Author Statement of Accessibility- Part 2- Permission for Internet Access

Name of Candidate: Sujesh Sharma
Degree: Master of Science (Chemistry)
Department/School: The University of the South Pacific
Institution/University: The University of the South Pacific
Thesis Title: Chemical and Biological Investigations of Melobesia from Namena odorata

Date of completion of requirements for award: 25/05/2010

1. I authorise the University to make this thesis available on the Internet for access by USP authorised users.  
   
2. I authorise the University to make this thesis available on the Internet under the International digital theses project.

Signed:  
Date: 25/05/2010

Contact Address
Flat 3,  
43 Varani Street,  
Laukala Bay, Suva.  
Ph: 9208784  
e-mail: sharma_suj@yahoo.com

Permanent Address
P.O. Box 1074,  
Ba.  

e-mail: sharma_suj@yahoo.com
CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF METABOLITES FROM MAMMEA ODORATA

By

Sujlesh Sharma

A thesis submitted in the fulfilment of the requirements for the degree of
Master of Science

Copyright © by Sujlesh Sharma

School of Biological and Chemical Sciences
Faculty of Science, Technology and Environment
University of the South Pacific

April, 2010
Declaration

I, Sujlesh Sharma, declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published, or substantially overlapping with material submitted for the award of any other degree at any institution, except where due acknowledgement is made in the text.

Ms. Sujlesh Sharma  
S11011928  
31 March, 2010  

The research in this thesis was performed under my supervision and to my knowledge is the sole work of Sujlesh Sharma.

Prof. Subramaniam Sotheeswaran  
(Principal supervisor)  
31/03/2010  

Date
Acknowledgements

I would like to profoundly acknowledge the following people and organisations that have provided me with immense support during my MSc. study and without whom this project would not have been completed.

This MSc. study was carried out under the guidance of my internal supervisors, Professor Subramaniam Sotheeswaran and Associate Professor Sadaquat Ali of University of the South Pacific (USP), and external supervisor, Dr. Rohan A. Davis of Eskitis Institute for Cell and Molecular Therapies, Griffith University (GU). I deeply acknowledge all of them for their support, words of advice, and sharing their knowledge with me throughout this study.

I would also like to thank Dr. Katherine Andrews of Queensland Institute of Medical Research for performing the anti-malarial assays, and Dr. Dhana Rao and PhD. researcher, Mr. Vinay Narayan of USP for their advice on the antimicrobial assays. Sincere thanks to Mrs. Pande of Institute of Applied Sciences and Ms. Shalini of Colonial War Memorial Hospital for providing the microorganisms for antimicrobial assays.

I also thank the staff of the South Pacific Regional Herbarium for the collection and taxonomic identification of the biota sample that I researched upon.

Furthermore, I would like to express my gratitude to Mr. Huan The Vu and Ms. Ann Lam of GU for the measurement of the high-resolution mass spectral data and 2D NMR spectral data respectively for some of my pure compounds.

I also appreciate the assistance of the technical staffs, Mr. Vas Deo, Mr. Shalvin Prasad, Mr. Shiva Padayachi and Ms. Kiran Lata of USP and Mr. Conway Lewis of Eskitis Institute.

My heartfelt thanks go to my fellow research students Roselyn, Mayuri, Pritesh, Ashweeta, Joslyn and Vipra at USP and Post doctoral researchers Min, Xinzhou and
Fredrick; research students Emma, Pouran and Trong; and Lekha and Yun at Eskitis Institute for their cherished friendship, never-ending support and fun-filled moments while researching in the laboratory. Above all, I thank Sheng, Post doctoral researcher, for being a mentor and a very good friend during my research work at Eskitis.

Moreover, I am grateful to USP for providing me with the Graduate Assistant scholarship to pursue my MSc. studies and Professor Ronald Quinn for the opportunity to use the state of the art facilities at the Eskitis Institute.

Finally, I greatly appreciate my parents, Mr. Jai and Mrs. Suman Sharma, sisters, Nikhita and Swastika, and grandparents for their love and support throughout my life and this study. I would also like to thank my uncle and aunt, Mr. Atma and Mrs. Jyotika Ram for the awesome moments during my stay in Brisbane. A special thank goes to Vinay for the motivation and encouragement throughout the ups and downs of this research.

Thank you all!
Abstract

This thesis focuses on the chemical and biological investigations of *Mammea odorata* (Clusiaceae), a plant indigenous to the South Pacific. Cold extraction of the stem bark collected from Deuba, Fiji Islands, resulted in methanol, ethyl acetate and dichloromethane extracts. Fractionation of the three extracts by employing flash chromatography and reverse phase HPLC separation techniques yielded a total of eleven compounds. A combination of spectral data mainly obtained through 1D and 2D NMR experiments (\(^1\)H and \(^1^3\)C NMR and gradient COSY, HMBC, HSQC and ROESY) and mass spectroscopy (HRESIMS and LRESIMS) were instrumental in structure elucidation of the isolated compounds.

Two new xanthone glycosides, 2,3-dihydroxyxanthone; 3-\(O\)-\(\beta\)-D-glucopyranoside (116) and 1,6,7-trihydroxyxanthone; 6-\(O\)-\(\beta\)-D-glucopyranoside (117) together with a known flavonol, (-)-epicatechin (115) were isolated from the methanol extract. Ethyl acetate extract yielded a new natural product, 2,3-dihydroxyxanthone (118) and six known xanthones, 2- and 4-hydroxyxanthones (83 and 84), 2,6-dihydroxyxanthone (94), 2-hydroxy-3-methoxyxanthone (119), 1,6,7-trihydroxyxanthone (88), and 1,6,7-trihydroxy-3-methoxyxanthone (120). A known coumarin, surangin B (34) was isolated from the dichloromethane extract.

*In vitro* antimalarial assays of the eleven compounds using 3D7 (chloroquine-sensitive) line of *Plasmodium falciparum* identified 34 to be the most potent of all the test compounds with an IC\(_{50}\) of 2.5 (\(\pm\)1.6) \(\mu\)M. Other compounds exhibited antimalarial activity in the range of 14.5% to 43.2% at 25.0 \(\mu\)M. *In vitro* antimicrobial susceptibility assays of the extracts (50 mg/mL) and the eleven compounds (10 mg/mL) against nine human pathogenic bacteria was performed using the disc diffusion method. The extracts were shown to inhibit the growth of Gram positive bacteria, *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* and *Enterococcus faecalis*, with the dichloromethane extract displaying the greatest zone of inhibition. The isolated compounds did not show any activity at the tested concentration.
Finally, this thesis reports the sole occurrence of xanthone glycosides 116 and 117 in the genus *Mammea*. The antimalarial activity of some compounds especially 34 has also been reported for the first time.
Abbreviations

br  broad
BSAC  British Society for Antimicrobial Chemotherapy
C₁₈ octadecyl bonded silica
COSY  correlation spectroscopy
d  doublet
dd  doublet of doublets
ddd  doublet of doublet of doublets
DPPH  2,2-diphenyl-1-picrylhydrazyl
HMBC  heteronuclear multiple-bond correlations
HPLC  high-pressure liquid chromatography
HRESIMS  high-resolution electrospray ionization mass spectrometry or spectrum
HSQC  heteronuclear single-quantum coherence
IC₅₀  concentration required to inhibit the growth of the test organisms by half
ID₅₀  Infectious dose to 50% of exposed individuals
IR  infra-red
LD₅₀  dosage required to kill 50% of the test organisms
LRESIMS  low-resolution electrospray ionization mass spectrometry or spectrum
m  multiplet
Me  methyl
MIC  minimum inhibitory concentration
MRSA  methicillin resistant *Staphylococcus aureus*
MSSA  Methicillin sensitive *Staphylococcus aureus*
Mₜ  molecular weight
NP  normal phase
NSAIDs  Non-steroidal anti-inflammatory drugs
NZRM  New Zealand Reference Culture Collection, Medical Section
PAG  polyamide gel
PDA  photo diode array
PMA  phosphomolybdic acid
q  quartet
Rᵣ  retention factor
ROESY  rotating-frame Overhauser effect spectroscopy
RP  reverse phase
s  singlet
SD  standard deviation
t  triplet
TFA  trifluoroacetic acid
TLC  thin layer chromatography
wt  weight
\(^nJ_{CH}\)  n-bond hydrogen to carbon correlation (n=1, 2, 3, or 4)
Table of Contents

Abstract ...................................................................................................................... iii
Abbreviations .............................................................................................................. v
List of Tables ............................................................................................................. ix
List of Figures ........................................................................................................... x
List of Schemes ......................................................................................................... xii

CHAPTER 1: Introduction ...................................................................................... 1
  1.1 The Significance of Ethnobotanic Medicine to Drug Discovery ............ 1
  1.2 Plants of the Genus Mammea L. (Clusiaceae) ........................................... 4
    1.2.1 Mammea odorata (Rafin.) Kosterm .............................................. 4
  1.3 Chemical Diversity of Mammea species ................................................. 6
    1.3.1 The Mammea Coumarins (12-81) ................................................. 6
    1.3.2 Xanthones (82-103) from Mammea species .................................. 11
    1.3.3 Flavonols (104-115) and Minor Compounds from
        Mammea Species ........................................................................... 13
  1.4 Biological Investigations of Metabolites from Mammea Species .......... 14
  1.5 The Plight of Malaria ............................................................................... 17
  1.6 The Objectives of this Study .................................................................... 18

CHAPTER 2: Experimental Methods ................................................................... 19
  2.1 General Experimental ............................................................................... 19
  2.2 Chemical Investigations of Mammea odorata ......................................... 20
    2.2.1 Collection and Extraction ................................................................ 20
    2.2.2 Isolation of Compounds (115-117) from MeOH Extract ............... 21
    2.2.3 Isolation of Compounds (83, 84, 88, 94, 118-120) from
        EtOAc Extract ............................................................................... 24
    2.2.4 Isolation of Surangin B (34) from DCM Extract ......................... 25
  2.3 Biological Investigations of Mammea odorata ......................................... 26
    2.3.1 In vitro Antimalarial Assays of Compounds .................................. 26
    2.3.2 Antimicrobial Susceptibility Assays of Crude Extracts
        and Compounds ........................................................................... 26
CHAPTER 3: Results and Discussion .......................................................... 28

3.1 Chemical Investigations of Metabolites from *Mammea odorata* .......... 28
   3.1.1 Determination of Suitable Parameters to Achieve Good HPLC Separation ................................................................. 28
   3.1.2 Isolation of Compounds (115-117) from MeOH Extract .......... 32
   3.1.3 Structure Elucidation of Compounds (115-117) ......................... 34
      3.1.3.1 (-)-Epicatechin (115) .................................................... 34
      3.1.3.2 2,3-Dihydroxyxanthone; 3-O-β-d-glucopyranoside (116) ................................................................. 38
      3.1.3.3 1,6,7-Trihydroxyxanthone; 6-O-β-d-glucopyranoside (117) ................................................................. 44
   3.1.4 Isolation of Known Oxygenated Xanthones (83, 84, 88, 94, 118-120) from EtOAc Extract .................................................. 48
   3.1.5 Structure Elucidation of Compounds (83, 84, 88, 94, 118-120) ................................................................. 50
      3.1.5.1 2,6-Dihydroxyxanthone (94) ........................................ 50
      3.1.5.2 2,3-Dihydroxyxanthone (118) ...................................... 53
      3.1.5.3 2-Hydroxyxanthone (83) .............................................. 55
      3.1.5.4 4-Hydroxyxanthone (84) ................................................ 58
      3.1.5.5 2-Hydroxy-3-methoxyxanthone (119) ......................... 60
      3.1.5.6 1,6,7-Trihydroxyxanthone (88) ......................... 62
      3.1.5.7 1,6,7-Trihydroxy-3-methoxyxanthone (120) ............... 64
   3.1.6 Isolation of Surangin B (34) from DCM Extract .................... 66
   3.1.7 Structure Elucidation of Surangin B (34) .................................... 68

3.2 Biological Investigations of Metabolites from *M. odorata* .......... 74
   3.2.1 *In vitro* Antimalarial Activity ........................................ 74
   3.2.2 *In vitro* Antimicrobial Activity ........................................ 78

CHAPTER 4: Conclusion ............................................................................. 80

References .................................................................................................. 81

Appendices .................................................................................................. 91
Appendix 1: $^1$H NMR spectrum of 2,3-dihydroxyxanthone; 3-0-β-D-glucopyranoside (116) in DMSO-d$_6$; 600 MHz .......................... 92

Appendix 2: $^{13}$C NMR spectrum of 2,3-dihydroxyxanthone; 3-0-β-D-glucopyranoside (116) in DMSO-d$_6$; 500 MHz ....................... 93

Appendix 3: gCOSY spectrum of 2,3-dihydroxyxanthone; 3-0-β-D-glucopyranoside (116) in DMSO-d$_6$; 600 MHz ....................... 94

Appendix 4: gHMBC Spectrum of 2,3-dihydroxyxanthone; 3-0-β-D-glucopyranoside (116) in DMSO-d$_6$; 600 MHz ....................... 95

Appendix 5: gHSQC spectrum of 2,3-dihydroxyxanthone; 3-0-β-D-glucopyranoside (116) in DMSO-d$_6$; 600 MHz ....................... 97

Appendix 6: gROESY spectrum of 2,3-dihydroxyxanthone; 3-0-β-D-glucopyranoside (116) in DMSO-d$_6$; 600 MHz ....................... 98

Appendix 7: $^1$H NMR spectrum of 1,6,7-trihydroxyxanthone; 6-0-β-D-glucopyranoside (117) in DMSO-d$_6$; 600 MHz ................. 99

Appendix 8: gCOSY spectrum of 1,6,7-trihydroxyxanthone; 6-0-β-D-glucopyranoside (117) in DMSO-d$_6$; 600 MHz ...................... 100

Appendix 9: gHMBC spectrum of 1,6,7-trihydroxyxanthone; 6-0-β-D-glucopyranoside (117) in DMSO-d$_6$; 600 MHz ...................... 101

Appendix 10: gHSQC spectrum of 1,6,7-trihydroxyxanthone; 6-0-β-D-glucopyranoside (117) in DMSO-d$_6$; 600 MHz ...................... 103

Appendix 11: gROESY spectrum of 1,6,7-trihydroxyxanthone; 6-0-β-D-glucopyranoside (117) in DMSO-d$_6$; 600 MHz ...................... 104

Appendix 12: $^1$H NMR spectrum of 2,3-dihydroxyxanthone (118) in DMSO-d$_6$; 600 MHz ............................................................... 105

Appendix 13: $^{13}$C NMR spectrum of 2,3-dihydroxyxanthone (118) in DMSO-d$_6$; 500 MHz ............................................................... 106

Appendix 14: gCOSY spectrum of 2,3-dihydroxyxanthone (118) in DMSO-d$_6$; 600 MHz ............................................................... 107

Appendix 15: gHMBC spectrum of 2,3-dihydroxyxanthone (118) in DMSO-d$_6$; 600 MHz ............................................................... 108

Appendix 16: gHSQC spectrum of 2,3-dihydroxyxanthone (118) in DMSO-d$_6$; 600 MHz ............................................................... 109

Appendix 17: gROESY spectrum of 2,3-dihydroxyxanthone (118) in DMSO-d$_6$; 600 MHz ............................................................... 110
List of Tables

Table 1: NMR data for (-)-epicatechin (115) .......................................................... 35
Table 2: NMR data for 2,3-dihydroxyxanthone;
    3-O-β-D-glucopyranoside (116) ................................................................. 39
Table 3: Some representatives of dihydroxyxanthone glucopyranosides ............. 43
Table 4: NMR data for 1,6,7-trihydroxyxanthone;
    6-O-β-D-glucopyranoside (117) .................................................................... 45
Table 5: NMR data for 2,6-dihydroxyxanthone (94) ........................................ 51
Table 6: NMR data for 2,3-dihydroxyxanthone (118) ..................................... 54
Table 7: NMR data for 2-hydroxyxanthone (83) .............................................. 56
Table 8: NMR data for 4-hydroxyxanthone (84) ............................................. 59
Table 9: NMR data for 2-hydroxy-3-methoxyxanthone (119) ...................... 61
Table 10: NMR data for 1,6,7-trihydroxyxanthone (88) .............................. 63
Table 11: NMR data for 1,6,7-trihydroxy-3-methoxyxanthone (120) .......... 65
Table 12: NMR data for surangin B (34) .......................................................... 69
Table 13: In vitro antimalarial activity displayed by compounds 34, 83, 84, 88, 94, 115-120 ................................................................. 76
Table 14: Zone of inhibition (mm) displayed by the extracts and compounds against human pathogenic bacteria ................................................. 77
List of Figures

Figure 1: Morphological features of *Mammea odorata* ........................................ 5
Figure 2: HPLC chromatograms obtained for various experiments .................... 30
Figure 3: HPLC chromatograms obtained for the isolation of 115-117 from MeOH extract ................................................................. 34
Figure 4: Position of protons in the two aromatic rings ....................................... 36
Figure 5: Key HMBC (→) and COSY (←) correlations in the two fragments of 115 .................................................................................... 37
Figure 6: ROE (↔) correlation in (-)-epicatechin .............................................. 38
Figure 7: Key HMBC (→), COSY (←) and ROE (↔) correlations seen for the aglycone ................................................................. 40
Figure 8: Key HMBC (→), COSY (←) and ROE (↔) correlations in glucose ..................................................................................... 42
Figure 9: Proton resonances in the glycosidic region of 1H NMR spectrum of 117 .................................................................................... 44
Figure 10: Key HMBC (→) and COSY (↔) correlations seen for the aglycone of 117 ..................................................................................... 47
Figure 11: Key HMBC (→) and ROE (↔) correlations seen for the glucose moiety ..................................................................................... 48
Figure 12: HPLC chromatogram for the isolation of 84, 88, 119 and 120 from EtOAc extract ................................................................. 50
Figure 13: Key HMBC (→), COSY (↔) and ROE (↔) correlations observed for 94 ..................................................................................... 53
Figure 14: Key HMBC (→), COSY (↔) and ROE (↔) correlations seen for 83 ..................................................................................... 57
Figure 15: Key HMBC (→), COSY (↔) and ROE (↔) correlations seen for 84 ..................................................................................... 60
Figure 16: Key HMBC (→), COSY (↔) and ROE (↔) correlations in 119 ..................................................................................... 62
Figure 17: Key HMBC (→), COSY (↔) and ROE (↔) correlations in 120 ..................................................................................... 66
Figure 18: HPLC chromatogram for the isolation of 34 from DCM extract ....... 68
Figure 19: Key HMBC (→) correlations in the structure determination of coumarin nucleus ................................................................. 71
Figure 20: Key HMBC (→), COSY (↔) and ROE (↔) seen for the 1-acetoxypropyl substituent ............................................................... 71
Figure 21: Key HMBC (→) and COSY (↔) seen for the geranyl substituent ...................................................................................... 72
Figure 22: ROE (↔) seen for the geranyl substituent ............................ 73
Figure 23: Key HMBC (→), COSY (↔) and ROE (↔) correlations in 2-methylbutyryl substituent ......................................................... 73
List of Schemes

**Scheme 1:** Formation of phenoxide ion by a polyphenol ....................................... 32

**Scheme 2:** Formation of oxonium ion by a polyphenol ......................................... 32

**Scheme 3:** Extraction and isolation procedure of MeOH extract

of *M. Odorata* ............................................................................................................. 33

**Scheme 4:** Isolation and extraction procedure for EtOAc extract

of *M. odorata* ............................................................................................................ 49

**Scheme 5:** Isolation and extraction procedure for DCM extract

of *M. odorata* ............................................................................................................. 67
CHAPTER 1: Introduction

1.1 The Significance of Ethnobotanic Medicine to Drug Discovery

Since the dawn of civilization, humans have relied upon the natural wealth bestowed upon them by the terrestrial and marine worlds to sustain their livelihood. In the process of providing for their basic needs, humans discovered the art of utilizing natural therapeutics to alleviate their health problems. For centuries, plants have formed the nucleus of such traditional medicinal practices to cure a myriad of diseases. People belonging to various societies of both the Eastern and Western worlds have acknowledged the health benefits associated with the medicines derived from plants or ethnobotanic medicine. The traditional use of ethnobotanic medicine by humans of either prehistoric or historic times has been instrumental in the discovery of modern day medicinal drugs. Out of the 119 drugs from 90 plant species used worldwide in the early 21st century, 74% had been isolated from plants used in traditional medicine.

The plant kingdom comprises approximately 250,000 to 500,000 species of plants, out of which a minute percentage (1-10%) is used as food source. It is estimated that 35,000 to 70,000 species of plants are attributed to medicinal use throughout the world. This is evidenced by numerous accounts of plant or plant derived therapeutics. Fossil records dating back 60,000 years ago to the Middle Palaeolithic age show the use of hollyhock by the Neanderthals for healing purposes. The present day Homo sapiens of varied cultural origins have perpetuated such practices, each possessing a unique collection of their own pharmacopoeia. The traditional Indian system of medicine or Ayurveda, which means the science of life, is one of the most ancient yet living traditions in India and its neighbouring countries. Its history goes back to 5000 years B.C., however, its concepts have been perfected between 2500 to 500 years B.C. Literary texts such as the Rig and Atharva Vedas, and the Ayurvedic pharmacopoeia show a dominance of plant based formulations as therapeutics. Records such as the Chakra Samhita (900 B.C.) and Sushutra Samhita (600 B.C.) document a total of 341 and 395 medicinal plants used in Ayurvedic practices respectively. Ramayana, a renowned Indian epic, describes the use of a
herb called *Sanjeevani* to resurrect person(s) from the state of death. Recent findings\(^{12,13}\) suggest that this mythical herb could be *Selaginella bryopteris*, a lycophyte, which has the ability to cure heat shocks. Extracts from *S. bryopteris* have also shown to prevent neuro-degeneration;\(^{12}\) whereas isolated bioflavonoids such as 7,4',7''-tri-O-methylamentoflavone (1) have demonstrated significant antiplasmodial activity (IC\(_{50}\) of 0.26 μM) against the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine).\(^ {14}\)

Additionally, Ayurvedic practices have led to the isolation of plant natural products that have proven noteworthy in drug discovery. Examples include: reserpine (2) from *Rauwolfia serpentina* (Sarpagandha), used as a hypertensive drug and tranquilizer;\(^ {7,15}\) curcumin (3) from *Curcuma longa* L. (Turmeric) used as an anti-inflammatory,\(^ {7}\) antioxidant and for cognitive disorders;\(^ {16}\) guggulsterone (4) and withanolide (5) from *Commiphora mukul* (Guggul) and *Withania somnifera* (Ashwagandha) respectively for treatment of arthritis.\(^ {8}\)

Another commendable medicinal system of the Eastern world belongs to the Chinese. It is also one of the most ancient practices and remains largely existent
throughout China.\textsuperscript{17} The oldest recorded form of Chinese \textit{Materia Medica} is the \textit{Wu Shi Er Bing Fang} (1100 B.C.), which contains 52 prescriptions. Works such as the Shannong Herbal (100 B.C.) and Tang Herbal (659 A.D.) containing 365 and 850 drugs respectively, were later documented.\textsuperscript{1} Quite a number of plant metabolites contributing to the modern drug discovery have been identified through the traditional Chinese medicinal practices. These are ephedrine (6) from \textit{Ephedra sinica} (Ma huang) used for the treatment of asthmatic and bronchial problems;\textsuperscript{1,4} artemisinin (7) from \textit{Artemisia annua} (Qinghao) used as an antimalarial drug;\textsuperscript{4,15} and a recently approved drug, arteether (8), which is a derivative of artemisinin and is also used as an antimalarial drug.\textsuperscript{5}

Contrary to the prominence of the traditional Chinese and Indian medicinal systems, it was the ancient Western system of medicine on which the foundation of modern drug discovery was laid. Hippocrates (late 500 B.C.) reported the use of 300 to 400 medicinal plants.\textsuperscript{6} The Greek philosopher and scientist, Theophrastus (300 B.C.), Greek physician, Dioscorides (100 B.C.), and Galen (130-200 A.D.), who taught pharmacy and medicine in Rome made substantial contributions towards the use of herbal drugs. \textit{De Materia Medica} written by Dioscorides formed the prototype for modern pharmacopoeia. On the basis of these and some other works, the \textit{London pharmacopoeia} that initiated the era of drug discovery and development, as the active constituents of commonly used medicinal plants could be isolated and synthesized, was published in 1618.\textsuperscript{1} As a result, morphine (9), the first pharmacologically active pure compound was isolated from \textit{Papaver somniferum} (Poppy) in the early 1800s.\textsuperscript{1,18} By 1990, about 80\% of the drugs were either natural products or analogs inspired by them; which provided treatment for a multitude of diseases. This has resulted in a current life expectancy of 77 years or more from about 40 years in the early 20th century.\textsuperscript{18}
1.2 Plants of the Genus *Mammea* L. (Clusiaceae)

The genus *Mammea* L. belongs to the family Clusiaceae (also known as Guttiferae) and subfamily Kielmeyeroideae. It is composed of 75 species of plants, with its centre of diversity in Madagascar. Some of the currently known plant species of this genus previously belonged to the genus *Ochrocarpus* or *Ochrocarpos*. Physical characteristics of the *Mammea* plants include being glabrous, woody, evergreen, and small to medium sized trees with perulate buds. Morphologically the leaves are rarely whorled, opposite with some variation in size and shape. On the basis of anatomical variation in the petiole and the lamina, different species of *Mammea* are categorised. The variation in the leaf petioles is due to a complex folding pattern of the vascular bundles; whereas variation in lamina is due to varying patterns of fibre motifs associated with the vascular tissue. A similar pattern of fibre motifs observed amongst the various species allow them to be identified to the four subgroups within *Mammea*. These are Americana, Eugenioides, Bongo, and Malcomberi groups.\textsuperscript{19} Chemical and biological investigations of some of the *Mammea* species have resulted in the isolation of structurally diverse, bioactive metabolites. These species are: *M. americana* (West Indies and United States) and *M. africana* (Africa) of the Americana group; and *M. acuminata* (Indonesia and Malaysia), *M. longifolia* (India), *M. odorata* (Fiji), and *M. siamensis* (Thailand) of the Eugenioides group.

1.2.1 *Mammea odorata* (Rafin.) Kosterm.

This section of the chapter describes *M. odorata* (Rafin.) Kosterm., which is the plant under investigation. *M. odorata* was previously known as *Ochrocarpus odoratus* (Rafin.).\textsuperscript{20} This plant is indigenous to the South Pacific, found only in Fiji, Guam and Papua New Guinea. It is commonly known as Vetao or Uvitao in Fijian and Chopak in Chamorro.\textsuperscript{20,21} The tree is found in the coastal areas growing amongst the mangroves. It grows up to 15 m in height, with close-grained wood. Morphological features include oppositely placed leathery, ovate leaves that are about 25 cm in length; fragrant, small flowers and oblong fruits that are 9 cm long (see Figure 1).\textsuperscript{22} Traditionally, the milky plant sap was utilized by the Fijians to dye
their hair an orange-brown colour.\textsuperscript{20,22} The plant bark has been reported to be used for medicinal purpose by the Chamorros from Guam.\textsuperscript{21}

\textbf{Figure 1}: Morphological features of \textit{Mammea odorata}.\textsuperscript{22}
1.3 Chemical Diversity of *Mammea* species

Chemical investigations of *Mammea* species was initiated by Morris and Pagán in 1953 through their work of isolating pure toxic compounds from the seeds of *M. americana* L. Since then, *M. americana* and various other species of *Mammea* such as *M. africana*, *M. acuminata*, *M. longifolia*, *M. odorata*, and *M. siamensis* have been studied for their chemical composition. Examination of a range of biota samples, for example, fruit pulp, seeds, flowers, leaves, twigs, bark, heartwood and roots have resulted in the isolation of more than 100 natural product compounds of varied structure classes, namely, coumarins, xanthones, flavonols, etc. Initially, a combination of column, analytical and preparative layer chromatography employing normal phase separations of the non polar extracts furnished coumarins; whereas xanthones were obtained from the polar extracts. Characterization of the isolated natural products was possible through degradative analyses together with 1D $^1$H NMR, IR, UV and mass spectral data. Comparison of the spectral data obtained for the natural product with that of its synthetic homologue complemented structure elucidation. However, such techniques involved large amounts of biota sample and time. Technological advancements such as the utility of normal and reverse phase liquid chromatography (HPLC and LCMS) and gas chromatography allowed such compounds to be isolated from minimum amounts of biota sample in a lesser time. Structure elucidation through degradative and synthetic studies was overcome by the use of 1D and 2D NMR spectral data. The following subsections provide a review of the chemical composition of *Mammea* species with a greater focus on the coumarin and xanadione diversity.

1.3.1 The *Mammea* Coumarins (12-81)

Coumarins are the principal secondary metabolite constituent of the *Mammea* species. The *Mammea* coumarins are oxygen-heterocyclic compounds comprising a 5,7-oxygenation pattern (10) or a phloroglucinol nucleus (11). On the basis of structural variation, the coumarins are categorised into various subgroups called the mammea series. Thus, the trivial nomenclature of the coumarins is a result of the series nomenclature. The mammea A, B, C, D and E series consist of a phenyl,
propyl, pentyl, 1-methylpropyl and 1-acetoxypropyl substituents respectively, on C-4 of the 5,7-dihydroxycoumarin nucleus. The substituents on the C-6 and C-8 are combinations of isoprenyl, geranyl, butyryl, 2-methylpropionyl, 2-methylbutyryl and 3-methylbutyryl moieties. Another set of series called the mammea cyclo D, E and F is formed by the 5,6-; 6,7-; and 7,8- annulated coumarins.

Mammein (12) was the first coumarin to be isolated as the toxic constituent of the seeds of *M. americana*. It was a unique representation of naturally occurring coumarin having a 4-propyl substituent. Re-examination of the seeds established mammein to be actually a mixture of 4-propyl coumarins, mammea B/BA (12), B/BB (13) and B/BC (14). Further studies on the *M. americana* seeds revealed three additional coumarins, mammea B/AA (isomammein) (15), mammea B/AB (16) and mammea B/AC (17) that are isomers to 12, 13 and 14 respectively. Concurrent studies by Carpenter et al. on the seeds of *M. africana* G. Don resulted in only 13, whereas both 13 and 16 were obtained from the bark. Another study on the seeds of *M. americana* and bark of *M. africana* furnished mammea B/BD (18) and B/AD (19). A final representative of the 4-propyl coumarin is surangin A (20) that was isolated from the roots of *M. longifolia* by Joshi et al. Some of these investigations also resulted in the isolation of another set of coumarins, whereby the isopropyl and acyl substituents on either C-6 or C-8 remained the same but propyl substituent on C-4 was replaced with a phenyl substituent. These are mammea A/AA (mammeisin) (21) from the fruit pulp and peelings; and mammea A/AB (22), A/BA (23) and A/BB (24) from the seeds of *M. americana*. The bark of *M. africana* also contained 21 and 22. Later, Thebtaranonth et al. showed the presence of mammea A/AC (25) in the flowers of *M. siamensis*.39
In addition to the 4-propyl and 4-phenyl coumarins, *Mammea* species have also proven to be in abundance of coumarins with other substituents on C-4. Coumarins with a 4-pentyl substituent are *Mammea* C/BB (26) and C/OB (27) from the seeds of *M. americana*\(^{27,28}\) and the bark of *M. africana*\(^{32,40}\) respectively. The 1-hydroxypropyl moiety is a rare substituent on the C-4 of *Mammea* coumarins and thus, does not belong to any of the mammea series. It is exemplified by surangin C (28) from the bark of *M. longifolia*\(^{41}\) and therapin B (29) from the seeds of *M. siamensis*.\(^{42}\) The isolation of toxic principles from the seeds of *M. americana* provided an impetus for isolation of numerous coumarins from the seeds. However, none of these coumarins accounted for the insecticidal nature of the light petroleum seed extract. Isolation of coumarins bearing a 1-acetoxypropyl substituent on C-4, proved otherwise. mammea E/BA (30), E/BB (31) and either of E/BC (32) or E/BD (33) from the seeds of *M. americana*, together with surangin B (34) from the roots of *M. longifolia* have shown significant insecticidal activity against mustard beetles and houseflies.\(^{43,44}\)
Being a remarkably rich source of over 20 biogenetically similar coumarins of the mammea A-C and E series, *Mammea* species also comprise the annulated coumarins belonging to the cyclo D, E and F series. The cyclo D series is formed by coumarins having a 2,2-dimethylchromene moiety resulting from the oxidative cyclisation between the ortho hydroxy group (C-5 or C-7) and the isoprenyl substituent (C-6 or C-8). Examples of 5,6-annulated cyclo D coumarins include, mammea A/BB cyclo D (35) from *M. americana*; and mammea E/BA cyclo D (36), E/BB cyclo D (37), E/BC cyclo D (38) and E/BD cyclo D (39) from *M. siamensis*. Examples of 7,8-annulated cyclo D coumarins include, mammea A/AA cyclo D (mammeigin) (40), mammea A/AB cyclo D (41), B/AA cyclo D (42), B/AB cyclo D (43), B/AC cyclo D (44), C/AA cyclo D (45) and C/AB cyclo D (46) from *M. americana* and *M. africana*; mammea A/AD cyclo D (47), B/AD cyclo D (48) from *M. americana*; and mammea E/AC cyclo D (49) from *M. siamensis*. The cyclo E series includes coumarins having 3-hydroxy-2,2-dimethylidihydropyran moiety formed by the hydroxy substituent on C-7 and isoprenyl substituent on C-6. Representatives, mammea B/BA cyclo E (50), B/BB cyclo E (51) and B/BC cyclo E (52) are found in the *M. americana* seed oil.
Furthermore, the mammea cyclo F series contains coumarins with a 2-(1-hydroxy-1-methyl-ethyl)dihydrofuran moiety formed between either C-5 and C-6 or C-7 and C-8.\textsuperscript{45} Representatives of the 5,6-annulated cyclo F coumarins are mammea B/BA cyclo F (53), B/BB cyclo F (54), B/BC cyclo F (55) and B/BA hydroxycyclo F (56) from \textit{M. americana};\textsuperscript{49,50} and siamenols A-D (57-60) and ochrocarpin E (61) from \textit{M. siamensis}\textsuperscript{51} and \textit{M. punctatus}\textsuperscript{52} respectively. The occurrence of peroxide derivatives of the cyclo F coumarins (62-64) from \textit{M. americana} has also been reported.\textsuperscript{49,50}
Numerous investigations have also yielded the 7,8-annulated cyclo F coumarins. These are mammea A/AB cyclo F (65) and B/AB cyclo F (66) from *M. americana*\(^{29,30}\) and *M. africana*,\(^{31,32}\) mammea A/AA cyclo F (67), A/AC cyclo F (68), A/AD cyclo F (69), B/AA cyclo F (70) and B/AC cyclo F (71) from *M. americana*;\(^{29,30}\) and ochrocarpins F (72) and G (73) from *M. punctatus*.\(^{52}\) Ochrocarpins A-D (74-77) from *M. punctatus*\(^{52}\) and pyranocoumarins 1-4 (78-81) from *M. siamensis*\(^{53}\) are recently isolated coumarins that do not belong to any of the Mammea series.

### 1.3.2 Xanthones (82-103) from *Mammea* species

Xanthone is another class of natural product compounds that is found in various *Mammea* species. Its dominance follows that of the coumarins, with over 20 naturally occurring xanthones isolated from *Mammea* species so far. These xanthones have a mono-, di-, tri-, and tetra-oxygenation pattern, whereby the hydroxyl and methoxyl groups are the common substituents. Despite the presence of
isoprenyl and geranyl substituents on Mammea coumarins, prenylated and glycosylated xanthones have not yet been isolated from or identified in any of the *Mammea* species. Investigations on the non-polar extract of the seeds of *M. americana* has largely furnished coumarins; however, the polar extracts of the seeds have generously yielded xanthones such as 2-methoxyxanthone (82), 38 2- and 4-hydroxyxanthones (83, 84) and 1,5- and 1,7-dihydroxyxanthones (85, 86).54 Likewise, studies by Carpenter et al. on the chloroform extract of the heartwood of *M. africana* has revealed 85 and 86 together with 1,5,6- and 1,6,7-trihydroxyxanthones (87, 88), 5-hydroxy-1-methoxyxanthone (89), and 1,3,5,6- and 1,3,6,7-tetrahydroxyxanthones (90, 91).55

In addition, Locksley and Murray in their examination of the heartwood of *M. odorata* (*O. odoratus*) isolated 3-hydroxy-2-methoxyxanthone (92) and 2,3,4-trihydroxyxanthone (93) along with 83, 85, 87, 90 and 91.56 Investigation of the timber extracts of *M. acuminata* led to the isolation of 82-86, 89 and 92 with 2,6-dihydroxyxanthone (94) and 1-hydroxy-7-methoxyxanthone (95).57 Later investigations on the bark and stem of *M. acuminata* revealed 2,7-dihydroxyxanthone (96).58 Acuminols A (97) and B (98), which are the only two representatives of furanoxanthones in *Mammea* species, were also isolated from the same source.59 A study on the twigs of *M. siamensis* by Poobrasert et al. furnished 1,2-dimethoxy-5-hydroxy- and 1,3-dimethoxy-5-hydroxyxanthones (99, 100), 2,5-dihydroxy-1-methoxy- and 3,5-dihydroxy-1-methoxyxanthones (101, 102), 1,3,7-trihydroxyxanthone (103) with 85 and 89.60
1.3.3 Flavonols (104-115) and Minor Compounds from Mammea Species

Some of the Mammea species contain flavonols in addition to coumarins and xanthones. Examples include quercetin and its derivatives (104-108), kaempferol and its derivatives (109-113), and proanthocyanidin oligomers (114) from the polar extracts of M. longifolia buds;\textsuperscript{61} and (-)-epicatechin (115) from the stems and bark of M. acuminata.\textsuperscript{58} Some of the minor compounds of Mammea species such as vitexin, meso-inositol, β-sitosterol 3-O-β-D-glucopyranoside and taraxerol have also been isolated from M. longifolia.\textsuperscript{41,61,62} Investigation of the flowers of M. siamensis has resulted in sterols, β-sitosterol and stigmasterol, and a terpenoid, friedelin, which was also isolated from M. americana.\textsuperscript{38,63}
1.4 Biological Investigations of Metabolites from *Mammea* Species

Investigations leading to a multitude of natural product compounds from *Mammea* species were inspired by the toxic activity of seeds of *M. americana* L. Contrary to the extent of isolated compounds from various species of *Mammea*, less than half of these compounds have been assayed for biological activities. The toxic constituent of *M. americana* seeds, which is 20% toxic compared to rotenone, had a LD₅₀ of 0.25 ppm in a guppy bioassay.²³ The toxic nature of the seed constituent was due to coumarins 12-14.²⁷,²⁸ Compounds 12-14 along with 21 demonstrated cytotoxicity against the Sarcoma 180 tumour cells with ID₅₀ of less than 0.1 μg/mL.⁶⁴ Sarcoma 180 inhibition activity was also displayed by xanthones 83-86, however, at higher concentrations (ID₅₀: 6.6-25 μg/mL).⁶⁵ Cytotoxic studies by Ouahouo et al. showed that 12, 13, 23, 27 and 65 inhibited 9-KB carcinoma cell activity in a range of 35-75% at 1 μM.⁶⁶ Screening of 12-14, 21 together with 18, 30-33, 40, 47, 53, 56 and...
by Yang et al. identified them to be active against human colon cancer cell lines: SW-480 (IC\textsubscript{50}: 13.9-88.1 \(\mu\)M), HT-29 (IC\textsubscript{50}: 11.2-85.3 \(\mu\)M) and HCT-116 (IC\textsubscript{50}: 10.7-76.7 \(\mu\)M).\textsuperscript{67} Moreover, \textsuperscript{28} and \textsuperscript{29} with respective IC\textsubscript{50} ranging from 0.78-2.56 \(\mu\)g/mL and 3.52-4.64 \(\mu\)g/mL were recently\textsuperscript{42} shown to be active against breast adenocarcinoma (MCF-7), human cervical cancer (HeLa), human oral cancer (KB) and HT-29. Compounds \textsuperscript{36}, \textsuperscript{37}, \textsuperscript{85} and \textsuperscript{89} proved to be inactive against all the cancer cell lines. Cytotoxicity against A2780 ovarian cancer cell line has been demonstrated by \textsuperscript{61}, \textsuperscript{72-77}.\textsuperscript{52} In another study, \textsuperscript{13} isolated from Calophyllum brasiliense (Clusiaceae) induced apoptosis in human leukaemia H-L60 cells by activating the caspase-9/caspase-3 pathway, indicating that \textsuperscript{13} might be noteworthy as an antitumour agent.\textsuperscript{68}

Moreover, \textsuperscript{21} and \textsuperscript{23} have both demonstrated antibacterial activity against Escherichia coli C600 (MIC: >256 \(\mu\)g/mL), methicillin sensitive Staphylococcus aureus, MSSA (MIC: 8 and 1 \(\mu\)g/mL respectively) and methicillin resistant \textit{S. aureus}, MRSA (MIC: 8 and 2 \(\mu\)g/mL respectively).\textsuperscript{69} Susceptibility assays of \textsuperscript{12}, \textsuperscript{13}, \textsuperscript{21} and \textsuperscript{65} have shown significant activity against \textit{S. aureus}, with zone of inhibitions measuring 15 to 18 mm.\textsuperscript{66} \textsuperscript{34} has also been reported to possess antibacterial and antifungal activity.\textsuperscript{44} Besides pure compounds, crude extracts of \textit{M. americana},\textsuperscript{69} \textit{M. africana}\textsuperscript{66,70} and \textit{M. siamensis}\textsuperscript{63} have shown to be bioactive against a number of Gram positive and Gram negative microbes, such as \textit{S. aureus}, \textit{E. coli}, Staphylococcus epidermidis, Enterococcus hirae, Bacillus subtilis and Candida albicans.

Mammea E coumarins (\textsuperscript{30}-\textsuperscript{33}) exhibited insecticidal activity against mustard beetles and houseflies at concentrations below 0.5 \(\mu\)g/ml. However, \textsuperscript{34} displayed better activity against houseflies than \textsuperscript{30}-\textsuperscript{33}, and was also toxic to mosquito larvae. The insecticidal nature of these coumarins may be due to the uncoupling of oxidative phosphorylation caused by the 1-acetoxypropyl substituents on C-4.\textsuperscript{43,44} In addition, antioxidant activity is also displayed by compounds from \textit{Mammea} species. The MeOH extract of \textit{M. longifolia}, which contained flavonols (\textsuperscript{104}-\textsuperscript{114}), showed radical scavenging properties, with the activity ratio of 3.2 mg of extract to 1 mg of rutin.\textsuperscript{61} Another investigation of the MeOH extract of \textit{M. longifolia} furnished \textsuperscript{115} that showed strong chemiluminescence and DPPH radical scavenging activities in
comparison with rutin. The MeOH extract together with aqueous EtOH extracts have proven to be active in numerous in vitro experiments such as DPPH scavenging assay; anti-lipid peroxidation assay; hydroxyl-, hydrogen peroxide-, and superoxide-radical scavenging assays. The aqueous EtOH extract is more potent than the MeOH extract; whereby its greater activity can be attributed to a higher amount of epicatechin equivalents (203 ± 3.18 mg/g) than that of the MeOH extract (152 ± 2.29 mg/g). The aqueous MeOH extract of *M. americana* has also exhibited antioxidant activity in DPPH scavenging assay (IC$_{50}$: 7.9 ± 2.7 µg/mL). Some coumarins (12, 14, 18, 21, 31, 32) have also shown antioxidant activity.

Other activities include antisecretory and/or gastrotective effects of the EtOH and DCM extracts of *M. americana* fruit. These extracts had the ability to protect gastric mucosa against detrimental effects resulting from necrotizing agents, hypothermic restraint stress, NSAIDs and pylorus ligation. In another investigation, isolated from *C. brasiliense*, was shown to inhibit the activity of gastric H$^+$,K$^+$-ATPase, which controls acid secretion with IC$_{50}$ of 109.5 ± 7.7 µM. The occurrence of in non-polar seed and fruit extracts of *M. americana* may account for the antiulcer effects of DCM extract in the previous study. The leaf extract of *M. americana* was shown to possess molluscicidal activity against *Biomphalaria glabrata* (100% lethality at 50 ppm).

Additionally, pure compounds as well as extracts from *M. africana* are potent inhibitors of enzyme activities. Examples include inhibition of the enzyme urease by 70, inhibition of β-lactamase activity by MeOH extract of the bark, and inhibition of transaminases and alkaline phosphatase activities by EtOH extract of the stem bark. Bioassay-guided fractionation of the DCM extract of the stem bark has identified coumarins, 12, 13 and 21 to possess some vasorelaxant properties, which might be useful for the treatment of hypertension. Another study by Nguelefack-Mbuyo et al., on the MeOH/DCM extract of the stem bark of *M. africana*, has shown that it has vasodilating properties, which traditionally may have been useful in the treatment of arterial hypertension.
1.5 The Plight of Malaria

Malaria is a grievous infectious disease, causing death of approximately 1-2 million people with 300-500 new clinical cases reported each year.\textsuperscript{81,82} It is highly prevalent in developing countries, whereby a total of 109 countries were endemic to malaria in 2008.\textsuperscript{83} Malaria is transmitted by the female \textit{Anopheles} mosquito, which is the vector for the malarial parasites \textit{Plasmodium falciparum}, \textit{P. vivax}, \textit{P. ovale} and \textit{P. malariae}. \textit{P. falciparum} is the most potent of the four \textit{Plasmodium} species and is responsible for the majority of the infections in Africa.\textsuperscript{84} Chloroquine, a synthetic and inexpensive drug inspired from the natural product, quinine, isolated from \textit{Cinchona succiruba}, has been effective in the treatment of malaria.\textsuperscript{81,83} However, the emergence of drug resistant strains of malarial parasites (especially to chloroquine) is a significant contributor towards serious health problems.\textsuperscript{83} As a result, the World Health Organisation (WHO) has recommended the use of combinatorial therapy to counteract resistance. At present, the ‘Artemisinin Combination Therapy’ is the treatment of choice but artemisinin-resistant \textit{in vitro} isolates of \textit{P. falciparum} have been found.\textsuperscript{85} Therefore, a quest for the development of new resistant antimalarial drugs, whether of a natural product or a synthetic origin, is indispensable.

Plants belonging to the genus \textit{Mammea} have been a generous source of bioactive secondary metabolites. Interestingly, none of the \textit{Mammea} compounds have been assayed for antimalarial activity. Only the crude extract of \textit{M. longifolia} (100 mg/mL) was shown to inhibit the growth of the malaria parasite by 86%.\textsuperscript{86} On the contrary, compounds isolated from genera \textit{Garcinia} and \textit{Calophyllum} (also members of Clusiaceae) have been assayed and shown to possess significant antimalarial activity. Examples include prenylated xanthones, cowaxanthone (IC\textsubscript{50} = 1.50 μg/mL), calothwaitesixanthone (IC\textsubscript{50} = 2.7 μg/mL) and mangostin (IC\textsubscript{50} = 17 μM) from \textit{G. cowa}, \textit{C. caledonicum} and \textit{G. mangostana}, respectively.\textsuperscript{81} Isolation of antimalarial compounds from \textit{Garcinia} and \textit{Calophyllum} suggests that \textit{Mammea} species such as \textit{M. odorata} may yield compounds having similar antimalarial activity.
1.6 The Objectives of this Study

The plants of genus *Mammea* are widespread through the world and are a source of more than 100 natural product compounds of enormous structural diversity. Some of these compounds have displayed significant biological activities. A species of *Mammea*, which is indigenous to the Pacific Islands, is *Mammea odorata*. Previous studies on the chloroform and acetone extracts of the heartwood of *M. odorata* have yielded seven hydroxy- and methoxy-substituted xanthurones, for which biological activities have not been reported. Therefore, the main aim of this study is to carry out chemical and biological investigations of metabolites from the stem bark of *Mammea odorata*.

The objectives of this study are to:

- obtain polar (MeOH and EtOAc) and non-polar (DCM) extracts of the stem bark of *M. odorata*;
- isolate new compounds from the three extracts using flash chromatography and HPLC separation techniques;
- characterize the isolated compounds using a combination of spectral data obtained through 1D and 2D NMR experiments (\(^1\)H and \(^{13}\)C NMR, gradient COSY, HMBC, HSQC and ROESY), mass spectroscopy (HRESIMS and LRESIMS) along with UV and IR data;
- perform *in vitro* antimalarial assays employing 3D7 (chloroquine-sensitive) strain of *P. falciparum* and;
- perform *in vitro* antimicrobial susceptibility assays of the MeOH, EtOAc, and DCM extracts and the isolated compounds against nine human pathogenic bacteria using the disc diffusion method.
CHAPTER 2: Experimental Methods

2.1 General Experimental

NMR spectra were recorded at 30 °C on either a Varian 500 MHz or 600 MHz Unity INOVA spectrometers. The 500 MHz and 600 MHz spectrometers were equipped with a 768AS Varian autosampler and a triple resonance cold probe respectively. The $^1$H and $^{13}$C NMR chemical shifts are expressed in ppm and were referenced to the solvent peak for DMSO-$d_6$ at $\delta_H$ 2.49 and $\delta_C$ 39.5, and for CDCl$_3$ at $\delta_H$ 7.27 and $\delta_C$ 77.2. Standard Varian parameters were used for the gradient COSY, ROESY, HSQC and HMBC experiments.

LRESIMS were recorded on a Mariner mass spectrometer and spectral analysis was performed using Data Explorer software. HRESIMS were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer equipped with an Apollo atmospheric pressure ionisation (API) source. Specific rotation, $[\alpha]_D$, was measured on a Jasco J-715 spectrophotometer. UV spectra were recorded on a Perkin Elmer Lambda 25 UV/Visible spectrometer while the IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer.

The stationary phase used for flash chromatography was Kieselgel 40-63 μm 60 Å Si and LiChroprep RP-18 40-63 μm C$_{18}$ bonded Si. PAG, Machery Nagel Polyamide CC6 (0.05-0.016 mm) was used for hydrolysable tannin and/or polyphenolic removal. Alltech Davisil 40-60 μm 60 Å C$_{18}$ bonded Si was used for pre-adsorption work. Alltech stainless steel cartridge (10 × 30 mm) was used for the loading of the pre-adsorbed material. Phenomenex (Luna) 5 μm C$_{18}$ (semi-preperative: 21.2 mm x 250 mm) and Thermo Hypersil-Keystone Betasil 5 μm C$_{18}$ (semi-preparative: 10.0 mm x 250 mm, and 21.2 mm x 150 mm) HPLC columns were used. A Waters 600 pump equipped with a Waters 996 PDA detector, Waters 717 autosampler and Gilson 204 fraction collector were used for HPLC separations. Waters HPLC system was operated using the Millennium 32 software. All HPLC fractions were monitored by UV detection at wavelengths 254, 280, 320, 350 and 380 nm on the PDA detector.
Analytical TLC was performed using Analtech Uniplate RSP-F$_{254}$ C$_{18}$ and Merck DC-Platten Kieselgel F$_{254}$ Si plates. TLC plates were visualized under UV light at 254 nm. Reagents used for the visualization of phenols were methanolic ferric chloride (1% FeCl$_3$ in 50% MeOH); and phosphomolybdic acid (8% PMA in 100% EtOH) followed by heating above 100 °C.

Pure isolates of all the microorganisms were obtained from the Institute of Applied Sciences, Suva, Fiji, except for MRSA, which was obtained from the Pathology Department of Colonial War Memorial Hospital, Suva, Fiji. These isolates were preserved in 80% glycerol at -70 °C prior to use. Mueller Hinton agar (MHA) was used for the susceptibility testing, whereas Mueller Hinton broth (MHB) was used for culturing of the isolates. Before using the media and the consumables for the antimicrobial assays, they were sterilized in an autoclave at 121 °C for 15 min. Aseptic techniques were strictly followed to minimize contamination.

2.2 Chemical Investigations of *Mammea odorata*

2.2.1 Collection and Extraction

The stem bark of *Mammea odorata* (Rafin.) Kosterm. was collected near the coastal region of Deuba, Viti Levu, Fiji during June, 2007. The biota sample was identified by the curator, Mr Marika Tuiwawa, of South Pacific Regional Herbarium.

Air-dried and ground bark sample (100.00 g dry wt) was transferred to a conical flask, 2 L of n-hexane added and the n-hexane/plant material mixture stirred on a magnetic stirrer for 24 h. The n-hexane extract was filtered under vacuum and kept aside for it largely constituted of lipids and was not investigated in this study. The defatted residue was sequentially extracted with DCM (2 L), EtOAc (2 L) and MeOH (2 L) each for 24 h under stirring on a magnetic stirrer at room temperature. The resulting DCM, EtOAc and MeOH extracts were individually dried under reduced pressure to yield 2.70 g of mustard yellow gum, 0.63 g of brown solid and 9.95 g of red solid respectively.
2.2.2 Isolation of Compounds (115-117) from MeOH Extract

Prior to the separation of the MeOH extract, four separate experiments were carried out to establish the effects of variables such as PAG and TFA on the resolution of HPLC chromatogram. To observe the effect of PAG, 500 mg of the extract was pre-adsorbed on C_{18} bonded Si gel, packed into a cartridge, which was subsequently attached to a Betasil C_{18} (21.2 mm x 150 mm) semi-preparative HPLC column. Isocratic conditions of 95% H$_2$O (0.1% TFA)/5% MeOH (0.1% TFA) were run for the first 1 min, then a linear gradient to 100% MeOH (0.1% TFA) was run over 49 min, followed by isocratic conditions of 100% MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty 1 min (9 mL) fractions were collected from time = 0 min. A second sample of the MeOH extract (500 mg) was suspended in MeOH (50 mL), loaded onto MeOH conditioned PAG (15 g) in a sintered glass column and washed with MeOH (500 mL). The resulting extract (128 mg) was taken and pre-adsorbed on C_{18} bonded Si gel. The pre-adsorbed material was packed into a cartridge and attached to the same HPLC column and subjected to the same HPLC separation conditions as that of the first sample.

To observe the effect of TFA on the HPLC chromatogram, the above two experiments were repeated without the use of TFA in the mobile phase solvents (H$_2$O and MeOH). A comparison of the HPLC chromatograms obtained from the four experiments showed that the use of PAG to remove tannins/polyphenols from the MeOH extract and the use of TFA as a modifier in the HPLC mobile phase gives better resolved peaks in HPLC separation. Thus these conditions were adapted for the following separations.

The MeOH extract (2.30 g) was suspended in MeOH (200 mL), loaded onto the MeOH conditioned PAG (80 g) and washed with MeOH (2 L). The resulting extract (644 mg) was pre-adsorbed on C_{18} bonded Si gel, packed into a cartridge, which was then attached to a Betasil C_{18} (21.2 mm x 150 mm) semi-preparative HPLC column. Isocratic conditions of 95% H$_2$O (0.1% TFA)/5% MeOH (0.1% TFA) were employed for 1 min, then a linear gradient to 100% MeOH (0.1% TFA) was run over 49 min, followed by isocratic conditions of 100% MeOH (0.1% TFA) for further 10 min, all at a flow rate of 9 mL/min. Sixty 1 min (9 mL) fractions were
collected from time = 0 min. These fractions were analysed by (+) and (-)-
LRESIMS. Fraction 21 contained a single molecular ion peak (m/z 290) showing the
presence of a pure compound, which was confirmed by its 1H NMR spectrum. Thus
fraction 21, eluting at 54% H₂O (0.1% TFA)/46% MeOH (0.1% TFA) yielded (-)-
epicatechin (115, 20.2 mg, 0.087% dry wt).

Fractions 28-34 (35.6 mg) contained ions at m/z 390 and 405 with an additional ion
at m/z 162. 1H NMR spectra of these fractions confirmed the presence of
glycosylated compounds. A further purification of fractions 28-34 (35.6 mg) was
carried out on the same HPLC column. Isocratic conditions of 60% H₂O (0.1%
TFA)/40% MeOH (0.1% TFA) were run for 1 min, a linear gradient to 20% H₂O
(0.1% TFA)/80% MeOH (0.1% TFA) was run for 49 min, then another linear
gradient to 100% MeOH was run for 5 min, followed by isocratic conditions of
100% MeOH for the final 5 mins, all at a flow rate of 9 ml/min. (+) and (-)-
LRESIMS showed ions of interest in fractions 13 and 21. Fraction 13 eluting at 49%
H₂O (0.1% TFA)/51% MeOH (0.1% TFA), yielded 2,3-dihydroxyxanthone; 3-O-β-
d-glucopyranoside (116, 5.7 mg, 0.025% dry wt) with m/z of 390. Fraction 21 (3.1
mg) was subjected to another semi-preparative HPLC separation utilizing a Betasil
C₁₈ (10.0 mm x 250 mm) column for the removal of minor impurities. Isocratic
conditions of 52% H₂O (0.1% TFA)/48% MeOH (0.1% TFA) for 15 min, then a
linear gradient to 100% MeOH for 10 mins and final isocratic conditions of 100%
MeOH for 5 min were employed with a flow rate of 2.5 mL/min. Thirty 1 min (2.5
mL) fractions were collected at time = 0 min. This afforded pure 1,6,7-
trihydroxyxanthone; 6-O-β-d-glucopyranoside (117, 2.2 mg, 0.010% dry wt), m/z
405, in fraction 16 eluting at 47% H₂O (0.1% TFA)/53% MeOH (0.1% TFA).

**Synthetic and Spectroscopic Data of New Compounds, 116 and 117**

2,3-Dihydroxyxanthone; 3-O-β-d-glucopyranoside (116): pale yellow crystals; [α]D
-52° (c 0.22, MeOH); UV (MeOH) λ<sub>max</sub>
241 (ε 18355; sh), 302 (ε 5919) and 354 nm (ε 4036); (MeOH + NaOH) λ<sub>max</sub>
251 (ε 14349; sh), 316 (ε 4724) and 399 nm (ε 3111); IR ν<sub>max</sub> (KBr) 3396, 1613, 1462,
1322, 1276, 1075 and 755 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 9.36 (1H, brs, 2-OH), 8.16 (1H, dd, 7.8, 1.8, H-8), 7.82 (1H, ddd, 8.4, 7.2, 1.8, H-6), 7.63 (1H, d, 7.8, H-5), 7.49 (1H, brs, H-1), 7.45 (1H, dd, 7.8, 7.2, H-7), 7.31 (1H, brs, H-4), 5.12 (1H, d, 7.2, H-1\(^¹\)), 3.75 (1H, d, 10.2, H-6\(^¹\)), 3.52 (1H, t, 7.2, H-5\(^¹\)), 3.49 (1H, d, 9.0, H-6\(^¹\)), 3.37 (1H, dd, 9.6, 7.2, H-2\(^¹\)), 3.34 (1H, t, 9.0, H-3\(^¹\)), 3.20 (1H, t, 9.0, H-4\(^¹\)); \(^1^3\)C NMR (DMSO-\(d_6\)) \(\delta\) 174.8 (C-9), 155.6 (C-4b), 152.0 (C-3), 150.3 (C-4a), 144.3 (C-2), 134.6 (C-6), 125.7 (C-8), 123.9 (C-7), 120.6 (C-8a), 117.9 (C-5), 115.5 (C-9a), 109.0 (C-1), 103.5 (C-4), 100.5 (C-1\(^¹\)), 77.2 (C-5\(^¹\)), 75.8 (C-3\(^¹\)), 73.1 (C-2\(^¹\)), 69.7 (C-4\(^¹\)), 60.7 (C-6\(^¹\)); (+)-LRESIMS \(m/z\) 391 (100%) [M+H]\(^{+}\) and 229 (23%) [M+H - C\(_6\)H\(_{11}\)O\(_5\)]\(^{+}\); (-)-LRESIMS \(m/z\) 389 (100%) [M-H]\(^-\); (+)-HRESIMS \(m/z\) 413.082 (calcd. for C\(_{19}\)H\(_{18}\)O\(_9\)Na [M+Na]\(^{+}\]); Anal. TLC \(R_f\) 0.48 (C\(_{18}\), H\(_2\)O/MeOH 7:3), homogenous spot (green, methanolic FeCl\(_3\); blue, PMA; purple, under 254 nm).

1,6,7-Trihydroxyxanthone; 6-O-\(\beta\)-D-glucopyranoside (117): bright yellow solid; [\(\alpha\)]\(_D\) -12\(^°\) (c 0.10, MeOH); UV \(\lambda_{max}\) 250 (\(\epsilon\) 1582; sh), 290 (\(\epsilon\) 763) and 382 nm (\(\epsilon\) 667); (MeOH + NaOH) \(\lambda_{max}\) 260 (\(\epsilon\) 1953; sh), 383 (\(\epsilon\) 543) and 412 nm (\(\epsilon\) 436); IR \(\nu_{max}\) (KBr) 3437, 1647, 1457,1204, 1139 and 1078 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 12.81 (1H, s, 1-OH), 9.52 (1H, s, 7-OH), 7.68 (1H, t, 8.4, H-3), 7.47 (1H, brs, H-8), 7.32 (1H, brs, H-5), 7.04 (1H, d, 8.4, H-4), 6.79 (1H, d, 8.4, H-2), 5.36 (1H, d, 4.2, 2\(^¹\)-OH), 5.14 (1H, d, 7.2, H-1\(^¹\)), 5.12 (1H, d, 4.2, 3\(^¹\)-OH), 5.09 (1H, d, 5.4, 4\(^¹\)-OH), 4.64 (1H, dd, 5.4, 4.8, 6\(^¹\)-OH), 3.74 (1H, dd, 10.2, 5.4, H-6\(^¹\)), 3.52 (m, H-5\(^¹\)), 3.48 (1H, dd, H-6\(^¹\)), 3.38 (m, H-2\(^¹\)), 3.34 (m, H-3\(^¹\)), 3.20 (m, H-4\(^¹\)); \(^1^3\)C NMR (DMSO-\(d_6\)) \(\delta\) 180.8 (C-9), 160.9 (C-1), 156.0 (C-4a), 155.0 (C-4b), 152.7 (C-6), 144.2 (C-7), 136.3 (C-3), 114.0 (C-8a), 109.6 (C-2), 108.0 (C-8), 107.6 (C-9a), 106.8 (C-4), 103.1 (C-5), 100.4 (C-1\(^¹\)), 77.1 (C-5\(^¹\)), 75.8 (C-3\(^¹\)), 73.0 (C-2\(^¹\)), 69.5 (C-4\(^¹\)), 60.5 (C-6\(^¹\)); (+)-LRESIMS \(m/z\) 407 (100%) [M+H]\(^{+}\) and 245 (24%) [M+H - C\(_6\)H\(_{11}\)O\(_5\)]\(^{+}\); (-)-LRESIMS \(m/z\) 405 (100%) [M-H]\(^-\); (+)-HRESIMS \(m/z\) 429.081 (calcd. for C\(_{19}\)H\(_{18}\)O\(_9\)Na [M+Na]\(^{+}\]); Anal. TLC \(R_f\) 0.39 (C\(_{18}\), H\(_2\)O/MeOH 7:3), homogenous spot (green, methanolic FeCl\(_3\); blue, PMA; purple, under 254 nm).
2.2.3 Isolation of Compounds (83, 84, 88, 94, 118-120) from EtOAc Extract

The EtOAc extract (580 mg) was pre-adsorbed and packed on top of the 90% H₂O/10% CH₃CN conditioned LiChroprep RP-18 40-63 μm C₁₈ bonded Si (20 g) in an open column. Flash chromatography was performed employing 5% stepwise gradient from 90% H₂O/10% CH₃CN to 100% CH₃CN affording seventy 40 mL fractions. Out of seventy, four of these fractions had deposited crystals upon standing. The crystals were triturated with cold acetone to yield pure compounds. Fraction 20 eluting with H₂O/CH₃CN (7:3) mixture, yielded 2,6-dihydroxyxanthone (94, 1.5 mg, 0.002% dry wt). Fractions 25 and 26, eluting with H₂O/CH₃CN (13:7) mixture, contained pure compounds exhibiting the same analytical TLC Rᵣ and ¹H NMR spectra, thus collectively yielded 2,3-dihydroxyxanthone (118, 8.8 mg, 0.010% dry wt). Pure 2-hydroxyxanthone (83, 4.3 mg, 0.005% dry wt) was obtained from fraction 38, eluting with H₂O/CH₃CN (3:2) mixture. (+) and (-) LRESIMS of 94, 118, and 83 displayed molecular ion peaks at m/z 228, 228 and 212 respectively. Fractions 30-39 were combined based on similar TLC pattern and were subjected to further HPLC purification. Fractions 30-39 (65 mg) was adsorbed on C₁₈ bonded Si and packed into a cartridge, which was then attached to Betasil C₁₈ (21.2 mm x 150 mm) semi-preparative HPLC column. Isocratic conditions of 65% H₂O (0.1% TFA)/35% MeOH (0.1% TFA) were run for 1 min, a linear gradient to 20% H₂O (0.1% TFA)/80% MeOH (0.1% TFA) was run over 49 min, then another gradient to 100% MeOH (0.1% TFA) for 5 min, and a final isocratic conditions of 100% MeOH was utilized for the further 5 min, all with a flow rate of 9 mL/min. Sixty 1 min (9 mL) fractions were collected from time = 0 min. Analysis of these fractions by (+) and (-) LRESIMS displayed single molecular ions peaks at m/z 212, 242, 244 and 274 in fractions 28, 33, 36 and 39 respectively. The purity of these fractions was confirmed by ¹H NMR spectra. Hence, these fractions were lyophilised to yield pure compounds. 4-hydroxyxanthone (84, 1.8 mg, 0.002% dry wt) was obtained from fraction 28 eluting at 39% H₂O (0.1% TFA)/61% MeOH (0.1% TFA). Fractions 33, 36 and 39 yielded 2-hydroxy-3-methoxyxanthone (119, 6.0 mg, 0.007% dry wt), 1,6,7-trihydroxyxanthone (88, 8.0 mg, 0.009% dry wt), and 1,6,7-trihydroxy-3-methoxyxanthone (120, 1.1 mg, 0.001% dry wt) respectively. These fractions eluted
between 35% H₂O (0.1% TFA)/65% MeOH (0.1% TFA) and 29% H₂O (0.1% TFA)/71% MeOH (0.1% TFA).

Synthetic and Spectroscopic Data for a New Natural Product, 118

2,3-Dihydroxyxanthone (118): pale yellow crystals; UV (MeOH) λ<sub>max</sub> 238 (ε 23641; sh), 313 (ε 9788) and 352 nm (ε 9825); (MeOH + 0.1 M NaOH) λ<sub>max</sub> 231 (ε 28209; sh) and 376 nm (ε 14414); IR ν<sub>max</sub> (KBr) 3396, 1614, 1485, 1320 and 748 cm<sup>-1</sup>; H NMR (DMSO-d<sub>6</sub>) δ 8.13 (1H, dd, 7.8, 1.2, H-8), 7.77 (1H, ddd, 8.4, 7.2, 1.2, H-6), 7.58 (1H, d, 8.4, H-5), 7.45 (1H, brs, H-1), 7.41 (1H, dd, 7.8, 7.2, H-7), 6.92 (1H, brs, H-4); C NMR (DMSO-d<sub>6</sub>) δ 174.5 (C-9), 155.4 (C-4b), 154.0 (C-4a), 151.0 (C-3), 143.8 (C-2), 134.2 (C-6), 125.6 (C-8), 123.7 (C-7), 120.8 (C-8a), 117.8 (C-5), 113.5 (C-9a), 108.7 (C-1), 102.7 (C-4); (+)-LRESIMS m/z 229 (100%) [M+H]<sup>+</sup>, 251 (25%) [M+23]<sup>+</sup>, and 479 (19%) [2M+23]<sup>+</sup>; (-)-LRESIMS m/z 227 (100%) [M-H]<sup>-</sup>; (+)-HRESIMS m/z 229.049 (calcd. for C<sub>13</sub>H<sub>9</sub>O<sub>4</sub> [M+H]<sup>+</sup>);

2.2.4 Isolation of Surangin B (34) from DCM Extract

The DCM extract (2.00 g) was pre-adsorbed and filled on top of the 100% n-hexane conditioned Kieselgel 40-63 µm 60 Å Si (60 g) open column. Flash chromatography was performed using mixtures of n-hexane and EtOAc as eluents. Stepwise elutions from 100% n-hexane to 100% EtOAc at 5% intervals afforded eighty-three 40 mL fractions. The fractions were grouped based on similar analytical TLC patterns. The combined fractions 23-36 (595 mg), collected between 60% n-hexane/40% EtOAc and 40% n-hexane/60% EtOAc was shown to contain prenylated compounds by H NMR, hence, were subjected to further HPLC purification. Fraction 23-36 (214 mg) was pre-adsorbed on C<sub>18</sub> bonded Si and packed into a cartridge, which was subsequently attached to a Phenomenex (Luna) C<sub>18</sub> (21.2 mm x 250 mm) semi-preparative HPLC column. Isocratic conditions of 20% H₂O (0.2% TFA)/80%
MeOH (0.2% TFA) were run for the first 1 min, a linear gradient 100% MeOH (0.2% TFA) was run over 49 min, and isocratic conditions of 100% MeOH (0.2% TFA) was employed for the final 10 min, all with a flow rate of 9 mL/min. Sixty 1 min (9 mL) fractions were collected from time = 0 min. Fraction 41, eluting at 3% H$_2$O (0.2% TFA)/97% MeOH (0.2% TFA) yielded pure surangin B (34, 19.4 mg, 0.07% dry wt) with $m/z$ of 498.

2.3 Biological Investigations of *Mammea odorata*

2.3.1 *In vitro* Antimalarial Assays of Compounds

The isolated compounds were submitted to Queensland Institute of Medical Research (QIMR) for *in vitro* antimalarial growth inhibition assays. *Plasmodium falciparum* 3D7, a chloroquine-sensitive line, was used following previously described procedures. Briefly, synchronous ring-stage infected erythrocytes (0.5% parasitemia and 2.5% hematocrit) were incubated in triplicate wells of 96 well culture dishes with different concentrations of compounds for 48 hr before adding 0.5 $\mu$Ci [$^3$H]-hypoxanthine. Following further incubation for 16-24 hr, cultures were harvested onto 1450 MicroBeta filter mats and $^3$H incorporation determined using a 1450 MicroBeta liquid scintillation counter. Percentage inhibition compared to matched DMSO controls (0.5%) was determined and IC$_{50}$ values calculated using linear interpolation of inhibition curves. Three independent experiments were carried out and the mean IC$_{50}$ ($\pm$SD) was established.

2.3.2 Antimicrobial Susceptibility Assays of Crude Extracts and Compounds

The *in vitro* antimicrobial susceptibility assays of the three crude extracts together with the eleven isolated compounds were performed using the modified BSAC disc diffusion method. A total of nine pathogenic microbes were used. These were three species of Gram positive bacteria, *Staphylococcus aureus* (NZRM 3022), MRSA (clinical isolate), *Enterococcus faecalis* (NZRM 1106) and six species of Gram negative bacteria, *Escherichia coli* (NZRM 916), *Enterobacter aerogenes*
(NZRM 798), *Pseudomonas aeruginosa* (NZRM 997), *Klebsiella pneumonia* (NZRM 482), *Salmonella menston* (NZRM 383), and *Proteus vulgaris* (NZRM 3475).

For the preparation of microbial cultures, MHB was inoculated with the test microbe and incubated at 37 °C for 18-24 hr. Prior to inoculation of the MHA plates, the turbidity of the broth cultures were adjusted to that of the 0.5 McFarland solution. The 0.5 McFarland solution was prepared by adding 0.5 mL of 0.048 M BaCl$_2$ to 99.5 mL of 0.18 M H$_2$SO$_4$ with constant stirring. The absorbance of this turbidity standard was measured to be in the range of 0.08-0.13 at $\lambda$ 625 nm, which would give 1-5 x $10^8$ colony forming unit (cfu)/mL. The turbidity adjusted broth cultures were instantly diluted 100 fold in MHB to give 1-5 x $10^6$ cfu/ml that would allow a semi-confluent growth to occur. The MHA plates were then inoculated with the broth cultures with sterile cotton swabs within 15 min of dilution. Subsequently, sterile, dry paper discs (6 mm) impregnated with 10 µL of either the crude extract (50 mg/mL) or pure compound (10 mg/mL) were aseptically applied onto the inoculated MHA plates. In addition, chloramphenicol and tetracycline (both prepared at 10 mg/mL based on their potency) discs were used as positive control; whereas DMSO and MeOH impregnated discs were used as negative control for crude extracts and pure compounds respectively. It was ensured that the paper discs were also applied within 15 min of inoculation. The plates were then incubated at 37 °C and the zone of inhibition (mm) was measured after 18 and 24 hr. The disc diffusion assays were carried out in duplicates and the activity was expressed as the mean of inhibition diameter.
CHAPTER 3: Results and Discussion

3.1 Chemical Investigations of Metabolites from *Mammea odorata*

Stem bark of *Mammea odorata* (Rafin.) Kosterm. collected near the coastal region of Deuba (Fiji) was sequentially cold extracted with DCM, EtOAc and MeOH after defatting with n-hexane. This furnished DCM, EtOAc and MeOH extracts that gave green and blue colouration to methanolic ferric chloride and PMA respectively. This indicated the presence of polyphenols in the extracts. Previous chemical investigation of this species, then known as *Ochrocarpos odoratus*, yielded only hydroxy and methoxy substituted xanthones. Therefore, a major focus of this study was to search for new compounds, especially glycosylated and prenylated xanthones.

This component of the chapter describes the extraction, isolation and structure elucidation of the two new compounds: 2,3-dihydroxyxanthone; 3-\(O-\beta-d\)-glucopyranoside (116) and 1,6,7-trihydroxyxanthone; 6-\(O-\beta-d\)-glucopyranoside (117); a new natural product, 2,3-dihydroxyxanthone (118); and eight known compounds, (-)-epicatechin (115), 2- and 4-hydroxyxanthones (83 and 84), 2,6-dihydroxyxanthone (94), 2-hydroxy-3-methoxyxanthone (119), 1,6,7-trihydroxyxanthone (88), 1,6,7-trihydroxy-3-methoxyxanthone (120), and surangin B (34) resulting from this study. The isolation of these compounds was achieved using flash chromatography and reverse phase HPLC separations. The structure elucidation was carried out using a combination of spectroscopic data obtained through LRESIMS and HRESIMS, 1D and 2D NMR experiments (\(^1\)H and \(^{13}\)C NMR, gradient COSY, HSQC, HMBC and ROESY), UV and IR spectroscopies. No degradative work was carried out to arrive at the final structures.

3.1.1 Determination of Suitable Parameters to Achieve Good HPLC Separation

In order to achieve a well-resolved HPLC chromatogram that ascertained good separation of compounds (115-117), suitable parameters were established. Four experiments were carried out, in which the two variables were the use of PAG to
cleanup MeOH extract prior to separation and the use of TFA in the HPLC mobile phase. A sample of MeOH extract without the PAG cleanup step was taken in the first experiment, whereas another sample of MeOH extract with PAG cleanup step was taken in the second experiment. In both the experiments, the samples of MeOH extract were subjected to HPLC separation involving the same Betasil C\textsubscript{18} column, mobile phase (H\textsubscript{2}O/MeOH with 0.1% TFA in both) and elution conditions. The third and fourth experiments were carried out emulating the procedures of the first two experiments, however excluding 0.1% TFA from the mobile phase (H\textsubscript{2}O/MeOH).

The two variables were shown to have significant effects on the resolution of the peaks in the HPLC chromatograms obtained from the four experiments (refer to Figure 2). PAG is used for the efficient removal of tannins/polyphenols from plant extracts. Tannins (121) are water-soluble polyphenolic compounds that have molecular weight between 500 to 3000 Da. They are simple esters of gallic acid, hence they contain six or more aromatic rings in their structure.\textsuperscript{98} Since aromatic rings absorb strongly at \( \lambda \) 254 and 280 nm, tannins are undesirable in the separation of plant extracts, which also have intense absorbances at similar wavelengths. In the first chromatogram, poorly resolved peaks were observed between the retention times of 12 and 35 min. Strong absorbance at \( \lambda \) 254 and 280 nm was also seen. The second chromatogram, which was obtained after cleanup with PAG, shows well resolved peaks between similar retention times. In addition, the absorbance at \( \lambda \) 254 and 280 nm has decreased; which could be due to the removal of tannin chromophores by PAG.
HPLC Chromatogram 1 for experiment 1: without the use of PAG and with the use of TFA

HPLC Chromatogram 2 for experiment 2: with the use of PAG and with the use of TFA

HPLC Chromatogram 3 for experiment 3: without the use of PAG and TFA

HPLC Chromatogram 4 for experiment 4: with the use of PAG and without the use of TFA

Figure 2: HPLC chromatograms obtained for various experiments.
The removal of tannins is crucial in the separation of MeOH extract in this study, as the compounds of interest (116 and 117) were obtained in fractions collected between retention times 28 and 34 min (see Figure 3). Tannins are adsorbed onto PAG by the formation of hydrogen bonds between the tannin polyphenolic hydroxyl groups and the amide units of the caprolactam monomers of PAG. Mallika and Dhar showed that almost 99% of non-tannin polyphenols are eluted from the PAG column, thus PAG clean up does not have harmful effects on the isolation of non-tannin secondary metabolites. In the experiments involving the passage of the MeOH extract over a PAG column, yields of approximately 28% were obtained. It can be inferred from these experiments that MeOH extract was rich in tannins and that a similar procedure may be applied to polar extracts of other plants.

Additionally, chromatograms obtained without the use of TFA in the mobile phase (3 and 4) did not have well shaped or resolved peaks when compared with those obtained with the use of TFA in the mobile phase (1 and 2). A marked difference is seen between chromatograms 2 and 4, which were obtained using same HPLC separation conditions, except for the absence of TFA in the mobile phase when the latter was obtained. In particular, chromatogram 2 has sharper peaks between retention times of 45 and 60 min. These experiments have discerningly demonstrated that the addition of TFA in the mobile phase greatly increases peak resolution and thus separation of compounds.

Partially ionized strong acid such as 0.1% TFA can function as pH stabilizers. At low pH, such as pH 3 or lower, the residual silanol groups on silica support of the column and the acidic solutes are fully protonated, therefore, they are not ionized. This prevents formation of tailing, broad peaks due to lack of electrostatic interactions. In this study, analytical TLC has shown the presence of polyphenols in the crude extracts. Phenols (or polyphenols) can act as weak acids and dissociate in dilute aqueous solutions to yield phenoxide ions, ArO⁻ (see Scheme 1). In experiments 1 and 2, the protonation of ArO⁻ ions by TFA present in the mobile phase produced neutral solutes, which were unable to interact electrostatically. This resulted in the formation of sharp and well shaped peaks that were seen in chromatograms 1 and 2. Chromatograms 3 and 4 had broad and ill shaped peaks due to the lack of TFA in the HPLC mobile phase.
Phenols (or polyphenols) can also act as weak bases and form oxonium ions (ArOH$_2^+$) in presence of strong acids (see Scheme 2). The ion pairing due to ionic interaction between the oxonium ion (cation) and the trifluoroacetate (CF$_3$COO$^-$) ion (anion) may also be responsible for the well resolved peaks seen in chromatograms 1 and 2.

**Scheme 2**: Formation of oxonium ion by a polyphenol.

3.1.2 Isolation of Compounds (115-117) from MeOH Extract

Reverse phase HPLC separation using C$_{18}$ semi-preparative column and H$_2$O (0.1% TFA)/MeOH (0.1% TFA) mobile phase compositions, involving isocratic and gradient elution conditions yielded (-)-epicatechin (115, 20.2 mg, 0.087% dry wt) in fraction 21. Evidence of an anomeric proton in the $^1$H NMR spectra resulted in further HPLC purification of later eluting fractions (28-34) (see Figure 3). This furnished two new compounds, 2,3-dihydroxyxanthone; 3-0-$\beta$-d-glucopyranoside (116, 5.7 mg, 0.025% dry wt) and 1,6,7-trihydroxyxanthone; 6-0-$\beta$-d-glucopyranoside (117, 2.2 mg, 0.010%) as shown in Scheme 3.
i. Extraction of the defatted biota sample with DCM, EtOAc and MeOH.

ii. Removal of tannins/polyphenols from MeOH extract (2.30 g) using PAG.

iii. RP HPLC using isocratic conditions of 95% H2O (0.1% TFA)/5% MeOH (0.1% TFA) for 1 min then gradient conditions to 100% MeOH (0.1% TFA) for 49 min.

iv. Semi-prep RP HPLC with isocratic of conditions 60% H2O (0.1% TFA)/40% MeOH (0.1% TFA) for 1 min then gradient conditions to 20% H2O (0.1% TFA)/80% MeOH (0.1% TFA) for 49 min.

v. Semi-prep RP HPLC with isocratic conditions of 52% H2O (0.1% TFA)/48% MeOH (0.1% TFA) for 15 min then gradient conditions to 100% MeOH (0.1% TFA) for next 15 min.

**Scheme 3**: Extraction and isolation procedure of MeOH extract of *M. Odorata.*
3.1.3 Structure Elucidation of Compounds (115-117)

3.1.3.1 (-)-Epicatechin (115)

(-)-Epicatechin (115) was obtained as a red solid with [α]D of -30° (c 0.08, MeOH). A molecular formula of C15H14O6 was assigned to 115 following the analysis of (+)-LRESIMS and 1H and 13C NMR data (see Table 1). The (+)-LRESIMS showed a pseudo-molecular ion at m/z of 290. The 1H NMR spectrum of 115 displayed nine unique signals, five of which were classified as aromatic protons. The interpretation
Table 1: NMR data for (-)-epicatechin (115).\(^a\)

<table>
<thead>
<tr>
<th>Position</th>
<th>(^{13}\text{C})</th>
<th>(^{1}\text{H}) (mult., (J), int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>78.1</td>
<td>4.73 (s, 1H)</td>
<td>3, 2', 6'</td>
<td>2, 8a, 1', 2', 6'</td>
<td>3, 6'</td>
</tr>
<tr>
<td>3</td>
<td>65.0</td>
<td>4.00 (s, 1H)</td>
<td>2, 4, 4</td>
<td>4a, 1'</td>
<td>2, 4, 4</td>
</tr>
<tr>
<td>3-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28.2</td>
<td>2.67 (dd, 16.2, 3.0, 1H)</td>
<td>3, 4</td>
<td>2, 3, 4a, 5, 8a</td>
<td>3, 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.47 (dd, 16.2, 4.2, 1H)</td>
<td>3, 4</td>
<td>2, 3, 4a, 5, 8a</td>
<td>3, 4</td>
</tr>
<tr>
<td>4a</td>
<td>98.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>155.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>95.2</td>
<td>5.88 (d, 1.2, 1H)</td>
<td>8</td>
<td>4a, 6, 7, 8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>156.6(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>94.2</td>
<td>5.72 (d, 1.8, 1H)</td>
<td>6</td>
<td>4a, 6, 8, 8a</td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>156.3(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>130.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>114.9</td>
<td>6.88 (brs, 1H)</td>
<td>2</td>
<td>2, 1', 2', 4', 6'</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>144.5(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3'-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>144.6(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>114.8</td>
<td>6.66 (d, 7.8, 1H)</td>
<td>6</td>
<td>1', 3', 5'</td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>118.0</td>
<td>6.64 (d, 9.0, 1H)</td>
<td>2, 5'</td>
<td>2, 2', 4'</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\) Spectra were recorded in DMSO-\(d_6\) at 30 °C.

\(^b\) Signals not observed.

\(^c, d\) Signals may interchange in some spectra.
of COSY data along with the $^1$H NMR data allowed the protons at $\delta_H$ 6.64 (d, 9.0) and 6.66 (d, 7.8) to be placed \textit{ortho} to each other, while proton at $\delta_H$ 6.88 (brs) was placed \textit{para} to proton at $\delta_H$ 6.66 in the aromatic ring. Further COSY correlations could not be observed for any of these three protons with other aromatic protons, thus, the remaining two protons were assigned to another aromatic ring. The protons at $\delta_H$ 5.72 (d, 1.8) and 5.88 (d, 1.2) did not display any COSY correlations but were \textit{meta} coupled, thus they were assigned \textit{meta} to each other in the other ring. This suggested the existence of two aromatic rings in the molecule, one of which is a tri-substituted ring (\textbf{Figure 4a}) and the other a tetra-substituted ring (\textbf{Figure 4b}).

The $^{13}$C NMR spectrum of \textbf{115} displayed fifteen unique signals altogether. Evaluation of $^{13}$C NMR and HSQC data allowed twelve of these signals to be assigned to the two aromatic rings. Out of these twelve carbons, five carbons had downfield chemical shifts $\delta_C$ 144.4, 144.6, 155.8, 156.3 and 156.6 indicating either hydroxyl substituted aromatic rings or heteroatom (such as oxygen) linkages. Strong $^3J_{CH}$ HMBC correlations from protons $\delta_H$ 6.64 and 6.88, and weak $^2J_{CH}$ HMBC correlations from proton $\delta_H$ 6.66 to $\delta_C$ 144.6 (C-4'), enabled a hydroxyl group to be attached \textit{meta} to the former two proton and \textit{ortho} to the latter. Similarly, a second hydroxyl group at $\delta_C$ 144.5 was attached \textit{meta} to $\delta_H$ 6.66 and \textit{ortho} to $\delta_H$ 6.88 following $^3J_{CH}$ and $^2J_{CH}$ HMBC correlations from protons $\delta_H$ 6.66 and $\delta_H$ 6.88 respectively to carbon $\delta_C$ 144.5 (C-3'). Mutual $^3J_{CH}$ HMBC correlations between $\delta_H$ 6.64 and $\delta_C$ 114.9 (C-2'), and $\delta_H$ 6.88 and $\delta_C$ 118.0 (C-6') further confirmed \textit{meta} placement of the two protons (\textbf{Figure 5b}). Additional mutual $^3J_{CH}$ HMBC correlations were seen between $\delta_H$ 5.72 and $\delta_C$ 95.2 (C6), and $\delta_H$ 5.88 and $\delta_C$ 94.2 (C-8). Both of these protons displayed strong $^3J_{CH}$ HMBC correlations to a quaternary carbon $\delta_C$ 98.6 (C-4a), while only $\delta_H$ 5.72 showed $^2J_{CH}$ HMBC
correlations to a quaternary carbon $\delta_C 156.3$ (C-8a). This allowed the assignment of two hydroxyl groups on remaining carbons $\delta_C 155.8$ (C-5) and 156.6 (C-7). Weak $^2J_{CH}$ HMBC correlations from $\delta_H 5.72$ and $\delta_H 5.88$ to C-7 and C-5 respectively, suggested meta placement of the hydroxyl groups in the aromatic ring (Figure 5a).

Further strong $^3J_{CH}$ HMBC correlations from protons $\delta_H 6.64$ and 6.88 to a methine carbon $\delta_C 78.1$ (C-2) established it to be the last substituent in the tri-substituted aromatic ring. The proton $\delta_H 4.73$ on C-2 showed COSY correlations to proton $\delta_H 4.00$ on another methine carbon $\delta_C 65.0$ (C-3). The downfield shifts of these carbons indicated either ether linkage or hydroxyl groups bonded to them. In addition, strong $^3J_{CH}$ HMBC correlations from $\delta_H 4.00$ and weak $^2J_{CH}$ HMBC correlations from $\delta_H 4.73$ to $\delta_C 130.7$ (C-1a) showed attachment of C-2 to C-1a in the aromatic ring. Additional strong $^3J_{CH}$ HMBC correlations from $\delta_H 4.73$ to C-8a (from the second aromatic ring) were seen. The downfield shifts of these carbons (C-2 and C-8a) suggested them to be linked through an ether linkage, connecting the two aromatic rings together. Proton $\delta_H 4.00$ showed additional COSY correlations to diastereotopic protons $\delta_H 2.47$ (dd, 16.2, 4.2) and 2.67 (dd, 16.2, 3.0) attached to methylene carbon $\delta_C 28.2$ (C-4). Since C-3 was already bonded to a proton $\delta_H 4.00$ and two carbons (C-2 and C-4), the downfield shift suggested a fourth hydroxyl group to be bonded. Strong $^3J_{CH}$ HMBC correlations from $\delta_H 4.00$ and weak $^2J_{CH}$ HMBC correlations from $\delta_H 2.47$ and 2.67 to C-4a indicated a bond between the methylene carbon, C-4 and aromatic quaternary carbon, C-4a forming a pyran-ring adjacent to the aromatic ring (Figure 5b). Strong $^3J_{CH}$ HMBC correlations from $\delta_H 2.47$ and 2.67 to C-5 and C-8a further supported this pyran-ring formation. On the
basis of these correlations a flavanol structure for 115 was established. Structure search through Dictionary of Natural Products\textsuperscript{103} revealed the molecular structure to be that of epicatechin. Comparison of the experimental $^1H$ and $^{13}C$ NMR data with the reported data confirmed this structure.\textsuperscript{104,105}

ROE correlations between the protons $\delta_H 4.00$ and 4.73 on chiral centres, C-3 and C-2 respectively indicated that the two protons were on the same face of the pyran ring. Specific rotation measurement of 115, $[\alpha]_D$ of -30 (c 0.08, MeOH) confirmed it to be (-)-epicatechin and not its enantiomer, (+)-catechin. Therefore the absolute stereochemistry of both C-2 and C-3 was established as S (Figure 6). (-)-Epicatechin has also been isolated from the stem and bark of \textit{M. acuminata}.\textsuperscript{58}

![Figure 6: ROE (↔) correlation in (-)-epicatechin.](image)

3.1.3.2 2,3-Dihydroxyxanthone; 3-$O$-$\beta$-$\d$-Glucopyranoside (116)

2,3-Dihydroxyxanthone; 3-$O$-$\beta$-$\d$-glucopyranoside (116) was obtained as pale yellow crystals with $[\alpha]_D$ of -52$^\circ$ (c 0.22, MeOH). The molecular formula, C$_{19}$H$_{18}$O$_9$ was derived from (+)-HRESIMS data, which had molecular ion at $m/z$ 390.082. (+)-LRESIMS data exhibited a molecular ion at $m/z$ 390 and a fragment ion at $m/z$ 228. A mass difference of 162 Da indicated a glycosyl moiety to be present in the molecule. Evaluation of the $^1H$ and $^{13}C$ NMR data of 116 (refer to Table 2) confirmed the presence of such a moiety due to an anomeric proton $\delta_H$ 5.12 (d, 7.2) on carbon $\delta_C$ 100.5 (C-1'), which formed O-linkage with the aglycone.\textsuperscript{106} Therefore, a molecular formula of C$_{13}$H$_8$O$_4$ with ten unsaturations was ascertained for the aglycone. In addition, the $^1H$ NMR spectrum showed thirteen other unique signals, six of which resonated in the aromatic region, one exchangeable proton and the rest belonging to the glycosyl moiety. The $^{13}C$ NMR spectrum showed one carbonyl
### Table 2: NMR data for 2,3-dihydroxyxanthone; 3-\(\beta\)-\(\alpha\)-glucopyranoside (116).\(^a\)

<table>
<thead>
<tr>
<th>Position</th>
<th>(^{13}C) (ppm)</th>
<th>(^{1}H) (mult., (J), int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109.0</td>
<td>7.49 (brs, 1H)</td>
<td>2, 3, 4, 4a, 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>144.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-OH</td>
<td></td>
<td>9.36 (brs, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>152.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OGlu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>103.5</td>
<td>7.31 (brs, 1H)</td>
<td>2, 3, 4, 4a, 9, 9a</td>
<td>1'</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>150.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>155.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>117.9</td>
<td>7.63 (d, 7.8, 1H)</td>
<td>6</td>
<td>4b, 8</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>134.6</td>
<td>7.82 (ddd, 8.4, 7.2, 1.8, 1H)</td>
<td>5, 7</td>
<td>4b, 8</td>
<td>5, 7</td>
</tr>
<tr>
<td>7</td>
<td>123.9</td>
<td>7.45 (dd, 7.8, 7.2, 1H)</td>
<td>6, 8</td>
<td>4b, 5, 6, 8a</td>
<td>6, 8</td>
</tr>
<tr>
<td>8</td>
<td>125.7</td>
<td>8.16 (dd, 7.8, 1.8, 1H)</td>
<td>7</td>
<td>4b, 6, 8, 9</td>
<td>7</td>
</tr>
<tr>
<td>8a</td>
<td>120.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>174.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>115.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>100.5</td>
<td>5.12 (d, 7.2, 1H)</td>
<td>2'</td>
<td>3, 5'</td>
<td>3', 5</td>
</tr>
<tr>
<td>2'</td>
<td>73.1</td>
<td>3.37 (dd, 9.6, 7.2, 1H)</td>
<td>1'</td>
<td>1', 3'</td>
<td>4'</td>
</tr>
<tr>
<td>3'</td>
<td>75.8</td>
<td>3.34 (t, 9.0, 1H)</td>
<td>4'</td>
<td>2', 4'</td>
<td>1', 5'</td>
</tr>
<tr>
<td>4'</td>
<td>69.7</td>
<td>3.20 (t, 9.0, 1H)</td>
<td>3', 5'</td>
<td>5', 6'</td>
<td>2', 6'</td>
</tr>
<tr>
<td>5'</td>
<td>77.2</td>
<td>3.52 (t, 7.2, 1H)</td>
<td>4'</td>
<td>1', 4'</td>
<td>1', 3'</td>
</tr>
<tr>
<td>6'</td>
<td>60.7</td>
<td>3.75 (d, 10.2, 1H)</td>
<td>6'</td>
<td>4'</td>
<td>6'</td>
</tr>
<tr>
<td></td>
<td>3.49 (d, 9.0, 1H)</td>
<td></td>
<td>6'</td>
<td>4', 5', 6'</td>
<td>6'</td>
</tr>
</tbody>
</table>

\(^a\) Spectra were recorded in DMSO-\(d_6\) at 30 °C
carbon, twelve aromatic carbons, four of which had downfield chemical shifts and six glycosyl carbons. The IR spectrum suggested the presence of conjugated carbonyl (1613 cm\(^{-1}\)), hydroxyl (3396 cm\(^{-1}\)), aromatic (1462, 1322, 1276 and 755 cm\(^{-1}\)) and ether (1075 cm\(^{-1}\)) functionalities.\(^{107,108}\) The UV spectrum in MeOH exhibited absorption bands at \(\lambda_{\text{max}}\) 241, 302 and 354 nm. Upon addition of NaOH, bathochromic shift was observed in the spectrum, indicating the presence of phenolic hydroxy group(s) in the molecule.

**Structure Determination of the Aglycone (2,3-Dihydroxyxanthone)**

Proton \(\delta_H\) 7.45 (dd, 7.8, 7.2) exhibited mutual COSY correlations with protons \(\delta_H\) 8.16 (dd, 7.8, 1.8) and 7.82 (ddd, 8.4, 7.2, 1.8), which also showed mutual COSY correlations with proton \(\delta_H\) 7.63 (d, 7.8). On the basis of these correlations and coupling constants, proton \(\delta_H\) 8.16 was placed *ortho* to \(\delta_H\) 7.45 and *meta* to \(\delta_H\) 7.82, while \(\delta_H\) 7.63 was placed *ortho* to \(\delta_H\) 7.82 and *meta* to \(\delta_H\) 7.45. No further COSY correlations were seen for these protons with the remaining two aromatic protons; hence they were assigned to another aromatic ring. Absence of COSY correlations within protons \(\delta_H\) 7.49 and 7.31 and their broad singlet splitting pattern, enabled them to be *para* positioned. Assignment of aromatic protons using COSY correlations agreed with the \(^2J_{CH}\) and \(^3J_{CH}\) HMBC correlations observed between protons (\(\delta_H\) 8.16, 7.82, 7.63 and 7.45) and aromatic carbons (C-5, C-6, C-7 and C-8; determined through HSQC correlations). Strong \(^3J_{CH}\) HMBC correlations from \(\delta_H\) 8.16 and \(\delta_H\) 7.49 to carbonyl carbon \(\delta_C\) 174.8 (C-9) suggested the two aromatic rings to be bonded by that carbonyl carbon forming an intermediate benzophenone structure (Figure 7a).

![Figure 7](image_url)

**Figure 7:** Key HMBC (→), COSY (↔) and ROE (↔) correlations seen for the aglycone.
Additional $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.49 to carbons $\delta_C$ 150.3 (C-4a) and 152.0 (C-3), and from $\delta_H$ 8.16 to $\delta_C$ 155.6 (C-4b) indicated attachment of either electronegative atoms or electron-withdrawing groups *meta* to $\delta_H$ 7.49 and $\delta_H$ 8.16. Since a strong $^3J_{CH}$ HMBC correlation was seen from the anomeric proton $\delta_H$ 5.12 to C-3, the glycosyl moiety was evident to be bonded to C-3. ROE correlations observed between protons $\delta_H$ 5.12 and 7.31 further supported this. Thus the remaining carbons, C-4a and C-4b were envisaged to be bonded through an ether linkage forming a dibenzo-γ-pyrene framework that was consistent with ten unsaturations. This molecular structure belonged to the xanthone class of secondary metabolites. Moreover, this structure was further supported by strong $^3J_{CH}$ and weak $^2J_{CH}$ HMBC correlations from $\delta_H$ 7.82 and $\delta_H$ 7.63 to C-4b, and weak $^2J_{CH}$ HMBC correlations from $\delta_H$ 7.31 to C-4a. $^2J_{CH}$ HMBC correlations from $\delta_H$ 7.49 to the downfield shifted carbon $\delta_C$ 144.3 (C-2) aided in assignment of an exchangeable proton, 9.36 (brs) to it. These correlations assisted in structure elucidation of 2,3-dihydroxyxanthone, the aglycone moiety for 116 (see Figure 7b).

**Structure Determination of the Glycosyl Moiety (β-α-Glucopyranose)**

The remaining six resonances in the glycosidic region of $^{13}$C NMR spectrum of 116 were evaluated to be one methylene and five methine carbons. Consequently the glycosyl moiety was determined to be an aldo-hexose sugar, which existed in a pyranose form. This was based on unique HSQC correlations shown by the diastereotopic protons $\delta_H$ 3.49 (d, 9.0) and 3.75 (d, 10.2) to methylene carbon $\delta_C$ 60.7 (C-6’). Due to mutual COSY correlations observed from proton $\delta_H$ 5.12 to 3.37 (dd, 9.6, 7.2), and from protons $\delta_H$ 3.34 (t, 9.0) and 3.52 (t, 7.2) to 3.20 (t, 9.0), protons $\delta_H$ 3.37 and 3.20 were assigned to positions H-2’ and H-4’ respectively. Designation of protons $\delta_H$ 3.34 as H-3’ and $\delta_H$ 3.52 as H-5’ was a result of distinct $^2J_{CH}$ HMBC correlations from H-2’ and H-6’ ($\delta_H$ 3.49) to methine carbons $\delta_C$ 75.8 (C-3’) and 77.2 (C-5’) respectively. All the COSY and HMBC correlations that assisted in the assignment of the seven protons to their respective carbons are shown in Figure 8a.

Once a planar structure for the glycosyl moiety was generated, ROE correlations were used to determine the relative stereochemistry at the various chiral centres in the pyranose ring. Since a vicinal coupling constant of 7.2 Hz was observed for the
anomeric proton $\delta_H 5.12$, a $\beta$ configuration was established for C-1'. This implied that -OH on C-1' was cis to -CH$_2$OH on C-5' and trans to -OH on C-2'. Therefore, -OH on C-1' and -CH$_2$OH on C-5' were placed on one plane (denoted by dashed bond) while –OH on C-2’ was placed on the opposite plane (denoted by solid bond) as shown in Figure 8b.

Accordingly, mutual ROE correlations displayed by H-1’, H-3’ and H-5’ allowed them to be placed on the opposite plane to OH-1’. Similar ROE correlations seen between H-2’, H-4’ and H-6’ ($\delta_H 3.49$) allowed them to be placed on the same plane as OH-1’. This representation of stereochemistry on the five chiral centres led the pyranose moiety to be resolved as $\beta$-glucose. On the same basis, a chair-conformation of $\beta$-glucose was worked out. The alternating cis and trans substitution pattern from C-1’ to C-5’ resulted in the substituents (4 –OHs and 1-CH$_2$OH) being equatorially positioned at all chiral centres. Since the dihedral angle between all the substituents is 180°, 1, 3-diaxial interactions between –OHs and –Hs is nullified. Due to this, steric strain is minimized and this becomes the most favoured conformation of $\beta$-glucose (see Figure 8c).

Figure 8: Key HMBC (→), COSY (↔) and ROE (↔) correlations in the glucose moiety.

Despite the thorough determination of relative stereochemistry of $\beta$-glucose, absolute stereochemistry could not be verified experimentally. This was because of insufficient amounts of 116 (5.7 mg, 0.025% dry wt) for it to be hydrolysed into constituent aglycone and glycosyl moieties. Very few dihydroxyxanthone glucopyranosides have been reported in the Literature. Table 3 shows some of the representatives of dihydroxyxanthone glucopyranosides that have been isolated from some species of plants. Moreover, those that have been reported all bear a $\beta$-d-
glucose moiety. On this basis, the β-glucose moiety in 2,3-dihydroxyxanthone; 3-O-β-glucopyranoside has been assigned the D configuration. The specific rotation of 116 was measured to be -52° (c 0.22, MeOH). Comparison of the experimental ¹H and ¹³C NMR data with reported data confirmed it to be β-D-glucose. Conclusively, 116 was found to be 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside, which is a new compound and is also the first xanthone glycoside to be isolated from the genus *Mammea*.

**Table 3:** Some representatives of dihydroxyxanthone glucopyranosides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Source</th>
<th>[α]D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,7-dihydroxyxanthone; 7-O-β-D-glucopyranoside</td>
<td><img src="image1" alt="Structure" /></td>
<td><em>Polygala caudata</em></td>
<td>-</td>
</tr>
<tr>
<td>7-hydroxy-1-methoxyxanthone; 7-O-β-D-glucopyranoside</td>
<td><img src="image2" alt="Structure" /></td>
<td><em>Polygala wattersii</em></td>
<td>-20.5° (c 0.6, MeOH)</td>
</tr>
</tbody>
</table>
3.1.3.3 1,6,7-Trihydroxyxanthone; 6-O-β-D-Glucopyranoside (117)

1,6,7-Trihydroxyxanthone; 6-O-β-D-glucopyranoside (117) was obtained as a yellow solid with [α]D -12° (c 0.10, MeOH). (+)-HRESIMS data displayed a pseudomolecular ion at m/z 406.081, for which a molecular formula of C19H18O10 was established. (+)-LRESIMS data showed a molecular ion at m/z 406 and a fragment ion at m/z 244. A mass difference of 162 Da suggested that 117 contained a similar glycosyl moiety as 116. This was confirmed by an anomeric proton at δH 5.14 (d, 7.2) in the 1H NMR spectrum of 117, which also showed seventeen other unique resonances. The additional four downfield shifts in glycosidic region of 117 (see Figure 9) that were unobserved in the 1H NMR spectrum of 116 were assigned to the –OH substituents in the glycosyl moiety. A total of nineteen unique resonances belonging to one carbonyl carbon, twelve aromatic carbons and six glycosyl carbons were worked out from the HSQC and HMBC spectra of 117 (see Table 4). Since the glycosidic carbon chemical shifts and proton to carbon correlations (determined by HSQC data) were comparable to that obtained for 116, the glycosyl moiety was postulated to be glucose forming O-linkage with the aglycone. Consequently, a molecular formula of C13H8O5 that was consistent with m/z of 244 was established for the aglycone.

Figure 9: Proton resonances in the glycosidic region of 1H NMR spectrum of 117.
Table 4: NMR data for 1,6,7-trihydroxyxanthone; 6-β-D-glucopyranoside (117).a

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C</th>
<th>$^1$H (mult., $J$, int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.9</td>
<td>12.81 (s, 1H)</td>
<td>1, 2, 9a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-OH</td>
<td></td>
<td>6.79 (d, 8.4, 1H)</td>
<td>3</td>
<td>1, 4, 9a</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>136.3</td>
<td>7.68 (t, 8.4, 1H)</td>
<td>2, 4</td>
<td>1, 4a</td>
<td>2, 4</td>
</tr>
<tr>
<td>3</td>
<td>106.8</td>
<td>7.04 (d, 8.4, 1H)</td>
<td>3</td>
<td>2, 4, 4a, 9a</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>156.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>155.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>103.1</td>
<td>7.32 (brs, 1H)</td>
<td>5, 6, 7, 9a</td>
<td>1'</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>152.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-OGlu</td>
<td></td>
<td>9.52 (s, 1H)</td>
<td>6, 7, 8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>144.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-OH</td>
<td></td>
<td>7.47 (brs, 1H)</td>
<td>6, 7, 9</td>
<td>7-OH</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>114.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>180.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>107.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>100.4</td>
<td>5.14 (d, 7.2, 1H)</td>
<td>2'</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>2'</td>
<td>73.0</td>
<td>3.38 (m)</td>
<td>1', 2'-OH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'-OH</td>
<td></td>
<td>5.36 (d, 4.2, 1H)</td>
<td>2'</td>
<td>1', 2', 3'</td>
<td>3'</td>
</tr>
<tr>
<td>3'</td>
<td>75.8</td>
<td>3.34 (m)</td>
<td>2'</td>
<td>2'-OH, 4'-OH</td>
<td></td>
</tr>
<tr>
<td>3'-OH</td>
<td></td>
<td>5.12 (d, 4.2, 1H)</td>
<td>2'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>69.5</td>
<td>3.20 (m)</td>
<td>4'-OH, 5'</td>
<td>5', 6'</td>
<td></td>
</tr>
<tr>
<td>4'-OH</td>
<td></td>
<td>5.09 (d, 5.4, 1H)</td>
<td>4'</td>
<td>4', 5'</td>
<td>3'</td>
</tr>
<tr>
<td>5'</td>
<td>77.1</td>
<td>3.52 (m)</td>
<td>5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>60.5</td>
<td>3.74 (dd, 10.2, 5.4, 1H)</td>
<td>6', 6'-OH</td>
<td>4'</td>
<td>6'</td>
</tr>
<tr>
<td>6'-OH</td>
<td></td>
<td>3.48 (dd, 1H)</td>
<td>6', 6'-OH</td>
<td>5'</td>
<td>6'</td>
</tr>
</tbody>
</table>

a Spectra were recorded in DMSO-$d_6$ at 30 °C.

b Obtained from gHSQC and gHMBC data as a good $^{13}$C NMR spectrum of 117 could not be attained.
A similar display of proton and carbon resonances in the non-glycosidic region of respective spectra of 116 and 117 indicated that the aglycone moiety for 117 was a xanthone. Strong absorption bands at 1647 cm\(^{-1}\) (carbonyl), 3437 cm\(^{-1}\) (hydroxyl), 1457, 1204, and 1139 cm\(^{-1}\) (aromatic) and 1078 cm\(^{-1}\) (ether) observed in the IR spectrum of 117 was in support of a xanthone structure. The UV spectrum showed absorbance maxima in MeOH at 250, 290 and 382 nm, which were different from those obtained for 116. This indicated that 117 had a different substitution pattern than 116. A bathochromic shift was observed upon addition of NaOH, which meant that 117 contained phenolic hydroxy group(s). The \(^1\)H NMR spectrum of 117 exhibited two exchangeable and five aromatic protons. Out of the twelve aromatic carbon resonances displayed by the HSQC and HMBC spectra, five had downfield chemical shifts. Comparison of this data with 116 that had only one exchangeable proton and four downfield carbon chemical shifts suggested that 117 was a tri-substituted xanthone. A combination of COSY, HSQC and HMBC correlations aided in the assignment of the three substituents on the xanthone aglycone moiety.

Protons \(\delta_H\) 6.79 (d, 8.4) and 7.04 (d, 8.4) were mutually coupled to \(\delta_H\) 7.68 (t, 8.4), thus the two were positioned \(ortho\) to \(\delta_H\) 7.68 and \(meta\) to each other on one aromatic ring of the xanthone moiety. Since no COSY correlations were observed with these protons to the remaining two uncoupled protons \(\delta_H\) 7.47 (brs) and 7.32 (brs), they were positioned \(para\) to each other on the second aromatic ring. The presence of a highly downfield shifted –OH proton \(\delta_H\) 12.81 (s) suggested it to be hydrogen bonded to the carbonyl group \(\delta_C\) 180.8 (C-9). Therefore, this –OH group was assigned to C-1. Strong \(^3J_{CH}\) HMBC correlations seen from 1-OH to \(\delta_C\) 109.6 (C-2) and \(\delta_C\) 107.6 (C-9a) and weak \(^2J_{CH}\) HMBC correlations to \(\delta_C\) 160.9 (C-1) were in agreement with the positioning. Strong \(^3J_{CH}\) HMBC correlations observed from \(\delta_H\) 7.04 to C-2 and from \(\delta_H\) 6.79 to \(\delta_C\) 106.8 (C-4) confirmed their \(meta\) positioning on the first aromatic ring. Further strong \(^3J_{CH}\) HMBC correlations from \(\delta_H\) 7.68 to C-1 and 156.0 (C-4a) allowed the assignment of \(\delta_H\) 6.79, 7.68 and 7.04 as H-2, H-3 and H-4 respectively. Conversely, strong \(^3J_{CH}\) HMBC correlations seen from \(\delta_H\) 7.47 to C-9 and downfield shifted carbons \(\delta_C\) 155.0 (C-4b) and 152.7 (C-6); and from \(\delta_H\) 7.32 to \(\delta_C\) 144.4 (C-7) and 114.0 (C-9a) aided in their assignment as H-8 and H-5.
respectively. Further $^3J_{CH}$ HMBC correlations observed from exchangeable proton $\delta_H$ 9.52 (s) to $\delta_C$ 108.1 (C-8) and C-6 and weak $^2J_{CH}$ HMBC correlations to $\delta_C$ C-7, allowed it to be positioned on C-7. Another $^3J_{CH}$ HMBC correlation from the anomeric proton $\delta_H$ 5.14 to C-6 confirmed the attachment of $\beta$-glucose to C-6 of the xanthone moiety. These significant correlations enabled the generation of a 1,6,7-trihydroxyxanthone structure for the aglycone (see Figure 10).

![Figure 10: Key HMBC (→) and COSY (↔) correlations seen for the aglycone of 117.](image)

**Characterization of the Glycosyl Moiety ($\beta$-d-Glucose)**

Comparison of the glycosidic proton and carbon resonances with the ones obtained for 116 had already confirmed the presence of a glucose moiety in 117. The vicinal coupling constant of 7.2 Hz for the anomeric proton $\delta_H$ 5.14, also suggested that it was $\beta$ conformer. Four additional downfield proton signals corresponding to the –OH substituents that were unassigned for 116, were assigned employing COSY, HMBC and ROE correlations. Mutual COSY correlations observed from protons $\delta_H$ 3.38 (H-2') to $\delta_H$ 5.36 (d, 4.2), from $\delta_H$ 3.20 (H-4') to $\delta_H$ 5.09 (d, 5.4), and from $\delta_H$ 3.74 (H-6') and 3.48 (H-6') to $\delta_H$ 4.64 (dd, 5.4, 4.8) enabled them to be positioned on C-2', C-4', and C-6' respectively. The remaining unassigned proton $\delta_H$ 5.12 (d, 4.2) was positioned on C-3'. Strong $^3J_{CH}$ and weak $^2J_{CH}$ HMBC correlations seen from OH-2', OH-3', -OH-4' and OH-6' that further confirmed these assignments are shown in Figure 11.

Moreover, ROE correlations observed for these additional proton signals were in agreement with the relative stereochemistry generated for $\beta$-glucose previously. H-3 exhibited mutual ROE correlations to OH-2' and OH-4' which indicated that they were placed cis to H-3 in $\beta$-glucose (see Figure 11). Additional ROE correlation from H-1' to H-5 verified the attachment of glycosyl moiety at C-6 of the aglycone.
Absolute stereochemistry could not be determined experimentally as insufficient amount of sample was present. The addition of a β-D-glucose moiety to 1,6,7-trihygroxyxanthone, which is a known compound, resulted in a new natural product compound. Thus, 117 is the second xanthone glycoside to be isolated from the genus *Mammea*. An analogue of 117 is 1,6-dihydroxy-7-methoxyxanthone; 6-O-β-D-glucopyranoside, which was isolated from *Poeciloneuron pauciflorum* (Clusiaceae). Occurrence of such an analogue suggests that 116 and 117 are not artefacts but authentic natural products; and that more such xanthones glycosides could be isolated from *Mammea* species.

![Figure 11: Key HMBC (→) and ROE (↔) correlations seen for the glucose moiety.](image)

### 3.1.4 Isolation of Known Oxygenated Xanthones (83, 84, 88, 94, 118-120) from EtOAc Extract

The EtOAc extract of *M. odorata* yielded seven xanthones, out of which one (118) was isolated as a secondary metabolite from a natural source for the first time, while six others were known compounds, 83, 84, 88, 94, 119 and 120 (refer to Scheme 4). Reverse phase flash chromatography of the EtOAc extract involving H₂O/CH₃CN mobile phase mixtures afforded 2,6-dihydroxyxanthone (94, 1.5 mg, 0.002% dry wt), 2,3-dihydroxyxanthone (118, 8.8 mg, 0.010% dry wt) and 2-hydroxyxanthone (83, 4.3 mg, 0.005% dry wt). Fractions 20, 25-26 and 38 had deposited 94, 118, and 83 respectively, which were triturated with cold acetone to yield pure compounds. Combination of fractions 30-39 and RP HPLC separation with H₂O (0.1% TFA)/MeOH (0.1% TFA) employing isocratic and gradient elution conditions yielded 4-hydroxyxanthone (84, 1.8 mg, 0.002% dry wt), 2-hydroxy-3-methoxyxanthone (119, 6.0 mg, 0.007% dry wt), 1,6,7-trihydroxyxanthone (88, 8.0
mg, 0.009% dry wt) and 1,6,7-trihydroxy-3-methoxyxanthone (120, 1.1 mg, 0.001% dry wt) (see Figure 12).

**Scheme 4**: Isolation and extraction procedure for EtOAc extract of *M. odorata.*
3.1.5 Structure Elucidation of Compounds (83, 84, 88, 94, 118-120)

3.1.5.1 2,6-Dihydroxyxanthone (94)

2,6-Dihydroxyxanthone (94), was obtained as white needles. Analysis of the (+)-LRESIMS data, which displayed a pseudomolecular ion at \( m/z \) 228, gave a molecular formula of \( \text{C}_{13}\text{H}_8\text{O}_4 \). The \(^1\text{H}\) NMR spectrum of 94 showed eight unique signals, consisting of two highly downfield shifted and six aromatic proton signals.

\[
\begin{array}{cccccc}
(83) & R_1 & R_2 & R_3 & R_4 & R_5 & R_6 \\
(84) & H & OH & H & H & H & H \\
(88) & OH & H & H & H & OH & OH \\
(94) & H & OH & H & H & OH & H \\
(118) & H & OH & OH & H & H & H \\
(119) & H & OH & OCH_3 & H & H & H \\
(120) & OH & H & OCH_3 & H & OH & OH \\
\end{array}
\]

Figure 12: HPLC chromatogram for the isolation of 84, 88, 119 and 120 from EtOAc extract.
Table 5: NMR data for 2,6-dihydroxyxanthone (94).a

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C</th>
<th>$^1$H (mult., $J$, int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108.7</td>
<td>7.44 (d, 3.0, 1H)</td>
<td>2, 3, 4a, 9</td>
<td></td>
<td>2-OH</td>
</tr>
<tr>
<td>2</td>
<td>153.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-OH</td>
<td></td>
<td></td>
<td>1, 2, 3</td>
<td></td>
<td>1, 3</td>
</tr>
<tr>
<td>3</td>
<td>123.2</td>
<td>7.23 (dd, 9.0, 3.0, 1H)</td>
<td>4</td>
<td>1, 2, 4a, 9a</td>
<td>2-OH, 4</td>
</tr>
<tr>
<td>4</td>
<td>118.7</td>
<td>7.47 (d, 9.0, 1H)</td>
<td>3</td>
<td>2, 4, 4a, 9, 9a</td>
<td>3</td>
</tr>
<tr>
<td>4a</td>
<td>149.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>157.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>101.5</td>
<td>6.83 (d, 1.8, 1H)</td>
<td>4b, 5, 6, 7, 8a, 9</td>
<td>6-OH</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>163.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td></td>
<td>10.86 (s, 1H)</td>
<td>4b, 5, 6, 7, 8a</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>113.4</td>
<td>6.88 (dd, 9.0, 1.8, 1H)</td>
<td>8</td>
<td>5, 6, 7, 8a</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>127.5</td>
<td>8.01 (d, 9.0, 1H)</td>
<td>7</td>
<td>4b, 6, 8, 9</td>
<td>7</td>
</tr>
<tr>
<td>8a</td>
<td>113.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>174.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>122.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Spectra were recorded in DMSO-$d_6$ at 30 °C.
The $^{13}$C NMR spectrum showed signals for 13 unique carbons. These were one carbonyl carbon $\delta_C$ 174.5 (C-9) and twelve aromatic carbons, out of which four were downfield shifted carbons $\delta_C$ 149.0 (C-4a), 157.4 (C-4b), 153.9 (C-2) and 163.2 (C-6). Comparison of the $^1$H and $^{13}$C NMR spectra with the ones for 116 and 117 indicated that 94 was also a xanthone. Structure elucidation was made possible through analysis of combination of 1D and 2D NMR data (see Table 5). Evaluation of $^1$H NMR spectrum, solely indicated that this was di-substituted xanthone. This is because of two exchangeable downfield protons $\delta_H$ 9.84 (s) and 10.86 (s) that were identified to belong to two hydroxy substituents on the two aromatic rings of the xanthone. However, these along with other six aromatic proton assignments were made with the aid of COSY spectral data.

COSY correlations were seen between mutually coupled protons $\delta_H$ 7.47 (d, 9.0) and 7.23 (dd, 9.0, 3.0), which also showed meta coupling to proton $\delta_H$ 7.44 (d, 3.0). Based on this, protons $\delta_H$ 7.47 and 7.23 were placed ortho to each other while being placed para and meta in respect to $\delta_H$ 7.44 on the first ring of the xanthone nucleus. Of the remaining three protons, $\delta_H$ 8.01 (d, 9.0) showed COSY correlations to mutually coupled proton $\delta_H$ 6.88 (dd, 9.0, 1.8) that also showed meta coupling to $\delta_H$ 6.83 (d, 1.8). Thus $\delta_H$ 6.83 was positioned meta and para to $\delta_H$ 6.88 and $\delta_H$ 8.01 respectively. On the basis of these correlations, it was determined that one –OH substituent belonged to each ring of the xanthone nucleus. However, positioning of these on individual carbons was determined using HSQC and HMBC correlations.

Strong $^3J_{CH}$ HMBC correlations to C-9 were seen from $\delta_H$ 7.44 and $\delta_H$ 8.01, which allowed them to be positioned on $\delta_C$ 108.7 (C-1) and 127.5 (C-8) respectively. Since an ortho and meta positions were vacant with respect to $\delta_H$ 7.44 and $\delta_H$ 8.01, the positioning of the two –OH groups were confirmed to be on C-2 and C-6. Strong $^3J_{CH}$ HMBC correlations from $\delta_H$ 8.01 to C-6, to which $\delta_H$ 10.86 (s) exhibited weak $^2J_{CH}$ HMBC correlations, aided in the assigning of $\delta_H$ 10.86 to C-6. Therefore, $\delta_H$ 9.84 (s) that showed strong $^3J_{CH}$ HMBC correlations to C-1 and $\delta_C$ 123.2 (C-3) and weak $^2J_{CH}$ HMBC C-2, was positioned on C-2. Additional $^3J_{CH}$ HMBC correlations from $\delta_H$ 7.47 and $^2J_{CH}$ HMBC correlations from $\delta_H$ 7.44 and 7.23 to C-2 and $\delta_H$ 6.83 and 6.88 to C-6 were observed. Finally strong ROE correlation from $\delta_H$ 9.84 to $\delta_H$ 7.44 and $\delta_H$ 7.23 and from $\delta_H$ 10.86 to $\delta_H$ 6.88 substantiated that 94 was 2,6-

52
dihydroxyxanthone (refer to Figure 13). 2,6-Dihydroxyxanthone has previously been isolated from the timber extract of *M. acuminata*, which is a source of many other mono- and di-hydroxyxanthones and furanoxanthones.\textsuperscript{57-59}

![Figure 13: Key HMBC (→), COSY (↔) and ROE (↔) correlations observed for 94.](image)

### 3.1.5.2 2,3-Dihydroxyxanthone (118)

2,3-Dihydroxyxanthone (118) was obtained as pale yellow crystals. (+)-HRESIMS data displayed \textit{m/z} at 228.049, which gave a molecular formula of C_{13}H_{8}O_{4} with 10 degree of unsaturations. The (+)-LRESIMS displayed a molecular ion at \textit{m/z} 228. Identical molecular ions and thus molecular formulae displayed by compounds 94 and 118 indicated they were constitutional isomers. \textsuperscript{1}H and \textsuperscript{13}C NMR of 118 exhibited the same number of aromatic protons and all the carbon resonances as that of 94, with the exception of two unobserved exchangeable protons. Therefore, 118 was postulated to be a di-hydroxyxanthone. Furthermore, 1D and 2D NMR data of 118 (see Table 6) were very similar to 1D and 2D NMR data obtained for the aglycone of compound 116 (see Table 2). The aglycone of 116 was determined to be 2,3-Dihydroxyxanthone (see Section 3.1.3.2). The IR spectrum of 118 exhibited almost identical absorbances for carbonyl (1614 cm\textsuperscript{-1}), hydroxyl (3396 cm\textsuperscript{-1}) and aromatic (1485, 1320 and 748 cm\textsuperscript{-1}) functionalities as that of 116. The UV \(\lambda_{\text{max}}\) in MeOH of 118 (238, 313 and 352 nm) resembled UV \(\lambda_{\text{max}}\) for 116 but had a bathochromic shift with the addition of NaOH. This indicated that an –OH group was attached either on C-3 or C-6, thus confirming the absence of a glucose moiety on C-3. On the basis of similarities between the spectral data of 118 and the aglycone of 116, 118 was identified to be 2,3-dihydroxyxanthone.
Table 6: NMR data for 2,3-dihydroxyxanthone (118).\(^a\)

<table>
<thead>
<tr>
<th>Position</th>
<th>(^1^C)</th>
<th>(^1^H) (mult., (J), int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108.7</td>
<td>7.45 (brs, 1H)</td>
<td></td>
<td>2, 3, 4a, 9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>143.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>151.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>102.7</td>
<td>6.92 (brs, 1H)</td>
<td></td>
<td>2, 3, 4a, 9, 9a</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>154.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>155.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>117.8</td>
<td>7.58 (d, 8.4, 1H)</td>
<td>6</td>
<td>4b, 7, 8a, 9</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>134.2</td>
<td>7.77 (ddd, 8.4, 7.2, 1.2, 1H)</td>
<td>5, 7, 8</td>
<td>4b, 5, 8</td>
<td>5, 7, 8</td>
</tr>
<tr>
<td>7</td>
<td>123.7</td>
<td>7.41 (dd, 7.8, 7.2, 1H)</td>
<td>6, 8</td>
<td>5, 7, 8a</td>
<td>6, 8</td>
</tr>
<tr>
<td>8</td>
<td>125.6</td>
<td>8.13 (dd, 7.8, 1.2, 1H)</td>
<td>7</td>
<td>4b, 6, 8, 9</td>
<td>7</td>
</tr>
<tr>
<td>8a</td>
<td>120.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>174.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>113.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Spectra were recorded in DMSO-\(_d_6\) at 30 °C.
\(^b\) Signals not observed.
The isolation of 2,3-dihydroxyxanthone from the bark of *M. odorata* is noteworthy as it was only known as a synthetic derivative up till now. Therefore, 2,3-dihydroxyxanthone is a new natural product. Comparison of the $^1$H and $^{13}$C NMR and IR data obtained for the natural product with that of the synthetic product verified the structure of 118.\(^{112}\)

The synthesis of 2,3-dihydroxyxanthone from 3,4-dimethoxyphenol as a starting material\(^{113}\) and through demethylation of either 2,3-dimethoxyxanthone\(^{109}\) or 3,4-dimethoxyxanthone\(^{112}\) has been reported. 2,3-dimethoxyxanthone has been previously isolated from the same source as 2,3-dihydroxyxanthone, that is from *M. odorata* (*O. odoratus*).\(^56\) This implies that the biosynthesis of 116 and 118 from 2,3-dimethoxyxanthone or vice versa in *M. odorata* is plausible.

### 3.1.5.3 2-Hydroxyxanthone (83)

2-Hydroxyxanthone (83) was obtained as yellow needles. The (+)-LRESIMS spectrum of 83 displayed a molecular ion at $m/z$ 212, which allowed a molecular formula of C$_{13}$H$_8$O$_3$ to be generated. $^1$H NMR spectrum of 83 showed a total of eight signals that accounted for all the protons in the molecule. These were one hydroxy and seven aromatic protons. The $^{13}$C NMR spectrum of 83 showed 13 unique signals, which belonged to one carbonyl carbon $\delta$C 175.8 (C-9) and 12 aromatic carbons. Out of the twelve carbons, three of them ($\delta$C 149.2, C-4a; 155.5, C-4b; and 153.9, C-2) resonated in the downfield aromatic region. The $^1$H and $^{13}$C NMR signals of 84 were characteristic of a mono-hydroxyxanthone. A combination 1D and 2D spectra (see Table 7) allowed the assignment of the protons onto the respective carbons.

Proton $\delta$H 7.85 (ddd, 8.4, 7.2, 1.8) showed mutual COSY correlations to protons $\delta$H 7.64 (d, 8.4) and 7.45 (dd, 7.8, 7.2), which also had COSY correlations to proton $\delta$H 8.18 (dd, 7.8, 1.2). Together with these correlations, *meta* coupling was observed between protons $\delta$H 7.85 and $\delta$H 8.18. This implied that these protons belonged to the same aromatic ring, whereby proton $\delta$H 7.85 was placed *ortho* to protons $\delta$H 7.64 and $\delta$H 7.45 that was also placed *ortho* to proton $\delta$H 8.18. Of the remaining three protons,
Table 7: NMR data for 2-hydroxyxanthone (83).\textsuperscript{a}

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C</th>
<th>$^{1}$H (mult., $J$, int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108.5</td>
<td>7.48 (d, 3.0, 1H)</td>
<td>2, 3, 4, 4a, 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>153.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-OH</td>
<td></td>
<td>9.94 (brs, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>124.5</td>
<td>7.33 (dd, 9.0, 3.0, 1H)</td>
<td>4</td>
<td>1, 2, 4a</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>119.4</td>
<td>7.56 (d, 9.0, 1H)</td>
<td>3</td>
<td>2, 4a, 9, 9a</td>
<td>3</td>
</tr>
<tr>
<td>4a</td>
<td>149.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>155.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>118.1</td>
<td>7.64 (d, 8.4, 1H)</td>
<td>6</td>
<td>4b, 7, 8a, 9</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>135.1</td>
<td>7.85 (ddd, 8.4, 7.2, 1.8, 1H)</td>
<td>5, 7</td>
<td>4b, 8</td>
<td>5, 7</td>
</tr>
<tr>
<td>7</td>
<td>123.9</td>
<td>7.45 (dd, 7.8, 7.2, 1H)</td>
<td>6, 8</td>
<td>4b, 5, 6, 8, 8a</td>
<td>6, 8</td>
</tr>
<tr>
<td>8</td>
<td>125.8</td>
<td>8.18 (dd, 7.8, 1.2, 1H)</td>
<td>7</td>
<td>4b, 6, 9</td>
<td>7</td>
</tr>
<tr>
<td>8a</td>
<td>120.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>175.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>121.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Spectra were recorded in DMSO-$d_6$ at 30 °C.
proton $\delta_H$ 7.56 (d, 9.0) displayed COSY correlations to $\delta_H$ 7.33 (dd, 9.0, 3.0). No COSY correlations were seen from proton $\delta_H$ 7.48 (d, 3.0). Therefore, protons $\delta_H$ 7.56 and $\delta_H$ 7.33 were placed ortho to each other, whereas proton $\delta_H$ 7.33 was placed meta to proton $\delta_H$ 7.48 and para to $\delta_H$ 7.56 on the second aromatic ring. Such an assignment identified the –OH substituent $\delta_H$ 9.94 (brs) to be attached to either of unassigned positions C-2 or C-3. Strong $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.48 and $\delta_H$ 8.18 to C-9, allowed the two protons to be assigned to $\delta_C$ 108.5 (C-1) and 125.8 (C-8) respectively. Since it was already established that the position ortho to $\delta_H$ 7.48 was unassigned for (C-2 or C-3), positioning of $\delta_H$ 7.48 onto C-1, allowed the –OH group to be placed on $\delta_C$ 153.9 (C-2). In addition, other strong $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.48 to $\delta_C$ 149.2 (C-4a) and $\delta_C$ 124.5 (C-3), from $\delta_H$ 7.33 to C-1 and C-4a, and from $\delta_H$ 7.65 to C-2 and $\delta_C$ 121.7 (C-9a) confirmed the positioning of the –OH group onto C-2.

Weak $^2J_{CH}$ HMBC correlations from $\delta_H$ 7.48 and $\delta_H$ 7.33 to C-2 were also seen. On the other hand, strong $^3J_{CH}$ HMBC correlations from $\delta_H$ 8.18 to $\delta_C$ 135.1 (C-6) and $\delta_C$ 155.5 (C-4b) and from $\delta_H$ 7.85 to $\delta_C$ 125.8 (C-8) and C-4b, confirmed the meta placement of protons $\delta_H$ 8.18 on C-8 and $\delta_H$ 7.85 on C-6 made through COSY correlations. Final strong $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.64 to $\delta_C$ 123.9 (C-7) and $\delta_C$ 120.4 (C-8a) and from $\delta_H$ 7.45 to $\delta_C$ 118.1 (C-5) and C-8a also confirmed their mutual meta positioning on C-5 and C-7 respectively. ROE correlations were seen from ortho coupled protons; however, any ROE correlations from $\delta_H$ 9.94 were unobserved. As a result of all the COSY, HMBC and ROE correlations (see Figure 14), 83 was deduced to be 2-hydroxyxanthone. 2-Hydroxyxanthone is a common metabolite of Genus Mammea, being isolated from $M$. odorata,56 $M$. americana54 and $M$. acuminata.57 A similar $^1H$ and $^13C$ NMR spectral data for 2-hydroxyxanthone as that obtained in this study has been reported.114

![Figure 14: Key HMBC (→→), COSY (→→) and ROE (→→) correlations seen for 83.](image)
3.1.5.4 4-Hydroxyxanthone (84)

4-Hydroxyxanthone (84) was obtained as white crystals. Its molecular ion in (+)-LRESIMS spectrum was seen at $m/z$ 212, which allowed a molecular formula of $\text{C}_{13}\text{H}_8\text{O}_3$ to be determined. Thus compounds 83 and 84 were considered to be constitutional isomers like compounds 94 and 118. The $^1$H NMR spectrum of 84 displayed eight signals (one hydroxy and seven aromatic) and the $^{13}$C NMR spectrum had 13 signal (one carbonyl and 12 aromatic signals). The $^1$H and $^{13}$C NMR spectra of 84 (see Table 8) were comparable to the ones obtained for 83 (see Table 7); hence 83 was confirmed to be a mono-hydroxyxanthone. Analysis of $^1$H NMR spectrum showed that four of the aromatic protons, $\delta_H$ 7.68 (d, 8.4), $\delta_H$ 7.88 (ddd, 8.4, 7.2, 1.2), $\delta_H$ 7.48 (dd, 7.8, 7.2), $\delta_H$ 8.19 (dd, 7.8, 1.2) had similar chemical shifts ($\Delta \delta_H \pm 0.04$ ppm) and coupling constants as that obtained for respective protons, H-5, H-6, H-7 and H-8 for 83. The 2D NMR data obtained for the four protons in 84 closely resembled the 2D data for the same four protons of 83. On the basis of these similarities of protons $\delta_H$ 7.68, 7.88, 7.48 and 8.19 of 84 to protons of 83, they were also assigned as H-5 to H-8 respectively.

On the contrary, of the remaining three aromatic protons, proton $\delta_H$ 7.27 (dd, 8.4, 7.8) was ortho coupled to protons $\delta_H$ 7.62 (dd, 8.4, 1.8) and $\delta_H$ 7.34 (dd, 7.8, 1.2); which were meta coupled to each other. COSY correlations were also seen between $\delta_H$ 7.27 and $\delta_H$ 7.62. This led to either a 1- or 4-hydroxanthone structure for 84. The downfield shift of $\delta_H$ 7.62 allowed it to be placed at a position deshielded by the carbonyl group $\delta_C$ 176.2 (C-9), which was C-1 ($\delta_C$ 115.2). Strong $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.62 to C-9 supported this and consequently $\delta_H$ 7.27 was assigned to C-2 ($\delta_C$ 124.0) and $\delta_H$ 7.34 to C-3 ($\delta_C$ 120.2). This allowed the hydroxy group $\delta_H$ 10.44 (s) to be assigned to C-4 ($\delta_C$ 146.6). Additional $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.62 to C-3 and $\delta_C$ 145.2 (C-4a); from $\delta_H$ 7.27 to C-4 and $\delta_C$ 122.2 (C-9a); and from $\delta_H$ 7.34 to C-1 and C-4a were also in support of the –OH substitution at C-4. Finally, ROE correlation seen from the –OH substituent to $\delta_H$ 7.34 confirmed 84 to be 4-hydroxyxanthone. All the key COSY, HMBC and ROE correlations that allowed structure determination are shown in Figure 15.
Table 8: NMR data for 4-hydroxyxanthone (84). *

<table>
<thead>
<tr>
<th>Position</th>
<th>(^{13}C)</th>
<th>(^{1}H) (mult., (J), int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>115.2</td>
<td>7.62 (dd, 8.4, 1.8, 1H)</td>
<td>2</td>
<td>3, 4a, 9</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>124.0</td>
<td>7.27 (dd, 8.4, 7.8, 1H)</td>
<td>1</td>
<td>4, 9a</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>120.2</td>
<td>7.34 (dd, 7.8, 1.2, 1H)</td>
<td>1, 4a</td>
<td></td>
<td>4-OH</td>
</tr>
<tr>
<td>4</td>
<td>146.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-OH</td>
<td></td>
<td>10.44 (s, 1H)</td>
<td>3</td>
<td>4a</td>
<td>3</td>
</tr>
<tr>
<td>4a</td>
<td>145.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>155.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>118.3</td>
<td>7.68 (d, 8.4, 1H)</td>
<td>6</td>
<td>4b, 7, 8a</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>135.4</td>
<td>7.88 (ddd, 8.4, 7.2, 1.2, 1H)</td>
<td>5, 7</td>
<td>4b, 5, 8</td>
<td>5, 7</td>
</tr>
<tr>
<td>7</td>
<td>124.2</td>
<td>7.48 (dd, 7.8, 7.2, 1H)</td>
<td>6, 8</td>
<td>5, 6, 8, 8a</td>
<td>6, 8</td>
</tr>
<tr>
<td>8</td>
<td>125.9</td>
<td>8.19 (dd, 7.8, 1.2, 1H)</td>
<td>7</td>
<td>4b, 6, 9</td>
<td>7</td>
</tr>
<tr>
<td>8a</td>
<td>120.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>176.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>122.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Spectra were recorded in DMSO-\(d_6\) at 30 °C.
4-Hydroxyxanthone has also been isolated from the seeds of *M. americana*. The $^1$H and $^{13}$C NMR spectral data obtained for 4-hydroxyxanthone are very similar to that reported previously.

![Figure 15: Key HMBC (→), COSY (↔) and ROE (↔) correlations seen for 84.](image)

### 3.1.5.5 2-Hydroxy-3-methoxyxanthone (119)

2-Hydroxy-3-methoxyxanthone (119) was obtained as pale yellow crystals. Its molecular formula was determined to be C$_{14}$H$_{10}$O$_4$ by means of (+)-LRESIMS spectrum, which exhibited a molecular ion at $m/z$ 242. The $^1$H NMR spectrum of 119 displayed six aromatic proton signals and one signal belonging to the chemically equivalent methoxy group protons. A total of nine protons were accounted for by these seven signals. Therefore, the unobserved proton signal was presumed to belong to an exchangeable hydroxy group. The $^{13}$C NMR spectrum of 119 had 14 unique resonances, which were one carbonyl, one methoxy and 12 aromatic carbon signals. A comparison of $^1$H and $^{13}$C NMR spectra of 119 (see Table 9) with those obtained for compounds 83, 84, 116, and 118 showed that protons $\delta_H$ 7.60 (d, 8.4), $\delta_H$ 7.81 (ddd, 8.4, 7.2, 1.8), $\delta_H$ 7.44 (dd, 7.8, 7.2) and $\delta_H$ 8.15 (dd, 7.8, 1.8) were attached to carbons $\delta_C$ 117.5 (C-5), $\delta_C$ 134.2 (C-6), $\delta_C$ 123.6 (C-7) and $\delta_C$ 125.3 (C-8) respectively. The remaining two aromatic protons, $\delta_H$ 7.46 (brs) and $\delta_H$ 7.19 (brs) did not show any coupling or COSY correlations; due to which they were para disposed. The deshielding effects of the carbonyl group $\delta_C$ 174.7 (C-9) led to the downfield shifted proton $\delta_H$ 7.46 being assigned to $\delta_C$ 109.9 (C-1), and hence the upfield shifted proton $\delta_H$ 7.19 was assigned to $\delta_C$ 100.0 (C-4). This was confirmed by strong $^3J_{CH}$ HMBC correlation from $\delta_H$ 7.46 to C-9.
Table 9. NMR data for 2-hydroxy-3-methoxyxanthone (119).\textsuperscript{a}

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C</th>
<th>$^1$H (mult., J, int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>107.9</td>
<td>7.46 (brs, 1H)</td>
<td>2, 3, 4a, 9</td>
<td></td>
<td>2-OH</td>
</tr>
<tr>
<td>2</td>
<td>144.4</td>
<td></td>
<td></td>
<td></td>
<td>1, 3-OCH$_3$</td>
</tr>
<tr>
<td>3</td>
<td>155.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OCH$_3$</td>
<td>56.0</td>
<td>3.95 (s, 3H)</td>
<td>3, 3-OH</td>
<td></td>
<td>2-OH, 4</td>
</tr>
<tr>
<td>4</td>
<td>100.0</td>
<td>7.19 (brs, 1H)</td>
<td>2, 3, 4a, 9, 9a</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>4a</td>
<td>155.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>150.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>117.5</td>
<td>7.60 (d, 8.4, 1H)</td>
<td>6</td>
<td>4b, 7, 8a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>134.2</td>
<td>7.81 (ddd, 8.4, 7.2, 1.8, 1H)</td>
<td>5, 7</td>
<td>4b, 8</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>123.6</td>
<td>7.44 (dd, 7.8, 7.2, 1H)</td>
<td>6, 8</td>
<td>5, 8a</td>
<td>6, 8</td>
</tr>
<tr>
<td>8</td>
<td>125.3</td>
<td>8.15 (dd, 7.8, 1.8, 1H)</td>
<td>7</td>
<td>4b, 6, 9</td>
<td>7</td>
</tr>
<tr>
<td>8a</td>
<td>120.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>174.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>114.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Spectra were recorded in DMSO-$d_6$ at 30 °C.

\textsuperscript{b} Signal not observed in $^1$H NMR but observed in ROESY spectrum.

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{H}_3\text{C} & \quad 3 \quad 4a \quad 8a \quad 8 \\
\text{O} & \quad \text{O} \\
\text{4b} & \quad 6
\end{align*}
\]
Accordingly, due to strong $^3J_{CH}$ HMBC correlations observed from –OCH$_3$ δ$_H$ 3.95 (s) to δ$_C$ 155.1 (C-3), the –OCH$_3$ was assigned to C-3; therefore, the –OH group was assigned to δ$_C$ 144.4 (C-2). Proton δ$_H$ 7.46 also showed strong $^3J_{CH}$ and weak $^2J_{CH}$ HMBC correlations to C-3 and C-2 respectively. In contrast, δ$_H$ 7.19 showed strong $^3J_{CH}$ HMBC correlations to C-2 and weak $^2J_{CH}$ HMBC correlations to C-3. Finally, mutual ROE correlations observed from 3-OCH$_3$ to H-4 and 2-OH and from 2-OH to H-1 (see Figure 16) strongly supported the 2-hydroxy-3-methoxyxanthone structure for 119. Earlier studies on the timber extract of Hypericum mysorense (Guttiferae)$^{115}$ and stem-bark extract of Dalbergia sissoides$^{116}$ have also resulted in the isolation of 2-hydroxy-3-methoxyxanthone. The $^1$H NMR spectral data of 2-hydroxy-3-methoxyxanthone was comparable to that reported previously, thus confirming the structure.$^{115}$

![Figure 16: Key HMBC (→), COSY (↔) and ROE (↔) correlations in 119.](image)

### 3.1.5.6. 1,6,7-Trihydroxyxanthone (88)

1,6,7-Trihydroxyxanthone (88) was obtained as bright yellow crystals. A molecular formula of C$_{13}$H$_8$O$_5$ that was consistent with m/z of 244 was derived from the (+)-LRESIMS spectrum. The $^1$H NMR spectrum of 88 displayed a total of eight unique resonances, which belonged to five aromatic and three hydroxy protons. Three out of the five aromatic protons were ortho coupled to each other and also showed mutual COSY correlations. The other two protons did not exhibit any coupling and COSY correlations, so were thought to be para disposed. The $^{13}$C NMR spectrum of 88 had thirteen signals altogether that accounted for one carbonyl and twelve aromatic carbons. The $^1$H and $^{13}$C NMR spectra of 88 (see Table 10) closely resembled the respective spectra for the aglycone of compound 117, 1,6,7-trihydroxyxanthone; 6-O-β-D-glucopyranoside (see Table 4 and Section 3.1.3.3).
Table 10: NMR data for 1,6,7-trihydroxyxanthone (88).a

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C</th>
<th>$^1$H (mult., $J$, int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-OH</td>
<td></td>
<td>12.97 (brs, 1H)</td>
<td>3</td>
<td>1, 4, 9a</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>109.5</td>
<td>6.74 (d, 8.4, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>136.1</td>
<td>7.63 (t, 8.4, 1H)</td>
<td>2, 4</td>
<td>1, 4a, 9a</td>
<td>2, 4</td>
</tr>
<tr>
<td>4</td>
<td>106.8</td>
<td>6.99 (d, 8.4, 1H)</td>
<td>3</td>
<td>2, 4a, 9, 9a</td>
<td>3</td>
</tr>
<tr>
<td>4a</td>
<td>155.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>155.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>102.6</td>
<td>6.92 (brs, 1H)</td>
<td></td>
<td>4b, 6, 7, 8a, 9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>151.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td></td>
<td>9.88 (brs, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>144.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-OH</td>
<td></td>
<td>10.87 (brs, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>107.6</td>
<td>7.42 (brs, 1H)</td>
<td></td>
<td>4b, 5, 6, 7, 9</td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>112.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>180.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>107.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Spectra were recorded in DMSO-$d_6$ at 30 °C.

![Structure of 1,6,7-trihydroxyxanthone (88)]
The 2D spectral correlations seen for 88 were also similar to those seen for the aglycone of 117, except for the absence of HMBC correlations from the –OH groups. However, an additional proton signal $\delta_H$ 9.88 (brs) was seen in the $^1$H NMR spectrum of 88 because of the replacement of the glucosyl moiety by an –OH substituent. As a result, proton $\delta_H$ 7.32 (brs; H-5) of 117 was shifted upfield to $\delta_H$ 6.92 (brs; H-5) in 88. On the basis of the spectral similarities, the structure of 88 was determined to be 1,6,7-trihydroxyxanthone. 1,6,7-Trihydroxyxanthone has been isolated from M. africana as well as Garcinia eugeniifolia (Guttiferae).

3.1.5.7 1,6,7-Trihydroxy-3-methoxyxanthone (120)

1,6,7-Trihydroxy-3-methoxyxanthone (120) was isolated as yellow brown crystals. Analysis of the (+)-LRESIMS spectrum gave a molecular formula of C$_{14}$H$_{10}$O$_6$, which was consistent with the molecular ion at m/z 274. An increment of 30 Da in the molecular weight of 120 from 244 Da of 88 indicated that a methoxy substituent was attached to the trihydroxyxanthone molecule. This was confirmed by the $^1$H NMR spectrum of 120 (see Table 11), which showed the presence of one methoxy, $\delta_H$ 3.87 (s); three hydroxy, $\delta_H$ 13.15 (brs), 10.74 (brs) and 9.81 (brs); and four aromatic protons, $\delta_H$ 6.33 (d, 1.8), 6.57 (d, 1.8), 6.88 (s) and 7.39 (s). The $^{13}$C NMR spectrum of 120 showed the presence of one methoxy ($\delta_C$ 55.6), one carbonyl ($\delta_C$ 178.6) and twelve aromatic carbon signals, out of which six were downfield shifted carbons ($\delta_C$ 162.0, 165.3, 157.1, 154.2, 150.9 and 143.8). Both the $^1$H and $^{13}$C NMR spectra supported a tri-hydroxy-mono-methoxyxanthone structure for 120, which was confirmed with the aid of HMBC and ROE correlations.

Protons $\delta_H$ 6.33 and $\delta_H$ 6.57 were meta disposed on one of the aromatic ring; whereas protons $\delta_H$ 6.88 and $\delta_H$ 7.39 were para disposed on the other aromatic ring. The presence of a highly downfield shifted –OH proton ($\delta_H$ 13.15) suggested it to be hydrogen bonded to the carbonyl group (C-9), which allowed it to be placed on C-1. This was evidenced by strong $^3J_{CH}$ and weak $^2J_{CH}$ HMBC correlations seen from proton $\delta_H$ 13.15 (H-1) to carbons $\delta_C$ 96.2 (C-2) and 162.0 (C-1) respectively.
Table 11. NMR data for 1,6,7-trihydroxy-3-methoxyxanthone (120).a

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C</th>
<th>$^1$H (mult., $J$, int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162.0</td>
<td>13.15 (brs, 1H)</td>
<td>1, 2, 9a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>96.2</td>
<td>6.33 (d, 1.8, 1H)</td>
<td>4</td>
<td>1, 4, 9a</td>
<td>3-OCH$_3$</td>
</tr>
<tr>
<td>3</td>
<td>165.3</td>
<td>3.87 (s, 3H)</td>
<td>3</td>
<td>3-OCH$_3$</td>
<td>2, 4</td>
</tr>
<tr>
<td>4</td>
<td>92.2</td>
<td>6.57 (d, 1.8, 1H)</td>
<td>2</td>
<td>2, 3, 4a, 9a</td>
<td>3-OCH$_3$</td>
</tr>
<tr>
<td>4a</td>
<td>157.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>154.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>102.3</td>
<td>6.88 (s, 1H)</td>
<td>4b, 6, 7, 8a, 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>150.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td></td>
<td>10.74 (brs, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>143.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-OH</td>
<td></td>
<td>9.81 (brs, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>107.7</td>
<td>7.39 (s, 1H)</td>
<td>4b, 6, 7, 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>111.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>178.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>102.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Spectra were recorded in DMSO-$d_6$ at 30 °C.
Weak $^2J_{CH}$ HMBC correlations shown by $\delta_H$ 6.57 (H-4) to $\delta_C$ 165.3 (C-3) and strong $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 3.87 to C-3 led to the assignment of the –OCH$_3$ group on to C-3. ROE correlations seen from 3-OCH$_3$ to H-2 and H-4 substantiated this (see Figure 17).

On the other hand, para displacement of protons ($\delta_H$ 6.88 and 7.39) allowed the two –OH groups to be placed on C-6 and C-7 on the second aromatic ring. On the basis of strong $^3J_{CH}$ HMBC correlations seen from $\delta_H$ 7.39 to C-9 and $\delta_C$ 150.9 (C-6), $\delta_H$ 7.39 was assigned as H-8, thus $\delta_H$ 6.88 was assigned as H-5. No HMBC or ROE correlations were observed from –OH protons $\delta_H$ 10.74 and 9.81; however, they were placed on C-6 and C-7 respectively. This was partly due to the respective chemical shifts of carbons $\delta_C$ 150.9 (C-6) and 143.8 (C-7); and proton chemical shift of the 6-OH and 7-OH substituents of 1,6,7-trihydroxyxanthone; 6-O-ß-d-glucopyranoside (117) and 1,6,7-trihydroxyxanthone (88). Therefore, the structure of 120 was determined to be 1,6,7-trihydroxy-3-methoxyxanthone. 1,6,7-Trihydroxy-3-methoxyxanthone, which is also known as Athyriol, had been previously isolated from the leaves of Athyrium mesosorum.$^{103}$

![Figure 17](image-url): Key HMBC (→), COSY (↔) and ROE (↔) correlations in 120.

### 3.1.6 Isolation of Surangin B (34) from DCM Extract

The DCM extract of the stem bark of *M. odorata* furnished Surangin B (34), a known coumarin, containing the phloroglucinol (5,7-oxygenation pattern) nucleus. Normal phase flash chromatography of the DCM extract involving mixtures of n-hexane and EtOAc as eluents afforded 83 fractions, which were combined together based on TLC pattern. Reverse phase HPLC separation of fractions 23-36 using H$_2$O/MeOH
(0.2% TFA in both) yielded surangin B (34, 19.4 mg, 0.07% dry wt) as shown in Scheme 5 and Figure 18.

![Chemical structure of surangin B](image)

### Scheme 5: Isolation and extraction procedure for DCM extract of *M. odorata.*

- **i.** Extraction of the defatted biota sample with DCM, EtOAc and MeOH.
- **ii.** NP Flash chromatography utilizing 5% stepwise gradient from 100% n-hexane to 100% EtOAc and combination of fractions using analytical tlc.
- **iii.** Semi-prep RP HPLC using isocratic conditions of 20% H2O (0.2% TFA)/80% MeOH (0.2% TFA) for 1 min then gradient conditions to 100% MeOH (0.2% TFA) for 49 min.

**Surangin B**
- **(11, 19.4 mg, 0.07% dry wt)**
- **Retention time = 41-42 min**
3.1.7 Structure Elucidation of Surangin B (34)

Surangin B (34) was obtained as colourless crystals with $[\alpha]_D$ of -33° (c 0.1, CHCl$_3$). The (+)-LRESIMS spectrum of 34 displayed a molecular ion at $m/z$ 498, from which a molecular formula of C$_{29}$H$_{38}$O$_7$ with 11 degrees of unsaturations was deduced. The $^1$H NMR in conjunction with HSQC spectra of 34 showed a total of 20 signals showing the presence of one hydroxy, one aromatic, two olefinic, two methine, three methylene, four diastereotopic and seven methyl protons. The $^{13}$C NMR spectra of 34 showed a total of 29 unique signals accounting for three carbonyl, eight aromatic, four olefinic, two methine, five methylene, and seven methyl carbons. $^1$H and $^{13}$C NMR spectral data (see Table 12), along with 11 unsaturations suggested that 34 belonged to a different class of secondary metabolite other than a xanthone.

Structure Determination of the Coumarin Nucleus

The presence of fewer aromatic proton signals than the aromatic carbon signals suggested that a highly substituted aromatic ring was present in the molecule. The downfield shift of the hydroxy proton $\delta_H$ 14.64 (s) indicated that it was a chelated hydroxy group, being hydrogen bonded to a carbonyl group. The integration pattern of the $^1$H NMR spectrum accounted for 37 protons in the molecule; therefore implying that another hydroxy group existed in the molecule. Proton $\delta_H$ 14.64 showed weak $^2J_{CH}$ HMBC correlations to downfield shifted aromatic carbon $\delta_C$ 166.1
Table 12: NMR data for surangin B (34).\(^a\)

<table>
<thead>
<tr>
<th>Position</th>
<th>(^1^C)</th>
<th>(^1^H)(mult., (J), int.)</th>
<th>gCOSY</th>
<th>gHMBC</th>
<th>ROESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>159.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>106.6</td>
<td>6.28 (s, 1H)</td>
<td>1', 2, 4, 4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>157.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-R'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>100.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>158.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>110.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-R&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>166.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-OH</td>
<td>14.64 (s, 1H)</td>
<td></td>
<td>1&quot;, 1&quot;, 5, 6, 7, 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>104.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-R&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>156.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>74.0</td>
<td>6.50 (d, 6.0, 1H)</td>
<td>2'</td>
<td>1'-OCO, 3, 4</td>
<td>3'</td>
</tr>
<tr>
<td>1'-OCOCH(_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'-OCO</td>
<td>170.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'-CH(_3)</td>
<td>21.2</td>
<td>2.18 (brs, 3H)</td>
<td>1', 1'-OCO, 1'-CH(_3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>29.0</td>
<td>1.96 (m, 1H)</td>
<td>1', 2', 3'</td>
<td>3', 4</td>
<td>2', 3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.68 (brs, 1H)</td>
<td>1', 2', 3'</td>
<td>3', 4</td>
<td>2', 3'</td>
</tr>
<tr>
<td>3'</td>
<td>10.3</td>
<td>1.01 (t, 7.2, 3H)</td>
<td>2'</td>
<td>1', 2', 3'</td>
<td>1', 2'</td>
</tr>
<tr>
<td>1&quot;</td>
<td>21.9</td>
<td>3.52 (d, 9.6, 2H)</td>
<td>2'</td>
<td>2&quot;, 3&quot;, 5, 6, 7</td>
<td>4&quot;</td>
</tr>
<tr>
<td>2&quot;</td>
<td>120.0</td>
<td>5.25 (t, 6.6, 1H)</td>
<td>1&quot;, 4&quot;</td>
<td>1&quot;, 4&quot;, 5, 6</td>
<td>5&quot;</td>
</tr>
<tr>
<td>3&quot;</td>
<td>142.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4&quot;</td>
<td>16.8</td>
<td>1.86 (brs, 3H)</td>
<td>2&quot;</td>
<td>2&quot;, 3&quot;, 5&quot;</td>
<td>1&quot;, 6&quot;</td>
</tr>
<tr>
<td>5&quot;</td>
<td>39.9</td>
<td>2.12 (s, 2H)</td>
<td>2&quot;, 5&quot;, 6&quot;</td>
<td>2&quot;</td>
<td></td>
</tr>
<tr>
<td>6&quot;</td>
<td>26.6</td>
<td>2.115 (s, 2H)</td>
<td>7&quot;</td>
<td>8&quot;</td>
<td>4&quot;</td>
</tr>
<tr>
<td>7&quot;</td>
<td>123.5</td>
<td>5.07 (brs, 1H)</td>
<td>6&quot;, 9&quot;, 10&quot;</td>
<td>5&quot;</td>
<td>10&quot;</td>
</tr>
<tr>
<td>8&quot;</td>
<td>132.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9&quot;</td>
<td>17.9</td>
<td>1.60 (brs, 3H)</td>
<td>7&quot;</td>
<td>7&quot;, 8&quot;, 10&quot;</td>
<td></td>
</tr>
<tr>
<td>10&quot;</td>
<td>25.9</td>
<td>1.68 (brs, 3H)</td>
<td>7&quot;</td>
<td>7&quot;, 8&quot;, 9&quot;</td>
<td>7&quot;</td>
</tr>
<tr>
<td>1&quot;&quot;</td>
<td>211.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2&quot;&quot;</td>
<td>47.2</td>
<td>3.94 (q, 6.6, 1H)</td>
<td>2&quot;&quot;, 3&quot;&quot;</td>
<td>1&quot;&quot;, 2&quot;&quot;, 3&quot;&quot;, 3&quot;&quot;, 4&quot;&quot;</td>
<td>2&quot;&quot;,-CH(_3)</td>
</tr>
<tr>
<td>2&quot;&quot;-CH(_3)</td>
<td>16.9</td>
<td>1.25 (d, 7.2, 3H)</td>
<td>2&quot;&quot;</td>
<td>1&quot;&quot;, 2&quot;&quot;, 2&quot;&quot;-CH(_3), 3&quot;&quot;</td>
<td>2&quot;&quot;</td>
</tr>
<tr>
<td>3&quot;&quot;</td>
<td>27.3</td>
<td>1.90 (m, 1H)</td>
<td>2&quot;&quot;, 3&quot;&quot;, 4&quot;&quot;</td>
<td>1&quot;&quot;, 2&quot;&quot;, 2&quot;&quot;-CH(_3), 4&quot;&quot;</td>
<td>3&quot;&quot;, 4&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.48 (m, 1H)</td>
<td>2&quot;&quot;, 3&quot;&quot;, 4&quot;&quot;</td>
<td>1&quot;&quot;, 2&quot;&quot;, 2&quot;&quot;-CH(_3), 4&quot;&quot;</td>
<td>3&quot;&quot;, 4&quot;&quot;</td>
</tr>
<tr>
<td>4&quot;&quot;</td>
<td>11.9</td>
<td>0.99 (t, 7.2, 3H)</td>
<td>3&quot;&quot;</td>
<td>2&quot;&quot;, 3&quot;&quot;, 4&quot;&quot;</td>
<td>3&quot;&quot;</td>
</tr>
</tbody>
</table>

\(^a\) Spectra were recorded in CDCl\(_3\) at 30 °C.  
\(^b\) Signal not seen.  
R = substituent
(C-7), onto which it was assigned. It also showed strong $^3J_{CH}$ HMBC correlations to upfield shifted carbons $\delta_C$ 104.5 (C-8) and 110.6 (C-6). HSQC data showed that no aromatic proton signals showing $meta$ coupling were attached to these carbons, thus the two carbons were assumed to contain electron donating substituents. Very weak $^4J_{CH}$ HMBC correlations seen from $\delta_H$ 14.64 to a carbonyl carbon $\delta_C$ 211.0 (C-1") and methylene carbon $\delta_C$ 21.9 (C-1") confirmed this. The existence of a carbonyl carbon $\delta_C$ 211.0 in the vicinity of $\delta_H$ 14.64 also confirmed its downfield shifting due to hydrogen bonding. Protons $\delta_H$ 3.52 (d, 9.6) located on C-1" showed $^3J_{CH}$ HMBC correlations to carbons $\delta_C$ 158.4 (C-5) and (C-7) and $^2J_{CH}$ HMBC correlations to C-6; based on which the second –OH substituent was placed onto C-5, $meta$ to $\delta_H$ 14.64. At this point, four out of eight aromatic carbon signals were assigned for.

Accordingly, an upfield shifted proton $\delta_H$ 6.28 (s) located on $\delta_C$ 106.6 (C-3) showed no coupling nor did it show any COSY correlations. This proton showed weak $^2J_{CH}$ HMBC correlations to downfield shifted carbons $\delta_C$ 157.5 (C-4); and strong $^3J_{CH}$ HMBC correlations to a quaternary carbon $\delta_C$ 100.7 (C-4a) and a methine carbon $\delta_C$ 74.0 (C-1'). Proton $\delta_H$ 6.50 (d, 6.0) positioned onto C-1' showed $^2J_{CH}$ HMBC correlations to C-4 and $^3J_{CH}$ HMBC correlation to C-3. This allowed a substituent to be placed on C-4. The quaternary nature of C-4a suggested that it was positioned $ortho$ to C-4 and C-5. Moreover, the absence of any more $^3J_{CH}$ HMBC correlations from $\delta_H$ 6.28 (H-3) to any other carbon suggested the presence of a heteroatom (oxygen in this case) within the ring. This could be well explained by the presence of a downfield shifted unassigned carbon $\delta_C$ 156.3, which could be linked to the oxygen atom, which in turn is bonded to $\delta_C$ 159.7 (C-2). Since no more –OH proton
signals were observed in the $^1$H NMR spectrum; C-2 was deduced to be a carbonyl carbon. Weak $^2J_{CH}$ HMBC correlations seen from H-3 to C-2, confirmed a heterocyclic moiety to be present in the molecule. The observed HMBC correlations were instrumental in the structure elucidation of a tri-substituted 5,7-dihydroxycoumarin (see Figure 19), which is a common constituent of the non polar extracts of numerous *Mammea* species.

![Figure 19: Key HMBC (→) correlations in the structure determination of coumarin nucleus.](image)

### Structure Determination of the Substituent Moieties

**1-Acetoxypropyl Substituent on C-4**

Proton $\delta_H$ 6.50 (H-1') showed mutual COSY correlations to diastereotopic protons $\delta_H$ 1.96 (m) and 1.68 (brs), which also showed COSY correlations to methyl protons $\delta_H$ 1.01 (t, 7.2). Based on these COSY and some HMBC and ROE correlations, a propyl chain was generated. In addition to showing weak $^2J_{CH}$ HMBC correlations to C-4, proton $\delta_H$ 6.50 H-1' showed strong $^3J_{CH}$ HMBC correlations to a carbonyl carbon $\delta_C$ 170.6 (C-1'-OCOCH$_3$). The downfield shift of carbon $\delta_C$ 74.0, on which H-1' is attached suggested that an electronegative atom was attached to it. Methyl protons $\delta_H$ 2.18 (brs) displayed weak $^2J_{CH}$ HMBC correlations to $\delta_C$ 170.6 and very weak $^4J_{CH}$ HMBC correlations to C-1'. As a result the structure for the substituent on C-4 was determined to be 1-acetoxypropyl as shown in Figure 20.

![Figure 20: Key HMBC (→), COSY (→) and ROE (↔) seen for the 1-Acetoxypropyl substituent.](image)
Geranyl Substituent on C-6

Methylene protons $\delta_H$ 3.52 (d, 9.6) on C-1” showed mutual COSY correlations to olefinic proton $\delta_H$ 5.25 (t, 6.6), which also showed COSY correlations to methyl protons $\delta_H$ 1.86 (brs). Protons $\delta_H$ 3.52 exhibited weak $^2J_{CH}$ HMBC correlations to olefinic carbon $\delta_C$ 120.0 (C-2”) and strong $^3J_{CH}$ HMBC correlations to a quaternary carbon $\delta_C$ 142.5 (C-3”). Similar HMBC correlations were observed from $\delta_H$ 5.25 and 1.86 (brs). A strong $^3J_{CH}$ HMBC correlation seen from $\delta_H$ 1.86 to methylene carbon $\delta_C$ 39.9 (C-5”), onto which protons $\delta_H$ 2.12 (s) were positioned. These protons showed weak $^2J_{CH}$ HMBC correlations to methylene carbon $\delta_C$ 26.6 (C-6”). Protons $\delta_H$ 2.115 (s) attached to C-6” showed COSY correlations to olefinic proton $\delta_H$ 5.07 (brs) positioned on $\delta_C$ 123.5 (C-7”). Mutual COSY correlations were also observed between protons $\delta_H$ 5.07 and methyl protons $\delta_H$ 1.60 (brs) and 1.68 (brs). A combination of these COSY correlations along with HMBC correlations confirmed the substituent on C-6 to be a geranyl moiety (see Figure 21).

![Figure 21](image_url)

**Figure 21**: Key HMBC (→) and COSY (↔) seen for the Geranyl substituent.

**Figure 22** shows the ROE correlations between protons of the geranyl moiety. Mutual ROE correlations seen between protons $\delta_H$ 3.52 (H-1”) and $\delta_H$ 1.86 (H-4”) allowed the methylene (C-1”) and methyl (C-4”) carbons to be placed on the same side of the double bond. ROE correlations seen between protons $\delta_H$ 5.25 (H-2”) and $\delta_H$ 2.12 (H-5”) allowed the olefinic proton (H-2”) and methylene carbon (C-5”) to be placed on the opposite side of the double bond with respect to H-1”. Similarly, ROE correlations seen between protons $\delta_H$ 5.07 (H-7”) and $\delta_H$ 1.68 (H-9”) allowed the olefinic proton (H-7”) and the methyl carbon (C-9”) to be placed on the same side of the second double bond and opposite to the methylene (C-6”) and methyl (C-10”) carbons. Based on these correlations the relative configuration at the two double bonds was established as trans or $E$. 

72
2-Methylbutyryl Substituent on C-8

Proton $\delta_H$ 3.94 (q, 6.6), attached to a methine carbon $\delta_C$ 47.2 (C-2’”) showed COSY correlations to methyl protons $\delta_H$ 1.25 (d, 7.2) and diastereotopic protons $\delta_H$ 1.90 (m) and 1.48 (m), which also showed COSY correlations to methyl protons $\delta_H$ 0.99 (t, 7.2). Weak $^2J_{CH}$ HMBC correlations from $\delta_H$ 3.94 to carbonyl carbon $\delta_C$ 211.0 (C-1’”), methylene carbon $\delta_C$ 27.3 (C-3’”) and methyl $\delta_C$ 16.9 (C-2’”-CH$_3$); and strong $^3J_{CH}$ HMBC correlations to methyl carbon $\delta_C$ 11.9 (C-4’”) allowed a 2-methylbutyryl substituent to be attached to C-8 of the coumarin nucleus as shown in Figure 23.

The attachment of a 1-acetoxypropyl, geranyl and 2-methylbutyryl on carbons 4, 6 and 8 of the 5,7-dihydroxycoumarin nucleus respectively led to the structure of 34 to be that of surangin B. Surangin B has been previously isolated from *M. longifolia*. Comparison of the $^1H$ NMR spectral data with that reported previously confirmed the structure. The experimental specific rotation, $[\alpha]_D$ of -33 ($c$ 0.1, CHCl$_3$) was comparable to the Literature value ($[\alpha]_D$ of -30°) as well. However, the absolute stereochemistry of the chiral centres present in the 34 could not be determined. Due to time constraints no further isolation was possible from the DCM extract. Figure 18 shows that the combined fraction 23-36 obtained from the fractionation of the DCM extract contain many other compounds that are yet to be isolated.
3.2 Biological Investigation of Metabolites from *M. odorata*

Eleven compounds isolated from the bark of *M. odorata* have been evaluated for antimalarial and antibacterial activities. Before performing any biological assays, all the isolated compounds were analysed for their purity and integrity using the nanostream microfluidics liquid chromatography (μPLC). Results obtained through the C18 μPLC work showed that all the compounds were stable and had a purity of greater than 96% except for 120, which was 90% pure.

### 3.2.1 *In vitro* Antimalarial Activity

The *in vitro* antimalarial activity displayed by the eleven compounds against the 3D7 (chloroquine-sensitive) line of *Plasmodium falciparum* is shown in Table 13. At a concentration of 25 μM, compounds 83, 84, 88, 94 and 115-120 showed activity in the range of 14.5% to 43.2%. At this concentration, 34 as well as chloroquine (positive control) were shown to inhibit the growth of the test organism by 100%. The IC50 of 34 was determined to be 2.5 (±1.6) μM, whereas the IC50 of chloroquine was 0.02 (±0.01) μM, which was almost 100-fold less than that obtained for 34.

Selectivity for *P. falciparum*, which is the most potent of the four species of the malarial parasite, may be due to the structural differences amongst the eleven compounds. This has been demonstrated by different activities of surangin B (34), a coumarin; (-) epicatechin (115), a flavanol; and oxygenated xanthones, 2- and 4-hydroxyxanthenes (83 and 84), 1,6,7-trihydroxyxanthone (88), 2,6-dihydroxyxanthone (94), 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside (116), 1,6,7-trihydroxyxanthone; 6-O-β-D-glucopyranoside (117), 2,3-dihydroxyxanthone (118), 2-hydroxy-3-methoxyxanthone (119), 1,6,7-trihydroxy-3-methoxyxanthone (120). Highest activity was shown by 34 (100%), whereas the lowest activity was shown by 84 (14.4%). Moderate activity was shown by 116 and 119, which had 43.1% and 43.2% of inhibition respectively. The rest of the compounds had percentage inhibition below 40%.
Xanthones have been reported to possess significant antimalarial activity, for which the oxygenation pattern on the xanthone moiety is of importance. The attachment of a hydroxy group on position 3 seems to increase the activity (such as in 118). However, the substitution of a methoxy or glucosyl group on the same position has a greater effect on activity (such as in 116 and 119). Higher activity shown by a 3-methoxy substitution has been reported. Hydroxylation on position 7 also contributes to higher antimalarial activity, however this was not observed for 88, 117 and 120 probably because they also had a hydroxy group on position 1 (peri), which causes decreased activity. The attachment of a hydroxy group on position 4 (peri) in 84 may be responsible for its lowest antimalarial activity. In addition, prenylated xanthones have also shown significant antimalarial activities. The attachment of isopentyl or geranyl moiety greatly increases the antimalarial activity of xanthones. Highest activity exhibited by 34 may be a result of its geranyl substituent, although no such experimental data for coumarins exist in the literature.

One of the key metabolic processes of the malarial parasite is the proteolysis of haemoglobin into toxic heme. Heme is detoxified through the heme polymerisation process to form hemozoin. It has been shown that hydroxyxanthones have the ability to inhibit heme polymerization and thus preventing hemozoin formation. This ability correlates well to its ability to inhibit the growth of P. falciparum. The attachment of hydroxy groups on peri positions (1, 4, 5 and 8) of the xanthone moiety is crucial to the inhibitory effects of the hydroxylated xanthone. This is possibly due to hydrogen bond formation, which reduces the xanthone’s affinity for the heme.

Quite a number of xanthones and a few coumarins showing antimalarial activity have been previously reported. A report on the antimalarial activity of most of the compounds, especially 34, which was the most potent of all, has been made for the first time in this study.
Table 13: *In vitro* antimalarial activity displayed by compounds 34, 83, 84, 88, 94, 115-120.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition of <em>P. falciparum</em> at 25 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3D7</td>
</tr>
<tr>
<td>34</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>83</td>
<td>30.2 (±12.70)</td>
</tr>
<tr>
<td>84</td>
<td>14.5 (±16.0)</td>
</tr>
<tr>
<td>88</td>
<td>33.7 (±4.40)</td>
</tr>
<tr>
<td>94</td>
<td>34.6 (±12.70)</td>
</tr>
<tr>
<td>115</td>
<td>17.2 (±17.1)</td>
</tr>
<tr>
<td>116</td>
<td>43.1 (±4.5)</td>
</tr>
<tr>
<td>117</td>
<td>23.6 (±6.6)</td>
</tr>
<tr>
<td>118</td>
<td>25.3 (±25.5)</td>
</tr>
<tr>
<td>119</td>
<td>43.2 (±14.2)</td>
</tr>
<tr>
<td>120</td>
<td>28.5 (±14.4)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> IC<sub>50</sub> = 2.5 (±1.6)

<sup>b</sup> IC<sub>50</sub> = 0.02 (±0.01)
Table 14: Zone of Inhibition (mm) displayed by the Extracts and Compounds against Human Pathogenic Bacteria.

<table>
<thead>
<tr>
<th>Compound/Extract</th>
<th>S. aureus NZRM 3022</th>
<th>MRSA Clinical isolate NZRM 1106</th>
<th>E. faecalis NZRM 916</th>
<th>E. coli NZRM 798</th>
<th>E. aerogenes NZRM 997</th>
<th>P. aeruginosa NZRM 482</th>
<th>K. pneumonia NZRM 383</th>
<th>S. menston NZRM 383</th>
<th>P. vulgaris NZRM 3475</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EtOAc</td>
<td>11</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DCM</td>
<td>19</td>
<td>25</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>83</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>84</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>88</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>94</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>115</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>116</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>117</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>118</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>119</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>120</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>32</td>
<td>31</td>
<td>37</td>
<td>28</td>
<td>31</td>
<td>20</td>
<td>36</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>40</td>
<td>38</td>
<td>30</td>
<td>31</td>
<td>29</td>
<td>8</td>
<td>36</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>
3.2.2 *In vitro* Antimicrobial Activity

The antimicrobial activities of the three extracts along with the eleven compounds are shown in Table 14 on the previous page. The extracts and compounds were evaluated using nine human pathogenic bacteria following the disc diffusion method. The extracts were tested at a concentration of 50 mg/mL or 500 µg/disc; whereas the isolated compounds and positive controls (chloramphenicol and tetracycline) were tested at 10 mg/mL or 100 µg/disc. At the tested concentration, the three extracts displayed modest activity only against the Gram positive bacteria: *S. aureus* (NZRM 3022), MRSA (clinical isolate), *E. faecalis* (NZRM 1106). The DCM extract was shown to be the most potent, followed by EtOAc and MeOH extracts in inhibiting the growth of three organisms. However, its activity was not comparable with that shown by chloramphenicol and tetracycline. In addition, chloramphenicol and tetracycline also showed significant activity against Gram negative bacteria: *E. coli* (NZRM 916), *E. aerogenes* (NZRM 798), *P. aeruginosa* (NZRM 997), *K. pneumonia* (NZRM 482), *S. menston* (NZRM 383), and *P. vulgaris* (NZRM 3475).

Furthermore, at a concentration of 10 mg/mL, none of the compounds were shown to possess any antibacterial activity. The very low activity of MeOH and EtOAc extracts may well explain for the lack activity of the compounds (83, 84, 88, 94 and 115-120) isolated from them, or apparent activity at a higher concentration. However, compound 34, which was isolated from DCM extract, also did not show any antimicrobial activity. This could be because the DCM extract contains many other coumarins or similar compounds that have yet to be isolated. These compounds may be responsible for the inhibition activity of DCM extract, either independently or synergistically.

The compounds could not be tested at a higher concentration for antimicrobial activity because of insufficient amounts being present. Accordingly, if a higher concentration of compounds is used then it would reflect on it being not that potent. In addition a bioassay guided fractionation of the DCM extract of *M. odorata* may prove noteworthy in the isolation of effective antimicrobials. However, antifungal evaluation of the extracts, isolated compounds or to be isolated compounds from *M. odorata* is also recommended as plants are more susceptible to fungi rather than
bacteria and malarial parasites. Bioassay-guided antifungal assays may result in the isolation of secondary metabolites, which the plants synthesize for their own protection.
CHAPTER 4.0: Conclusion

In conclusion, the aim of the study, which was to carry out chemical and biological investigations of the metabolites from *Mammea odorata*, was successfully achieved. Chemical investigations resulted in the isolation of eleven compounds from the three different extracts of the stem bark. The MeOH extract yielded two new compounds, 2,3-dihydroxyxanthone; 3-\(O\)-\(\beta\)-D-glucopyranoside (116) and 1,6,7-trihydroxyxanthone; 6-\(O\)-\(\beta\)-D-glucopyranoside (117) and a known compound, (\(-\))-epicatechin (115). The EtOAc extract yielded a new natural product, 2,3-dihydroxyxanthone (118) and six known compounds, 2- and 4-hydroxyxanthones (83 and 84), 2,6-dihydroxyxanthone (94), 2-hydroxy-3-methoxyxanthone (119), 1,6,7-trihydroxyxanthone (88), and 1,6,7-trihydroxy-3-methoxyxanthone (120). Surangin B (34), a known compound, was isolated from the DCM extract. This study reported the first time isolation of xanthone glycosides from the genus *Mammea*.

Biological investigations of the isolated compounds using the *in vitro* antimalarial assays identified 34 to be the most potent of all the other compounds with an IC\(_{50}\) of 2.5 \(\mu\)M. A possible mode of action of antimalarial potency of hydroxylated xanthones was also discussed. Antimicrobial susceptibility assays of the three extracts and eleven compounds demonstrated that the DCM extract showed highest growth inhibition of *S. aureus*, MRSA and *E. faecalis*. None of the compounds showed any antimicrobial activity at 10 mg/mL. It may prove noteworthy if a bioassay guided fractionation of the dichloromethane extract was carried out in order to investigate the antimalarial and antimicrobial activities potential of its metabolites. Antifungal evaluations of the extracts and compounds are also recommended.

Finally, the isolation of eleven compounds from the bark of *M. odorata* in this study has been significant since it has proven that *M. odorata* is a rich source of secondary metabolites. At present a total of seventeen compounds, which comprises one coumarin, one flavanol and fifteen xanthones have been isolated from the stem bark and heartwood of *M. odorata*. Further investigation of the dichloromethane extract may yield new coumarins, whereas the methanol extract may be a potential source of new xanthone glycosides.
References


the 6-acyl family, B/AA (Isomammein) and B/AB. *Tetrahedron Lett.* 1970, 46, 3979-3982.


Reynertson, K. A.; Weinstein, I. B.; Kennelly, E. J., Antioxidant and cytotoxic
852-860.

68. Ito, C.; Murata, T.; Itoigawa, M.; Nakao, K.; Kaneda, N.; Furukawa, H.,
Apoptosis induced activity of 4-substituted coumarins from *Calophyllum
brasiliense* in human leukaemia HL-60 cells. *J. Pharm. Pharmacol.* **2006**, 58,
975-980.

69. Yasunaka, K.; Abe, F.; Nagayama, A.; Okabe, H.; Lozada-Peréz, L.; López-
Villafranco, E.; Muñiz, E. E.; Aguilar, A.; Reyes-Chilpa, R., Antibacterial
activity of crude extracts from Mexican medicinal plants and purified

Minyem, C.; Mbing, J. N.; Ngassam, P.; Tih, R. G.; Sodengam, B. L.; Bodo,
B., The in-vitro antimicrobial activities of some medicinal plants from

71. Rao, L. J. M.; Yada, H.; Ono, H.; Ohnishi-Kameyama, M.; Yoshida, M.,
Occurrence of antioxidant and radical scavenging proanthocyanidins from the
Indian minor spice nagkesar (*Mammea longifolia* planch and triana syn).

72. Rathee, J. S.; Hassarajani, A.; Chattopadhyay, S., Antioxidant activity of

73. Einbond, L. S.; Reynertson, K. A.; Luo, X.-D.; Basile, M. J.; Kennelly, E. J.,
Anthocyanin antioxidants from edible fruits. *Food Chem.* **2004**, 84,

74. Toma, W.; Hiruma-Lima, C. A.; Guerrero, R. O.; Brito, A. R. M. S.,
Preliminary studies of *Mammea americana* L. (Guttiferae) bark/latex extract
point to an effective antiulcer effect on gastric ulcer models in mice.

Kauffman, F. C.; Sanchez, R. I.; Mesia-Vela, S., Inhibition of gastric H⁺, K⁺-
ATPase activity by flavonoids, coumarins and xanthones isolated from

76. Atta-ur-Rahman; Choudhary, M. I., Bioactive natural products as a potential
73 (3555-560).

Azebaze, A.; Pegnyemb, D. E.; Watcheung, J.; Goffin, C.; Galleni, M.,
Screening of some medicinal plants from Cameroon for β-Lactamase inhibitory

78. Antia, B. S.; Okkon, J. E.; Nwidu, L. L.; Jackson, C. L., Effect of subchronic
administration of ethanolic stembark extract of *Mammea africana* Sabine on


APPENDICES
Appendix 1: $^1$H NMR spectrum of 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside (116) in DMSO-$d_6$; 600 MHz
Appendix 2: $^{13}$C NMR spectrum of 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside (116) DMSO-$d_6$; 500 MHz
Appendix 3: gCOSY spectrum of 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside (116) in DMSO-$d_6$; 600 MHz
Appendix 4: gHMBC spectrum of 2,3-dihydroxyxanthone; 3-O-gluco-\(\beta\)-glucopyranoside (116) in DMSO-\(d_6\); 600 MHz
Appendix 5: gHSQC spectrum of 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside (116) in DMSO-d$_6$; 600 MHz
Appendix 6: gROESY spectrum of 2,3-dihydroxyxanthone; 3-O-β-D-glucopyranoside (116) in DMSO-$d_6$; 600 MHz
Appendix 7: $^1$H NMR spectrum of 1,6,7-trihydroxyxanthone; 6-O-β-D-glucopyranoside (117) in DMSO-$d_6$; 600 MHz
Appendix 8: gCOSY spectrum of 1,6,7-trihydroxyxanthone; 6-\(O\)-\(\beta\)-D-glucopyranoside (117) in DMSO-\(d_6\); 600 MHz
Appendix 9: gHMBC spectrum of 1,6,7-trihydroxyxanthone; 6-O-β-D-glucopyranoside (117) in DMSO-$d_6$; 600 MHz
Appendix 10: gHSQC spectrum of 1,6,7-trihydroxyxanthone; 6-O-β-D-glucopyranoside (117) in DMSO-\textit{d}_6; 600 MHz
Appendix 11: gROESY spectrum of 1,6,7-trihydroxyxanthone; 6-O-β-D-glucopyranoside (117) in DMSO-d$_6$; 600 MHz
Appendix 12: $^1$H NMR spectrum of 2,3-dihydroxyxanthone (118) in DMSO-$d_6$; 600 MHz
Appendix 13: $^{13}$C NMR spectrum of 2,3-dihydroxynanthone (118) in DMSO-$d_6$, 500 MHz
Appendix 14: gCOSY spectrum of 2,3-dihydroxyxanthone (118) in DMSO-$d_6$; 600 MHz
Appendix 15: gHMBC spectrum of 2,3-dihydroxanthone (118) in DMSO-$d_6$: 600 MHz
Appendix 16: gHSQC spectrum of 2,3-dihydroxyxanthone (118) in DMSO-$d_6$; 600 MHz
Appendix 17: gROESY spectrum of 2,3-dihydroxyxanthone (118) in DMSO-\textit{d}_6; 600 MHz