in this study.

The antimicrobial activity of the extracts and compounds [***1***]-[***7***] isolated from the leaves of *S. macrophylla* were also evaluated against four species of bacteria and one species of yeast. The methanol and dichloromethane extracts were active against the Gram-positive bacteria tested, *Staphylococcus aureus* and *Bacillus subtilis,* and were moderately or inactive against the Gram-negative bacteria tested, *Escherichia coli* and *Pseudomonas aeruginosa*. The methanol extract also showed antifungal properties. On the other hand, the *n*-hexane extract and compounds [***1***]-[***7***] were found inactive towards all the microorganisms tested.

CHAPTER 1

INTRODUCTION

1.1 General

Natural products have been exploited by humans for centuries as medicines, dyes, foods, poisons and many other uses (Verpoorte, 2007). Among the various uses, natural products are prominent as a rich source of agents of medicinal value (Gordaliza, 2007). The constituents that played an important role in producing the medicinal properties of plants are mostly secondary metabolites, which are produced by plants to protect themselves against insect attacks and diseases. Secondary metabolites are usually unique in their structural features and found in only specific plants or groups of plants. They are usually described as an expression of individuality (Paul, 2002). Among the well-known secondary metabolites which showed medicinal properties are terpenoids, alkaloids and phenolics (Verpoorte, 2007).

In modern medicine, compounds originating from plant sources are used as the ingredients of coronary heart disease drugs, laxatives, anticancer agents, hormones, diuretics, antibiotics, decongestants, analgesics and antiparasitic compounds (Cragg *et al*., 1997). Around one in four of all prescription drugs dispensed by pharmacists are likely to contain ingredients derived from plants (Cragg *et al*., 1997; Oksman-Caldentey *et al*., 2004). To date, over 1000 phytochemicals have been employed to treat human ailments and diseases such as cancer, heart disease and microbial infections, with sixty percent of anticancer drugs or anti-infectives are natural products or analogs (Zhang & Demain, 2005).

1.2 Meliaceae

The family Meliaceae is the mahogany family of flowering plants, of the order Sapindales. The plants range in size from magnificent forest trees to small shrubs. It consists of 50 genera and about 800 species, which are mostly found in the tropical and sub-tropical regions of both hemispheres (Styles *et al*., 1991).

Economically, Meliaceae is important, especially for its high-quality timber. It is considered as one of the most valuable source of timber in the world. Some of the more important timber genera in the family, especially mahogany (*Swietenia*) and tropical cedar (*Cedrela*), which are prestige for their high-quality timber, have received considerable attention (Mayhew & Newton., 1998).

The family is also known to have wide-ranging uses in ethno-medicine, such as in the treatment of malaria, anemia, diarrhea, kidney pain, fevers and many other diseases, which prompting extensive research on them (Mulholland *et al*., 2000).

Chemically, the family Meliaceae showed a high level of phytochemical diversity. Compounds isolated from the members of this family include monoterpenoids, diterpenoids, sesquiterpenoids, triterpenoids, coumarins, chromones, lignans, flavonoids and phenolics (Muholland *et al*., 2000).

Among the various phytochemicals, Meliaceae is well-known for its presence of limonoids, a group of highly oxygenated triterpenes which characterized members of the family (Amit & Shailendra., 2006). Limonoids have been found in almost every member of the family studied. Elsewhere, they are very restricted in

occurrence. Characterization of limonoids from the Meliaceae family has been of great interest to the phytochemical research community and a large number of compounds have been identified. Hitherto, more than 300 limonoids have been isolated and identified from 54 species belonging to 23 genera of the Meliaceae family (Sohail *et al*., 2006). Many new limonoids, some from new species, are subsequently being discovered.

1.3 *Swietenia*

Swietenia is a member of the Meliaceae family and usually referred to as Mahogany. It is found in the neotropics. This genus comprises three species, *Swietenia humilis*, *Swietenia macrophylla* and *Swietenia mahogany*, which are geographically separated (Pennington, 1981).

Swietenia are medium-sized to large trees up to 20 to 45 m tall. They have pinnately-compound leaves composed of 4 to 6 ovate-acuminate leaflets without a terminal leaflet. The bark has vertical scales. The flowers are small, yellow-white. The fruit is a pear-shaped five-valve capsule 8 to 20 cm long, containing numerous winged seeds about 5 to 9 cm long (Scott, 1999).

This genus is famed as an important source of high quality timber for the furniture industry (Mayhew & Newton., 1998). Other than that, the bark of *Swietenia* is also used as a dye to color cotton threads (Munoz *et al*., 2000). In Malaysia, *Swietenia macrophylla* is the most commonly found species. It is planted for shade along road side.

1.4 *Swietenia macrophylla* King.

Swietenia macrophylla (Table 1.1; Plates 1.1 and 1.2) is also known as big-leaf mahogany. It is an evergreen tree from the Meliaceae family, which is up to 30 to 35 m tall, and is native to Central and South America (Styles *et al*., 1991).

Table 1.1 Taxonomy classification of *Swietenia macrophylla* King. (Gillies *et al*., 1999)

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	*Swietenia*
Scientific name	*Swietenia macrophylla*
Species authority	King
Common names	Big-leaf mahogany, Large-leaved mahogany, Honduras mahogany, Caoba, Acajou

The bark of the tree is grey and smooth when young, turning into dark brown, ridged and flaky when old. The leaves (Fig. 1.1) are up to 35 to 50 cm long, with 4 to 6 pairs of leaflets, each leaflet 9 to 18 cm long. The flowers (Fig. 1.1) are small, white colored, found in a 10 to 20 cm long, branching panicle. The fruits (Fig. 1.1) are 5-lobed capsules, erect, 12 to 15 cm long. The outer valves are woody, 5 to 7 mm thick. In the centre of the fruit is a woody, five angled columella extending to the apex. The fruits split open from the apex or base when they are ripe and dry. The seeds (Fig. 1.1) are hanging from the columella by their wing, leaving conspicuous scars after their release. Usually 35 to 45 seeds can be found per fruit. The seeds are brown, oblong, compressed, crested and extended into a wing at the attachment end, 7.5 to 15 cm long (inclusive of wing) with extensive air spaces. They are usually dispersed by wind (Lars & Dorthe, 2000).

Plate 1.1 *Swietenia macrophylla* (Penang, Malaysia, 2007)

Plate 1.2 Leaves of *Swietenia macrophylla* (Penang, Malaysia, 2007)

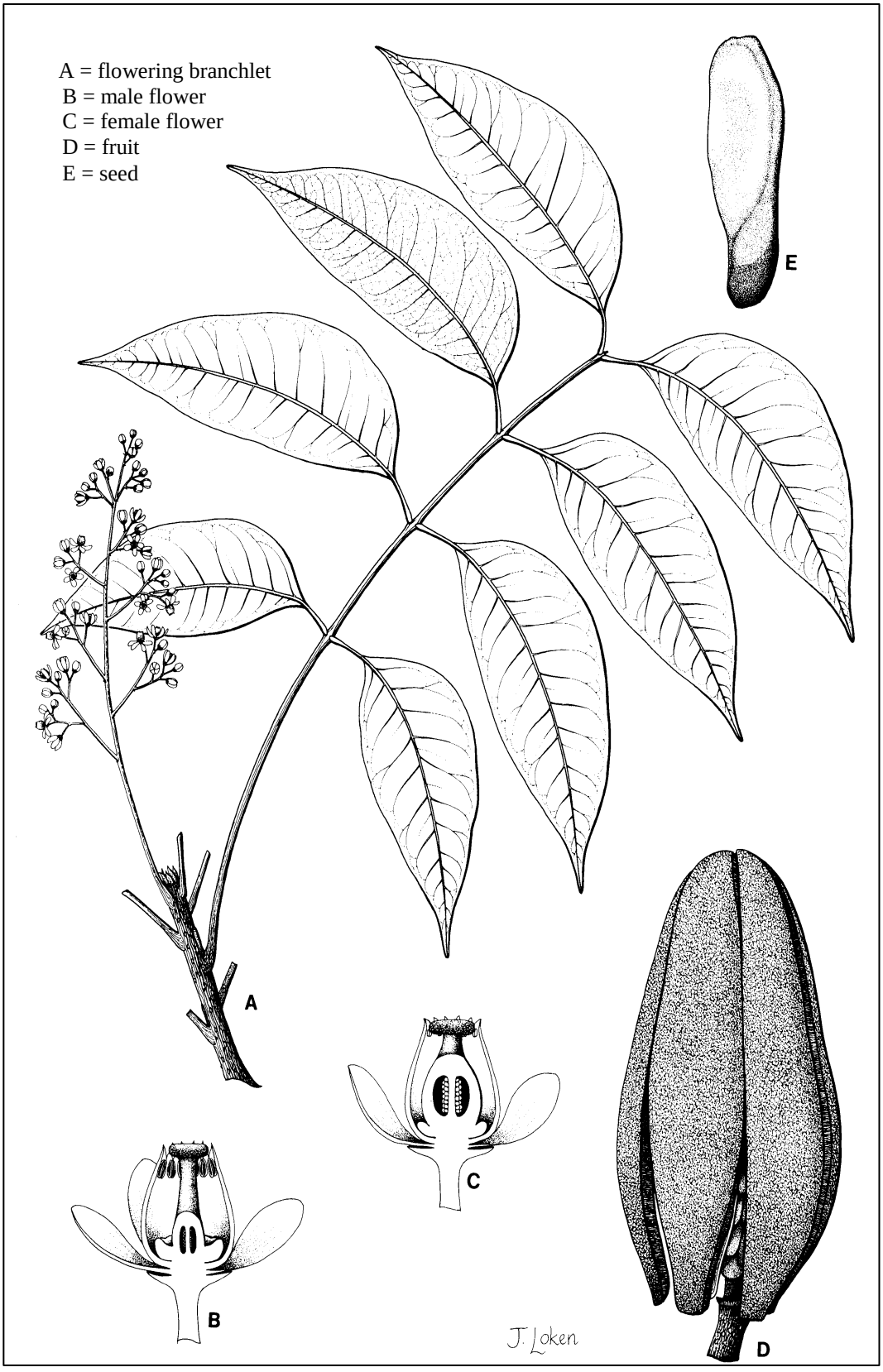


Figure 1.1 Flowers, leaves, fruit and seed illustration of *Swietenia macrophylla* (Pennington, 1981)

1.4.1 Medicinal uses

The traditional medicinal uses of *S. macrophylla* in various countries are summarized in Table 1.2.

Table 1.2 Traditional medicinal uses of *S. macrophylla* King.

Country	Plant part	Medicinal uses
Malaysia	Seeds	High-blood pressure and diabetes (Chan *et al*., 1976).
Bolivia	Seeds	Abortion, wounds and skin ailments (Munoz *et al.*, 2000).
India	Seeds	Diarrhea (Maiti *et al*., 2007a).
Indonesia	Seeds	Malaria (Tri *et al*., 2005).
Mexico	Bark	Diarrhea and fevers (Rocas, 2003)

Traditionally, the natives and the common folks of Malaysia chew and then swallow the seeds of *S. macrophylla* as a cure for high-blood pressure and diabetes (Chan *et al*., 1976). In Bolivia, the decoction from the crushed seeds is drunk in order to induce abortion (Munoz *et al*., 2000). Apart from that, the crushed seeds are also mixed with *Attalea phalerata* seed oil and applied in the form of a poultice on the skin to heal wounds and various skin ailments, such as skin allergy in children (Munoz *et al*., 2000). In West-Bengal, India, the local healers use the seeds to cure diarrhea (Maiti *et al*., 2007a). In Indonesia, the seeds extract of *S. macrophylla* is used for the treatment of malaria (Tri *et al*., 2005). Meanwhile, in Mexico, the infusion made with the bark of *S. macrophylla* is used to treat diarrhea and fevers (Rocas, 2003).

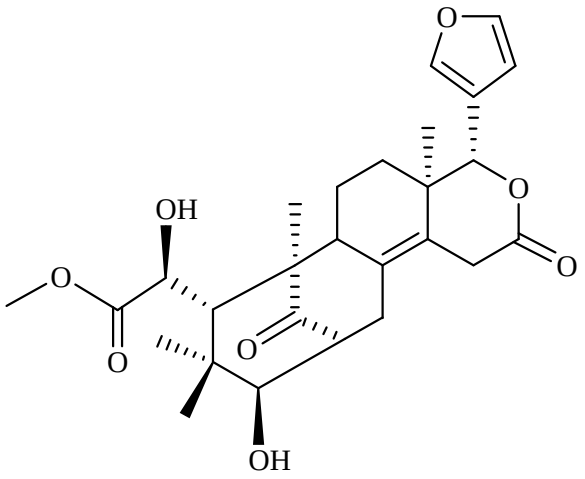
To provide scientific validation for the acclaimed traditional use of the plant, several studies have been done on the extracts obtained from the tree. In a study done by Guevara *et al*. (1996), the crude ethanol extract of the seeds was tested for anti-inflammatory, antimutagenic and antitumor-promoting activities. The

extract showed positive effects in all of the activities tested. A subsequent research by Soediro *et al*. (1990) showed that the methanolic bark extract of *S. macrophylla* displayed high antimalarial activity, both *in vivo* and *in vitro*. Further studies done by Tri *et al*. (2005) and Maiti *et al*. (2007c) showed that the water extract of the seeds possessed strong antimicrobial, antimalarial and antibabesial properties. In another study done by Maiti *et al*. (2007a), it was observed that the petroleum ether extract of the seeds has remarkable anti-diarrhea activity. In a more recent study, the methanol extract of *S. macrophylla* seeds was found to be a potent anti-diabetic (Maiti *et al*., 2007b). It was reported that the methanol extract has significantly reduced blood glucose level in diabetic rats.

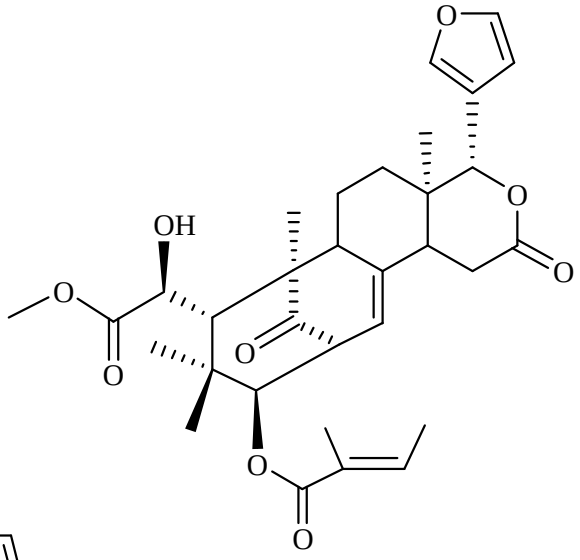
1.4.2 Previous studies on *Swietenia macrophylla* King.

A survey of literature showed that earlier phytochemical investigations on *S. macrophylla* were primarily focused on the seeds. Limonoids, which are highly oxygenated, modified terpenoids, were the principal type of compounds isolated from the seed extracts.

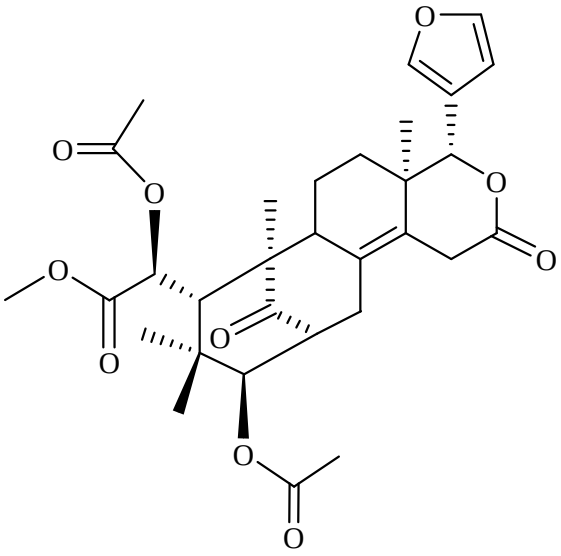
The earliest phytochemical investigation on *S. macrophylla* started in the 1940s. The first two compounds isolated from the seeds were simply identified as a bitter principle and a non-bitter residue by Chakravarty & Guha-Sircar (1947). Later, Guha-Sircar & Chakravarty (1951) identified the aforementioned compounds as mexicanolide-type limonoids, named swietenolide (**1**) and swietenine (**2**). A subsequent study by Chan *et al*. (1976) isolated another limonoid from the seeds, named swietenolide diacetate (**3**), by using the Soxhlet technique.



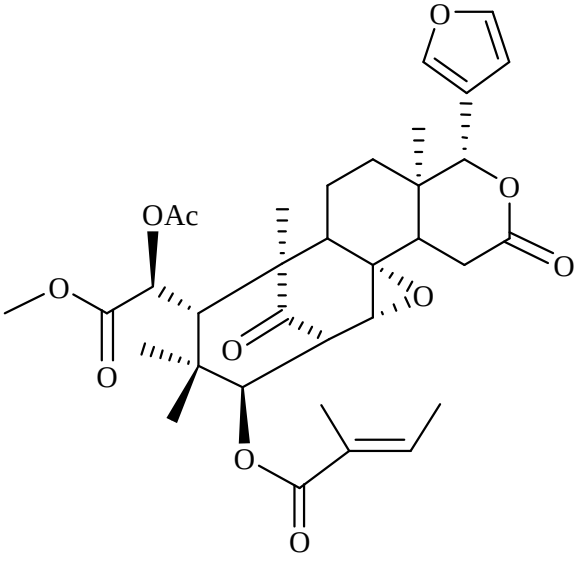
(**1**)



(**2**)

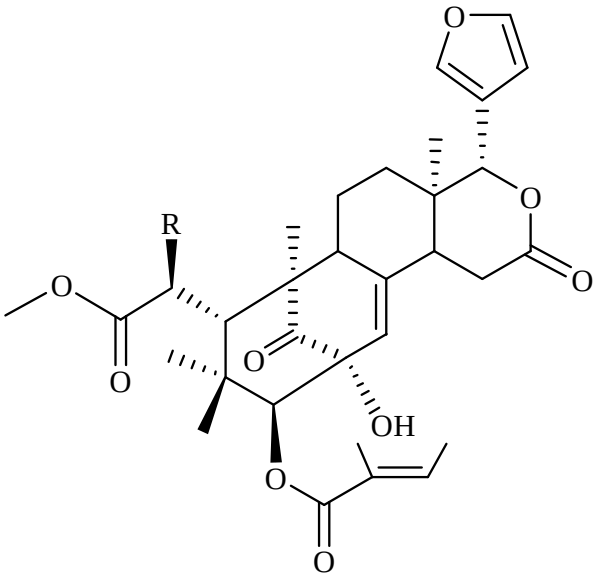


(**3**)

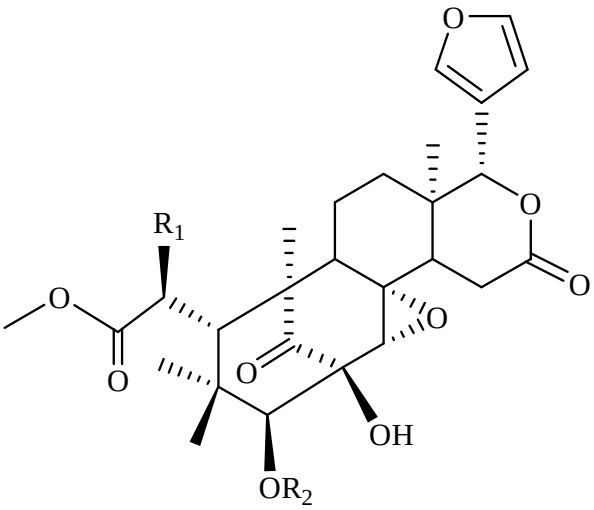


(**4**)

Another mexicanolide-type limonoid, named 8,30-epoxyswietenine acetate (**4**) was isolated from the hexane extract of the seeds by Taylor & Taylor (1983) using HPLC. A further research on the seeds by Kojima *et al*. (1998) had successfully isolated five limonoids, including 2-hydroxyswietenine (**5**), methyl 3*β*-tigloyloxy-2-hydroxy-1-oxo-meliac-8(30)-enate (**6**), methyl 3*β*-tigloyloxy-2-hydroxy-8,30-epoxy-1-oxo-meliacate (**7**), methyl 3*β*-acetoxy-2,6-dihydroxy-8,30-epoxy-1-oxo-meliacate (**8**) and methyl 3*β*-isobutyryloxy-2,6-dihydroxy-8,30-epoxy-1-oxo-meliacate (**9**).



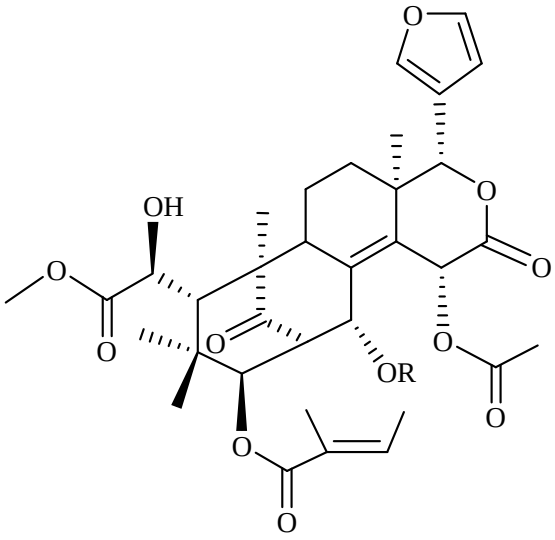
(**5**) R = OH
(**6**) R = H



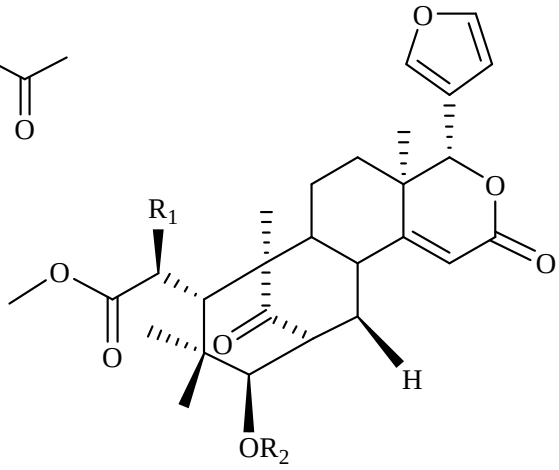
(**7**) R_1 = H, R_2 = $COC(CH_3)CH(CH_3)$
(**8**) R_1 = OH, R_2 = $COCH_3$
(**9**) R_1 = OH, R_2 = $COCH(CH_3)_2$

Mootoo *et al*. (1999) had re-investigated the seeds of *S. macrophylla* and managed to identify several limonoids, including augustineolide (**10**), 3*β*,6-dihydroxydihydrocarapin (**11**), 7-deacetoxy-7-oxogedunin (**12**), andirobin (**13**),

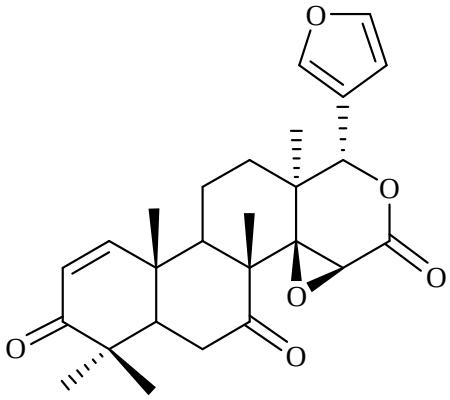
proceranolide (**14**), 6-*O*-acetyl-swietenolide (**15**), khayasin T (**16**), swietemahonins E (**17**), swietemahonin G (**18**), 6-deoxyswietenine (**19**), 3*β*,14-dihydroxymexicanolide (**20**) and 3-*O*-tigloyl-swietenolide (**21**).



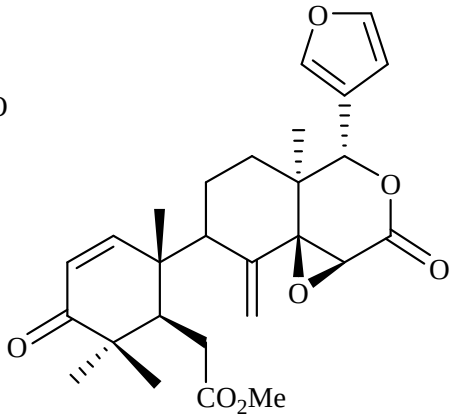
(**10**) R = $COCH(CH_3)_2$



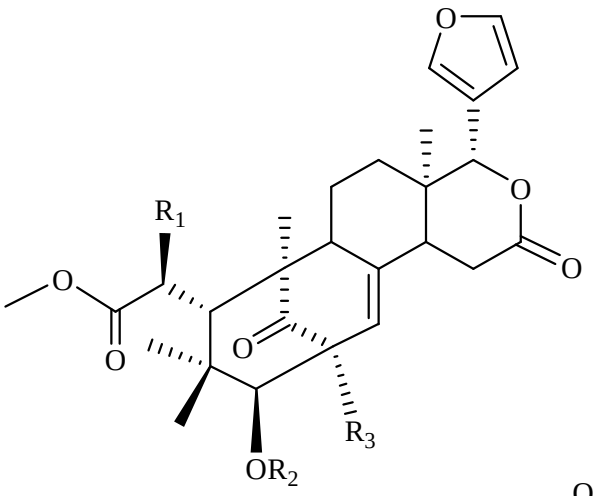
(**11**) R_1 = OH, R_2 =H
(**16**) R_1 = H, R_2 = $COC(CH_3)CH(CH_3)$



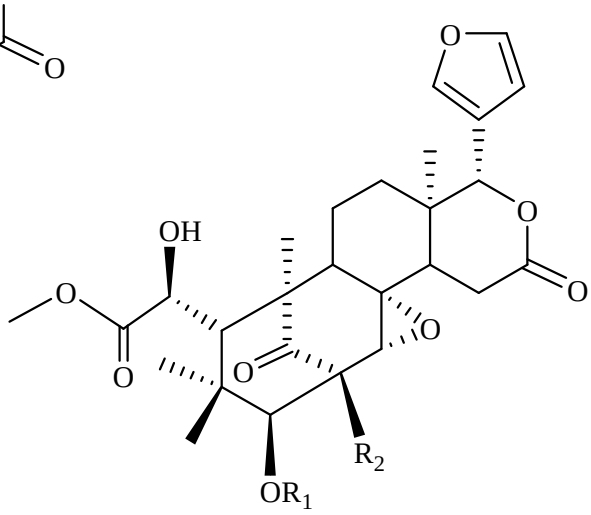
(**12**)



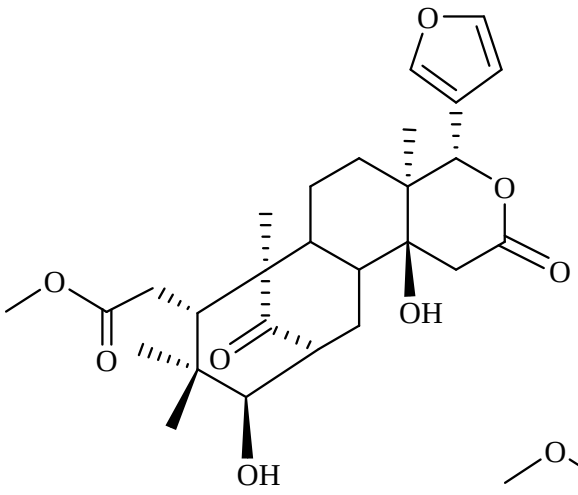
(**13**)



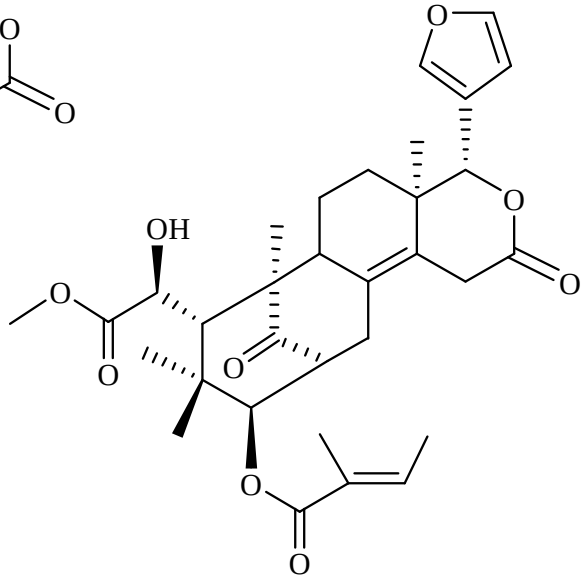
(**14**) R_1 = H, R_2 = $COCH(CH_3)CH_2CH_3$
(**15**) R_1 = OAc, R_2 = H
(**19**) R_1 = H, R_2 = $COC(CH_3)CH(CH_3)$



(**17**) R_1 = $COC(CH_3)CH(CH_3)$, R_2 = H
(**18**) R_1 = $COC(CH_3)CH(CH_3)$, R_2 =OH

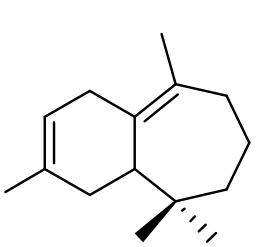


(**20**)

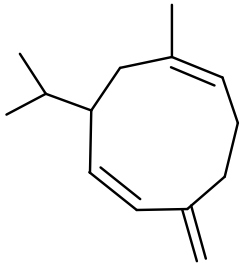


(**21**)

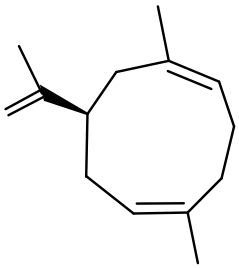
Apart from limonoids, the volatile compositions of *S. macrophylla* leaves have also been studied. Marisi *et al*. (2003) had extracted essential oils from the terminal shoots, mature and senescent leaves of *S. macrophylla* by the steam distillation method. Himachalene (**22**), germacrene-D (**23**), germacrene-A (**24**), cadina-1,4-diene (**25**), hexadecanoic acid (**26**), and ethyl hexadecanoate (**27**) were the major constituents found in the essential oils of *S. macrophylla* leaves.



(**22**)



(**23**)



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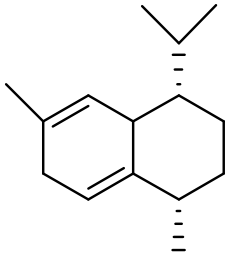
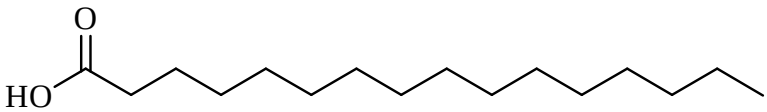
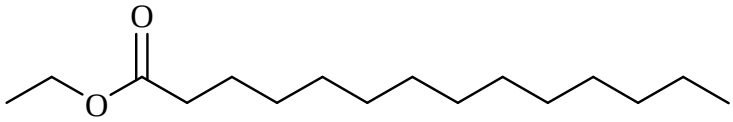


(**25**)



(**26**)



(**27**)

Besides that, Munguia *et al*. (1949) had studied the oil compositions of *S. macrophylla* seeds collected in Mexico. The oil was found to consist of linoleic acid (48.4 %), oleic acid (24.5 %), saturated glycerides (25.7 %) and unsaponifiables (1.4 %). The fatty acid compositions of *S. macrophylla* seeds oil were re-examined by Chowdhury *et al*. (1954), and were found to contain palmitic

acid (12.50 %), stearic acid (16.42 %), arachidic acid (0.56 %), oleic acid (25.30 %), linoleic acid (33.87 %) and linolenic acid (11.32 %).

In another study, the gum exudate of *S. macrophylla* was analyzed and found to contain polysaccharides, which include uronic acid, galactose, arabinose and rhamnose (Leon *et al*., 1996).

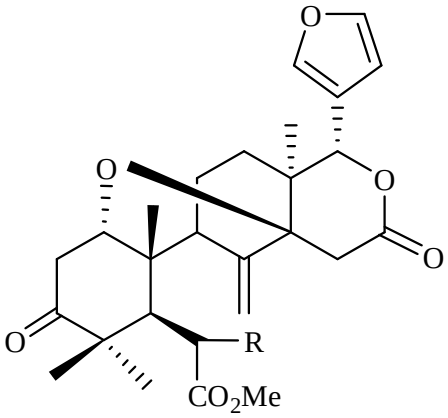
1.5 Studies on other *Swietenia* species

The other two species from the genus *Swietenia* have also been studied phytochemically. More than 30 limonoids have been isolated from different plant parts of the *S. mahogany and S. humilis*, with the mexicanolide-type limonoids as the most commonly found ones, followed by the phragmalin-type limonoids.

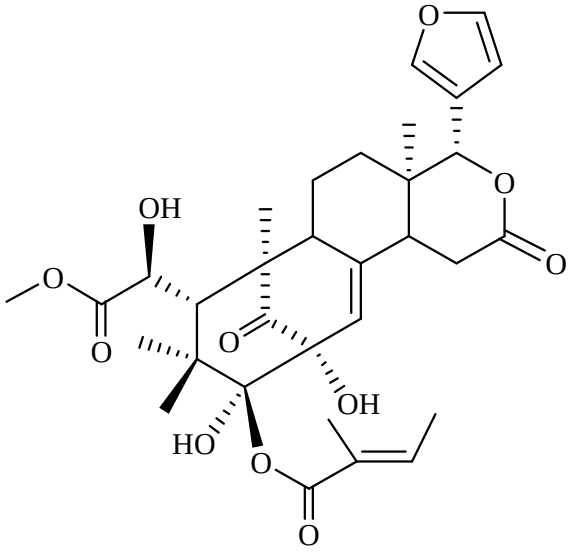
From the seeds of *S. mahogany*, several mexicanolide-type limonoids, including methyl angolensate (**28**), methyl 6-hydroxyangolensate (**29**), swietenine (**2**), methyl-2,3,6-trihydroxy-meliac-8(30)-enate-3-tiglate (**30**) and 2-hydroxy-swietenin (**5**) were isolated (Taylor, 1969; Daily *et al*., 1985).

Another two limonoids, named 6-acetylswietenine (**31**) and 6-acetyl-3-tigloylswietenolide (**32**), which showed antifungal properties were isolated from *S. mahogany* by Govindachari *et al*. (1999).

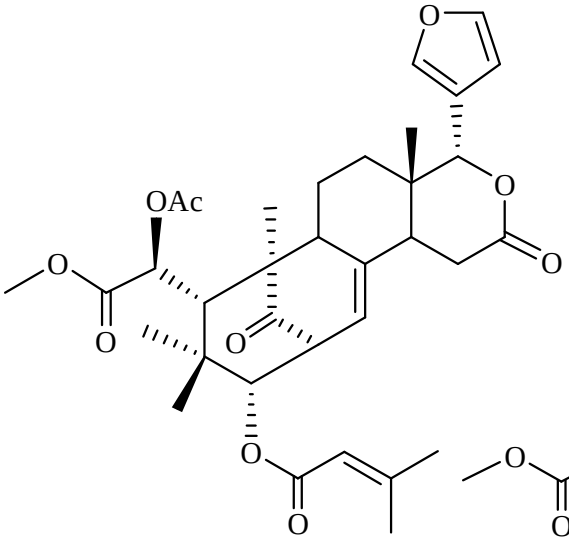
From the leaves of *S. mahogany*, swietenolide (**1**), swietenolide-diacetate (**3**) and 3-acetate-swietenolide (**33**) were isolated *by* Kikuchi *et al*. (1990). These three compounds were found active in inhibiting blood platelet aggregation.



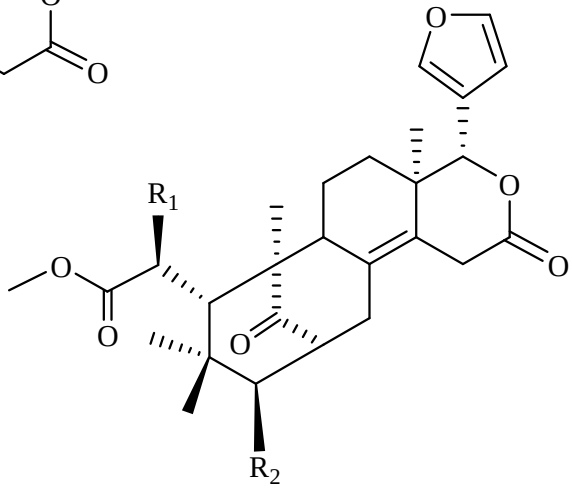
(**28**) R = H
(**29**) R = OH



(**30**)



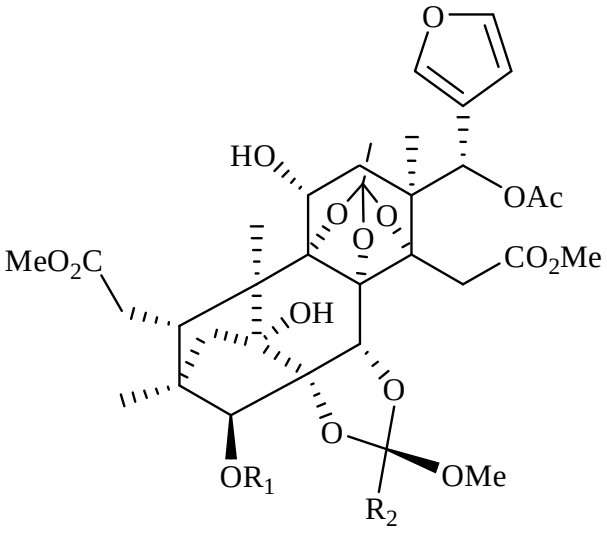
(**31**)



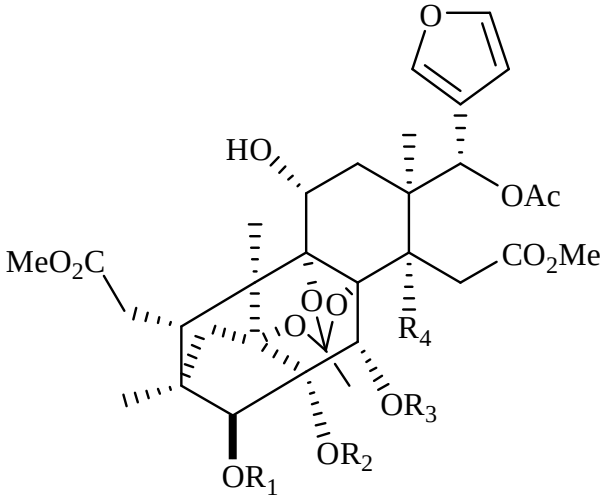
(**32**) R_1 = OAc, R_2 = $OCOC(CH_3)CH(CH_3)$
(**33**) R_1 = OH, R_2 = OAc

A further study on the stem bark of *S. mahogany* by Saad *et al*. (2004) managed to isolate three novel ring-D open phragmalin-type limonoids with an 8,9,14-*ortho* acetate group, named swietenialides A-C (**34**-**36**), together with two ring-D open

phragmalin-type limonoids with an 1,8,9-*ortho* acetate group, named swietenialide D (**37**) and swietenialide E (**38**).



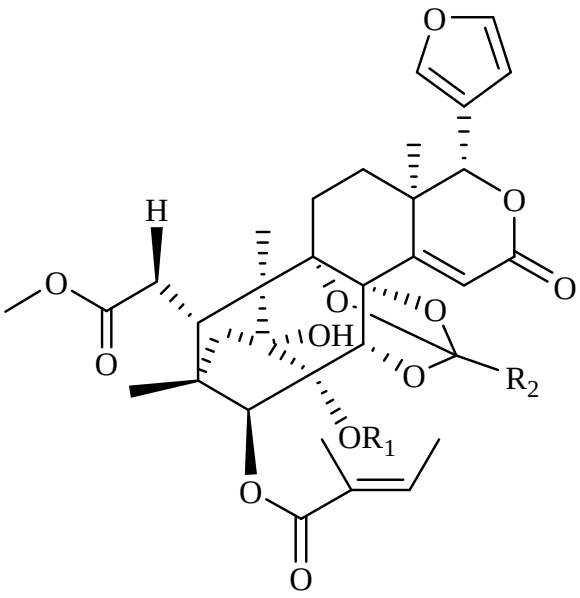
(**34**) R_1 = $COC(CH_3)CH(CH_3)$, R_2 = CH_3
(**35**) R_1 = $COC(CH_3)CH(CH_3)$, R_2 = CH_2CH_3
(**36**) R_1 = $COCH_3$, R_2 = CH_2



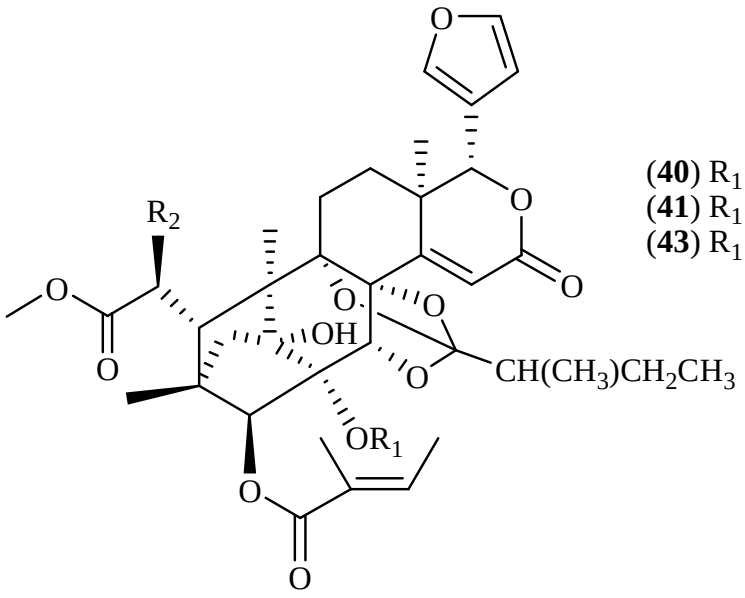
(**37**) R_1 = $COCH_3$, R_2 = H, R_3 = $COCH_2CH_3$, R_4 = H
(**38**) R_1 = R_2 = R_3 = $COCH_3$, R_4 = OH

A continual phytochemical investigation on the leaves of *S. mahogany* by Abdelgaleil *et al*. (2006) had successfully isolated seven phragmalin-type

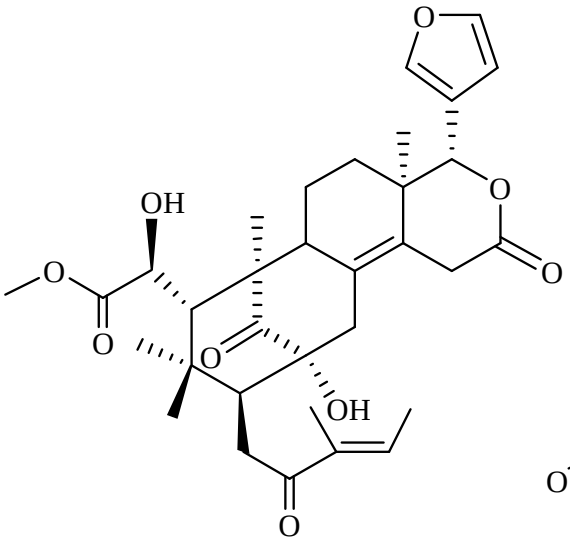
limonoids, named swietephragmins A-G (**39-45**), together with 2-hydroxy-3-*O*-tigloylswietenolide (**46**), deacetylsecomahoganin (**47**), methyl 6-hydroxyangolensate (**29**), swietemahonin G (**18**) and 7-deacetoxy-7-oxogedunin (**12**). In a more recent study, two new limonoids**,** namely swiemahogin A (**48**) and swiemahogin B (**49**) were isolated from the twigs and leaves of *S. mahogany* by Chen *et al*. (2007).



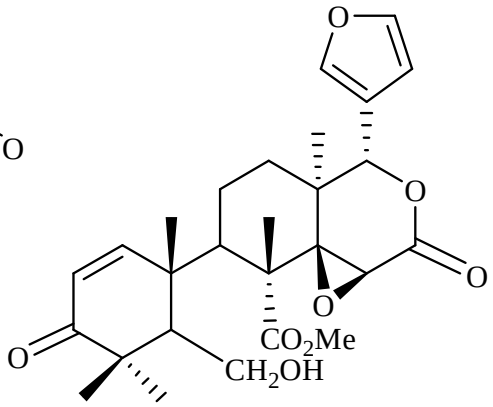
(**39**) $R_1 = COCH_3$, $R_2 = CH(CH_3)_2$
(**42**) $R_1 = H$, $R_2 = CH(CH_3)_2$
(**44**) $R_1 = H$, $R_2 = CH_2CH_3$
(**45**) $R_1 = H$, $R_2 = CH_3$



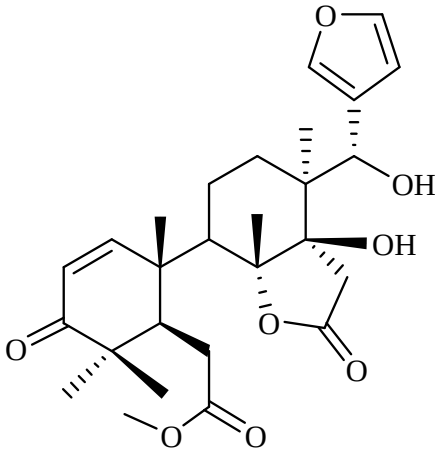
(**40**) $R_1 = Ac$, $R_2 = H$
(**41**) $R_1 = H$, $R_2 = H$
(**43**) $R_1 = H$, $R_2 = OH$



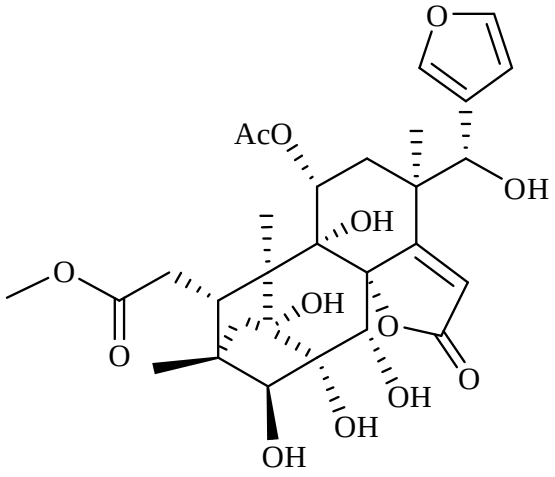
(**46**)



(**47**)



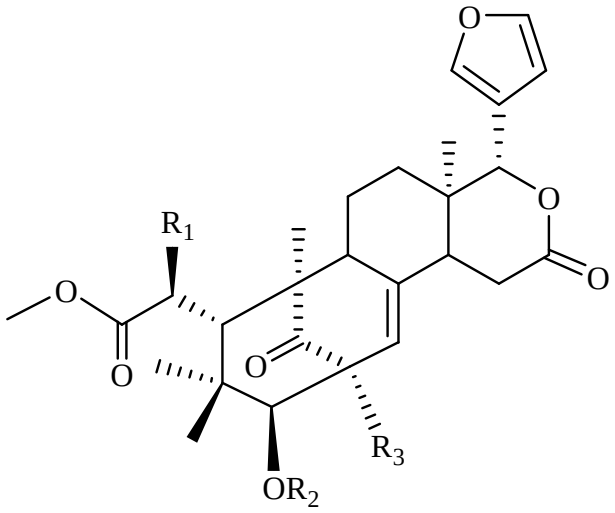
(**48**)



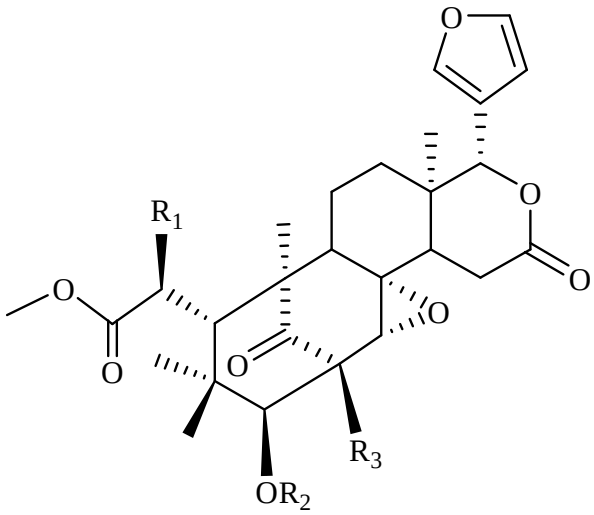
(**49**)

Phytochemically, *S. humilis* is the least studied species in the genus *Swietenia*. The earliest phytochemical study on *S. humilis* was done by Okorie & Taylor (1971), where two limonoids, named swietenin C (**50**) and humilin B (**51**), were isolated from the seeds. Later, a research by Segura-Correa & Mata (1993) on the seeds of *S. humilis* found humilinolides A-D (**52**-**55**). A subsequent study by

Jimenez *et al*. (1997 & 1998) on the seeds isolated humilinolides E-F (**56-57**) and swietemahonin C (**58**).



(**50**) R_1 = OH, R_2 = $COCH(CH_3)_2$, R_3 =H
(**54**) R_1 = H, R_2 = $COC(CH_3)CH(CH_3)$, R_3 = OAc
(**55**) R_1 = OAc, R_2 = $COCH_3$, R_3 = H
(**56**) R_1 = OAc, R_2 = $COC(CH_3)CH(CH_3)$, R_3 = OH



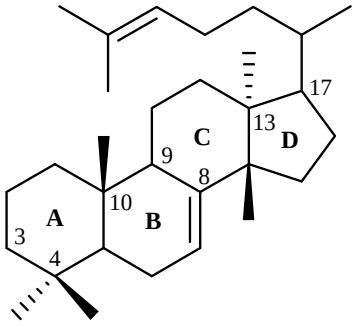
(**51**) R_1 = H, R_2 = $COCH(CH_3)_2$, R_3 = OH
(**52**) R_1 = OH, R_2 = $COCH(CH_3)_2$, R_3 = OH
(**53**) R_1 = OAc, R_2 = $COCH(CH_3)_2$, R_3 = OH
(**57**) R_1 = OAc, R_2 = $COC(CH_3)CH(CH_3)$, R_3 = OAc
(**58**) R_1 = OAc, R_2 = $COCH(CH_3)_2$, R_3 = H

1.6 Limonoids

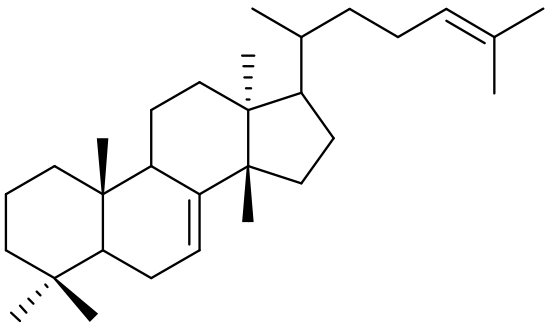
1.6.1 Introduction

Limonoids, first reported in 1864 (Emerson, 1948), are a group of highly oxygenated triterpenes, classified as tetranortriterpenoids. They are moderately polar, insoluble in water but soluble in hydrocarbons, alcohols and ketones, and are normally bitter in taste (Aliero, 2003).

This group of compounds are usually described as modified triterpenes having a 4,4,8-trimethyl-17-furanylsteroid skeleton (Taylor, 1984). They have a prototypical structure that originated from biogenetic precursors such as euphane (**59**) or tirucallane (**60**) (Amit & Shailendra, 2006). The oxidative degradation occurring at the C-17 side chain of these biogenetic precursors results in the loss of four carbon atoms, and thus leading to the formation of *β*-substituted furan ring, which is a typical feature found in all naturally occurring limonoids (Fraser *et al*., 1997). Besides that, limonoids also have characteristic oxygen containing functional groups at C-3, C-7, C-16 and C-17 (Amit & Shailendra, 2006).



Euphane (**59**)



Tirucallane (**60**)

The structures of limonoids are usually complex and diverse due to a high degree of rearrangements and oxidations occurring in their parent skeletons. The skeletal rearrangements and oxidations occurring in one or more of their ring structures, designated as A, B, C and D, resulting in different groups of limonoids.

To date, 14 groups of limonoids have been identified, including intact limonoid, degraded limonoid, highly modified limonoid, highly cleaved limonoid, ring-A-*seco* limonoid, ring-A,C,D-intact-ring-B-*seco*-limonoid, ring-B,D-*seco*-limonoid, ring-C cleaved limonoid, ring-D-lactone-limonoid, ring-C-*seco* limonoid, gama-lactone side chain limonoid, mexicanolide-type limonoid, trijugin-type limonoid and phragmalin-type limonoid (Fig. 1.2) (Amit & Shailendra, 2006).

It is interesting to note that limonoids are secondary metabolites found exclusively in the plant families of the order Rutales (Amit & Shailendra, 2006). They are most frequently found in the family Meliaceae followed by the family Rutaceae. They are also found occasionally in the family Cneoraceae of Simaroubaceae (Waterman & Grundon, 1983). Limonoids from the Meliaceae are known as meliacins. They are usually complex with a high degree of structural variations (Amit & Shailendra, 2006).

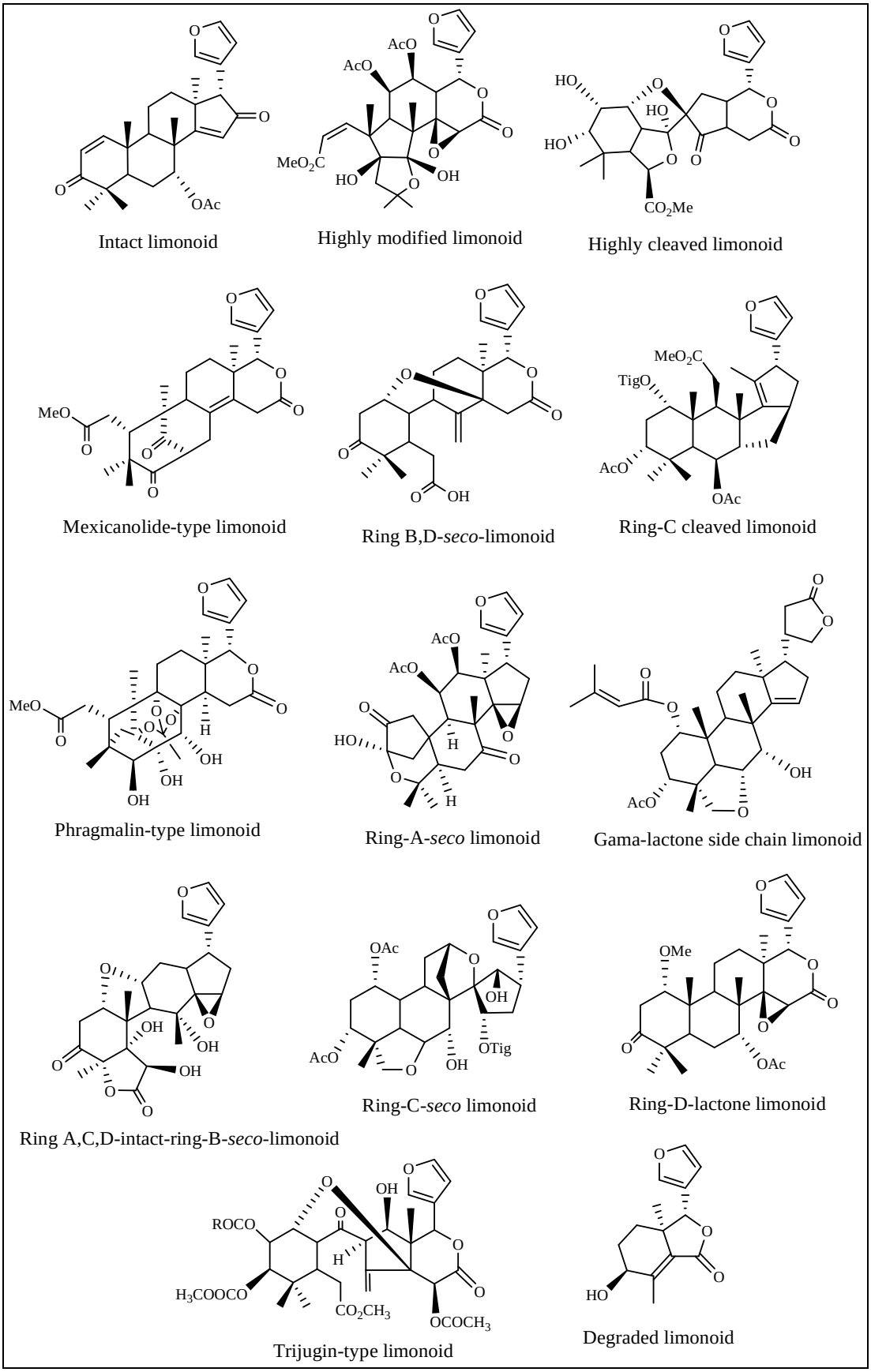


Figure 1.2 Skeletal arrangements of 14 groups of limonoids (Amit & Shailendra, 2006)

1.6.2 Biological activities of limonoids

Compounds belonging to this group have recently attracted attention as they have exhibited a vast range of biological activities and pharmacological activities in humans. Although best known for their insecticidal properties, such as insect antifeedant and growth inhibiting characteristics (Nihei *et al*., 2002; Abdelgaleil *et al*., 2001; Nakatani *et al*., 2004; Luo *et al*., 2002), limonoids possess other biological properties that can benefit the human race.

Many experimental evidences have revealed that limonoids, such as limonin-17-*β*-D-glucopyranoside (**61**), limonin (**62**), nomilin (**63**), obacunone (**64**), ichangensin (**65**), deoxylimonin (**66**) and deacetylnomilinicacid-17-*β*-glucopyranoside (**67**) have cancer chemo-preventive properties. They have been shown to inhibit chemically induced carcinogenesis, decrease colon tumor-genesis and suppress a series of human cancer cell lines, and also have exceptional cytotoxicity against lung, colon, oral and skin cancers in animal test models (Miyagi *et al*., 2000; Silalahi, 2002; Tanaka *et al*., 2000 & 2001; Miller *et al*., 2004; Lam *et al*., 2000; Poulose *et al*., 2005). It is also reported that dietary supplementation with limonin (**62**) and nomilin (**63**) suppressed carcinogenesis in the liver and small intestine of rats (Lam *et al*., 1989; Miller *et a*l., 1989; Miyake *et al*., 1992).

Apart from anticancer activities, limonoids also show anti-HIV properties. Limonin (**62**) and nomilin (**63**), isolated from the citrus, have been reported to inhibit the replication of HIV-1 in different types of cellular systems (Battinelli *et al*., 2003). A novel limonoid, named clausenolide-1-ethyl-ether (**68**), isolated from

Clausena excavate has also showed remarkable HIV-1 inhibitory activity (Sunthikawinsakul *et al*., 2003).

Besides the aforementioned biological activities, other biological activities displayed by a great number of limonoids belonging to different groups include antimalarial, antimicrobial, cholesterol-lowering, antioxidant, platelet aggregation inhibiting, anti-inflammatory, anti-gastric ulcer, anti-arthritic and antipyretic (Jean *et al*., 2000; Nsiama, *et al*., 2008; Ekimoto *et al*., 1991; Poulose *et al*., 2005; Biswas *et al*., 2002).

From the intensive studies done on limonoids, this group of compounds have been shown to be free of toxic effects towards animal test systems. This observation indicates the potential for the use of limonoids against human diseases either directly from natural sources such as fruits and vegetables, or in foods enriched with limonoids, or even in purified forms of specific limonoids (Sohail *et al*., 2006).

Thus far, limonoids exhibited promising health benefits. Properties such as cancer chemo-preventive, insecticidal, antimalarial, anti-HIV and many other biological activities suggested that more extensive biological screenings are warranted for this interesting group of compounds (Amit & Shailendra, 2006).