ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM SELECTED MANGROVE ENDOPHYTIC FUNGI ALONG THE KENYA COAST

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A THESIS SUBMITTED TO THE SCHOOL OF APPLIED AND HEALTH SCIENCES IN THE DEPARTMENT OF PURE AND APPLIED SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY (CHEMISTRY) OF TECHNICAL UNIVERSITY OF MOMBASA

2023

DECLARATION

Declaration by the Student

This thesis is my original work and has not been presented for examination in any University.

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This thesis has been submitted with our approval as University supervisors.

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This thesis is dedicated to God Almighty and my family.

ACKNOWLEDGEMENT

Every departure must be accompanied by a destination in the far or near future. The long academic journey of my PhD has finally come to a good end after six years of hard work. First: I am grateful to the Technical University of Mombasa (TUM) for giving me the opportunity to study chemistry, and for granting me study leave. . Secondly, National Research Fund (NRF) Kenya for financial support through the post-graduate research grant NRF/R/2016/2017. Thirdly, Western Indian Ocean Marine Science Association (WIOMSA) for their financial support through the Marine Research Grant (MARG-1) Programme that supplemented my research budget. My sincere gratitude to Prof. Martin Onani, University of the Western Cape, South Africa and Prof. Matthias Heydenreich - Institut für Chemie, Postdam, Germany for all the nuclear magnetic resonance (NMR) analysis conducted in this study.

I trace my PhD journey to a conference sponsored by Kenya Coastal Development Project (KCDP) held at Voi Lodge, Taita Taveta County in March, 2016. This is where I met Dr. Elisha Mrabu of Kenya Marine and Fisheries Research Institute (KMFRI) and Dr. Joyce Jefwa of the National Museums of Kenya who inspired me to delve into the study of mangrove endophytic fungi. My sincere gratitude to these two for their inspiration. I would like to sincerely thank Prof. Josiah Ochieng Odalo, the main supervisor who has consistently and tirelessly provided support since the conceptualization of this project, through funding acquisition and to this end. I am truly grateful. I would wish to extend my gratitude to Prof. Paul Mwashimba Guyo, Pwani University for his guidance and support in providing valuable links during the PhD Programme. Thank you Dr. Cosmas Nzaka Munga for being a mentor. You have walked with me throughout proposal writing, funds acquisition, analysis and manuscript writing. Last but not least, my gratitude to Dr. Cromwell Kibiti for his guidance and support in thesis and manuscript writing. Thanks for always raising the red flag, it was elemental.

My sincere thanks to Dr. Mackenzie Nzaro, Chairperson in the Department of Pure and Applied Sciences and Dr. Udu Rahma, the former Dean School of Applied and Health Sciences for their great support. Laboratory technologists; Kennedy Agoi and Mercy Nyaribo of TUM, and Mackmillan Odhiambo of KMFRI for their support. I am grateful for all the support given to me by my colleagues in the Department of Pure and Applied Sciences.

I acknowledge and appreciate the support given to me by my family. First to my father, Mr. Simon Kiti Mbura for providing me and my siblings with equal opportunities for basic education. God bless you baba. My late mum, Jedilia Majala from whom I first learned that God answers the prayers of mortals and that victory belongs to everyone who believes. My husband Masai Mwawira for all your support. God bless your life. To our children for being a source of inspiration. Special thanks to Samuel Mazera, differently abled and for being the rate determining individual in all my undertakings; Esther Masai and Chao, always hopeful and cheerful, and Miriam Masai, though not understanding many things but conscious in many aspects that this study was taking a toll on me. Thank you, Miriam, for believing without understanding that somehow "mama" was doing the right things. For both the moral and financial support from my family, I am grateful. To my brother Samuel Jumaa, my sisters, Mrs. Ruth Wambua, Mrs. Claris Mbega, Mrs. Peninah Chesitoni for cheering me and believing in me. Thank you Rev. Silas Kazungu, Mrs. Lucy Mwandisha, Elizabeth Chacha, and Janet Kingi for your prayer support.

Finally, my uttermost gratitude goes to the Almighty God, the giver of life, the creator of the heavens and the earth. I am grateful for good health, the peace of mind, opening doors for funding, and causing the right people to come my way to either lead me, correct me or just encourage me along. I am forever grateful Lord. Thank you, Jesus.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION III

ACKNOWLEDGEMENT IV

TABLE OF CONTENTS IV

LIST OF FIGURES XII

LIST OF TABLES VI

LIST OF ABBREVIATIONS XX

ABSTRACT XXIII

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background Information 1

1. 2 Problem Statement 3

1.3 Justification of the Study 3

1.4 Significance of the Study 5

1.5 Objective 6

1.5.1 Overall Objective 6

1.5.2 Specific Objectives 6

1.6 Research Questions 7

1.7 Scope of the Study 7

CHAPTER TWO 8

LITERATURE REVIEW 8

2.1 Introduction 8

2.2 Infectious Diseases and Drug Resistance 8

2.2.1 Pathogenesis of Staphylococcus Aureus 10
2.2.1.1 Treatment and Control of *Staphylococcus Aureus* 10
2.2.2 Pathogenesis of *Escherichia coli* 11
Treatment and Control of *Escherichia coli* 12
2.3 Natural Bioactive Compounds 12
2.4 Sources of Natural Bioactive Compounds 14
2.4.1 Animal Sources of Natural Bioactive Products 14
2.4.2 Plants as Sources of Natural Bioactive Compounds 15
2.4.2.1 Alkaloids 16
2.4.2.2 Terpenoids 17
2.4.2.3 Saponins 18
2.4.2.4 Phenolics 18
2.4.2.5 Lipids 19
2.4.2.6 Polyketides 20
2.4.2.6.1 Biosynthesis of Beta Polyketides 21
2.4.2.7 Carbohydrates and Their Derivatives 23
2.4.2.8 Glycosides 24
2.4.3 Microorganisms as Sources of Natural Bioactive Compounds 24
2.4.3.1 Fungi 29
2.4.3.2 Endophytes 31
2.4.3.3 Endophytic Fungi 33
2.4.3.4 Fungi as Sources of Bioactive Compounds 34
2.4.3.5 Endophytic Fungi as Sources of Natural Bioactive Compounds 35
2.4.3.5.1 Aspergillus as a Source of Natural Bioactive Compounds 36
2.5 Mangroves as Reservoirs of Endophytic Fungi 37
CHAPTER THREE 42
MATERIALS AND METHODS 42
3.1 Introduction 42

3.2 Description of the Study Area 42
3.3 Preparation for Field and Laboratory Works 44
3.4 Experimental Procedures 44
3.5 Sampling Techniques and Illustrations 47
3.6 Ethical consideration 47
3.7 Sampling of Twigs from Selected Mangroves Species of Coastal Kenya 47
3.8 Sample Preparation 48
3.9 Isolation and Purification of Mangrove Fungal Endophytes 48
3.10 Morphological Diversity of Mangrove Fungal Endophytes 49
3.10.1 Cultural Identification of Fungal Isolates 50
3.10.2 Morphological Identification 50
3.11 Antimicrobial Properties of Mangrove Fungal Isolates 50
3.11.1 Small Scale Fermentation and Extraction of Fungal Metabolites 51
3.11.2 Preparation of Muller Hinton Agar Plates 52
3.11.3 In *vitro* Antimicrobial Susceptibility Test by Disk Diffusion Method 53
3.11.4 Determination of Minimum Inhibition Concentration (MIC) Using Micro Dilution Method 55
3.12 Molecular Phylogenetic Characterization of the Active Fungal Endophytes
3.12.1 Genomic DNA Extraction 56
3.12.2 Gel Electrophoresis 57
3.12.3 DNA Quantification 57
3.12.4 Polymerase Chain Reaction (PCR) 58
3.12.5 Quality Control 58
3.13 Extraction, Purification, Isolation and Characterization of Secondary Metabolites of Aspergillus flavus of Coastal Kenya 58
3.13.1 Mass Culture and Extraction of Secondary Metabolites 59
3.13.2 Chemical Isolation and Purification of Secondary Metabolites A. flavus 59
3.13.2.1 Thin Layer Chromatography of the Crude Extracts 60

3.13.2.2 Silica Gel Column Chromatography 60
3.14 Data Analysis 61
3.14.1 Determination of Colonization Rate, Isolation Rate and Relative Frequency 61
3.14.2 Determination of the Diversity of Fungal Isolates 62
3.14.3 Antimicrobial Data Analysis 63
3.14.4 DNA Sequence, Editing and Phylogenetic Analyses of the Endophytes 63
3.14.5 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Crude Ethyl Acetate Extracts *Aspergillus flavus* (MZ314728) 64
3.14.6 Nuclear Magnetic Resonance (NMR) Analysis of Pure Compounds 65
CHAPTER FOUR 67
RESULTS 67
4.1 Introduction 67
4.1.1 Culturally Identified Mangrove Fungal Endophytes 67
4.1.2 Morphologically Identified Mangrove Fungal Endophytes 68
4.1.3 Colonization and Isolation Rate 74
4.2 Biological Characteristics of Mangrove Fungal Endophytes 76
4.2.1 Preliminary Screening of Mangrove Fungal Extracts 76
4.2.2 Minimum Inhibition Concentration (MIC) of Crude Extracts 81
4.3 Molecular Characteristics of Selected Mangrove Fungal Endophytes 82
4.4 Extracted, Purified and Characterized Secondary Metabolites from *Aspergillus Flavus* 87
4.4.1 Crude Extracts of Aspergillus Flavus 87
4.4.2 Constituents of Ethyl Acetate Crude Extracts of *Aspergillus flavus* by GC-MS Analysis 88
4.8. Results of GC-MS analysis of ethyl acetate crude extracts of *Aspergillus flavus*
4.4.3 TLC Characterized Methanolic and Ethyl Acetate Crude Extracts of *A.*

lavus 89
4.5 Secondary metabolites of *Aspergillus flavus* 93
4.5.1 Methyl (E)-octadec-3-enoate (4.1) 93
4.5.2 Methyl (E)-tetracos-3-enoate (4.2) 100
4.5.3 Methyl 3-hydroxynonadecanoate (4.3) 108
4.5.4 (E)-octadec-3-enoic acid (4.4) 114
4.5.5 Methyl (E)-3-hydroxypentadec-5-enoate (4.5) 121
4.5.6 Flavulactone A (4.6) 128
4.5.7 Flavulactone B (4.7) 134
4.5.8 Flavulactone C (4.8) 140
4.5.9 Methyl (E)-5-hydroxynonadec-9-enoate (4.9) 146
4.5.10 Flavulactone D (4.10) 151
4.5.11 Flavulactone E (4.11) 157
CHAPTER FIVE 160
DISCUSSION 160
5.1 Diversity of Mangrove Fungal Endophytes 160
5.1.1 Host Recurrence of Mangrove Endophytic Fungi 162
5.1.2 Spatial Heterogeneity of Mangrove Endophytic Fungi 163
5.2 Biological Characteristics of Mangrove Fungal endophytes of Coastal Kenya
5.4 Mangrove Fungal Endophytes of Coastal Kenya 166
5.5 Secondary Metabolites from Crude Extracts of *Aspergillus flavus* 166
5.5.1 Secondary Metabolites from *Aspergillus flavus* 167
CHAPTER SIX 172
CONCLUSIONS AND RECOMMENDATIONS 172
6.1 Conclusions 172
6.2 Recommendations 173
REFERENCE 175

LIST OF APPENDICES 196

Appendix I: TUM ERC Certificate 196

Appendix II. Manuscript 1 197

Appendix III: Manuscript 2 198

Appendix IV: GC-MS fragmentation for 4.1- 4.8 199

LIST OF FIGURES

Figure 2.1: Schematic diagram of the reaction occurring in polyketide synthases (PKSs); ACP, acyl career protein; AT, acyltransferase; KS, ketosynthase; KR, ketoreductase; DH, dehydratase; ER, enoylreductase (*Adopted from Risdian et al., 2019*) 23

Figure 2.2.The fungal families (*Source, https://www.chegg.com 30/3/2022*)..... 30

Figure 3.1 A map showing the Mangrove forests of Gazi Bay, Tudor and Mida creeks along Kenya coast……..43

Figure 3.2 A section of degraded mangroves forest of Gazi Bay Kwale County..44

Figure 3.3 Schematic diagram of isolation, determination of bioactivity, phylogenetic analysis and characterization of mangrove……....46

Figure 3.4 A schematic diagram of isolation of fungal isolates from selected mangroves of coastal Kenya……...49

Figure 3.5 A schematic diagram of small-scale fermentation and extraction of Mangrove fungal isolates from selected mangrove species of Coastal Kenya….52

Figure 3.6 Bioassay and preliminary GC-MS analysis of secondary metabolites……..54

Figure 4.1.A section of the culturally identified mangrove fungal endophytes from the twig cuttings of 4 mangrove species of coastal Kenya (a) *A. marina* (b) *C. tagal* (c) *R. mucronata* (d) *S. alba*..... 68

Figure 4.2.Morphologically identified mangrove endophytic fungal genera from selected mangrove species of coastal Kenya; (h) *Aspergillus*; (i) *Penicillium*; (j)

Fusarium; (k) *Cephalosporium*; (l) *Blastomyces* 69

Figure 4.3. Colony morphology of *Aspergillus*: A) front view; and B) Microscopic features of *Aspergillus* (x100) 71

Figure 4.4. Colony morphology of *Penicillium*: A) front view; and B) Microscopic features of *Penicillium* (x100) 71

Figure 4.5. Colony morphology of *Fusarium*: A) front view; and B) Microscopic features of *Fusarium*(x100) 72

Figure 4.6.Colony morphology of *Cephalosporium*: A) front view; and B) Microscopic features of *Cephalosporium*(x100) 72

Figure 4.7. Colony morphology of *Blastomyces*: A) front view; and B) Microscopic features of *Blastomyces*(x100) 73

Figure 4.8.spatial distribution of mangrove endophytic fungal genera in (e) Tudor Creek, (f) Gazi Bay and (g) Mida Creek 74

Figure 4.9. Colonization of mangrove fungal endophytes sampled along the Kenya coast..... 75

Figure 4.11. Mean inhibition diameters of ethyl acetate crude extracts against *Staphylococcus aureus* (SA) and *Escherichia coli* (EC) 81

Figure 4.12.PCR Amplicons (approximately 500 base pairs) of its region of endophytic fungi isolated from selected mangrove species of Coastal Kenya..... 83

Figure 4.13. Phylogenetic tree of 9 unique fungal isolates of Coastal Kenya rooted from *Fusarium oxysporum* 85

Figure 4.14.(A) A brown gummy ethyl acetate crude extract (B) a brown waxy solid of ethyl acetate crude extract 88

Figure 4.15. Silica gel column chromatography fractions of the methanolic crude extracts of *Aspergillus flavus* 91

Figure 4.16. Silica gel column chromatography fractions of the ethyl acetate crude extracts of *Aspergillus flavus* 92

Figure 4.17. Important HMBC correlation diagram of methyl (E)-octadec-3-enoate (4.1)..... 94

Figure 4.18.MS fragmentation for methyl (E)-octadec-3-enoate (4.1) 95

Figure 4.19.^{1}H NMR spectrum of methyl (E)-octadec-3-enoate (4.1)..... 97

Figure 4.20.^{13}C NMR and DEPT NMR spectra of methyl (E)-octadec-3-enoate (4.1) 98

Figure 4.21.HCOSY plot for methyl (E)-octadec-3-enoate (4.1) 99

Figure 4.22.HSQC plot for methyl (E)-octadec-3-enoate (4.1)..... 99

Figure 4.23. HMBC plot for methyl (E)-octadec-3-enoate (4.1)..... 100

Figure 4.24.Important HMBC Correlation diagram of methyl (E)-tetracos-3-enoate (4.2)..... 102

Figure 4.25. MS fragmentation of methyl (E)-tetracos-3-enoate (4.2) 102

Figure 4.26.^{1}H NMR spectrum of methyl (E)-tetracos-3-enoate (4.2) 104

Figure 4.27.^{13}C NMR and DEPT NMR spectra of methyl (E)-tetracos-3-enoate (4.2).... 105

Figure 4.28.^{1}H COSY plot for methyl (E)-tetracos-3-enoate (4.2)..... 106

Figure 4.29.HMBC plot for methyl (E)-tetracos-3-enoate (4.2)..... 107

Figure 4.30.Important HMBC Correlation for methyl 3-hydroxynonadecanoate (4.3) . 109

Figure 4.31. MS fragmentation for methyl 3-hydroxyoctadecanoate (4.3) 109

Figure 4.32.^{1}HNMR spectrum of methyl 3-hydroxyoctadecanoate (4.3)..... 111

Figure 4.33. ^{13}C NMR and DEPT NMR spectral data of methyl 3-hydroxyoctadecanoate (4.3) 112
Figure 4.34. ^{1}H COSY plot for methyl 3-hydroxyoctadecanoate (4.3) 113
Figure 4.35. HSQC plot for methyl 5-hydroxyoctadecanoate (4.3) 113
Figure 4.36. HMBC plot for methyl 3-hydroxyoctadecanoate (4.3) 114
Figure 4.37: HMBC Correlation diagram of (E)-octadec-3-enoic acid (4.4) 115
Figure 4.38.MS fragmentation for (E)-octadec-3-enoic acid (4.4) 116
Figure 4.39.^{1}H NMR spectrum of (E)-octadec-3-enoic acid (4, 4) 118
Figure 4.40.^{13}C NMR and DEPT NMR spectra for (E)-octadec-3-enoic acid (4.4) 119
Figure 4.41.^{1}H COSY plot for (E)-octadec-3-enoic acid (4.4) 120
Figure 4.42. HSQC plot for (E)-octadec-4-enoic acid (4.4) 120
Figure 4.43.HMBCplot for (E)-octadec-3-enoic acid (4.4) 121
Figure 4.44.Important HMBC Correlation of methyl (E)-3-hydroxypentadec-5-enoate (4.5). 122
Figure 4.45. MS fragmentation for methyl (E)-3-hydroxypentadec-5-enoate (4.5). 123
Figure 4.46.^{1}H NMR spectrum of methyl (E)-3-hydroxypentadec-5-enoate (4.5) 125
Figure 4.47.^{13}C NMR and DEPT NMR spectra of Methyl (E)-3-hydroxypentadec-5-enoate (4.5) 126
Figure 4.48. ^{1}H COSY plot for methyl (E)-3-hydroxypentadec-5-enoate (4.5) 127
Figure 4.49. HSQC plot for methyl (E)-3-hydroxypentadec-5-enoate (4.5) 127
Figure 4.50. HMBC plot for methyl (E)-3-hydroxypentadec-5-enoate (4.5) 128
Figure 4.51. Important HMBC correlations for Flavulactone A (4.6) 129

Figure 4.52.^{1}H NMR for Flavulactone A (4.6) 131

Figure 4.53.^{13}C NMR and DEPT NMR for Flavulactone A (4.6) 132

Figure 4.54.^{1}H COSY plot for Flavulactone A (4.6) 133

Figure 4.55. HSQC plot for Flavulactone A (4.6) 133

Figure 4.56. HMBC plot for Flavulactone A (4.6) 134

Figure 4.57.Important HMBC correlations of Flavulactone B (4.7) 135

Figure 4.58.^{1}H NMR spectrum of Flavulactone B (4.7) 137

Figure 4.59.^{13}CNMR and DEPT NMR spectra of Flavulactone B (4.7) 138

Figure 4.60.^{1}H COSY plot for Flavulactone B (4.7) 139

Figure 4.61.HSQCplot for compound Flavulactone B (4.7) 139

Figure 4.62.^{1}HMBC plot for Flavulactone B (4.7) 140

Figure 4.63. Important HMBC correlations of Flavulactone C (4.8) 141

Figure 4.64.^{1}H NMR for Flavulactone C (4.8) 143

Figure 4.65.^{13}C NMR and DEPT NMR spectral data of Flavulactone C (4.8) 144

Figure 4.66.^{1}H COSY plot for Flavulactone C (4.8) 145

Figure 4.67. HSQC plot for Flavulactone C (4.8) 145

Figure 4.68. HMBC plot for Flavulactone C (4.8) 146

Figure 4.69.HMBC correlations of Methyl (E)-5-hydroxynonadec-9-enoate (4.9) 147

Figure 4.70.^{1}H NMR for methyl (E)-5-hydroxynonadec-9-enoate (4.9) 149

Figure 4.71.^{1}H COSY of methyl (E)-5-hydroxynonadec-9-enoate (4.9) 150

Figure 4.72: HSQC correlations of methyl (E)-5-hydroxynonadec-9-enoate (4.9) 150

Figure 4.73. HMBC correlations of methyl (E)-5-hydroxynonadec-9-enoate (4.9) 151

Figure 4.74: HMBC correlations for Flavulactone D (4.10) 152

Figure 4.75: ^{1}H NMR for Flavulactone D (4.10) 154

Figure 4.76: ^{13}C NMR of Flavulactone D (4.10) 155

Figure 4.77:^{1}H, H- COSY NMR for Flavulactone D (4.10) 156

Figure 4.78: HSQC correlations for Flavulactone D (4.10) 156

Figure 4.79. HMBC correlations of Flavulactone D (4.10) 157

Figure 4.80. HMBC correlations of methyl (E)-5-(4-hydroxy-2-((E)-3-hydroxytetradec-1-en-1-yl)-6-oxotetrahydro-2H-pyran-3-yl) pent-3-enoate (4.11) 158

Figure 5.1: Biosynthetic pathway for methyl-3-hydroxyoctadecanoate (4.3) 168

Figure 5.2 Joint biosynthetic pathway of compound 4.1 to 4.5 169

Figure 5.3: Folding and cyclization of 5-hydroxyhexadecan-2-one in flavulactone B formation 170

Figure 5.4: Suggested biogenetic pathway for compounds 4.6, 4.7 and 4.8 171

LIST OF TABLES

Table 2.1: Selected mangrove species of Kenya and their uses (*Source*Bandaranayake, 1998) ..... **Error! Bookmark not defined.**9
Table 4.1: Morphological characteristics of fungal mangrove endophytes isolated from selected study sites in coastal Kenya 70
Table 4.2. Colonization and isolation rates of mangrove fungal endophytes sampled along the Kenyacoast 75
Table 4.3. Antibacterial activities of mangrove fungal isolates against selected pathogens from coastal Kenya 78
Table 4.4. Mean inhibition diameters for *S. aureus* and *E. coli* at different concentrations 80
Table 4.5. Minimum inhibition concentration in mg/ml of ethyl acetate extract of *Aspergillus flavus* from Coastal Kenya 82
Table 4.6: Concentration and Purity of DNA Extract 83
Table 4.7. Mangrove Fungal Isolates Identified by Molecular Techniques 86
4.8. Results of GC-MS analysis of ethyl acetate crude extracts of *Aspergillus flavus* 89
Table 4.9. ^{1}H NMR and ^{13}C NMR(400 MHz) spectral data for methyl (E)-octadec- 96
Table 4.10. ^{1}H NMR and ^{13}C NMR(400 MHz) spectral data for methyl 3-ydroxyoctadecanoate (4.2) 103
Table 4.11. ^{1}H NMR and ^{13}C NMR(400 MHz) spectral data for (E)-octadec-3-enoic acid(4.3) 110
Table 4.12.^{1}H NMR and ^{13}C NMR(400 MHz) spectral data for methyl (E)-3-h 117
Table 4.13. ^{1}H NMR and ^{13}C NMR(400 MHz) spectroscopic data for Flavulactone A (4.6) 124
Table 4.14: ^{1}H NMR and ^{13}C NMR(400 MHz) spectra for Flavulactone B (4.7) 130
Table 4.15: ^{1}H NMR and ^{13}C NMR(400 MHz) spectral data of Flavulactone C (4.8) 136
Table 4.16. ^{1}H NMR and ^{13}C NMR(400 MHz) spectroscopic data for methyl (E)-5-hydroxynonadec-9-enoate (4.9) 142

Table 4.17 ^{1}H NMR and ^{13}C NMR(400 MHz) spectroscopic data ofFlavulactone D (4.10)
Table 4.18. ^{1}H NMR and ^{13}C NMR(400 MHz) spectroscopic data formethyl (E)-5-(4-hydroxy-.....153
Table 4.19. ^{1}H NMR and ^{13}C NMR (400 MHz) spectroscopic data formethyl (E)-5-(4-hydroxy-2-(E)-3-hydroxytetradec-1-en-1-yl)-6-oxotetrahydro-2H-pyran-3-yl) pent-3-enoate(4.11)……...159

LIST OF ABBREVIATIONS

MFEs	Mangrove fungal endophytes
µ	Micro
µg	Microgram
µL	Microliter
^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{13}C	Carbon thirteen isotope
1D	One dimensional NMR
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{1}H	Proton
2D	Two-dimensional NMR
3α-HSD	3α-hydroxysteroid dehydrogenase
ACD	Advance chemistry development
AF_{1-16}	*Aspergillus flavus*1-16
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
ATCC	American type culture collection
BLAST	Basic alignment search tool
CFU	Colony Forming Units
CO_2	Carbon Dioxide
COSY	Correlation Spectroscopy
COVID	Corona Virus Disease
COX	Chemiluminescent Cyclooxygenase
CTAB	Cetyl Trimethylammonium Bromide
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid

EAE	Ethyl Acetate Extract
FAME	Fatty acid methyl ester
EtOH	Ethanol
g	gram
GC-MS	Gas chromatography-Mass spectrometry
H1N1	Influenza virus
Ha	Hectare
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Simple Quantum Correlation
HUS	Hemolytic uremic syndrome
IDs	Infectious Diseases
IR	Infrared
ITS	Internal transcribed spacer
IZDs	Inhibition zone diameters
KMFRI	Kenya Marine and Fisheries Research Institute
MDG	Millennium development goal
MDR	Multi Drug Resistance
ME	Methanolic extract
MEGA	Molecular evolutionary genetics analysis
MeOH	Methanol
MHA	Muller Hinton Agar
MIC	Minimum inhibition concentration
mL	Milli Litre
MS	Mass Spectrometry
MUSCLE	Multi sequence Alignment
NA	Nutrient Agar
NACOSTI	National Commission for Science, Technology and Innovation
$NaHCO_3$	Sodium hydrogen sulphate
NC	Non-Clavicipitaceous

NCBI	National center for biological information
NMR	Nuclear magnetic resonance
NRF	National Research Fund
^{o}C	Degrees Celsius
PCR	Polymerase chain reaction
PDA	Potato dextrose Agar
PDB	Potato dextrose broth
SARS	Severe acute respiratory syndrome
SD	Standard deviation
SE	Standard error
TLC	Thin layer chromatography
TUM	Technical University of Mombasa
TUM ERC	Technical University of Mombasa Ethical Review Committee
UV	Ultraviolet
WHO	World Health Organization
WIOMSA	Western Indian Ocean Marine Science Association
ZIDs	Zone Inhibition Diameters
SDG	Sustainable development goals
MDH	Malate dehydrogenase
TMS	Tetramethyl silane
MRSA	Methicillin-resistant *Staphylococcus aureus*
PKS	Polyketide Synthase
MHB	Mycorrhiza helper bacteria

ABSTRACT

Due to the global spread of resistant bacteria and fungi, antibiotics are no longer as effective as they once were, and microbial illnesses are once more a threat to human life. This situation is offering a continuous opportunity for research of alternative novel bioactive molecules to address the problem. In this study we isolated and characterized bioactive compounds from selected endophytic fungi originating from common mangroves of the Kenya coastline namely; *Avicennia marina*, *Ceriops tagal*, *Rhizophora mucronata* and *Sonneratia alba* against *Staphylococcus aureus* and *Escherichia coli* that could potentially be used for drugs development. Isolation and purification using potato dextrose agar (PDA), potato dextrose broth (PDB) gave 19 mangrove fungal endophytes (MFEs). Morphological identification resulted in 18 MFEs belonging to 5 fungal genera namely; *Aspergillus, Penicillium, Fusarium, Cephalosporium* and *Blastomyces*. Molecular identification gave 9 successfully characterized species belonging to the genus *Aspergillus* namely; *Aspergillus flavus, Aspergillus niger, Aspergillus nomius, Aspergillus tubingensis, Aspergillus oryzae, Aspergillus awamori, Aspergillus aculeatus, Aspergillus brunneoviolaceus* and *Aspergillus welwitchiae.* Ethyl acetate extract (EAE) and methanolic extract (ME) were produced through fermentation and extraction with ethyl acetate and ethyl acetate in 10% methanol. The extracts contained alkaloids, phenols, steroids, tannins, and terpenoids, according to preliminary phytochemical screening. *Aspergillus flavus* ethyl acetate extracts showed maximum activity when tested for susceptibility via disk diffusion on Muller Hinton Agar, with inhibition zone diameters (IZDs) Standard deviation (SD) of (17.1 ± 2.45) and (15.9 ± 2.45) against S. *aureus* and *E. coli*, respectively. *Aspergillus flavus* methanolic extracts had a lower inhibition activity (9.2 ± 0.75) and no action against *E. coli* at the investigated doses as compared to the positive control. The minimum inhibitory concentration of *A. flavus* crude extract against *S. aureus* and *E. coli* was (0.82 ± 0.05) and (0.91 ± 0.05) mg/ml, respectively. Results of one-way ANOVA test indicated no significant difference in the inhibition between the two test pathogens ($p > 0.05$). Results of Gas Chromatography coupled with Mass Spectrometry (GC-MS) analysis of ethyl acetate crude extracts of *A. flavus* revealed four known active compounds namely; Lactic acid, Isopropyl alcohol, Semi carbazone and Corydaldine. All of them were active against a broad spectrum of pathogens including *S. aureus* and *E. coli* in this study. Fractionation using Silica gel (60-120) mesh column chromatography of the antimicrobial *A. flavus* methanolic and ethyl acetate extract, gave 11 compounds which when characterized by TLC resulted in pure compounds with different RF values. The isolates were established to be the new δ-lactones Flavulactone B, Flavulactone C, Flavulactone D, Flavulactone E, and Flavulactone F which were all new compounds alongside the known 6-tridecyloxan-2-one now named Flavulactone A. The other oxy-compounds were the new fatty acid methyl esters; methyl (E) - octadec-3-enoate, methyl (E)- tetracos-3-enoate and Methyl (E)-3-hydroxypentadec-5-enoate, methyl (E)-5-hydroxynonadec-9-enoate and the known methyl 3-hydroxynonadecanoate and (E)-octadec-3-enoic acid. The study confirms that

bioactive metabolites indeed reside in endophytic fungi inhabiting selected mangroves from coastal Kenya.