



Faculty of Resource Science and Technology

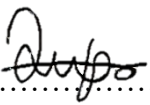
Secondary Metabolites from *Calophyllum soulattri* and *Calophyllum gracilentum* and Their Antibacterial Activity

Dayang Nurul Anisa binti Abang Heilman

**Master of Science
2024**

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.


.....

Signature

Name: Dayang Nurul Anisa binti Abang Heilman

Matric No.: 22020221

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date : 27/9/2024

ACKNOWLEDGEMENT

First and foremost, I want to take this opportunity to express my sincere appreciation to my supervisor, Dr Nor Hisam binti Zamakshshari, for her guidance and advice throughout the study. I am thankful for her unselfishness in leading me in performing this project with success. I gained much extra knowledge from her during the research period. I also would like to express my gratitude to Associate Professor Dr Vivien Jong Yi Mian for her guidance and support during my attachment at Universiti Teknologi MARA, Sarawak. I am grateful for her concern and understanding about the hard times I faced during the research period.

Besides that, I also want to express my gratitude to the staff of the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, for their assistance and guidance in operating the instruments, which made this project a success. Moreover, special thanks to my laboratory mates, Nur Fazlin Zafirah, Mas Atikah and Sheril Haziani, who are willing to share their knowledge, provide unconditional support and discuss scientific issues, which helped a lot in my research.

In addition, I would like to offer my heartfelt gratitude and respect to my loving family for always providing me with better motivational support. Finally, I want to convey my appreciation to those who are not named here but have played a key role in providing me with the insights that have sparked my enthusiasm and opened the doors to limitless knowledge.

ABSTRACT

Antibiotic resistance has emerged as a substantial threat to global public health, necessitating an urgent investigation and evaluation of novel antimicrobial agents to combat this escalating issue. Plants are valuable resources for the development of novel pharmaceutical products. *Calophyllum* species are known for having excellent biological activities such as antimicrobial and antiviral due to their secondary metabolites, which led to the selection of two *Calophyllum* species, *Calophyllum soulattri* and *Calophyllum gracilentum* for this study. The stem bark of *C. soulattri* and *C. gracilentum* were studied for their phytochemical and biological activities. The powdered stem bark of both *Calophyllum* species were extracted at room temperature sequentially with hexane, ethyl acetate and methanol. Each of the extracts were filtered and solvents removed by rotary evaporator to give hexane, ethyl acetate and methanol extracts. The extracts were isolated using various chromatography techniques such as column chromatography and thin layer chromatography which led to the isolation of a new xanthone, soulaxanthone (**62**), along with four other known metabolites, euxanthone (**63**), calopolyanolide E (**19**), calanolide E (**3**) and friedelin (**60**). The structures of these compounds were identified and elucidated using spectroscopic techniques such as MS, IR, 1D and 2D NMR. The extracts showed inhibition against Gram-negative bacteria but showed no inhibition against Gram-positive bacteria. For compounds, there were no inhibition demonstrated against *B. cereus*, *L. plantarum* and *S. marcescens*. Ethyl acetate extract of *C. soulattri* demonstrated moderate activity against *P. aeruginosa* (MIC = 125 µg/mL). Meanwhile, the hexane extract from both plants exhibited moderate activity against *E. cloacae* (MIC = 250 µg/mL). For isolated compound, soulaxanthone (**62**) exhibited moderate activity against *P. aeruginosa*

with an MIC value of 25 µg/mL. Calopolyanolide E (**19**) and friedelin (**60**) demonstrated moderate activity against *E. cloacae* with the MIC values of 25 and 50 µg/mL, respectively. Both compounds showed bactericidal activity against *E. cloacae* with the similar value to ampicillin (MBC = 50 µg/mL). The significance of outcomes from this study is the isolation of a new xanthone, soulaxanthone from the stem bark of *C. soulattri*. For the future study, soulaxanthone could be isolated with higher percentage of yield by doing standardise method.

Keywords: *Calophyllum soulattri*, *Calophyllum gracilentum*, xanthone, soulaxanthone, antibacterial

*Metabolit Sekunder daripada Calophyllum soulattri dan Calophyllum gracilentum dan
Aktiviti Antibakterianya*

ABSTRAK

*Rintangan antibiotik telah muncul sebagai ancaman besar kepada kesihatan awam global, yang memerlukan penyelidikan segera dan penilaian agen antimikrob baharu untuk memerangi isu yang semakin meningkat ini. Tumbuhan adalah sumber yang berharga untuk pembangunan produk farmaseutikal baharu. Spesies Calophyllum terkenal dengan aktiviti biologi yang sangat baik seperti antimikrob dan antivirus disebabkan oleh metabolit sekundernya, yang membawa kepada pemilihan dua spesies Calophyllum, Calophyllum soulattri dan Calophyllum gracilentum untuk kajian ini. Kulit batang C. soulattri dan C. gracilentum telah dikaji untuk aktiviti fitokimia dan biologi mereka. Serbuk kulit batang kedua-dua spesies Calophyllum telah diekstrak pada suhu bilik secara berurutan dengan heksana, etil asetat dan metanol. Setiap ekstrak telah ditapis dan pelarut dikeluarkan oleh penyejat berputar untuk memberikan ekstrak heksana, etil asetat dan metanol. Ekstrak telah diasingkan dengan menggunakan pelbagai teknik kromatografi seperti kromatografi lajur dan kromatografi lapisan nipis yang membawa kepada pengasingan xanton baharu, soulaxanthone (**62**), bersama empat metabolit lain yang diketahui, euxanthone (**63**), calopolyanolide E (**19**), calanolide E (**3**) dan friedelin (**60**). Struktur sebatian ini dikenal pasti dan dijelaskan menggunakan teknik spektroskopi seperti MS, IR, 1D dan 2D NMR. Ekstrak menunjukkan perencatan terhadap bakteria Gram-negatif tetapi tidak menunjukkan perencatan terhadap bakteria Gram-positif. Untuk sebatian, tiada perencatan yang ditunjukkan terhadap B. cereus, L. plantarum dan S. marcescens. Ekstrak etil asetat C. soulattri menunjukkan aktiviti sederhana terhadap P.*

aeruginosa (MIC = 125 µg/mL). Manakala, ekstrak heksana daripada kedua-dua tumbuhan menunjukkan aktiviti yang sederhana terhadap E. cloacae (MIC = 250 µg/mL). Manakala, untuk sebatian yang diasingkan, *soulaxanthone* (62) menunjukkan aktiviti yang sederhana terhadap P. aeruginosa dengan nilai MIC 25 µg/mL. *Calopolyanolide E* (19) dan *friedelin* (60) menunjukkan aktiviti sederhana terhadap E. cloacae dengan nilai MIC masing-masing 25 dan 50 µg/mL. Kedua-dua sebatian menunjukkan aktiviti bakteria terhadap E. cloacae dengan nilai yang serupa dengan *ampicillin* (MBC = 50 µg/mL). Kepentingan hasil daripada kajian ini ialah pengasingan xanton baharu, *soulaxanthone* daripada kulit batang C. soulattri. Untuk kajian masa depan, *soulaxanthone* boleh diasingkan dengan peratusan hasil yang lebih tinggi dengan melakukan kaedah piawai.

Kata kunci: Calophyllum soulattri, Calophyllum gracilentum, xanton, *soulaxanthone*, antibakteria

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
ABSTRAK	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xvi
CHAPTER 1: INTRODUCTION	1
1.1 Background of Study	1
1.2 Genus <i>Calophyllum</i>	3
1.3 Problem Statement	5
1.4 Aim and Objectives of Research	6
CHAPTER 2: LITERATURE REVIEW	7
2.1 Introduction	7
2.2 Secondary Metabolites from <i>Calophyllum</i> spp.	7
2.2.1 Coumarins	8
2.2.2 Xanthones	14
2.2.3 Chromanones	21

2.2.4	Triterpenes	25
2.3	Antibacterial Activity of Plant Extracts from <i>Calophyllum</i> spp.	27
2.4	Antibacterial Activity of Secondary Metabolites from <i>Calophyllum</i> spp.	28
CHAPTER 3: MATERIALS AND METHODS		31
3.1	Materials	31
3.1.1	Plant materials	31
3.1.2	Chemicals	31
3.2	Extraction and Isolation of Secondary Metabolites from <i>Calophyllum soulattri</i> and <i>Calophyllum gracilentum</i>	32
3.2.1	Preparation of crude extract	32
3.2.2	Isolation of secondary metabolites from <i>Calophyllum soulattri</i>	32
3.2.3	Isolation of secondary metabolites from <i>Calophyllum gracilentum</i>	36
3.3	Chromatographic Methods	38
3.3.1	Column chromatography	38
3.3.2	Thin Layer Chromatography (TLC)	39
3.4	Instrumentations	40
3.5	Antibacterial Assay	41
3.5.1	Preparation of media Mueller Hinton Agar (MHA)	41
3.5.2	Preparation of McFarland Standard and bacterial suspension	41
3.5.3	Agar well diffusion method	42
3.5.4	Minimum Inhibitory Concentration (MIC)	42

3.5.5	Minimum Bactericidal Concentration (MBC)	43
3.5.6	Statistical analysis	43
3.6	Physical and Spectral Data of Secondary Metabolites from <i>Calophyllum soulattri</i> and <i>Calophyllum gracilentum</i>	44
3.6.1	Calanolide E (3)	44
3.6.2	Calopolyanolide E (19)	45
3.6.3	Soulaxanthone (62)	46
3.6.4	Euxanthone (63)	47
3.6.5	Friedelin (60)	47
CHAPTER 4: RESULTS AND DISCUSSION		50
4.1	Isolation of Chemical Constituents from <i>Calophyllum soulattri</i>	50
4.1.1	Characterisation of calanolide E (3)	50
4.1.2	Characterisation of calopolyanolide E (19)	63
4.1.3	Characterisation of soulaxanthone (62)	76
4.2	Isolation of Chemical Constituents from <i>Calophyllum gracilentum</i>	89
4.2.1	Characterisation of euxanthone (63)	89
4.2.2	Characterisation of friedelin (60)	100
4.3	Antibacterial Activities	106
CHAPTER 5: CONCLUSION AND RECOMMENDATIONS		111
5.1	Conclusion	111
5.2	Recommendations	112

REFERENCES	113
APPENDICES	125

LIST OF TABLES

		Page
Table 2.1	Structure of pyranochromanone derivatives	23
Table 2.2	Antibacterial activity of <i>Calophyllum</i> extracts	28
Table 2.3	Antibacterial activity of compounds isolated from <i>Calophyllum</i> species against several bacterial strains reported from 2000 to 2023	30
Table 4.1	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C NMR (125 MHz, CDCl ₃). Assignments of calanolide E (3)	53
Table 4.2	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C NMR (125 MHz, CDCl ₃). Assignment of calopolyanolide E (19)	66
Table 4.3	¹ H NMR (400 MHz, acetone- <i>d</i> ₆) and ¹³ C NMR (100 MHz, acetone- <i>d</i> ₆). Assignment of soulaxanthone (62)	79
Table 4.4	¹ H NMR (500 MHz, acetone- <i>d</i> ₆) and ¹³ C NMR (125 MHz, acetone- <i>d</i> ₆). Assignment of euxanthone (63)	91
Table 4.5	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C NMR (125 MHz, CDCl ₃). Assignment of friedelin (60)	102
Table 4.6	Antibacterial activities of crude extracts	108
Table 4.7	Antibacterial activities of isolated compounds	109
Table 4.8	The MIC and MBC of crude extracts and isolated compounds	110

LIST OF FIGURES

		Page
Figure 1.1	(A) The leaves of <i>Calophyllum soulattri</i> , (B) The leaves of <i>Calophyllum gracilentum</i>	4
Figure 2.1	General structure of coumarin	8
Figure 2.2	Structures of isolated coumarins from <i>Calophyllum</i>	13
Figure 2.3	General structure of xanthone	15
Figure 2.4	Structures of isolated xanthenes from <i>Calophyllum</i>	19
Figure 2.5	Structures of chromanone derivatives	24
Figure 2.6	Structures of triterpene derivatives	26
Figure 3.1	Flowchart of the purification process of calanolide E (3)	34
Figure 3.2	Flowchart of the purification process of calopolyanolide E (19) and soulaxanthone (62)	35
Figure 3.3	Flowchart of the purification process of euxanthone (63)	37
Figure 3.4	Flowchart of the purification process of friedelin (60)	38
Figure 3.5	Column chromatography	39
Figure 3.6	The developed TLC plate	40
Figure 3.7	Flow diagram of the experimental	49
Figure 4.1	Structure of calanolide E (3)	50
Figure 4.2	HMBC correlation in calanolide E (3)	52
Figure 4.3	FTIR spectrum of calanolide E (3)	55

Figure 4.4	LC-MS spectrum of calanolide E (3)	56
Figure 4.5	¹ H NMR spectrum of calanolide E (3) (500 MHz, CDCl ₃)	57
Figure 4.6	¹³ C NMR spectrum of calanolide E (3) (125 MHz, CDCl ₃)	58
Figure 4.7	¹³ C DEPT spectrum of calanolide E (3)	59
Figure 4.8	COSY spectrum of calanolide E (3)	60
Figure 4.9	HSQC spectrum of calanolide E (3)	61
Figure 4.10	HMBC spectrum of calanolide E (3)	62
Figure 4.11	Structure of calopolyanolide E (19)	63
Figure 4.12	HMBC correlation in calopolyanolide E (19)	65
Figure 4.13	FTIR spectrum of calopolyanolide E (19)	68
Figure 4.14	LC-MS spectrum of calopolyanolide E (19)	69
Figure 4.15	¹ H NMR spectrum of calopolyanolide E (19) (500 MHz, CDCl ₃)	70
Figure 4.16	¹³ C NMR spectrum of calopolyanolide E (19) (125 MHz, CDCl ₃)	71
Figure 4.17	¹³ C DEPT spectrum of calopolyanolide E (19)	72
Figure 4.18	COSY spectrum of calopolyanolide E (19)	73
Figure 4.19	HSQC spectrum of calopolyanolide E (19)	74
Figure 4.20	HMBC spectrum of calopolyanolide E (19)	75
Figure 4.21	Structure of soulaxanthone (62)	76
Figure 4.22	HMBC correlation in soulaxanthone (62)	78
Figure 4.23	FTIR spectrum of soulaxanthone (62)	80

Figure 4.24	LC-MS spectrum of soulaxanthone (62)	81
Figure 4.25	¹ H NMR spectrum of soulaxanthone (62) (400 MHz, acetone- <i>d</i> ₆)	82
Figure 4.26	¹³ C NMR spectrum of soulaxanthone (62) (100 MHz, acetone- <i>d</i> ₆)	83
Figure 4.27	¹³ C DEPT spectrum of soulaxanthone (62)	84
Figure 4.28	COSY spectrum of soulaxanthone (62)	85
Figure 4.29	HSQC spectrum of soulaxanthone (62)	86
Figure 4.30	HMBC spectrum of soulaxanthone (62)	87
Figure 4.31	Expansion HMBC spectrum of soulaxanthone (62)	88
Figure 4.32	Structure of euxanthone (63)	89
Figure 4.33	HMBC correlation in euxanthone (63)	90
Figure 4.34	FTIR spectrum of euxanthone (63)	92
Figure 4.35	LC-MS spectrum of euxanthone (63)	93
Figure 4.36	¹ H NMR spectrum of euxanthone (63) (500 MHz, acetone- <i>d</i> ₆)	94
Figure 4.37	¹³ C NMR spectrum of euxanthone (63) (125 MHz, acetone- <i>d</i> ₆)	95
Figure 4.38	¹³ C DEPT spectrum of euxanthone (63)	96
Figure 4.39	COSY spectrum of euxanthone (63)	97
Figure 4.40	HSQC spectrum of euxanthone (63)	98
Figure 4.41	HMBC spectrum of euxanthone (63)	99
Figure 4.42	Structure of friedelin (60)	100
Figure 4.43	¹ H NMR spectrum of friedelin (60) (500 MHz, CDCl ₃)	104

Figure 4.44	^{13}C NMR spectrum of friedelin (60) (125 MHz, CDCl_3)	105
Figure 4.45	Samples of pictures for agar well diffusion assay	109

LIST OF ABBREVIATIONS

ACN	Acetonitrile
AMR	Antimicrobial resistance
ANOVA	Analysis of Variance
AR	Analytical Research
BaCl ₂	Barium chloride
br. s	Broad singlet
CDCl ₃	Deuterated chloroform
CD ₃ COCD ₃	Deuterated acetone
CH ₂	Methylene
CH ₃	Methyl
C ₂ H ₄	Ethylene
C=C	Alkene
COOH	Carboxyl group
COOMe	Methoxycarbonyl group
COSY	Correlation spectroscopy
C=O	Carbonyl group
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	Dimethyl sulfoxide
d	Doublet
dd	Doublet of doublets

EIMS	Electron Ionisation Mass Spectroscopy
FeCl ₃	Iron(III) chloride
FTIR	Fourier-Transform Infrared Spectroscopy
GCC	Gravity Column Chromatography
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High-Performance Liquid Chromatography
HSD	Honestly Significant Difference
HSQC	Heteronuclear Single Quantum Coherence
HREIMS	High-Resolution Electron Ionisation Mass Spectroscopy
H ₂ SO ₄	Sulphuric acid
<i>J</i>	Coupling constant in Hertz
LC-MS	Liquid chromatography-Mass Spectroscopy
MBC	Minimum Bactericidal Concentration
MeOH	Methanol
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-susceptible <i>Staphylococcus aureus</i>
m	Multiplet
<i>m/z</i>	Mass-to-charge ratio
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect

OAc	Acetoxy group
OH^{-1}	Hydroxide
-OH	Hydroxyl group
ppm	Part per million
q	Quartet
s	Singlet
TLC	Thin Layer Chromatography
t	Triplet
UV-Vis	Ultraviolet-Visible
1D NMR	One Dimension Nuclear Magnetic Resonance
2D NMR	Two Dimension Nuclear Magnetic Resonance
^1H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
δ_{C}	Chemical shift of carbon
δ_{H}	Chemical shift of hydrogen
λ_{max}	Lambda max

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Antimicrobial resistance (AMR) is currently considered a significant global health menace, presenting substantial obstacles to prevent and treat chronic diseases (Prestinaci et al., 2015; Sharma et al., 2022). Numerous bacterial resistances should be a cause for concern, including the fact that it frequently leads to treatment failure and can have profound implications, particularly in critically ill patients (Tenover, 2006). The capability of bacteria to evolve resistance to antibacterial agents complicates the treatment of bacterial infections. Antimicrobial medicines that are efficient are essential for both curative and preventive therapies, protecting patients from possibly fatal diseases and allowing complex procedures such as chemotherapy and surgery to be performed securely. Therefore, the development of antibiotics is regarded as one of the most significant advances in modern science.

People have utilised plants to treat and cure illnesses since the beginning of civilisation (Jamshidi-Kia et al., 2018). Antimicrobials, including antivirals, antibiotics, antiparasitic and antifungals are medicines utilised to inhibit and cure infectivity in humans, animals and plants. The genus *Calophyllum* has been identified as a promising source of lead compounds for pharmacological research and development. *Calophyllum* species is rich in secondary metabolites with a distinct spectrum of pharmacological activities (Zailan et al., 2022). Xanthonenes, coumarins, triterpenes and flavonoids are examples of secondary metabolites that occur in this genus. Several studies have shown that

secondary metabolites from *Calophyllum* possess a broad range of interesting biological properties such as anti-inflammatory (Sundur et al., 2014), antibacterial (Aminudin et al., 2016), anticancer (Kurniawan et al., 2021), antioxidant (Abbas, 2019) and antiviral (Sundur et al., 2014). Numerous genus *Calophyllum* plants are utilised as folk medicines to cure health conditions such as infections, inflammation, malaria, tumours, eye diseases, and hypertension. In Vietnam, *Calophyllum inophyllum* seed oil has been utilised as a traditional medicine to cure insomnia, burns and rheumatic diseases (Nguyen et al., 2017). Zou et al. (2005) stated that the stem and bark of *Calophyllum membranaceum* have been used in Chinese folk medicine for the treatment of arthritis, wounds and lumbago. In Brazil, *Calophyllum brasiliense* is used to treat inflammation, ulcers and varicose haemorrhoids (Filho et al., 2009).

One of the species chosen for this study, *Calophyllum soulattri*, has been reported to possess numerous secondary metabolites such as soulamarin (Ee et al., 2011), soulattrin (Mah et al., 2011), phylattrin (Mah et al., 2012), airtangins A and B (Tanjung et al., 2018). Previous study by Khan et al. (2002) stated that the methanol extract of leaves, stem and root barks from *C. soulattri* were fractionated with petrol, dichloromethane and ethyl acetate. 13 Gram-positive and 12 Gram-negative bacteria were used for antibacterial assay. All the extractives showed a range of activities against all the tested bacteria. Petrol fraction of the root bark demonstrated stronger antibacterial activity than the other fractions. Another species chosen for this study is *Calophyllum gracilentum*. However, *Calophyllum gracilentum* is a new species and there is no antimicrobial activity being done.

1.2 Genus *Calophyllum*

The genus *Calophyllum* is classified under the Calophyllaceae family. This genus consists of about 180 to 200 species, with some exclusively found only in the tropical rainforest of Sarawak, Malaysia (Lim et al., 2019). The habitat of the species ranges from drier regions at higher elevations to the lowlands' moist tropical rainforest (Gupta & Gupta, 2020). The Malay local name for the *Calophyllum* genus is “bintangur” (Whitemore, 1973). According to Gupta and Gupta (2020), the genus possesses an array of distinctive taxonomical characteristics, such as opposing leaves with closely parallel veins and the outer bark with red-coloured and diamond-shaped fissures.

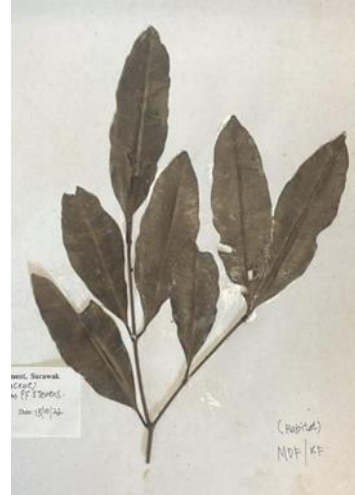
Calophyllum soulattri is a species of flowering plant which can be found in Southeast Asia, northern Australia and the Solomon Islands (Pelser et al., 2011 onward). It is a tall tree which can grow up to a height of 20 m and the length of branch-free stem is up to 35 m. The texture of its wood is slightly rough to rough, and uneven. Meanwhile, the direction of wood fibre is highly integrated and sometimes wavy (Kaliky et al., 2020).

Calophyllum gracilentum is one of the *Calophyllum* species that grows in Malaysia. The native range of this species is in Peninsular Malaysia (Kew, 2024). This tree normally grows in lowland dipterocarp forests and swampy areas. The leaves are elliptical with dark green colour. The flowers of this species are fragrant, with white or cream colour. It has round drupe fruits with hard shells (Kew, 2024).

Figure 1.1 below displays the leaves of *Calophyllum soulattri* (NParks Flora & Fauna Web, 2020) and *Calophyllum gracilentum*.



(A)



(B)

Figure 1.1: (A) The leaves of *Calophyllum soulattri*, (B) The leaves of *Calophyllum gracilentum*

1.3 Problem statement

Nowadays, the latest efficient antimicrobial compounds are important to be created as pathogens evolve resistance toward medicine. For instance, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as the major general antimicrobial resistant bacteria (AMR) (Alghamdi et al., 2023). MRSA has developed resistance to beta-lactam drugs like penicillin (methicillin and oxacillin) and cephalosporin (Kırmusaoğlu 2017, Vestergaard et al., 2019; Nandhini et al., 2022). Medicinal plants are abundant in secondary metabolites and are widely utilised as drug in the pharmaceutical industry. Malaysia is rich in flora and fauna which have high biological activities and may have probable to be used as a drug. However, most of the flora and fauna in Malaysia have not yet been discovered. Numerous plants of the genus *Calophyllum* are used as traditional medicine. For example, seed oil from *Calophyllum inophyllum*, *Calophyllum apetalum* and *Calophyllum soulattri* has been used for leprosy, skin infection, rheumatic problems and pain (Watt, 2014; Sundur et al., 2016). Hence, it leads to investigating secondary metabolite and antibacterial activities of two *Calophyllum* species: *Calophyllum soulattri* and *Calophyllum gracilentum*. It is believed that the secondary metabolites from *Calophyllum* species possess various therapeutic activities and interact directly with cell membranes, receptors and nucleic acids, leading to antibacterial activities. These secondary metabolites can disrupt bacterial cell membranes, leading to leakage of cellular contents and cell death. Besides, they can inhibit essential bacterial enzymes and interfere with DNA replication and protein synthesis (Mitra et al., 2023). Studies have demonstrated the effectiveness of *Calophyllum* extracts against various bacterial strains, including both Gram-positive and Gram-negative bacteria (Aminudin et al., 2019; Abbas & Minarti, 2020). The antibacterial activities of the compounds and the extracts were tested against