CHEMICAL CONSTITUENTS AND CYTOTOXICITY OF THE STEM BARK *Calophyllum lanigerum* var. austrocoriaceum (Whitemore) P. F. STEVENS AND *Calophyllum andersonii* P. F. STEVENS

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

C-H	Alkane
C=C	Alkene
AR Grade	Analytical Research Grade
&	And
APG	Angiosperm Phylogeny Group
‘ ’	Apostrophe
*	Asterisk
β	Beta
br tt	Broad triplet of triplets
C	Carbon
CO_2	Carbon Dioxide
^{13}C NMR	Carbon Nuclear Magnetic Resonance
C-O	Carbonyl
$CHCl_3$	Chloroform
cm	Centimetre
:	Colon
,	Comma
COSY	Correlation spectroscopy
J	Coupling constant
˚C	Degree Celcius
δ	Delta
$CDCl_3$	Deuterated Chloroform

DMSO	Dimethyl Sulfoxide
d	Doublet
dd	Doublet of doublets
ddq	Doublet of doublet of quartets
dq	Doublet of quartets
DMEM	Dulbecco's Modified Eagle Medium
EMEM	Eagle's Minimum Essential Medium
EI-MS	Electron-Impact Mass Spectrometry
EA	Ethyl Acetate
=	Equal
FBS	Fetal Bovine Serum
FT-IR	Fourier Transform Infrared
.	Full stop
GC-MS	Gas Chromatography-Mass Spectrometry
g	Gram
GCC	Gravity Column Chromatography
IC_{50}	Half-Maximal Inhibitory Concentration
Hz	Hertz
HMBC	Heteronuclear Multiple-Bond Coherence
HSQC	Heteronuclear Single Quantum Coherence
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HL-7702	Human Normal Liver cell line
H	Hydrogen

O-H	Hydroxyl
-	Hyphen
IR	Infrared
kg	Kilogram
λ	Lambda
LC-MS	Liquid Chromatography-Mass Spectrometry
L	Litre
MS	Mass Spectrometer
m/z	Mass-to-charge ratio
MHz	Mega Hertz
MeOH	Methanol
m	Metre
µg/mL	Microgram per millilitre
µL	Microlitre
µM	Micromole
mg	Milligram
mg/mL	Milligram per millilitre
mm	Millimetre
–	Minus
M^+	Molecular mass (positive charge)
m	Multiplet
nm	Nanometre
NMR	Nuclear Magnetic Resonance
O	Oxygen

( )	Parentheses
ppm	Parts per million
Pen-Strep	Penicillin-Streptomycin
%	Percent
PBS	Phosphate-Buffered Saline
+	Plus
±	Plus-Minus
^{1}H NMR	Proton Nuclear Magnetic Resonance
qd	Quartet of doublet
“ ”	Quotations marks
cm^{-1}	Reciprocal centimetre
C-18	Reversed-Phase Chromatography
;	Semicolon
s	Singlet
/	Slash
SD	Standard Deviation
TLC	Thin Layer Chromatography
t	Triplet
tt	Triplet of triplets
UV-Vis	Ultraviolet-Visible
UiTM	Universiti Teknologi Mara
2D	2-Dimensional
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

LIST OF APPENDICES

SEBATIAN KIMIA DAN KESITOTOKSIKAN DARI KULIT BATANG KAYU *Calophyllum lanigerum* var. austrocoriaceum (Whitemore) P. F. STEVENS DAN *Calophyllum andersonii* P. F. STEVENS

ABSTRAK

Tumbuhan dari genus *Calophyllum*, telah menarik minat ahli fitokimia kerana penemuan baharu yang menarik tentang konstituen bioaktifnya. Fitokonstituen seperti kumarin, kromanon, xanton, dan triterpenoid ialah kumpulan fitokimia yang kerap ditemui dalam genus ini. Fitokimia ini terbukti mempunyai potensi kepentingan farmakologi yang penting bagi saintis untuk aplikasi farmakoterapeutik dalam merawat penyakit yang disasarkan. Sebagai contoh, digunakan untuk tujuan farmakoterapeutik dalam merawat HIV, kanser, dan penyakit berkaitan keradangan. Dalam kajian ini, kulit batang *Calophyllum lanigerum* dan *Calophyllum andersonii* telah dikaji dengan mengekstrak, mengasing, dan mencirikan fitokimia yang berkemungkinan terdapat di dalamnya serta menilai aktiviti sitotoksik masing-masing. Lima fenolik dan dua triterpenoid telah diasingkan daripada kulit batang kayu *C. lanigerum*. Sebatian fenolik telah dikenalpasti sebagai *caloteysmannic acid* (**A**), *isocalolongic acid* (**B**), *calolongic acid* (**C**), *euxanthone* (**D**), *calanone* (**E**), dan dua triterpenoid biasa, *friedelin* (**F**) dan *stigmasterol* (**G**). Sementara itu, *calanone* (**E**) dan isomer konstitusi, isocalanone (**H**) serta soulattrolide (**1**) dan friedelin (**F**) telah diperoleh dari kulit batang *C. andersonii*. Asid kromanon (sebatian **A** dan **B**) telah dilaporkan buat kali pertama di dalam *C. lanigerum* di Sarawak. Struktur kimia sebatian-sebatian ini telah dikenalpasti menggunakan teknik spektroskopi terperinci

seperti NMR (1D dan 2D), MS, UV-Vis, FTIR. Ekstrak-ekstrak mentah (*n*-heksana, kloroform, etil asetat) dan sebatian tulen (**A**, **B**, **E** dan **H**) daripada kedua-dua spesis *Calophyllum* telah diuji sitotoksisitinya terhadap sel hati HeLa Chang (karsinoma serviks manusia) dan sel HL-7702 (hati normal manusia). Hanya ekstrak etil asetat dari *C. lanigerum* yang menunjukkan sitotoksisiti yang menjanjikan, dengan nilai IC_{50} sebanyak 34.13 ± 3.82 µg/mL, manakala ekstrak *n*-heksana dan kloroform masing-masing menunjukkan sitotoksisiti sederhana terhadap sel hati HeLa Chang [93.84 ± 9.35 µg/mL dan 81.33 ± 2.41 µg/mL]. Kesan sitotoksik yang diperhatikan daripada ekstrak etil asetat menunjukkan potensinya untuk pembangunan lanjut sebagai ejen antikanser. Penyelidikan yang meluas ke atas sifat farmakologi dan toksikologi ekstrak ini adalah penting untuk menerangkan aplikasi farmakoterapeutik optimalnya. Penyelidikan ke atas spesies ini boleh dilihat dan dipelajari dalam pelbagai domain penyelidikan, termasuk fitokimia, biologi, dan farmakologi. Pendekatan yang melibatkan pelbagai bidang ini bukan hanya membantu dalam mendapatkan data tambahan, tetapi juga berfungsi sebagai pemangkin untuk memberi inspirasi kepada penyelidik masa depan untuk membangunkan cara-cara inovatif dan praktikal untuk merungkai lebih lanjut misteri di sebalik spesies ini.

CHEMICAL CONSTITUENTS AND CYTOTOXICITY OF THE STEM BARK *Calophyllum lanigerum* var. Austrocoriaceum (Whitemore) P. F. STEVENS AND *Calophyllum andersonii* P. F. STEVENS

ABSTRACT

Plants from the genus *Calophyllum*, have gained the interest of phytochemists due to the exciting new discoveries of its bioactive constituents. Phytoconstituents such as coumarins, chromanones, xanthones, and triterpenoids are frequently discovered phytochemical groups in this genus. These phytochemicals proven to possess potential pharmacological importance that are essential for scientists to subject it in pharmacotherapeutic applications in treating targeted diseases. For example, used for pharmacotherapeutic purpose in treating HIV, cancer, and inflammatory related diseases. In this study, the stem bark of *Calophyllum lanigerum* and *Calophyllum andersonii* were investigated by extracting, isolating, and characterising the possible phytochemicals that present and assessing their cytotoxic activity, respectively. Five phenolics and two triterpenoids have been isolated from the stem bark of *C. lanigerum.* The phenolic compounds were identified as caloteysmannic acid (**A**), isocalolongic acid (**B**), calolongic acid (**C**), euxanthone (**D**), calanone (**E**), and two common triterpenoids, friedelin (**F**) and stigmasterol (**G**). Meanwhile, calanone (**E**) and its constitutional isomer, isocalanone (**H**) together with soulattrolide (**I**) and friedelin (**F**) were isolated from the stem bark of *C. andersonii*. Chromanone acids (Compounds **A** and **B**) were reported for the first time in *C. lanigerum* in Sarawak region. The chemical structures of these isolated compounds were elucidated using detailed spectroscopic techniques including NMR (1D, 2D), MS, UV-Vis, FTIR. Extracts (*n*-

hexane, chloroform, ethyl acetate) and compounds (**A**, **B**, **E**, and **H**) from both *Calophyllum* species were subjected for their cytotoxicity against HeLa Chang liver (human cervix carcinoma) and HL-7702 (human normal liver) cell lines. Only ethyl acetate extract from *C. lanigerum* exhibited promising cytotoxicity, with IC_{50} value of 34.13 ± 3.82 µg/mL, while *n*-hexane and chloroform extracts showed moderate cytotoxicity against Hela Chang liver cell line [IC_{50}: 93.84 ± 9.35 µg/mL and 81.33 ± 2.41 µg/mL, respectively]. The observed cytotoxic effects of the ethyl acetate extract suggest its potential for further development as an anticancer agent. Extensive research on the pharmacological and toxicological properties of this extract is essential to elucidate its optimal pharmacotherapeutic applications. The investigation of these species can be viewed and learned various research domains, including phytochemical, biological, and pharmacological properties. This multi-faceted approach not only aids in the acquisition of additional data but also serves as a catalyst for inspiring future researchers to develop innovative and practical ways to unravel the mystery behind these species.

CHAPTER 1

INTRODUCTION

1.1 Plants as sources of new chemical entities

When asking the elder generation for advice on how to keep healthy and look younger than their age, they frequently remarked that they are used to consuming certain plants to stay young-looking and fit. They even mentioned that their advice had been handed down through the generations. Scholars have been curious on how these elder people learned that some plants have "special ingredients" to aid with their bodies' issues, even before the existence of modern technologies like what is being used today. According to statistics reported by the World Health Organization (WHO), primary healthcare for around 65% of the world's population is provided by medicines derived from plant (Srivastav et al. 2020). As time passes, more technologies are developed, and people are utilizing them to study in more detail.

Research has consistently demonstrated that plants create a significant variety of chemical constituents known as secondary metabolites, many of which have been linked to defence mechanisms against pathogens and predators. For instance, through creating colour (which draws pollinators and shields plants from animal attack), signalling, and controlling the primary metabolic processes, all of which aid the plant in maintaining balance with its surroundings (Priya and Satheeshkumar 2020). The chemical constituents found in some plants interest chemists from all over the world, inspiring them to conduct numerous studies to learn more about the fascinating structural variations, stereochemical configurations, conformations, and molecular structures of these compounds. Plants producing secondary metabolites have a

complex structure, as well as provide a significant pharmaceutical value such as glycoside, alkaloids, flavonoids, volatile oils, and more (Srivastay et al. 2020).

Novel chemical entities were found and thoroughly explored for their phytochemistry and biological activities until today. Since environmental factors play a significant role in isolating lead compounds, new chemical entities may also be identified in previously discovered plant species in addition to new species. The ability to establish new chemical entities isolated from plants is what makes this field of study fascinating. Scientists need to use their analytical, spectroscopic, as well as biology knowledge in order to confirm it chemical and physical properties of these novel chemical entities. It is undeniably tough, yet the importance of these studies towards future generations are incredibly invaluable.

Calophyllum was once recognised as a member of the Guttiferae/Clusiaceae family but is now recategorised in Calophyllaceae family. It is placed under the APG lll system, the Angiosperm Phylogeny Group's third iteration of floral plant categorisation. There are 14 genera and about 475 species updated so far (Christenhusz et al. 2016). Calophyllaceae is a family of tropical tree with simple opposite leaves with delicately parallel lateral veins and yellow-coloured latex. The flowers have a variety of stamens. The current family were discovered for a long time, and there have been phytochemical and pharmacological investigations conducted for some of the species. Examples of extant species from this family that could be beneficial the the world and have been successfully documented in the phytochemical database are *C. lanigerum* and *C. andersonii*.

The characterisation and elucidation of the isolated pure compounds can be accomplished by advanced spectroscopic methods including NMR, MS, and X-ray

crystallographic analyses. To pinpoint the biological activities such as anti-cancer, anti-inflammatory, and antibacterial, more research can be conducted, and all the knowledge in this field can be updated for future reference.

1.2 Problem statements

The anti-HIV activity of calanolides A and B was initially documented in 1992 by Kashman and colleagues in their first study on *C. lanigerum* (Kashman et al. 1992). Subsequently, Tee and colleagues conducted the pioneering investigation on *C. andersonii*, reporting the discovery of several known xanthones (Tee et al. 2018a). Despite Steven's initial report on the existence of this species in 1980, no comprehensive reports on its phytochemical composition or biological activities were available until 2018.

We live in a time where both technology and human knowledge are advancing rapidly. Hence, we must take advantage of the opportunity to research more about the phytochemistry of plants. This offers a platform to unravel the chemical constituents of numerous yet undiscovered plants, encouraging the identification of novel chemical entities with distinct capabilities. This persuit is important, especially for burgeoning future researchers to contribute to the field, as it not only boosts the progression of ongoing research but also enriches the reservoir of phytochemical knowledge.

Despite being from the same species, published information shows that they are not identical to one another, making it impossible to continue ongoing research on specific isolated phytochemicals from these species. Thus, it is possible that the diverse geographical distribution of the same species led to the discovery of different groups of phytochemicals.

Due to the limited research on the genus *Calophyllum*, the discovery of new phytochemical with bioactive qualities from *C. lanigerum* and *C. andersonii* will be of enormous value not only to the development of new drugs but as well benefits in updating the phytochemical database for scientific exploration. In addition, the findings from the methods employed to isolate the phytochemicals and assess their bio-activity would be highly helpful, especially to phytochemists for further research projects. Therefore, due to their limitations, it is necessary to explore the phytochemistry of these kinds.

1.3 Objectives of the study

The objectives of this study are as below:

1. To extract and isolate the phytochemicals from the stem bark of *C. lanigerum* and *C. andersonii*.

2. To characterise and elucidate the chemical structures of the isolated pure compounds by advanced spectroscopic methods (NMR, MS, UV-Vis, FTIR & HPLC).

3. To determine the potential of selected compounds (caloteysmannic acid, isocalolongic acid, calanone, isocalanone) and extracts (*n*-hexane, chlororform, ethyl acetate) for cytotoxic activities against cancerous and non-cancerous cell lines using MTT colorimetric assay technique.

1.4 Significance of Study

The significance of this study are as below:

1. To provide more information on *C. lanigerum* and *C. andersonii* especially on the phytochemistry, as well as methods used in extraction and isolation parts. These species might have much more capabilites that have not been discovered yet.

2. Younger generation can access to this study easily, which could help them understand the characterisation and elucidation of phytochemicals better. They will indirectly be parts of future generations that have the ability to spread awareness about the benefits of herbal plants in the future.

3. The information provided from this study can be referred by other scientist of similar field. The outcomes from the cytotoxic activities part in this study is crucial, especially for further applications such as in the pharmaceutical and medicinal fields.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany of plants studied

2.1.1 The genus *Calophyllum*

Calophyllum has taxonomic features including red colour cracks in diamond-shaped outer bark. The leaves consist of opposite narrow and tight parallel veins. The flowers appear at the end of branches or in the axils of leaves and produce white or yellow latex. The hermaphrodite floral arrangement is made up of the sepals and petals of species from this genus. This genus's fruit is a drupe with a big seed and a thin covering of flesh areas (Gupta and Gupta 2020).

Trees and shrubs in this genus are exceptionally tall, but majority are medium-sized trees. Many species in this genus live in the moist tropical rainforest's lowlands. Some species, however, can be found at higher altitudes, in flooded areas, and in drier areas (Gupta and Gupta 2020).

Some of the species in this genus have been discovered in the tropical rainforest of Sarawak, Malaysia. Genus *Calophyllum* has attracted a lot of attention because of its unique biological and chemical profiles. The phytochemistry of *Calophyllum* species has shown a number of secondary metabolites, including xanthones (Mah et al. 2015), coumarins (Spino et al. 1998), chromanone (Lim et al. 2015), and others.

2.1.2 The species *C. lanigerum*

The Greek words *kalos* and *phyllon* imply “beautiful” and “leaf” respectively. As a result, it is referring to the plant’s finely veined leaf blades. *Lanigera,* on the other hand, comes from the Latin word that means woolly, which refers to the species’ delicate hairs. *Bentangur pasir* is the Indonesian name for this species. The tree can reach a height of 21 m tall. It has densely leathery leaf blades that are narrowly egg-shaped to oblong, and long-stalked leaves. Its flowering branches are 3-7 cm long and contain 7-21 white blooms apiece. Green-yellow fruits range in size from spherical to egg-shaped, measuring 18-29 by 17-24 mm. it has 11-21 mm long seeds (NParks Flora & Fauna 2022).

C. lanigerum is a tropical forest tree native to Borneo, particularly in the Malaysian state of Sarawak. Anti-HIV active calanolides have been discovered in this species. Calanolide A, for example, was isolated from this species and has been shown to impede HIV-1 replication (Kashman et al. 1992).

Table 1. Taxonomy of *C. lanigerum.*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Calophyllaceae
Genus	*Calophyllum*
Species	*Calophyllum lanigerum*

Taken from natureloveyou.sg

Taken from Global Biodiversity Information Facility

Figure 1. Stem barks and leaves of *C. lanigerum*

2.1.3 The species *C. andersonii*

C. andersonii is a tropical forest tree found on the island of Borneo with a local name of *Kayu mahadingan*. The tree can grow to be 18 m tall, 12 cm wide, free branching, and 12 m above the ground. The bark is brownish-grey on the outside and reddish-brown on the interior, with a thickness of 0.3 mm. It has a golden sap and a gleaming green leaf, according to the Global Biodiversity Information Facility website.

Tee and his colleagues have reported the isolation of many novel xanthones from *C. andersonii* in 2018. The compounds are caloxanthone I, pyrnojacareubin, macluraxanthone, caloxanthone C, and euxanthone (Tee et al. 2018a).

Table 2. Taxonomy of *C. andersonii.*

Kingdom	**Plantae**
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Calophyllaceae
Genus	*Calophyllum*
Species	*Calophyllum andersonii*

Taken from Global Biodiversity Information Facility

Figure 2. Stem bark and leaves of *C. andersonii*

2.2 Phytochemistry of *Calophyllum* species

A significant number of studies on the phytochemicals of *Calophyllum* species have been conducted. Numerous phytochemicals, including coumarins, chromanones, and xanthones have been found as a result of the research conducted. The basic structure of coumarin, chromanone, and xanthone are shown below as **Figures 3-5**, respectively.

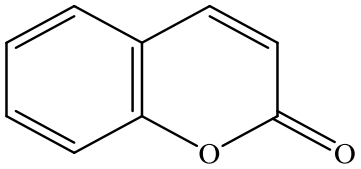


Figure 3. Basic structure of coumarin

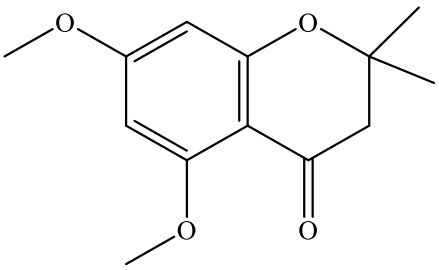


Figure 4. Basic structure of chromanone

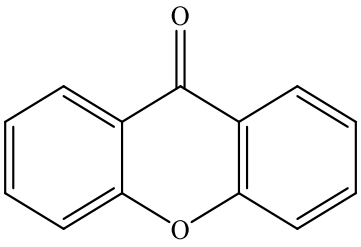


Figure 5. Basic structure of xanthone

2.2.1 Chemistry (Coumarins)

Coumarin (2H-1-benzopyran-2-one) is a chemical compound originating from plants with molecular formula of $C_9H_6O_2$. It is made up of α-pyrone rings and fused benzene (Venugopala et al. 2013). According to Sarker and Nahar (2017), coumarins can be categorised as simple, simple geranylated, simple prenylated, pyrano, furano, sesquiterpenyl, and oligomeric. There are various substitutions in the core structure of coumarins that results in diverse structures which affect the chemical and physical properties as well as biological activities (Ruiz-Marcial et al. 2007; Srivastav et al. 2020). For instance, calanolides A and B, which belong to the pyrano type of coumarin (angular type) are the first isomers identified and extracted from *C. lanigerum* leaves in 1992, have progressed to clinical development and have been shown to be protective against HIV-1 replication (Venugopala et al. 2013; Zailan et al 2022). The other published data on the *Calophyllum* species from numerous research, as well as the chemical structures and derivatives of coumarins are shown in **Table 3** and **Figure 6**.

Table 3. Coumarins isolated from *Calophyllum* species

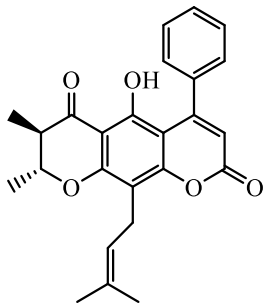
***Calophyllum* species**	**Compounds**	**Part of plants**	**References**
C. austraiianum	Calaustralin (**10**)	Stem bark	Breck et al. 1969
C. benjaminum	Benjaminin (**11**)	Stem bark	Sahimi et al. 2015
C. blancoi	Isorecedensolide (**12**) Recedesolide (**13**)	Seeds	Shen et al. 2004
C. brasiliense	(-) mammea A/BB (**14**) (-) mammea B/BB (**15**) Brasimarins A (**16**) Brasimarins B (**17**) Brasimarins C (**18**) Calocoumarin A (**19**)	Leaves, stem bark	Pires et al. 2014 Ito et al. 2003
C. cordato-oblongum	Cordatolides A (**20**) Cordatolides B (**21**) Oblongulide (**22**)	Leaves,	Dharmaratne et al. 1984
C. dispar	Isodispar B (**23**) Disparinol D (**24**) Disparpropylinol B (**25**) Dispardiol B (**26**) Mammea A/AB cyclo E (**27**) Mammea A/AB dioxalanocyclo F (**28**) Mammea A/BA cyclo F (**29**) Mammea A/BB cyclo F (**30**) Mammea A/BC cyclo F (**31**) Isodisparfuran A (**32**) Disparfuran B (**33**) Disparacetylfuran A (**34**) Mammea A/AA deshydrocyclo F (**35**) Mammea A/AA methoxycyclo F (**36**) Mammea A/AA cyclo F (**37**) Mammea A/AB cyclo F (**38**) Mammea A/AC cyclo F (**39**)	Fruits, stem bark	Guilet et al. 2001a Guilet et al. 2001b
C. ferrugineum	Isocalanone (**40**)	Stem bark	Noh et al. 2020
C. hosei	Hoseimarin (**41**)	Stem bark	Daud et al. 2014
C. incrassatum	Incrassamarin A (**42**) Incrassamarin B (**43**) Incrassamarin C (**44**) Incrassamarin D (**45)**	Stem bark, leaves	Aminudin et al. 2016

Table 3. Continued

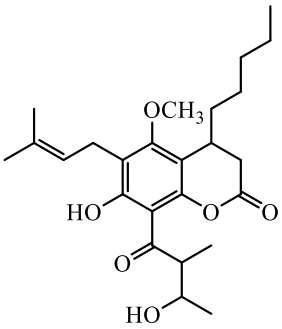
***Calophyllum* species**	**Compounds**	**Part of plants**	**References**
C. inophyllum	(−)-12-methoxyinophyllum A (**46**) (+)-12-methoxyinophyllum H-1 (**47**) (−)-12-methoxyinophyllum H-2 (**48**) Inophyllum J (**49**) Inophyllum A (**50**) Inophyllum B (**51**) Inophyllum C (**52**) Inophyllum E (**53**) Soulattrolide (**54**) Inophyllum P (**55**) Inophyllum G-1 (**56**) Inophyllum G-2 (**57**) Calophyllolide (**58**) Inophyllum D (**59**) Calocoumarin-A (**60**) Calocoumarin-B (**61**) Calocoumarin-C (**62**) Inocalophyllin A (**63**)	Leaves, nut, aerial part, seeds	Li et al. 2016 Patil et al. 1993 Yimdjo et al. 2004 Itoigawa et al. 2001 Shen et al. 2003
C. lanigerum	Inocalophyllin B (**64**) Calanolide A (**65**) Calanolide B (**66**) Calanolide C (**67**) Calanolide D (**68**) 12-Acetoxycalanolide A (**69**) 12-Methoxycalanolide A (**70**) 12-Methoxycalanolide B (**71**) Calanolide E1 (**72**) Calanolide E2 (**73**) Cordatolide E (**74**)	Fruits, twigs, stem bark	Kashman et al. 1992 McKee et al. 1996
C. moonii	Inophyllum A (**50**)	Leaves	Bandara et al. 1986
C. mucigerum	Mucigerin (**75**)	Stem bark	Ee et al. 2004
C. polyanthum	Calopolyanolide C (**76**) Calopolyanolide D (**77**)	Seeds	Ma et al. 2004
C. soulattri	Soulamarin (**78**) Soulattrolide (**54**)	Stem bark	Ee et al. 2011 Gunasekera et al. 1977

Table 3. Continued

***Calophyllum* species**	**Compounds**	**Part of plants**	**References**
C. symingtonianum	Inophyllum D (**59**) Inophyllum H (**79**)	Stem bark, leaves	Aminudin et al. 2015
C. teysmannii	Calanone (**80**) Costatolide (**81**) Soulattrolide (**54**) Teysmanone A (**82**) Teysmanone B (**83**)	Latex, stem bark	Gustafson et al. 1994 Cao et al. 1997a
C. wallichianum	Wallimarin T (**84**) Calanolide E (**85**)	Stem bark	Tee et al. 2018b



(10)



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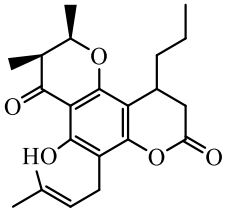

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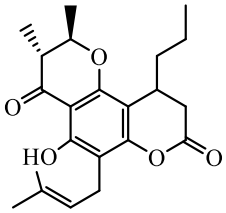

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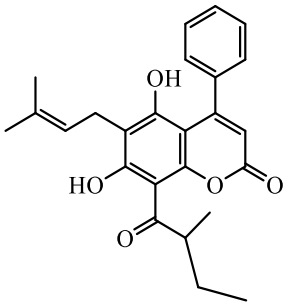

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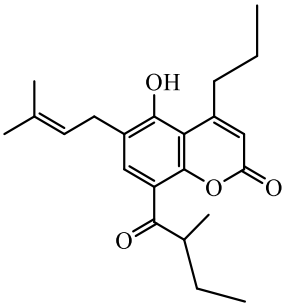

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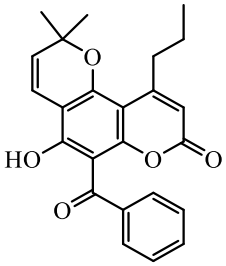

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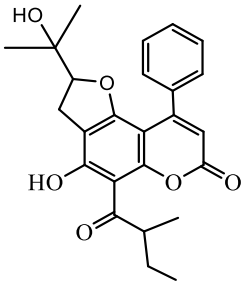

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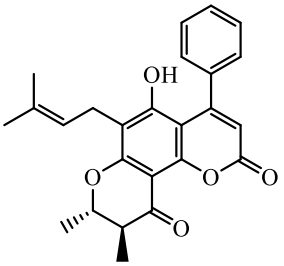

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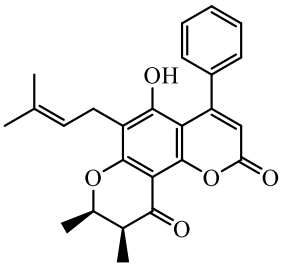

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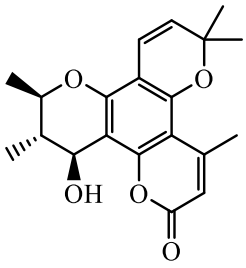

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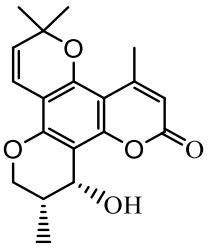

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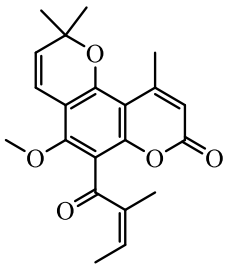
Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3**



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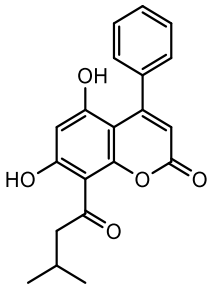

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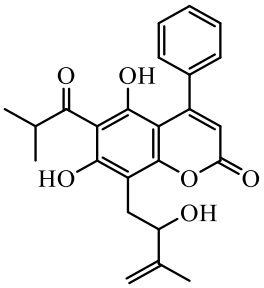

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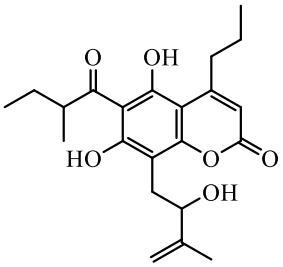

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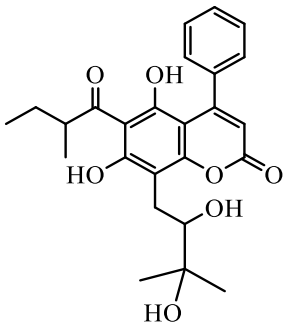

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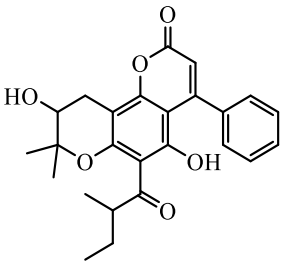

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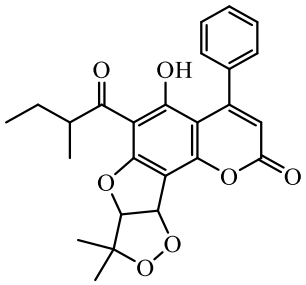

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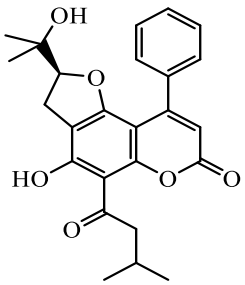

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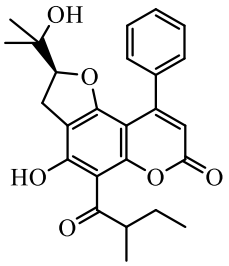

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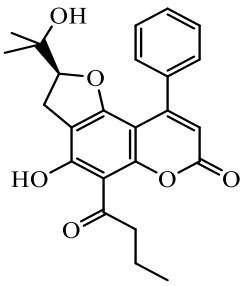

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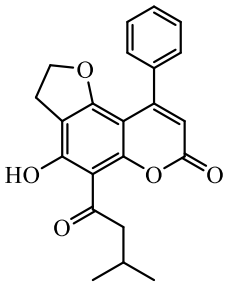

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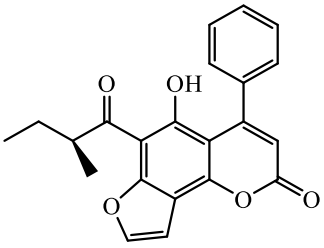

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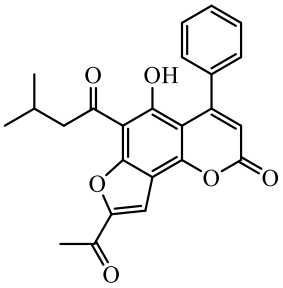
Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3** (continued)



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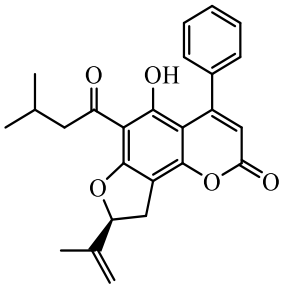

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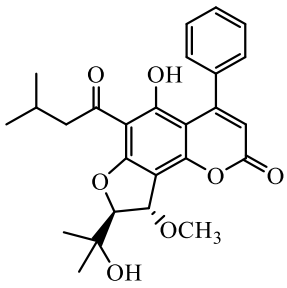

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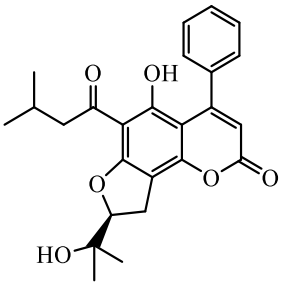

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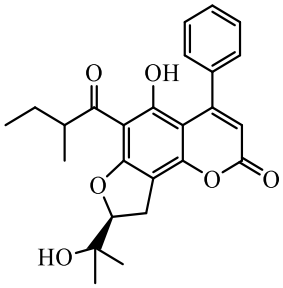

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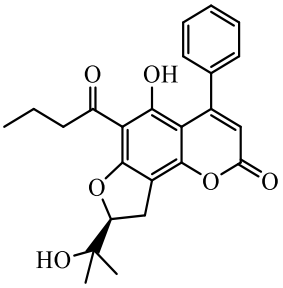

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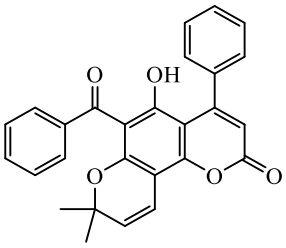

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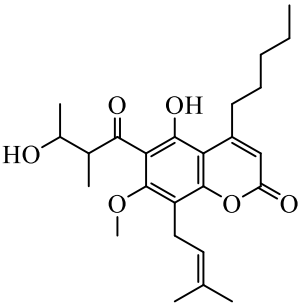

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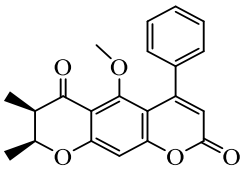

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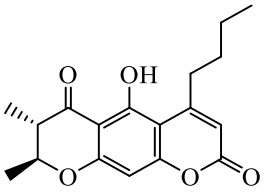

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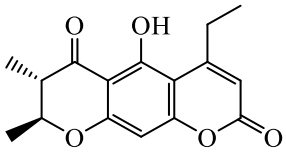

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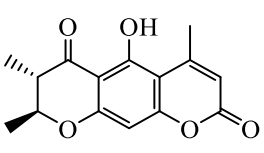

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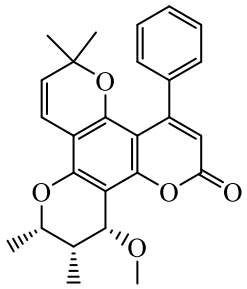
Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3** (continued)



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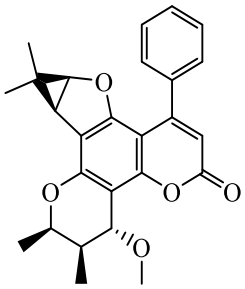

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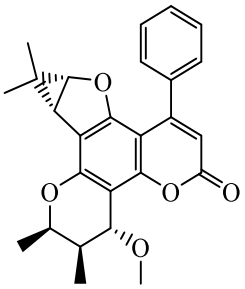

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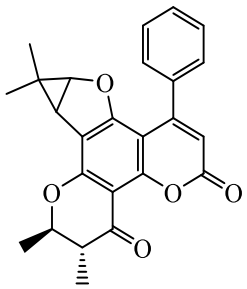

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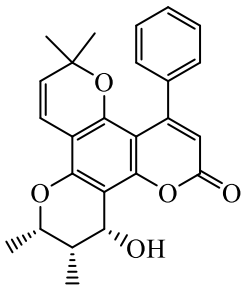

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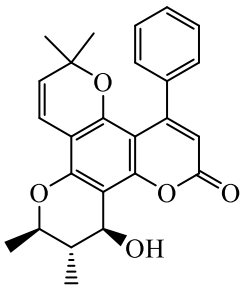

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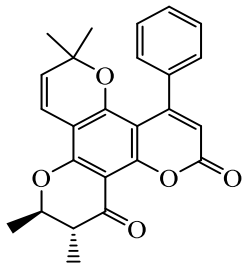

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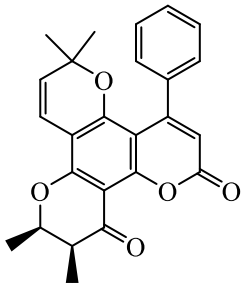

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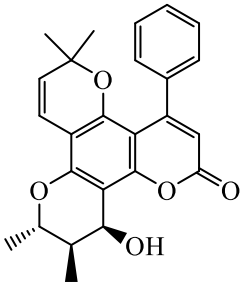

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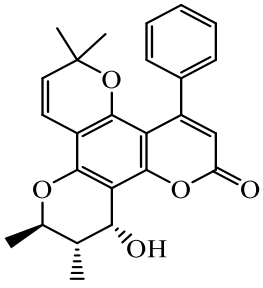

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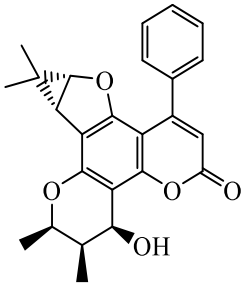

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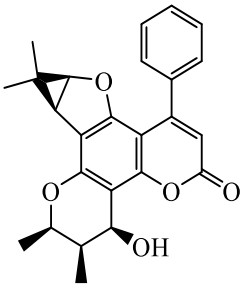

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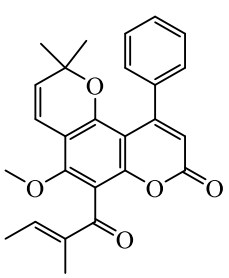
Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3** (continued)



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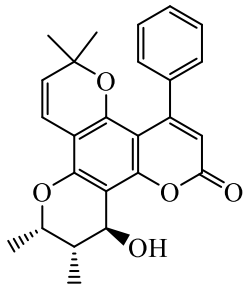

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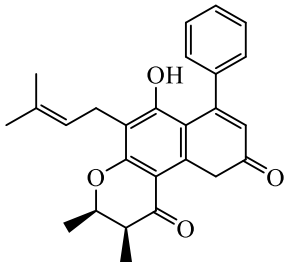

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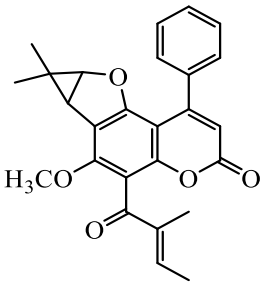

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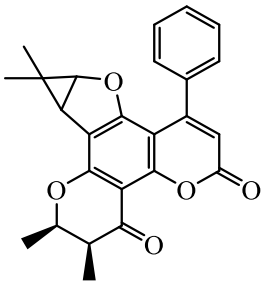

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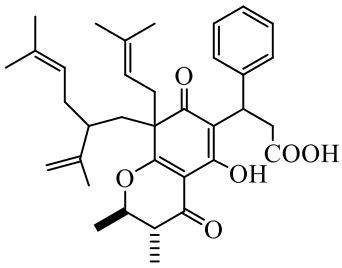

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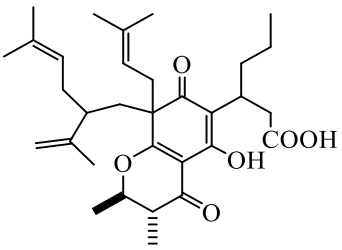

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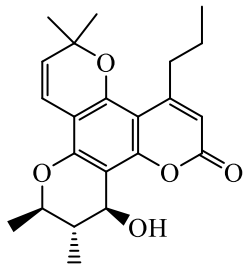

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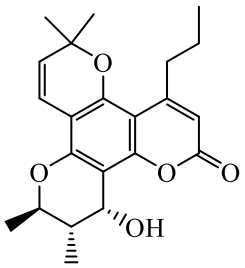

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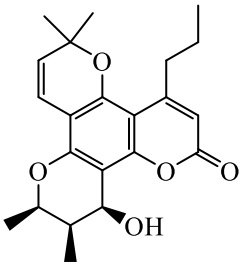

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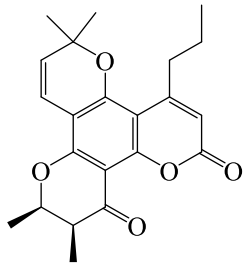
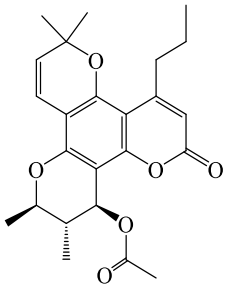
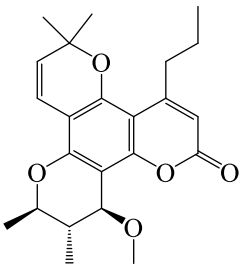


(68)



(69)

Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3** (continued)



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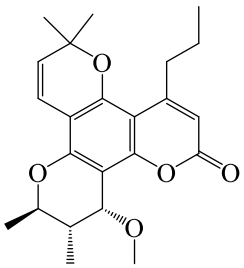

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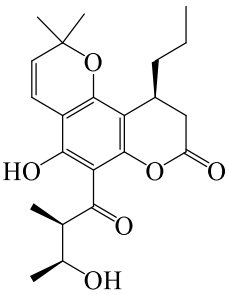

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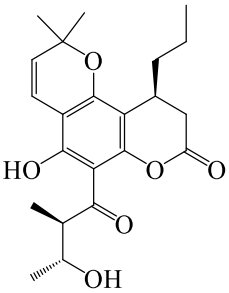

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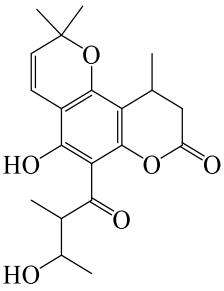

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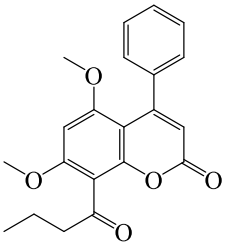

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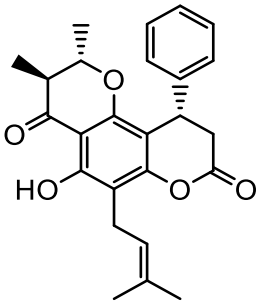

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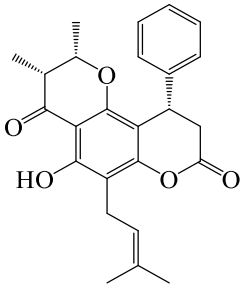

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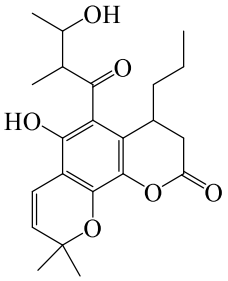

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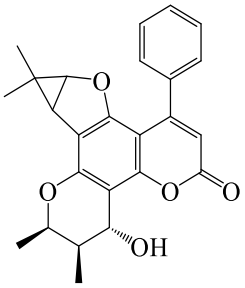

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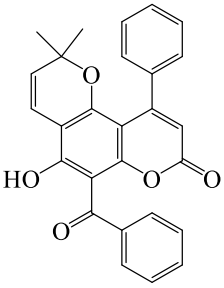

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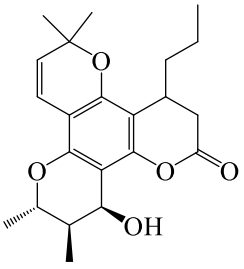
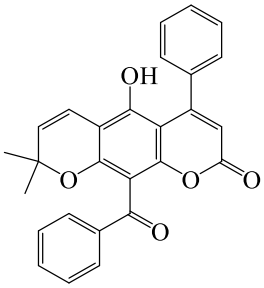


(81)

Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3** (continued)



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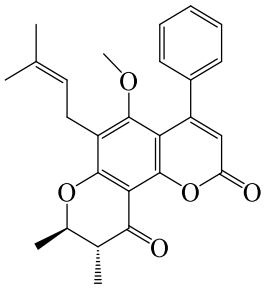

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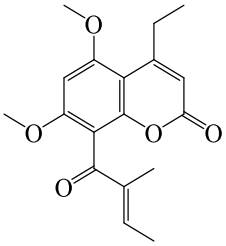

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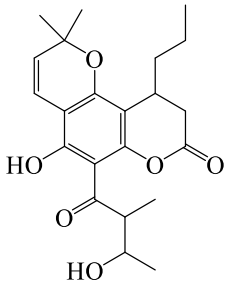

(85)

Figure 6. The chemical structures of coumarins isolated from the species mentioned in **Table 3** (continued)

2.2.2 Chemistry (Chromanones)

Chromanone, with the molecular formula of $C_9H_6O_2$ is also a part of phenolic compounds that has been isolated before from genus *Calophyllum*. It comprises of two stereocenters at carbon positions of C-2 & C-3 (2,3-dimethylchromanone ring) as well as stereocenters on the associated alkyl side chain (5 to 8 carbons), along with a linked carboxyl group (acidic side chain) (Nugroho et al. 2017). For example, in the case of apetalic acid (Ha et al. 2012), blancoic acid (Plattner et al. 1974), and caloteysmannic acid (Lim et al. 2015). Phytochemists tend to focus primarily on the configuration of chromanones, particularly at the two carbons (C-2 &C-3) in 2,3-dimethylchromanone ring, and may overlook the carbon from the acidic side chain, which could cause confusion (Nugroho et al. 2017).

In 2015, Lim and colleagues isolated several chromanone acids including caloteysmannic acid, and diastereoisomers named isocalolongic acid, and calolongic acid from the stem bark of *C. teysmannii*. The difference regarding the chemical structures between caloteysmannic acid and both diastereoisomers is at the configuration of C-2 and C-3, as well as the existence of 3-phenylpropanoic acid instead of hexanoic acid. Caloteysmannic acid was found for the first time in the same study and in comparison to other chromanone acids, it has the strongest inhibitory activity against HeLa Chang liver cell line (Lim et al. 2015). Other chromanones that were isolated from *Calophyllum* species were tabulated in **Table 4** and **Figure 7** below, to show their chemical structures and its derivatives, along with references from previous studies.

Table 4. Chromanones isolated from *Calophyllum* species

***Calophyllum* species**	**Compounds**	**Part of plants**	**References**
C. blancoi	Apetalic acid (**86**) Isoapetalic acid (**87**) Apetalic acid methyl ester (**88**) Apetalic acid 5-*O*-acetate (**89**) Isoapetalic methyl ester (**90**) Isoapetalic acid 5-*O*-acetate (**91**)	Seeds	Shen et al. 2004
C. brasiliense	Brasiliensophyllic acid A (**92**) Isobrasiliensophyllic acid A (**93**) Brasiliensophyllic acid B (**94**) Isobrasiliensophyllic acid B (**95**) Brasiliensophyllic acid C (**96**) Isobrasiliensophyllic acid C (**97**)	Bark	Cottiglia et al. 2004
C. castaneum	Isoblancoic acid (**98**)	Stem bark	Lim et al. 2019a
C. decipiens	Apetalic acid (**86**) Decipic acid (**99**) 12-acetyl apetalic acid (**100**)	Bark	Ajithabai et al. 2012
C. incrassatum	Calofolic acid B (**101**) Apetalic acid (**86**)	Stem bark	Hasanah et al. 2019
C. inophyllum	Caloinophyllin A (**102**)	Root	Ponguschariyagul et al. 2018
C. polyantum	Calopolyanic acid (**103**) Isocalopolyanic acid (**104**) Isorecedensic acid (**105**) Apetalic acid (**86**) Blancoic acid (**106**) Chapelieric acid (**107**) Methyl isoapetalate (**108**) Isoapetalic acid (**87**) Isocalolongic acid (**109**)	Pericarps	Wang et al. 2010
C. scriblitifolium	Calofolic A (**110**) Calofolic B (**101**) Calofolic C (**111**) Calofolic D (**112**) Calofolic E (**113**) Calofolic F (**114**)	Stem bark	Nugroho et al. 2017
C. symingtonianum	Isocordato-oblongic acid (**115**)	Stem bark	Aminudin et al. 2015
C. teysmannii	Isocalolongic acid (**109**) Caloteysmannic acid (**116**) Calolongic acid (**117**)	Stem bark	Lim et al. 2015